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Morphological Mechanism of the Development of Miosatellitocytes from Structural Elements of Muscle Fiber under Conditions of Increased Functional Activity of Skeletal Muscles

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As is commonly known, myosatellitocytes (MSC) develop from promyoblasts, the sole source of development of muscle tissue [1]. The appearance of mature MSC is preceded by structural-metabolic transformations of the initial cells, which take place simultaneously with the development of muscle fibers (MF). The final differentiation occurs as the muscle matures as an organ, after which the MSC are retained under the basal membrane of the MF [6]. Under extreme conditions of the development of muscle tissue (regeneration after injury, atrophy, circulatory disorders in the muscle, denervation, hypertrophy), MSC act as the muscular cambium [8,12-14]. Moving into the interstitial space, MSC are transformed into regenerative myoblasts [5]. It has been shown that MSC can become part of MF [6]. Their cytoplasm thereupon merges with the sarcoplasm of the MF, while the nuclei of the incorporated MSC, losing the capacity for proliferation, become component elements of the myosymplast.

A hypothesis of discontinuity, or the formation of the cellular phase of myogenic tissue from the acellular (myosymplastic) phase, has been put forward [3,9,10,15]. Several investigations have been carried out to verify this hypothesis [4,7,10]. The results reported, however, are not very convincing. They seem to prove not the process of new formation of MSC, but rather different stages of ejection of satellite cells from MF or their incorporation into the myosymplasts. On the other hand, it is not to be excluded that a mechanism exists for the new formation of MSC from components

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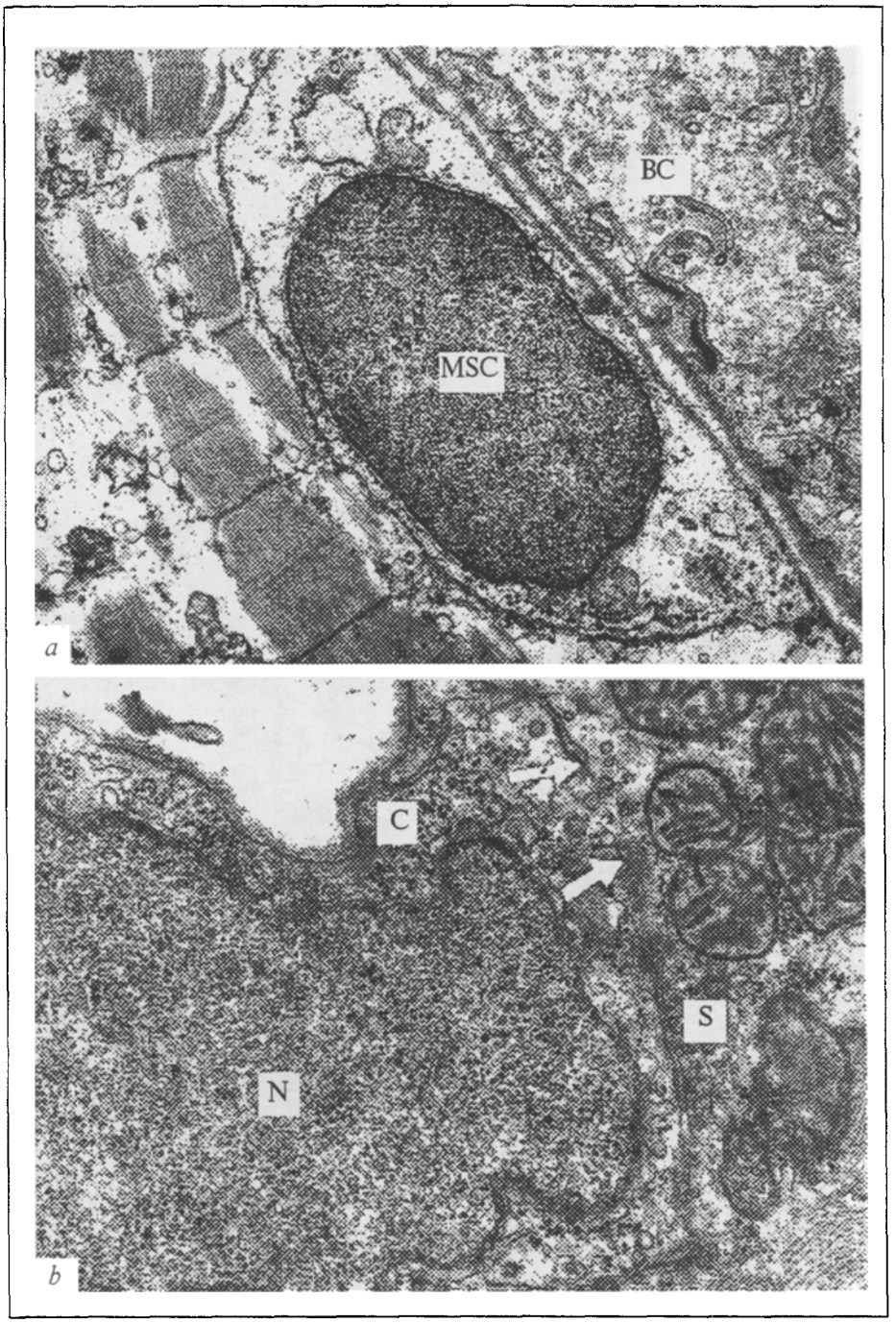

Fig. 1. Varieties of myosatellitocytes and their interactions with blood capillaries and myosymplast in the vertical thigh muscle of trained rats. *a*) gap junction between type 2 myosatellitocytes and blood capillaries (MSC – myosa-tellitocytes; BC – blood capillaries); *b*) incorporated type 1 MSC as a part of myosymplast (N – nucleus of MSC; C – cytoplasm of MSC; S – sarcoplasm of MF). The start of dismantling of MSC cytolemma and the merging of its cytoplasm with the MF sarcoplasm are indicated by arrows. Magnification: a) ×8000; b) ×20,000

of MF (the nuclei and sarcoplasms). This is a quiestion that needs to be further investigated [2].

The purpose of the present study was to ascertain whether MSC can develop from components of MF (the nuclei and sarcoplasm) under conditions of increased functional activity of skeletal muscles.

MATERIALS AND METHODS

The vertical thigh muscle of white mongrel male rats weighing 95.4±3.9 g was used in our experiment. To increase the functional activity of their skeletal muscles, the rats were made to run on a treadmill. The animals were subjected to the exercise every day for a period of 30 days. The imposed physical loads ranged from 150 to 900 meters at a running speed of 15-30 m/min. For electron-microscopic examination the specimens were treated with a 1% solution of OsO_4 with 0.1 M calcium phosphate as a buffer at pH 7.4. The solution was embedded in a mixture of Epon 812 and araldite. Ultrathin sections were contrasted with lead citrate and then examined under an electron microscope (JEM-7A).

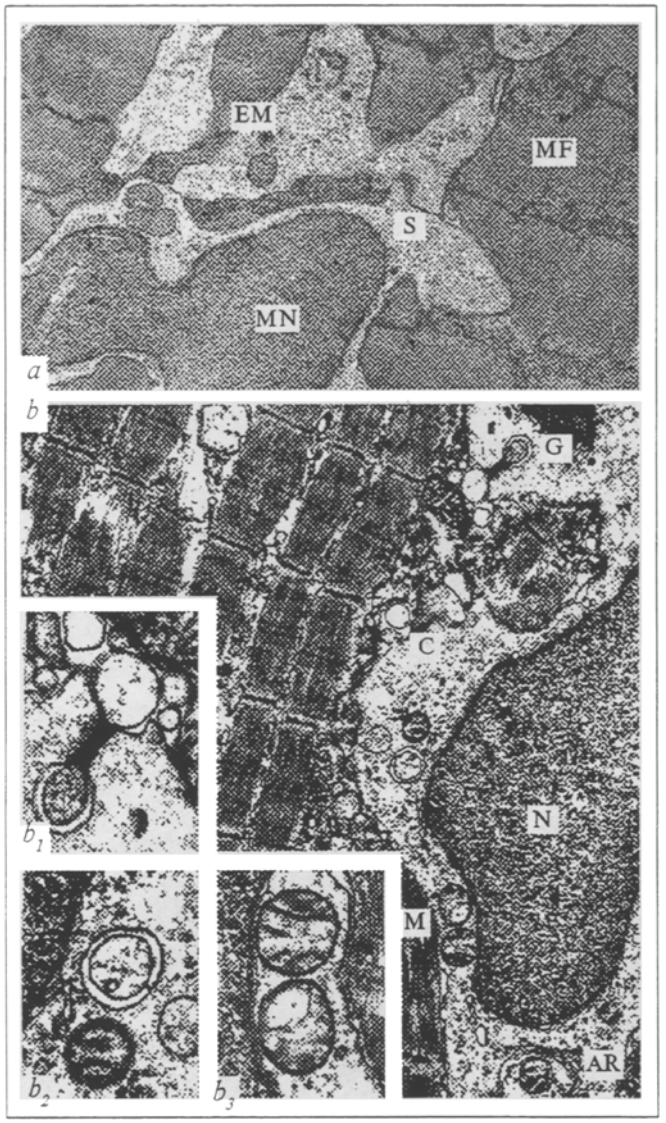

Fig. 2. Stages of development of myosatellitocytes from structural elements of muscle fiber. *a*) segregation of disintegrated sections of muscle fiber with myonucleus by elementary membrane (MN – myonucleus; S – sarcoplasm; EM – elementary membrane; MF – myofibril); *b*) development of organelles in the precellular formation – "sarcocyte" (N – nucleus of "sarcocyte"; C – cytoplasm of "sarcocyte"; M – mitochondria; G – glycogen; AR – agranular endoplasmic reticulum); b_1) emperipolesis of microcorpuscle (magnified section of Fig. 2, *b*); b_2) microcorpuscle having a two-membrane contour (magnified section of Fig. 2, *b*). b_3) young mitochondria (magnified section of Fig. 2, *b*). Magnification: *a*) ×6000; *b*) ×2000.

RESULTS

The MSC in the vertical thigh muscle of the intact and experimental animals from a heterogeneous population which include "free" satellite cells (localized in the interstitial space) and MSC lying under the basal membrane of MF. The latter MSC are in various stages of functional activity, and thus they can be arbitrarity divided into two groups: type 1 MSC and type 2 MSC [5]. Type 2 satellite cells come into close contact with blood capillaries, ensuring the active transport of pinocytotic vesicles to MF (Fig. 1, *a*).

In MF of the trained animals active migration of type 2 MSC to the interstitial space and an increase in their proliferative activity were observed. A considerable number of type 2 MSC were incorporated into MF; this brought about an increase in the concentration of myonuclei (Fig. 1, *b*).

In some MF large areas were observed where disintegration of the myofibrillar apparatus had taken place. This process could affect 2-5 sarcomeres in length and 4-5 myofibrils in width. The area of these microregions was 15-25 μ^2. Micronuclei, which had apparently existed from the very start of the proliferative linkage of the functionally different nuclei, migrated to the disintegrated sections of MF [6]. The appearance of muscle nuclei in the disintegrated sections of MF triggered the mechanism of segregation of the microregions by an elementary membrane formed from the surfaces of the sarcoplasmic reticulum and the triade system (Fig. 2, *a*).

The further development of the segregated regions with the micronucleus was related to the formation and differentiation of cytoplasmic organelles (mitochondria, free ribosomes and polyribosomes, granular and agranular endoplasmic reticula). In other words, processes characteristic of the period of development and differentiation of cells were underway. At this stage we were able to trace the pattern of development of the mitochondria in the precellular formation. Microcorpuscles were formed near the elementary membrane and, owing to emperiopolesis [11], migrated into the cytosol of the "sarcocyte", the precellular formation (see the magnified fragments in Fig. 2, *b*). The incorporated microcorpuscles had a two-membrane contour, which consisted of the membrane itself and a fragment of the plasmalemma. The next stage of the evolution of the microcorpuscles was related to the formation of cristae and matrix. At the stage of 3-5 cristae the outer membrane contour became blurred and young mitochondria entered into close contact with the cytosol of the "sarcocyte". During the active formation of organelles a considerable number of trophic inclusions were found in the cytoplasm. These inclusions were in the form of α and β particles of glycogen deposited near the smooth endoplasmic reticulum.

The new growth of organelles took place with the active participation of the nucleus. It may be assumed that expression of the genes which direct the differentiation of the "sarcocyte" became pos-

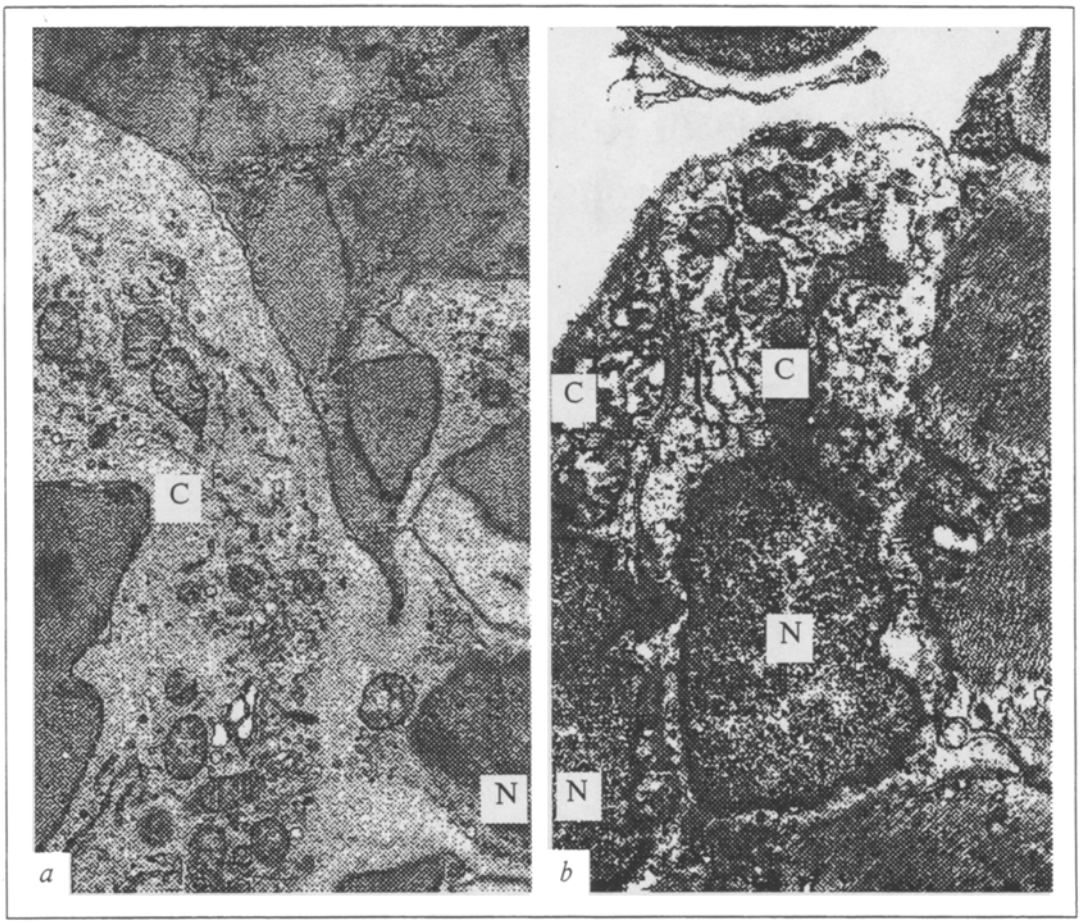

Fig. 3. Ultrastructure of newly formed myosatellitocytes at the later stages of development. *a*) ultrastructure of cytoplasm of newly formed satellite cell (N – nucleus of MSC; C – cytoplasm of MSC); *b*) mature MSC underlying basal membrane of MF. Magnification: *a*) ×8000; *b*) ×10,000

sible after there was close structural-metabolic interaction between the nucleus and the segregated sarcoplasm. On the other hand, the formation of cells from structural elements of MF got underway apparently owing to induction, i.e., owing to the effect arising under the influence of prognostic metabolites, effects which via the plasm led to the expression of different genes capable of responding to these inductive factors. Thanks to these interactions, the "sarcocyte" developed intensively and at a certain stage had well-differentiated cytoplasmic inclusions (Fig. 3, *a*).

In the next stage of development the "sarcocyte" migrated toward the basal membrane of MF, where its structural-functional maturation finally took place. As a result, a satellite cell appeared which, judging from the ultrastructural features, sould be considered to be a type 2 MSC (Fig. 3, *b*).

The results obtained confirm the hypothesis that MSC can be formed from components of striated MF under conditions of increased functional activity of skeletal muscles. They also broaden our understanding of the cambial properties of muscle tissue.

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