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Electron Microscopic Observations of Satellite Cells with Special Reference to the Development of Mammalian Skeletal Muscles*

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Using the electron microscope, MAURO (1961) found certain cells intimately associated with muscle fibers in the skeletal muscles of frogs and called them "satellite cells". The satellite cell lies between the plasma membrane and the basement membrane of the muscle fiber, and thus is enveloped by the basement membrane of the muscle fiber.

Similar cells were observed in some mammalian skeletal muscles (see MAURO, 1961; VENABLE, 1964) and in brachyuran cardiac muscles (MIDZUKAMI, 1964).

Such cells, closely associated with the muscle fibers, have not been reported so far by light microscopy. Indeed, with the light microscope, the nuclei of the satellite cells appear to be indistinguishable from those of the muscle fibers proper.

Although MAURO proposed several hypotheses, the origin and role of the satellite cell are unknown. The work reported here deals with the satellite cells with special reference to the development of mammalian skeletal muscles using the electron microscope.

I. Materials and Methods

Gastrocnemius muscles from human fetuses, young and adult humans, cats, and dogs were used for this study. Specimens were carefully dissected out and fixed for three hours in cold one per cent osmium tetroxide dissolved in sodium phosphate buffer with the pH 7.3 according to the method described by MILLONIG (1962). Most of the specimens were pre-fixed for three hours in 0.6 M glutaraldehyde in the phosphate buffer. During pre-fixation, the materials were cut into small blocks. The specimens were dehydrated through a series of alcohol or acetone and embedded in Epon 812 (LUFT, 1961). Thin sections were stained with lead hydroxide or uranyl acetate, and examined with a Hitachi HU-11A electron microscope.

II. Observations

1. Satellite cells in mature skeletal muscles

The satellite cells seen in the human mature muscles (3, 4, 5, 10, 13 and 22 years of age) are similar in appearances to those described by MAURO (1961) in frog muscles. These cells are the small cells which are intimately associated with muscle fibers and contain no cytoplasmic fibrous elements. The cytoplasm is remarkably scanty in comparison to the nucleus. The satellite cell is wedged between the plasma membrane and the basement membrane of the muscle fiber. In other words, the satellite cell touches the muscle fiber without the intervention of a basement membrane, and is enveloped by the basement membrane common to itself and the muscle fiber (Fig. 1). This cell could not be identified in the light micrographs of our mature muscles.

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In mature skeletal muscles, the nuclei of the muscle fibers occur much more frequently in sections than those of the satellite cells. The latter seems to be rather sparsely scattered throughout the muscle fibers. High-powered electron micrographs indicate that the plasma membranes of the satellite cell and the muscle fiber are separated from each other by an interspace of about 150 Å. These plasma membranes are either wavy or straight in appearance. On the inner surface of the satellite cell, its plasma membrane is in apposition with that of

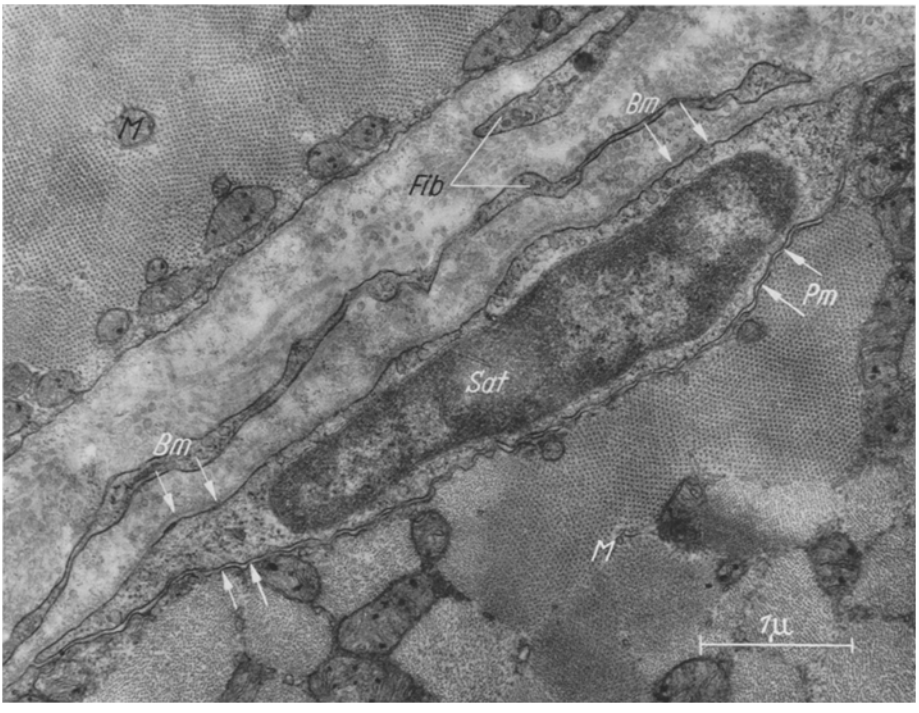


Fig. 1. Electron micrograph of a satellite cell *Sat* in a mature muscle. 10 year old boy. The satellite cell lies between the plasma membrane *Pm* and the basement membrane *Bm* of a muscle fiber *M*, and protrudes inward, pushing the myofibrils of the muscle fiber aside. *Fib* Fibroblast. Osmium tetroxide fixation. Lead stained. ×20000

the muscle fiber, but there is neither a desmosome-like structure nor a cytoplasmic fusion between these two types of cell. Not only on the outside surface of the satellite cell, but also on its inner surface, many caveolae intracellulares or pinocytotic vesicles are seen. In addition, the cell contains a small number of mitochondria and the vestiges of a granular endoplasmic reticulum. Occasionally centrioles and Golgi substances are also observed in the cytoplasm. The nucleus occupies the major part of the cell, is ovoid to slightly elongated in shape, and is oriented lengthwise to the muscle fiber. The nucleoplasm is rather unevenly dispersed, more so than that of the muscle fiber; otherwise there seems to be no significant difference between the nucleoplasm of these two cells.

2. Satellite cells in developing skeletal muscles

In mature skeletal muscles, it is accepted that a satellite cell is a small cell which is in intimate contact with the muscle fiber and is enveloped by the base-

ment membrane which is common to both the muscle fiber and the satellite cell. For this reason, it is only possible to identify the satellite cell after the basement membrane is formed around the muscle fiber. From this point of view, our observations did not deal with the muscle in its earliest embryonic development, but started at the early muscle fiber stage shortly before the basement membrane became electron-microscopically visible in stained sections.

a) Early muscle fiber stage. The term "early muscle fiber" has been used to designate a mononucleated or multinucleated elongated cell which has the general

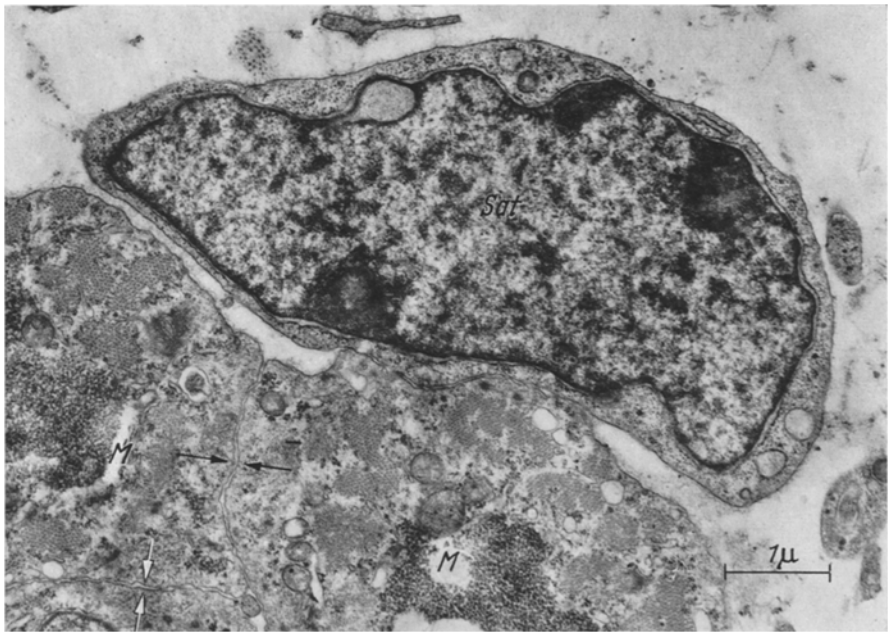


Fig. 2. A fibroblast-like cell, a presumable satellite cell *Sat*, closely associated with a muscle cell *M*. 10 week old human fetus. The fibroblast-like cell is different in appearance from the muscle cell. A basement membrane can be barely be identified. Several muscle cells *M* are in close contact with each other to form a bundle. Note the apposing plasma membranes of the muscle cells (arrows). Glutaraldehyde and osmium tetroxide fixation. Lead stained. ×14000

form of a fiber but whose cytoplasm is not filled up with myofibrils (HAY, 1963). This muscle cell is traditionally referred to as a "myotube" (see BOYD, 1960).

The gastrocnemius muscle of a human fetus 40 mm in C. R. length (10 week old embryo) is in an early muscle fiber stage. Cylindrical muscle cells show centrally placed nuclei and peripheral myofibrils. Five to ten, sometimes more than twenty muscle cells are grouped into a bundle. In electron micrographs, one can clearly see the apposition of the plasma membranes between adjacent muscle cells. They are in close contact with each other separated only by a 150 Å interspace. Thus, between the apposed plasma membranes the presence of a basement membrane is not possible because of the narrow interspace between the membranes. Although the apposed plasma membranes, in longitudinal sections, are occasionally found sectioned tangentially or appear to be discontinuous possibly due to artificial breakage, no cytoplasmic fusion between the adjacent myotubes

can be observed. However, the confusion arising from tagentially sectioned apposed plasma membranes regarding cytoplasmic fusion does not occur in cross-sectioned material.

In the same stage, the basement membrane can barely be identified along the side of the muscle fiber, while at the end of the fiber it is more easily identifiable. Both light and electron micrographs indicate that several fibroblast-like

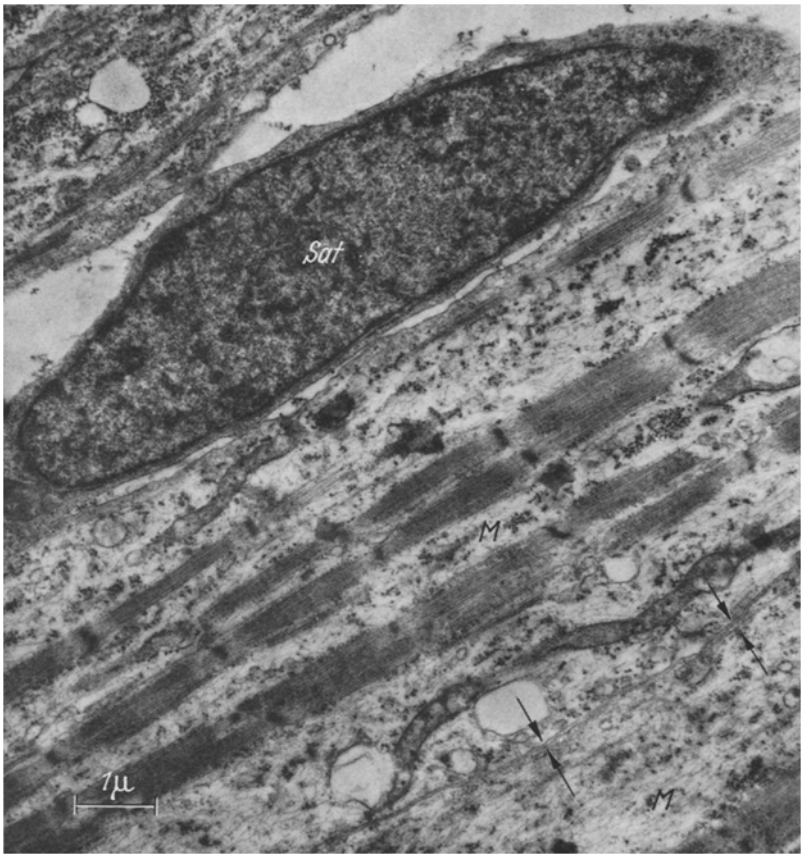


Fig. 3. A longitudinal view of a fibroblast-like cell *Sat*. 10 week old human fetus. Note the apposing plasma membranes (arrows) of two adjacent muscle cells *M*. Uranyl acetate stained. ×10000

cells with denser nuclei than the muscle cells are seen closely associated with the bundle of the muscle cells (Figs. 2 and 3). These fibroblast-like cells are similar in appearances to the fibroblasts existing in the peripheral loose connective tissue; that is, they have deeply and unevenly stained nuclei with a dense appearance. These cells also contain a somewhat well developed granular endoplasmic reticulum and numerous free ribosomes; however, cytoplasmic fibrillar components or glycogen particles are barely discernible in the cytoplasm. In contrast, the myotubes proper have clear nuclei with evenly distributed nucleoplasm and prominent nucleoli, and are rich in glycogen particles as well as free ribosomes, myofibrils, fibrillar structures in the cytoplasm and vesicular structures. However, the myotubes are very poor in granular endoplasmic reticulum. The associated

fibroblast-like cell can, thus, be definitely distinguished from the muscle cell. The former does not seem to be essentially different in appearance from the mesenchymal cell. Sometimes, a few fibroblast-like cells lie one upon another in apposition to the myotube. A single fibroblast-like cell may be closely attached to one or two myotubes. Such a cell frequently intervenes between the myotubes. Occasional fibroblast-like cells are seen deep inside the bundle of the myotubes

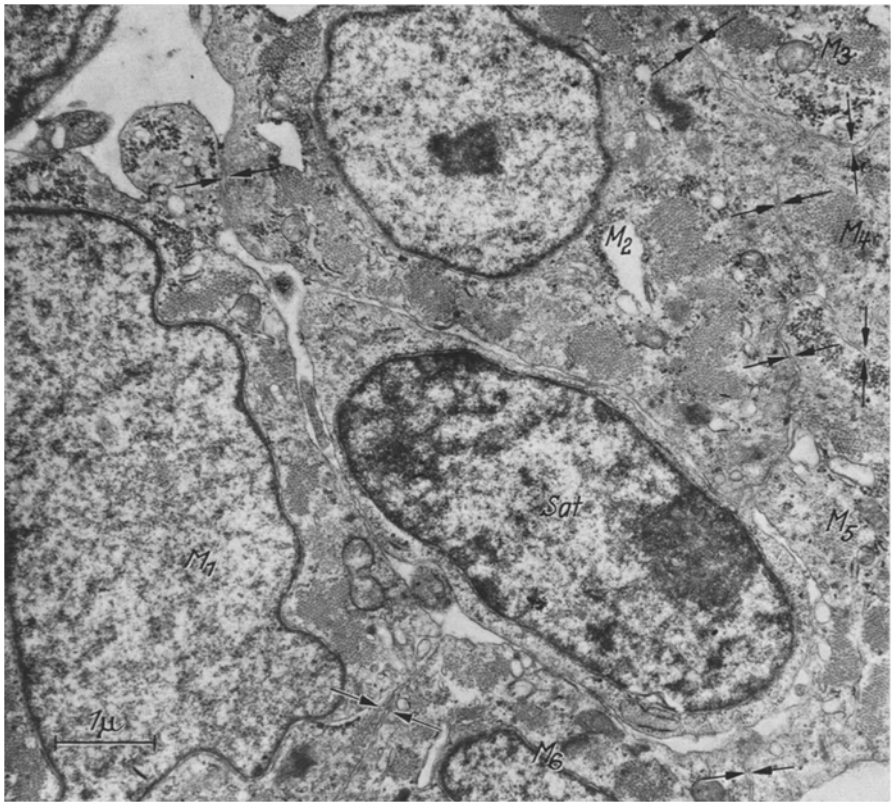


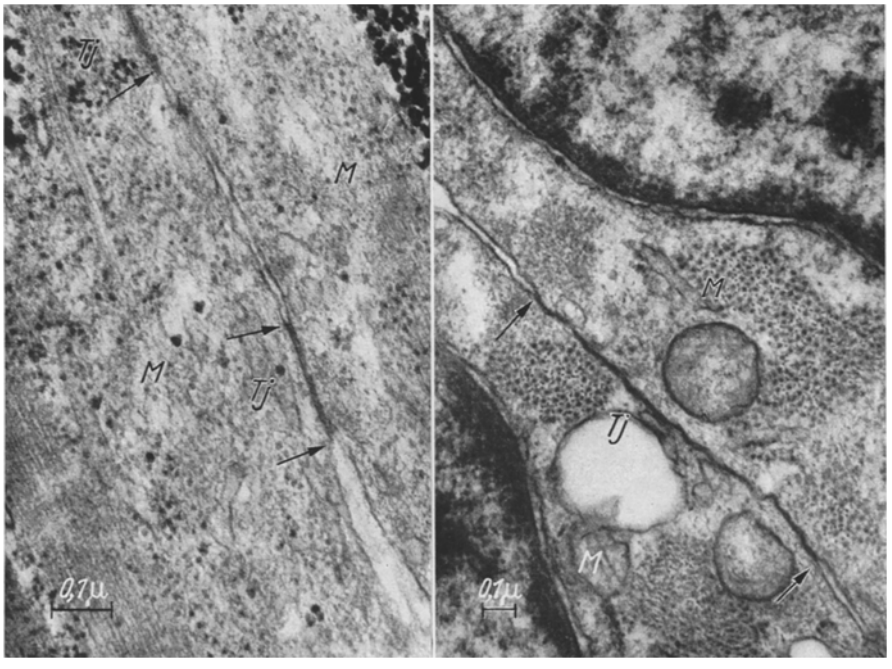
Fig. 4. A fibroblast-like cell *Sat* seen deep inside the bundle of muscle cells *M*. 10 week old human fetus. Note the opposing plasma membranes of adjacent muscle cells (arrows). × 13000

(Fig. 4). At this stage, a basement membrane can hardly be detected along the side of the muscle cells. Therefore, it can simply be said that the fibroblast-like cells are in contact with the myotubes. There seems to be neither simple adhesion nor cytoplasmic fusion between these two types of cell. These cells are delimited by a definite plasma membrane with a narrow interspace so that one cannot expect the presence of a basement membrane between them, as in the case of the myotubes themselves.

In this stage, the question arises whether or not these fibroblast-like cells closely associated with the myotubes can be regarded as "satellite cells". In the subsequent course of the development of the muscle, as described below, a number of cells showing similar appearances and located at similar positions are observed associated with the muscle fibers closely enough as to be accepted as "satellite

cells". Thus, it seems to be reasonable to conclude that the satellite cells may be derived from these fibroblast-like cells.

In the 13-week period (70 mm C. R. length), the myotube has acquired a greater diameter. The myofibrils are more abundant and thicker, but the central portion which corresponds to the pale axial core in light micrographs is occupied by an abundance of glycogen particles. Several muscle fibers are in intimate contact with each other to form a bundle. Intercellular junctions are rarely found between the apposed plasma membranes of adjacent muscle fibers similar to



a b

Fig. 5a and b. Tight, quintuple-layered junctions *Tj* seen between the apposed plasma membranes of two adjacent muscle fibers *M*. 13 week old human fetus. A longitudinal section *a* and a transverse section *b* of the muscle fibers. Glutaraldehyde and osmium tetroxide fixation. Lead stained. *a* × 75000. *b* × 37000

those first noticed in the cardiac muscles of the guinea-pig and the mouse by SJÖSTRAND, ANDERSSON-CEDERGREN and DEWEY (1958). In our specimens fixed by both glutaraldehyde and osmium tetroxide, this structure shows quintuple layers, and the total width of it is about 110 Å (Fig. 5a and b). This finding occurs only in the early muscle fiber stage. Some fibroblast-like cells adhere to the surfaces of the myotubes, others intervene between two or more myotubes, as seen in the previous stage (Fig. 6). These fibroblast-like cells do not contain glycogen particles in the cytoplasm. The basement membrane component of the muscle fiber begins to appear. The indistinct presence of the basement membrane can be detected in places along the side of the muscle fiber. However, it is not evident at this stage that the basement membrane also envelopes the fibroblast-like cell in common with the related muscle cell.

In the leg muscles of human fetuses, 10 and 13 weeks of age, certain muscle cells with the cytoplasmic alteration which was described with the light microscope

as physiologic degeneration (e. g. GODLEWSKI, 1902; HÄGGQVIST, 1931; GLÜCKMANN, 1934) or retrograde metamorphosis (e. g. BARDEEN, 1900; SPEIDEL, 1938) are frequently found among the intact myotubes. In the cytoplasm, the structure which corresponds to the dark-staining material with iron hematoxylin in light micrographs is found to be composed of fine fibrils of about 50 Å in thickness, closely packed into bundles or masses. Such fibrous dense materials show various degrees of irregularity in arrangement, but may exhibit continuity with the myofibrils in the same cytoplasm. The nuclei are pyknotic in appearance. Vesiculation occurs especially beneath the plasma membrane which then shows

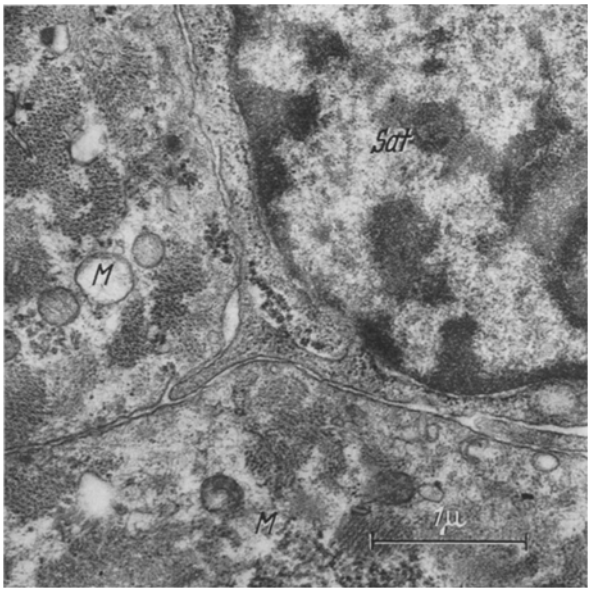


Fig. 6. Portion of a fibroblast-like cell *Sat* intervening between two adjacent muscle fibers *M*. 13 week old human fetus. × 22000

a ruffled appearance. A detailed description will be made in a separate paper. Fibroblast-like cells are also applied to the muscle cell with these "degenerative" changes.

b) Formed muscle fiber stages. According to HAY (1963), the formed muscle fiber is defined as a cell in which myofibrils fill most of the cytoplasm.

At the 15th-week (100 mm fetal length), groups of muscle fibers begin to be separated into individual fibers. However, two or more of the fibers are still in close contact with each other. The muscle fibers which had formed a bundle in the previous stage lie together and are surrounded by loose connective tissue, resulting in a muscle fascicle in a subsequent stage of development. The muscle fiber begins to be filled with myofibrils throughout its cytoplasm. The nuclei of the muscle fiber show a tendency to move toward the periphery. The basement membrane becomes distinct along the side of the muscle fiber. The fibroblast-like cells are found closely associated with the muscle fibers and are usually enveloped by a common basement membrane (Fig. 7). Since such an intimate connection between the fibroblast-like cell and the muscle fiber satisfies the

criteria for the classification of "satellite cell" presented by MAURO (1961), the fibroblast-like cell may be regarded as a "satellite cell" from this stage of development onward. The satellite cells contain granular endoplasmic reticulum, Golgi materials, centrioles and numerous free ribosomes, but neither fibrous structures nor glycogen particles in the cytoplasm. They have several projections, which extend along the side of the muscle fiber. The cell body and the projections

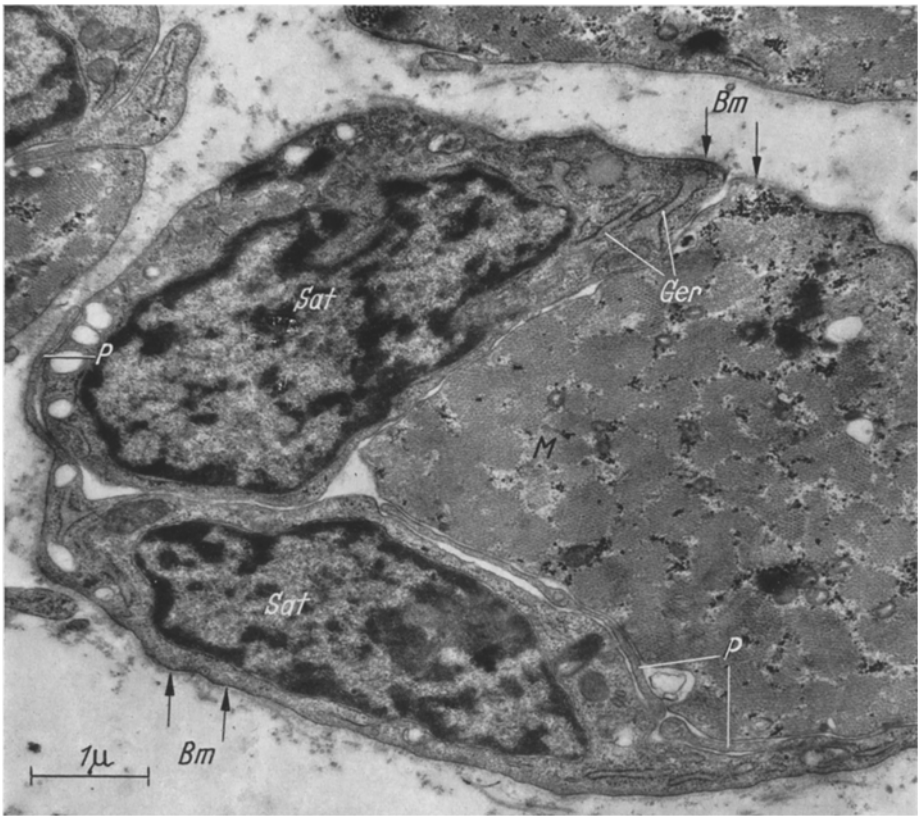


Fig. 7. Two satellite cells *Sat* in apposition side by side to a muscle fiber *M*. 15 week old human fetus. They are all enveloped by a single basement membrane *Bm*. Note well developed granular endoplasmic reticulum *Ger* and projections *P* of the satellite cells. × 16000

are seen intervening between two adjacent muscle fibers. Occasionally, two satellite cells are in apposition, side by side to a muscle fiber in cross-section. In this case both satellite cells are in direct contact with each other without the interposition of a basement membrane between them (Fig. 7). It seems that this may result from the mitosis of a satellite cell precursor in a certain earlier embryonic stage.

In the 18- (140 mm), 19- (147 mm) and 20-week (160 mm C. R. length) fetuses, the muscle fibers have become still larger in diamter and filled with well-developed myofibrils. Generally, therefore, the ratio of the size of the satellite cell to that of the muscle fiber has become smaller, since the former seems to remain the same size as it was in the previous stages of development. Two or three muscle fibers

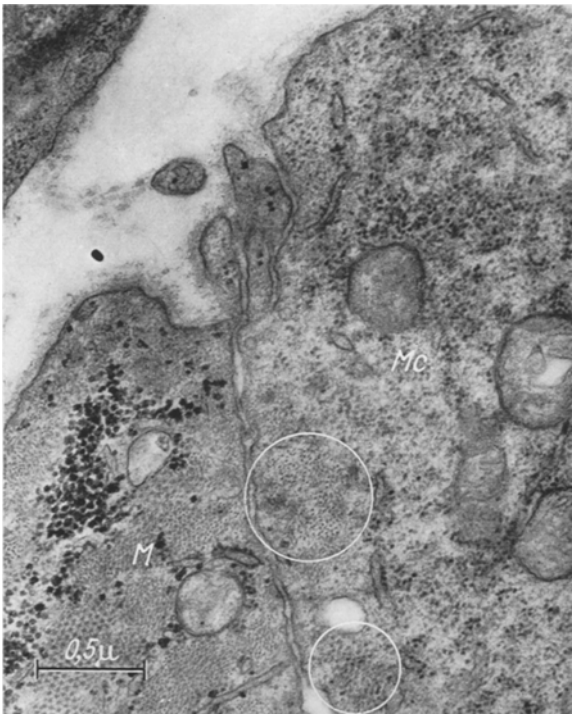


Fig. 8. A muscle cell *Mc* showing inmature cytoplasm in close contact with a well developed muscle fiber *M*. 23 week old human fetus. The inmature cytoplasm contains peripheral less-developed myofibrils (circles), numerous free ribosomes and a few glycogen particles. × 29000

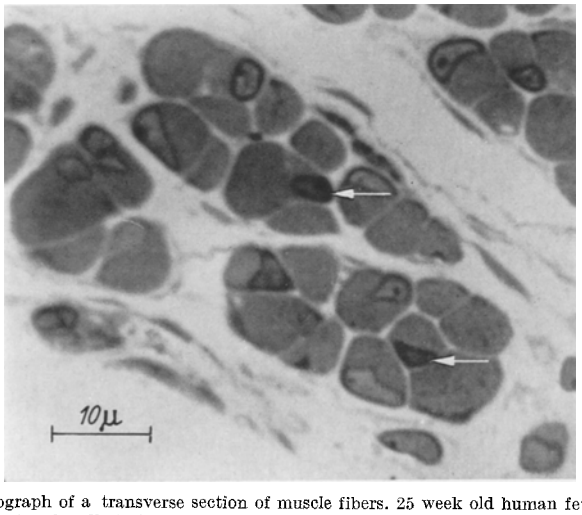


Fig. 9. Photomicrograph of a transverse section of muscle fibers. 25 week old human fetus. Five to ten muscle fibers are grouped into a bundle to form a muscle fascicle. In close contact with the muscle fibers, several deeply stained nuclei (arrows) are found. An Epon section stained with toluidine blue. × 1300

are frequently in close contact with each other. Several interdigitations of the opposed plasma membranes between two adjacent muscle fibers are occasionally seen in cross-sections but not between the satellite cell and the muscle fiber.

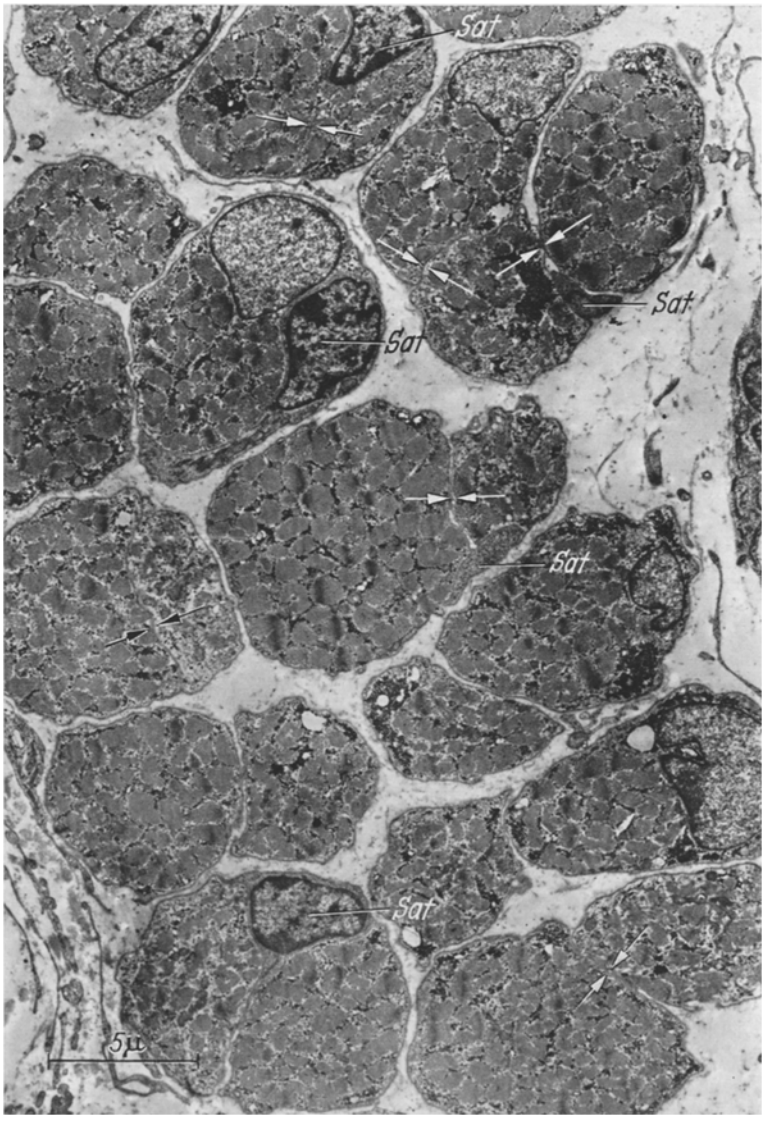


Fig. 10. Low-powered electron micrograph of a transverse section of muscle fibers. 25 week old human fetus. Several satellite cells *Sat* are seen closely associated with each other (arrows). Glutaraldehyde and osmium tetroxide fixation. Lead stained. × 4000

At the 23rd-week (175 mm C. R. length), many satellite cells are also found. The nuclei of the muscle fibers show clear nucleoplasm and distinct nucleoli, while those of the satellite cells appear darker without any prominent nucleoli. Cells, similar to the satellite cell and containing less-developed myofibrils in the cytoplasm, are rarely found (Fig. 8).

In the 25-week period (220 mm C. R. length) the light micrographs of muscle cross-section reveal five to ten muscle fibers grouped into a bundle, called a muscle fascicle. Most nuclei of the muscle fibers are located at the periphery of

each fiber, and have a clear appearance with prominent nucleoli. In close contact with the muscle fibers, several deeply stained nuclei, solid in appearance, are also clearly identified in the light micrographs (Fig. 9). Therefore, one can easily distinguish such solid nuclei from those of the muscle fiber proper. The solid nuclei and the related cytoplasm are very often interposed between two adjacent muscle fibers. Such nuclei seem to be regarded as those of endomysial connective tissue cells, but are so closely associated to the muscle fiber that one might take them for another type of nuclei of muscle fibers.

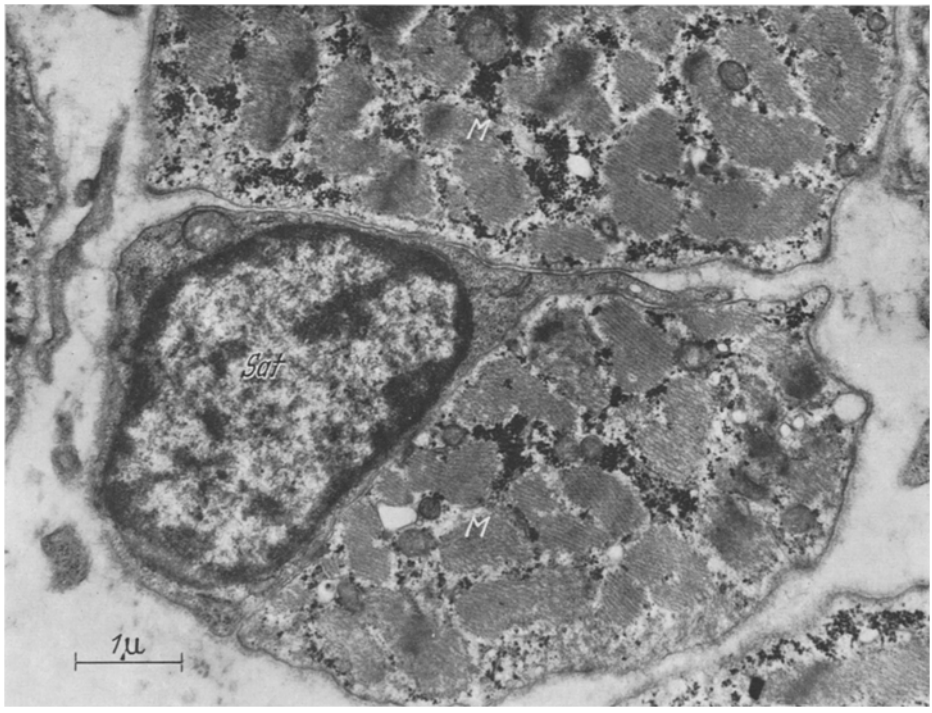


Fig. 11. A satellite cell *Sat* intervening between two adjacent muscle fibers *M*. 25 week old human fetus. × 15000

In low-powered electron micrographs, the fibroblast-like cells which can be distinguished from the muscle fibers as described above are seen enveloped by basement membranes common to both types of cells (Fig. 10). Thus, these cells are referred to as satellite cells. In this stage, the satellite cell is still rich in cytoplasm relative to the size of the nucleus. Two or rarely three muscle fibers still are in contact with each other even in this stage. One or two satellite cells are seen interposed partially or completely between two adjacent muscle fibers (Figs. 11 and 12). These findings are more evident in cross-sections of tissue. Two muscle fibers are enveloped by a continuous, common basement membrane which also covers the outside surfaces of the intervening satellite cell. As such, various degrees of intervention by the satellite cells between adjacent muscle fibers seem to suggest that the former may participate in separating the latter into individual muscle fibers. The satellite cells contain a granular endoplasmic reticulum. The cisternae lie parallel to each other or are irregularly distributed somewhat like

that in typical fibroblasts (see CHAPMAN, 1962) (Fig. 13). The satellite cells are generally elongated in shape, and may reach 30 microns in length. They have winged or tongue-like projections which extend between the basement membrane and the plasma membrane of the muscle fiber. Some cells are round in shape and measure less than 10 microns in length.

In the 27- (230 mm) and 31-week (260 mm C. R. length) human fetuses, satellite cells are also found, but occur less frequently.

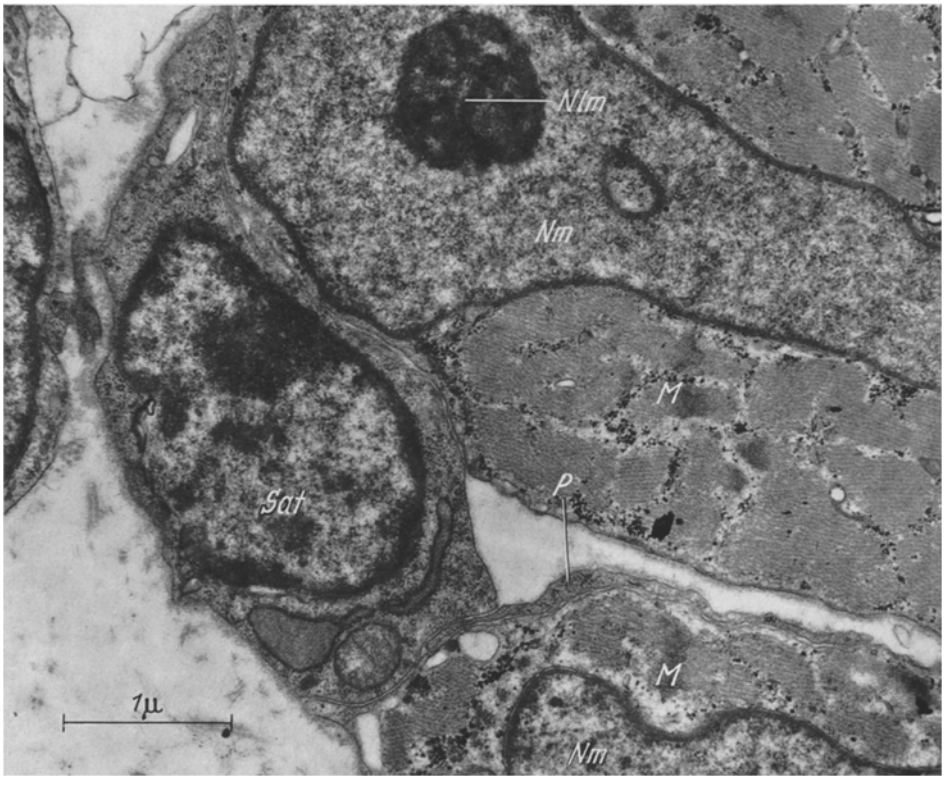


Fig. 12. A satellite cell *Sat* bridging two muscle fibers *M*. 25 week old human fetus. The nucleus *Nm* of the muscel fiber shows a clear nucleoplasm and a distinct nucleolus *Nlm*, while that of the satellite cell appears darker without a prominent nucleolus. *P* Projection of the satellite cell. × 23000

Satellite cells in the gastrocnemius muscles from some other young and adult animals investigated (cats and dogs) show similar findings to those in human specimens. In a 7 day old cat, the muscle fibers are seen separated into individual fibers, and thus each muscle fiber is surrounded by endomysial connective tissue. Satellite cells are frequently found closely associated with the muscle fibers. They usually show well developed granular endoplasmic reticulum without glycogen particles in the cytoplasm, and they protrude slightly inward pushing the myofibrils of the muscle fibers aside. Rarely, satellite cells are observed bridging two muscle fibers situated apart from each other (Fig. 14). In this material, there are also occasionally seen muscle cells containing less-developed myofibrils, few glycogen particles and poor granular endoplasmic reticulum in close

Fig. 13. Longitudinal section of muscle fibers, showing a satellite cell *Sat* intervening between two adjacent muscle fibers *M*. 25 week old human fetus. The satellite cell is elongated in shape and contains a somewhat well developed granular endoplasmic reticulum *Ger*. Note the extreme poles of the satellite cell (arrows). × 10000

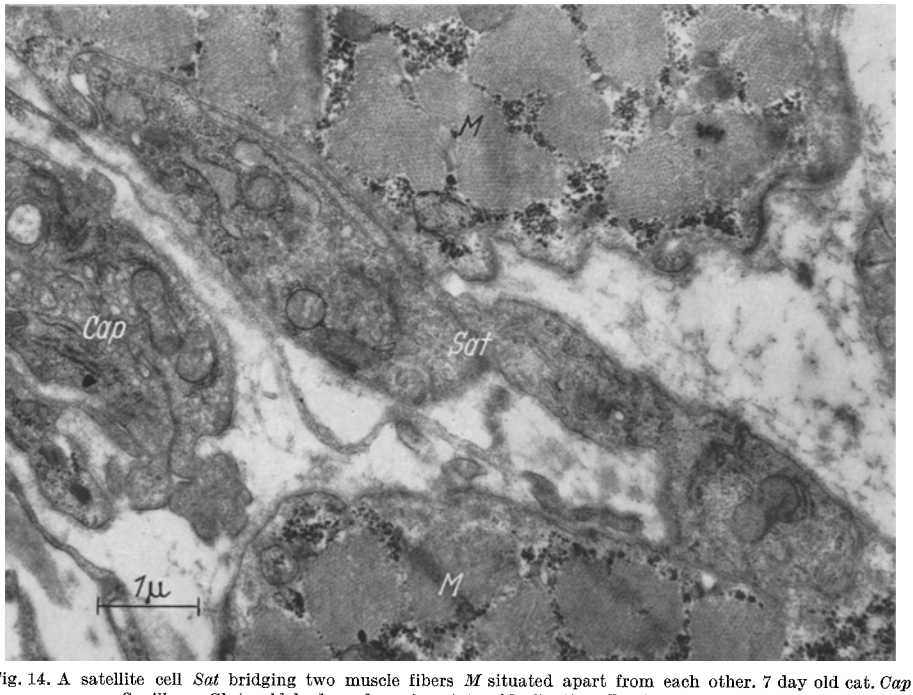


Fig. 14. A satellite cell *Sat* bridging two muscle fibers *M* situated apart from each other. 7 day old cat. *Cap* Capillary. Glutaraldehyde and osmium tetroxide fixation. Lead stained. × 13000

contact with more mature muscle fibers. It remains to be seen whether or not the satellite cell may be transformed into a muscle cell.

III. Discussion

The term "satellite cell" was used by MAURO (1961) to describe the small cell which is enveloped by a basement membrane common to both itself and a muscle fiber and which does not contain any contractile fibrils in its cytoplasm. On the criteria mentioned above, many "satellite cells" have been observed in our material from embryonic developing muscles. However, embryologically, even if there are certain cells intimately associated with the muscle cells, it is difficult to identify them as satellite cells until the basement membranes become visible.

In early muscle fiber stages, the basement membrane component can barely be identified along the side of the muscle cell; the membrane is more easily seen at the end of the muscle cell (ISHIKAWA, 1965). Many fibroblast-like cells which can be distinguished from muscle cells are found closely applied to the myotubes. Now it is only possible to say that the fibroblast-like cells are closely associated with the myotubes by such narrow intercellular spaces that basement membrane layers can not be expected to be present.

The question arises whether or not the fibroblast-like cells can be accepted as satellite cells or satellite cell precursors. During the subsequent development of the muscle, however, fibroblast-like cells, which show similar appearances and situated at similar positions to those seen in the early muscle fiber stage, are observed enveloped by the basement membranes of the muscle fibers. It is, therefore, reasonable to assume that some of these fibroblast-like cells in the early muscle fiber stage may subsequently grow into satellite cells.

MAURO (1961) has presented several possible hypotheses concerning the origin of the satellite cell. First, the satellite cells might be remnants from the embryonic development of the multinucleated muscle cells which result from the process of fusion of individual myoblasts. Thus, satellite cells might be merely dormant myoblasts that failed to fuse with other myoblasts. However, the literature remains contradictory and confusing regarding the mode of formation of the multinucleated muscle cells (see HÄGGQVIST, 1931 and 1956; ADAMS, DENNEY-BROWN and PEARSON, 1953; and BOYD, 1960). Many light-microscopists have made observations in sections and tissue cultures to support the syncytium theory. BOYD (1960), however, states that it is doubtful whether the early tight packing of myoblasts is due to the formation of true cytoplasmic fusion. In view of the subsequent history of the myoblasts, syncytial formation probably does not occur. DE RÉNYI and HOGUE (1934) reported an interesting experiment, obtaining no evidence that fusion actually took place between muscle fibers in tissue culture. With the aid of a micropipette, saline solution was injected into the "complex fiber" at the point of supposed fusion. The sarcoplasm was liquefied but only in a restricted area of the "complex fiber". The other part appeared unaffected by the injection, thus signifying the presence of a distinct fiber. Although several electron microscopic observations seem to favor the fusion of myoblasts (WILDE, 1958; ALLBROOK, 1962; and HAY, 1963), sufficient structural evidence has not yet been reported to support this view.

Furthermore, the satellite cells in developing muscles have fibroblastic characteristics rather than myoblastic ones. They seem, therefore, to be distinct from myoblasts. The fine structure of a myoblast has been described by several investigators (HAY, 1959 and 1963; GILËV, 1960; ALLBROOK, 1962; and HOJIRO,

1963). Among them, HAY (1963) gave a detailed description of the myoblast in differentiating muscles in Salamander tails. The myoblast has a rounded and diffusely granular nucleus containing prominent nucleoli. The ribonucleoprotein granules in myoblasts are predominantly free in the cytoplasm. HAY states that the myoblasts can easily be identified in various stages of development as distinctly different in their fine structure from mesenchymal cells. The satellite cells seen in developing muscles are similar in appearances to mesenchymal cells. However, muscle cells are very rarely found containing less-developed myofibrils in their cytoplasm. These muscle cells are somewhat similar in appearance to the satellite cells. In our material, the satellite cell does not contain any glycogen particles, whereas the muscle cell is rich in glycogen. HOJIRO (1963) has observed numerous glycogen particles in both young and differentiated myoblasts seen in the regenerating limb blastemata of adult newts at 60 days after amputation. However, ENGEL (1961), who histochemically studied the occurrence of glycogen in cultured skeletal muscles, reported that no glycogen was found in the mononuclear myoblast stage but in the multinucleated myoblast stage glycogen occurred. The relationship between the occurrence of glycogen and the differentiation of myoblasts must be studied further in embryonic material. Finally, it can not yet be denied or affirmed that satellite cells in developing muscles might be undifferentiated myoblasts, despite the fact that satellite cells possess some morphological features of mesenchymal cells.

Secondly, it is said, in the course of muscle regeneration, that surviving nuclei in a damaged multinucleated muscle cell give rise to single cells by "gathering up" cytoplasm from the sarcoplasm of the muscle cell. MAURO (1961) suggested, therefore, that in the resting state some cells might be produced at a slow rate by the above mechanism and reside just outside the plasma membrane of the muscle cell. However, the cells which can be regareded as "satellite cells" are already identified in the early development of muscle fibers. The appearance of the satellite cell in the developing muscle seems to be different from that of the dedifferentiating muscle cell described by HAY (1959) in regenerating Amblystoma limbs.

MAURO (1961) has suggested that satellite cells might be pertinent to the regeneration of the skeletal muscle. It is quite safe to assume that these cells may supply some of the free cells present in the sarcolemmal tube in the early stage of regeneration. However, since the satellite cells occur rarely in sections from mature mammalian skeletal muscles, it is not likely that the satellite cells are the chief source of the free cells in regeneration. Further investigation is needed to clarify the role of this cell in the regeneration and in the degenerative diseases of skeletal muscle.

The third hypothesis is that satellite cells might be "wandering cells" which have penetrated the basement membranes of the muscle fibers. From our observations, presumptive satellite cell precursors can be seen closely associated with the muscle cells before the formation of a definite basement membrane. Although small round satellite cells rarely occur in developing muscles, most of the satellite cells are quite elongated and spindle-shaped.

It is equally possible that certain fibroblast-like cells — presumable mesenchymal cells from the same origin as muscle cells rather than wandering cells —

may become enveloped by the basement membranes of muscle cells under certain conditions and then identified as "satellite cells". Basement membranes are generally regarded as connective tissue components, and perhaps the main function of the basement membrane is that of attaching parenchyma to stroma (see PEASE, 1960). From this point of view, the satellite cells are different in cell nature from fibroblasts proper. The satellite cells seen in developing muscles are generally large in size, and therefore they face the connective tissue space with a relatively broad surface. Considering that the outside surface of the satellite cell is also enveloped by the basement membrane, it is reasonable to think that there may be reasons why the satellite cells themselves must be separated from the stroma. There are other situations in which a single basement membrane may be shared by two types of cell.

In developing muscles, two or more muscle fibers are seen in close contact with each other without the intervention of a basement membrane and with only their outside surface enveloped by a continuous, common basement membrane. This finding may be of importance in considering the nature and the role of the basement membrane.

VENABLE (1964) has found satellite cells in the levator ani muscle of mice and rats, and thought that the satellite cells might be morphologically identical to and interchangeable with pericytes, perivascular cells surrounding blood vessels.

Another possibility is that the satellite cells may maintain their fibroblastic nature, but have become enveloped by the basement membranes of the muscle fibers in the course of muscle development only because of the close contact of the satellite cells with the muscle fibers. Indeed, even in cat newborn muscles, satellite cells still contain a rather rich granular endoplasmic reticulum in their cytoplasm and winged cytoplasmic projections, an indication of continued activity. Such an active appearance of the satellite cells will be discussed later.

Most investigators (see ADAMS et al., 1953; HÄGGQVIST, 1956; and BOYD, 1960) believe that there is an increase in the number of muscle fibers in a muscle during development. Two main theories appear in the literature to explain this remarkable increase in number. One is that new muscle fibers are formed by the splitting or even budding of differentiated muscle fibers. CUAJUNCO (1942) stated that the longitudinal splitting of a muscle fiber was seen for the first time in a twelve-week human embryo and continued until the fourteenth or fifteenth week. The similar finding of the splitting of a muscle fiber was observed in tissue culture (see MURRAY, 1960). Our observations with the electron microscope did not reveal any process of the longitudinal splitting of a single muscle fiber in the fetal stage of development. Although the longitudinal splitting of muscle cells appears to occur in some light micrographs, the electron micrographs indicate that two adjacent muscle cells are separated by their respective continuous plasma membrane with an interspace of nearly 150 Å. As such, it seems that the light microscope is not adequate for fine details in developmental cytology.

The second theory is that the increase in the number of muscle fibers is due to recruitment by the differentiation of undifferentiated cells or interstitial, fibroblastic-like cells related to or surrounding the "primary" muscle fibers. Light microscopically, therefore, many investigators have noted uninucleated

cells closely associated with developing muscle fibers (MORPURGO, 1898; BARDEEN, 1900; MEVES, 1909; COUTEAUX, 1941; and some others). There are, however, some differences in the literature concerning the nature of these cells. MORPURGO (1898) described the not yet differentiated elements which were located between and on the well-developed muscle fibers in newborn and very young rats. Similar cells were regarded as myoblasts by MEVES (1909) in chick embryos, but some of their nuclei seem to be different in appearance from those of developing muscle cells in his illustrations. COUTEAUX (1941, cited in BOYD, 1960) also observed that "primary" muscle fibers were covered with numerous uninucleated elements, which multiplied by mitotic division on the surfaces of muscle fibers. According to him, it is from some of these so-called "satellite cells" (at the light microscopic level) that new fibers are added to the developing muscle until the full number is achieved.

Some earlier workers have made interesting observations on the nuclei of developing muscle fibers (MACCALLUM, 1898; and ASAI, 1915). MACCALLUM pointed out that the muscle cells in pig fetuses of 64 to 75 mm and in human fetuses of 130 to 150 mm in C. R. length contained two types of nuclei — a centrally placed vesicular nucleus and a peripherally placed nucleus with a solid appearance. The nuclei of developing muscle fibers underwent changes, and the muscle fibers which at first had contained only centrally placed nuclei acquired the peripheral nuclei. It was possible that the latter nucleus was derived from the centrally placed nucleus which was eventually "lost". ASAI (1915) also described the nuclei of muscle fibers in the later period of development in mouse muscles to be situated either centrally or peripherally and the peripheral nuclei to be different in appearance from the centrally placed ones.

As seen with the light microscope, developing muscle fibers showing various stages of myogenesis form a bundle. A similar appearance of bundle formation can be observed in electron micrographs. Although muscle cells showing inmature peripheral cytoplasm were rarely observed in close contact with well developed muscle fibers, our electron microscopic observations did not reveal structural evidence for the differentiation of satellite cells into muscle fibers. In addition, many satellite cells still exist obviously different in appearance from muscle fibers not only late in fetal life but even in a newborn cat. It is, therefore, not likely that "true" satellite cells differentiate into muscle fibers. In a careful comparison between light and electron micrographs of developing muscles, it is likely that some of the "not yet differentiated cells", "satellite cells" and/or "peripherally placed solid nuclei" previously described in light microscopic studies may be identical to the satellite cells reported in this paper. HAY (1963) has also described that cells with some of the morphological features of mesenchymal cells are occasionally seen in close association with myoblasts in differentiating salamander muscles.

Frequently two or more muscle fibers are seen in close contact with each other in the fetal stage of development. However, there is no cytoplasmic fusion between them. In transverse sections, if each fiber is sectioned at the level of its nucleus, the number of muscle fibers could accurately be estimated with the light microscope. If not, one might make a mistake in calculating the number. In longitudinal sections, the incomplete separation — partial apposition — of

two adjacent muscle fibers might be regarded as longitudinal splitting of a muscle fiber. Furthermore, it is not likely that muscle fibers form elongated processes or branches. As MACCALLUM (1898) himself pointed out, it is emphasized that estimations of the number of fibers in a muscle reported in the literature were not those of the number of fibers in the muscle as a whole, but merely those found in cross-sections. In addition, we have no exact knowledge of the period which muscle fibers continue to increase in number. MACCALLUM concluded that there is no longer an increase in number in human fetuses larger than 170 mm in C. R. length. In contrast, MORPURGO (1898) found new fibers in the newborn rats. It can not be denied that the increase in number of muscle fibers may occur even in the latter half of embryonic development, but electron microscopic proof for it is still lacking.

As development proceeds, muscle fibers showing a tight packing have a tendency to be separated completely into individual fibers. In mature skeletal muscles it is difficult to find two adjacent muscle fibers enveloped by a common basement membrane.

In our materials from human skeletal muscles at the myotube stage of development, "tight junctions" were found between the apposed plasma membranes of adjacent muscle cells. This type of intercellular junction was first noticed in the cardiac muscles of the guinea-pig and the mouse by SJÖSTRAND, ANDERSSON-CEDERGREN and DEWEY in 1958. Similar structures have been described in striated muscle cells seen in thoracic and lung veins of the mouse (KARRER, 1960), and in some other tissues (see FARQUHAR and PALADE, 1963; and BRIGHTMAN and PALAY, 1963). Such structures have so far not been reported in skeletal muscles. This structure is concerned with the tight packing of muscle cells in the early stage of muscle development, but its significance is quite unknown.

As development proceeds, generally, the number of satellite cells diminishes in sections. Satellite cells in developing muscles are found very frequently, but rarely in mature muscles. It may be reasonable to conclude that, because of the striking growth in size and length of the muscle fibers, the satellite cells are rather sparsely scattered throughout the muscle without a change in their total number.

From the results of our observations, it is very interesting to speculate on the role of the satellite cell. The tight packing of inmature muscle fibers subsequently becomes separated into individual muscle fibers. This separation may presumably be promoted by the satellite cells closely associated with the muscle fibers. Some investigators (see HÄGGQVIST, 1956), utilizing the light microscope, have described the mesenchymal cells penetrating between the muscle fibers. According to our observations, satellite cells are seen intervening in varying degrees between two muscle fibers which are in close contact with each other. The satellite cell shows an active appearance in developing muscles, e. g. the well developed granular endoplasmic reticulum, Golgi material, and the elongated or winged cytoplasmic projections. De RÉNYI and HOGUE (1934) pointed out the relationship between fibroblasts and muscle fibers in tissue culture. Many fibroblasts were found in tissue culture but only a few were attached to muscle fibers. The fibroblast adhering to the outer surface of the sarcolemma could move along

the fiber either distally or proximally. In the experiment in which the cell was separated from the muscle fiber, the attached fibroblast was destroyed but its branches remained adherent to the sarcolemma. Whether these fibroblasts in tissue culture correspond to the satellite cells is not yet certain, but it is possible that the satellite cell with its active appearances can move beneath the basement membrane of the muscle fiber. In mature muscles, the satellite cell seems to persist as an inactive small cell. In other words, the satellite cell which may have played a certain role in muscle development seems to remain as a dormant cell in mature muscles. However, it also remains to be shown whether the satellite cells in mature muscles are really resting and inactive cells.

Summary

Satellite cells intimately associated with muscle fibers in various fetal stages of development of human gastrocnemius muscles (40 mm to 260 mm C. R. length human fetuses) were studied with the electron microscope.

In the early muscle fiber stage, several muscle cells were grouped into a bundle. No cytoplasmic fusion between adjacent muscle cells could be found, but rarely, tight, quintuple-layered junctions occurred between the apposed plasma membranes. The basement membrane began to appear along the side of the muscle cell. Many fibroblast-like cells were found closely applied to the muscle cells with an interspace so narrow that a basement membrane layer could not be expected to be present. The fibroblast-like cell had a deeply and unevenly stained nucleus without a prominent nucleolus, and contained a rather well developed granular endoplasmic reticulum. In its cytoplasm, cytoplasmic fibrillar components or glycogen particles could not be found. The basement membrane barely enveloped this cell in common with the related muscle cell. The fibroblast-like cells were frequently seen intervening between adjacent muscle cells or deeply inside a bundle of muscle cells. It seemed that the satellite cells seen in a later stage of development might be derived from such fibroblast-like cells.

In the formed muscle fiber stage, groups of muscle fibers began to be separated into individual fibers. However, two or more muscle fibers were still in close contact with each other. The nuclei of the muscle fiber moved toward the periphery. As development proceeded, the basement membrane of the muscle fiber became more visible and distinct. The fibroblast-like cell and muscle fiber in this stage were enveloped by a basement membrane common to both. Therefore, the fibroblast-like cell in this period of development were regarded as a satellite cell. The satellite cells were easily distinguished from the related muscle fibers. In sections, satellite cells were seen intervening partially or completely between adjacent muscle fibers. Satellite cells were rarely observed bridging two muscle fibers situated a distance from each other. The satellite cells were usually elongated in shape and were up to 30 microns in length. They had winged or tongue-like projections, which extended between the basement membrane and the plasma membrane of the muscle fiber.

The possible origin and role of satellite cells were discussed with reference to the development of skeletal muscles.

References

ADAMS, R. D., D. DENNEY-BROWN, and C. M. PEARSON: Diseases of muscle, p. 3—51. London-Toronto-Melbourne-Sydney-Wellington: Cassell 1953.

ALLBROOK, D.: An electron microscopic study of regenerating skeletal muscle. J. Anat. (Lond.) **96**, 137—152 (1962).

ASAI, T.: Beiträge zur Histologie und Histogenese der quergestreiften Muskulatur der Säugetiere. Arch. mikr. Anat. **86**, 8—68 (1915).

BARDEEN, C. R.: The development of the musculature of the body wall in the pig, including its histogenesis and its relation to the myotomes and to the skeletal and nervous apparatus. Johns Hopk. Hosp. Rep. **9**, 367—400 (1900).

BOYD, J. D.: Histogenesis of striated muscle. In: Structure and function of muscle (ed. by G. H. BOURNE), vol. 1, p. 72—82. New York and London: Academic Press 1960.

BRIGHTMAN, M. W., and S. L. PALAY: The fine structure of ependyma in the brain of the rat. J. Cell Biol. **19**, 415—439 (1963).

CHAPMAN, J. A.: Fibroblasts and collagen. Brit. med. Bull. **18**, 233—237 (1962).

CUAJUNCO, F.: Development of the human motor end plate. Carneg. Inst. Wash. Publ. 541, Contr. Embryol. **30**, 127—152 (1942).

ENGEL, W. K.: Cytological localization of glycogen in cultured skeletal muscle. J. Histochem. Cytochem. **9**, 38—43 (1961).

FARQUHAR, M. G., and G. E. PALADE: Junctional complexes in various epithelia. J. Cell Biol. **17**, 375—412 (1963).

FERRIS, W.: Electron microscope observations of the histogenesis of striated muscle. Anat. Rec. **133**, 275 (1959).

GILËV, V. P.: Die Untersuchung einiger Elemente des Muskelgewebes in seiner Histogenesis und Regeneration. IV. Intern. Kongr. für Elektronenmikroskopie, II, S. 321—324. Berlin-Göttingen-Heidelberg: Springer 1960.

GLÜCKSMANN, A.: Über die Entwicklung der quergestreiften Muskulatur und ihre funktionelle Beziehungen zum Skelet in der Onto- und Phylogenie der Wirbeltiere. Z. Anat. Entwickl.-Gesch. **103**, 303—370 (1934).

HÄGGQVIST, G.: Gewebe und Systeme der Muskulatur. In: Handbuch der Mikroskopischen Anatomie des Menschen, hrsg. von W. v. MÖLLENDORFF, Bd. II/3. Berlin: Springer 1931.

— Gewebe und Systeme der Muskulatur. In: Handbuch der Mikroskopischen Anatomie des Menschen, hrsg. von W. BARGMANN, Bd. II/4. Berlin-Göttingen-Heidelberg: Springer 1956.

HAY, E. D.: Electron microscopic observations of muscle dedifferentiation in regenerating Amblystoma limbs. Develop. Biol. **1**, 555—585 (1959).

— The fine structure of differentiating muscle in the salamander tail. Z. Zellforsch. **59**, 6—34 (1963).

HOJIRO, O.: Electron microscope observations on the myoblast of the regenerating forelimb of the adult newt. J. Electronmicroscopy (Chiba) **12**, 223—235 (1963).

ISHIKAWA, H.: The fine structure of myo-tendon junction in some mammalian skeletal muscles. Arch. histol. jap. **25**, 275—296 (1965).

KARRER, H. E.: The striated musculature of blood vessels. II. Cell interconnections and cell surface. J. biophys. biochem. Cytol. **8**, 135—150 (1960).

LUFT, J. H.: Improvement in epoxy embedding methods. J. biophys. biochem. Cytol. **9**, 409—414 (1961).

MACCALLUM, J. B.: On the histogenesis of the striated muscle fibre, and the growth of the human sartorius muscle. Bull. Johns Hopk. Hosp. **9**, 208—215 (1898).

MAURO, A.: Satellite cell of skeletal muscle fibers. J. biophys. biochem. Cytol. **9**, 493—495 (1961).

MEVES, F.: Über Neubildung quergestreifter Muskelfasern nach Beobachtung an Hühnerembryo. Anat. Anz. **34**, 161—165 (1909).

MIDZUKAMI, M.: Electron microscopic studies of satellite cells in the cardiac muscle of brachyura. Okajimas Fol. anat. jap. **40**, 173—185 (1964).

MILLONIG, G.: Further observations on a phosphate buffer for osmium solutions in fixation. 5th Intern. Congr. for Electron Microscopy, vol. 2, P-8. New York and London: Academic Press 1962.

Morpurgo, B.: Über die postembryonale Entwicklung der quergestreiften Muskeln von weißen Ratten. Anat. Anz. **15**, 200—206 (1898).

Murray, M. R.: Skeletal muscle tissue in culture. In: Structure and function of muscle, ed. by G. H. Bourne, vol. 1, p. 111—136. New York and London: Academic Press 1960.

Pease, D. C.: The basement membrane: Substratum of histological order and complexity. In: 4th Intern. Congr. for Electron Microscopy, vol. 2, p. 139—155. Berlin-Göttingen-Heidelberg: Springer 1960.

Rényi, G. S., de, and M. J. Hogue: Studies on skeletal muscle growth in tissue cultures. Arch. exp. Zellforsch. **16**, 167—186 (1934).

Sjöstrand, F. S., E. Andersson-Cedergren, and M. M. Dewey: The ultrastructure of the intercalated disc of frog, mouse and guinea-pig cardiac muscle. J. Ultrastruct. Res. **1**, 271—286 (1958).

Speidel, C. C.: Studies of living muscle. I. Growth, injury and repair of striated muscle, as revealed by prolonged observations of individual fibers in living frog tadpoles. Amer. J. Anat. **62**, 179—235 (1938).

Venable, J.: The cytology of hypertrophy in a skeletal muscle. Anat. Rec. **148**, 347 (1964).

Wilde jr., C. E.: The fusion of myoblasts, a morphogenetic mechanism in striated muscle differentiation. Anat. Rec. **132**, 517—518 (1958).

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