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ELECTRON-MICROSCOPIC INVESTIGATION OF SATELLITE CELL FORMATION IN SKELETAL MUSCLE DURING PHYSICAL EXERTION

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The content of condensed chromatin is increased in nuclei of skeletal muscle fibers of rats after repeated physical exertion continued until exhaustion. Some muscle fiber nuclei, together with part of the sarcoplasm, were separated from the muscle fibers. Satellite cells formed from the separated parts of the muscle fibers.

KEY WORDS: *Physical exertion; muscle fibers; satellite cells.*

Much attention has recently been paid to the satellite cells of skeletal muscles. The main reason for this is that these cells are regarded as the principal sources of myoblasts [4, 8, 10]. The role of trophic cells has also been ascribed to them [3].

Satellite cells have been investigated in different phases of ontogeny and under experimental and clinical pathological conditions. Electron-microscopic studies have shown that satellite cells are formed during disturbance of the circulation or cooling of a muscle [9] and after traumatic injury or denervation of muscle [1, 2, 5, 11].

No description of the formation of these cells during physical exertion could be found in the accessible literature, and it was accordingly decided to investigate this problem.

EXPERIMENTAL METHOD

Male Wistar albino rats weighing 200-220 g were used as experimental animals. The physical exertion consisted of swimming in water at a temperature of 30°C carrying a load equivalent to 2-3% of the body weight. The interval between the first and second physical exertions was 24 h, so that the second took place in the phase of increased working capacity.

Pieces of the gracilis muscle were taken during the first and second periods of exertion at a time when the animals were in a state of extreme fatigue, and also 24 and 48 h after the first and second exertions. The material was fixed in 4% formaldehyde solution (prepared from paraformaldehyde) in phosphate buffer at pH 7.4 with the addition of 5% su-

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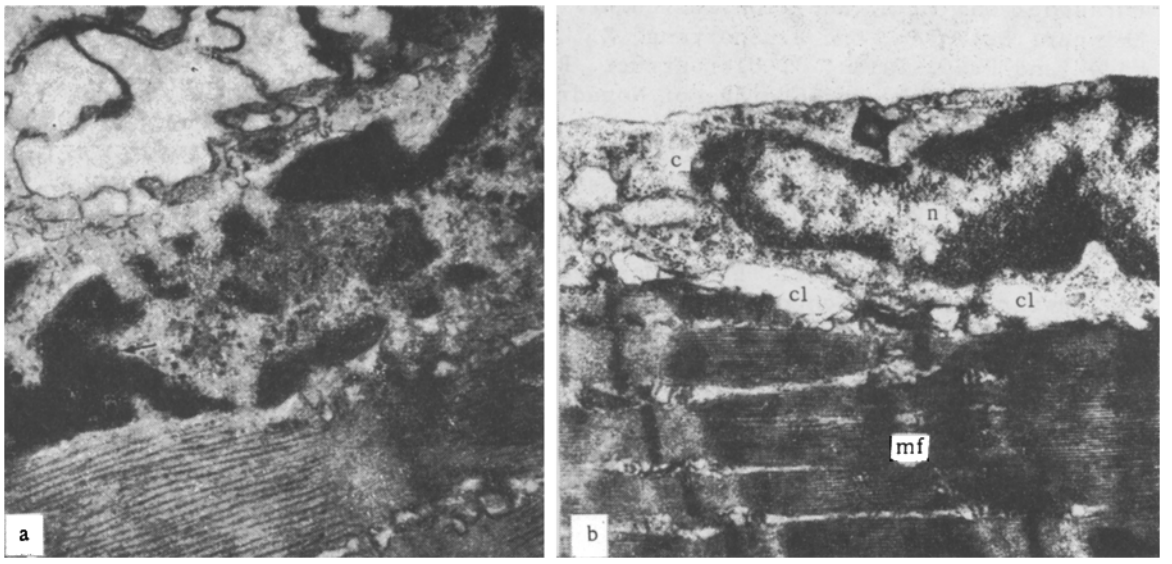

Fig. 1. Section through gracilis muscle of rat (period of fatigue during repeated physical exertion): a) increased content of condensed chromatin in nucleus of muscle fiber (arrow), b) formation of clefts between detached area of muscle fiber and its residual mass. n) Nucleus, c) cytoplasm of detached area, mf) muscle fiber, cl) cleft; 25,000×.

crose. After fixation in formaldehyde the material was washed in buffer and postfixed in 1% osmium tetroxide in the same buffer, then dehydrated in acetone and embedded in a mixture of Epon 812 and Araldite. Ultrathin sections were stained with lead acetate and examined in the UÉMV 100V and IEM-100B electron microscopes.

EXPERIMENTAL RESULTS

Under conditions of severe fatigue during the second period of physical exertion and also 24 and 48 h thereafter, the ultrastructure of most nuclei in the muscle fibers was changed. The content of condensed chromatin in the nuclei distributed near the nuclear membrane and as clumps everywhere in the nucleoplasm was increased (Fig. 1a). The layer of condensed chromatin lying next to the nuclear membrane could be of considerable thickness; it contained small round formations, the contents of which were less electron-dense than chromatin. These formations were evidently nuclear pores cut tangentially. Appreciable dilatation of the nuclear pores could be seen in transverse sections through the muscle fibers.

Projections of sarcoplasm penetrated to a considerable depth into some nuclei of the muscle fibers, and as a result the nuclei in some sections appeared to consist of separate fragements. Much of the nucleoplasm of these fragments could be filled with condensed chromatin.

Small cavities bounded by a membrane, irregular in shape, and frequently arranged in a chain appeared in the sarcoplasm near some of the nuclei described above with an increased content of condensed chromatin; these were probably derivatives either of the T system or of the sarcoplasmic reticulum. Clefts of various sizes, in which severed fragments of membranes could be seen, also were found near these nuclei (Fig. 1b).

Besides the formation of clefts and cavities, deep invaginations of sarcolemma could be found between the nucleus and the residual part of the muscle fiber. The invaginations of sarcolemma, clefts, and cavities could be separated from one another only by narrow bridges of sarcoplasm. At this stage of satellite cell formation a nucleus-containing region of the muscle fiber largely isolated from the rest of its mass was thus formed. The cytoplasm of the detached part of the fiber contained many ribosomes and polysomes. Small cisterns of the granular cytoplasmic reticulum also were found.

The next stage of satellite cell formation was its separation over a large extent from the muscle fiber, probably as a result of fusion of the clefts and cavities in the sarcoplasm and invaginations of the sarcolemma described above. As a result a future satellite

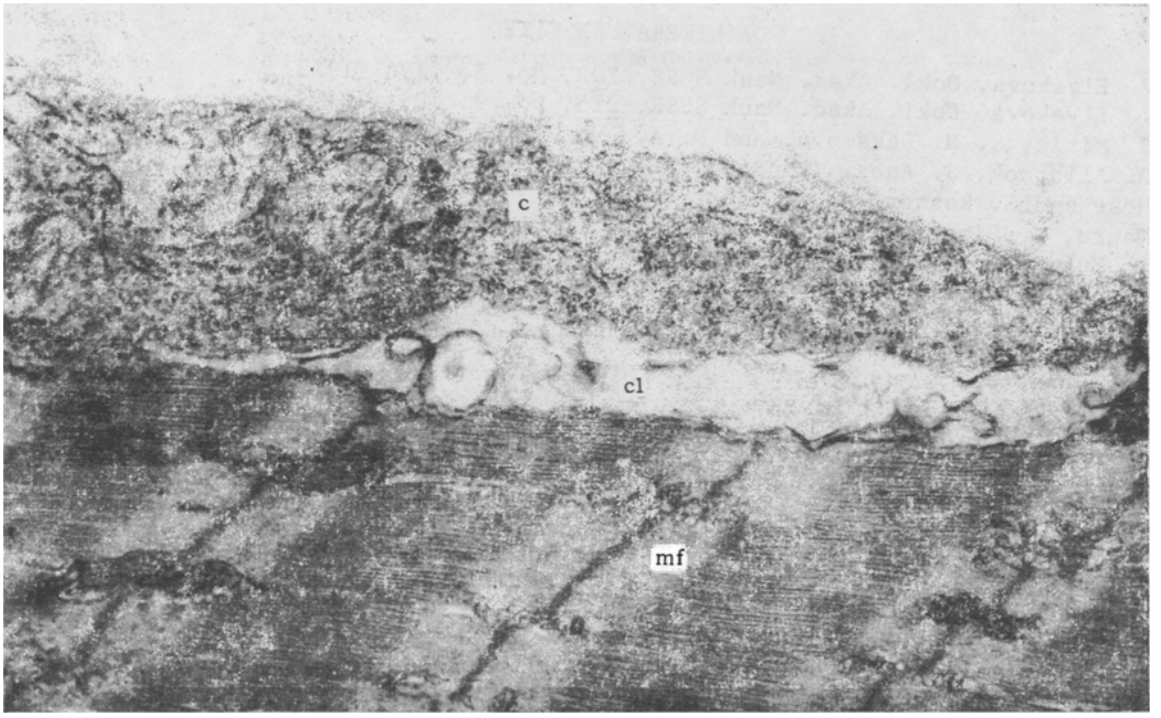

Fig. 2. Section through gracilis muscle of rat (period of fatigue during repeated physical exertion): newly formed satellite cell, detached over a large extent from muscle fiber; its cytoplasm contains many polysomes and narrow tubules of granular cytoplasmic reticulum. mf) Muscle fiber, cl) cleft between muscle fiber and newly formed satellite cell, c) cytoplasm of satellite cell; 25,000×.

cell, still connected at one place with the mother fiber, but elsewhere separated from it was formed (Fig. 2). A characteristic feature of the cytoplasm of the satellite cell at this stage of its formation was the presence of many free-lying ribosomes and polysomes. It also contained many narrow tubules of the granular cytoplasmic reticulum. In some cases the lumen of the tubules was dilated. The mitochondria had clearly outlined cristae. The matrix of the mitochondria was somewhat translucent.

Later the satellite cell separated completely from the muscle fiber. However, it still remained beneath its basement membrane. No myofilaments were found in the cytoplasm of the newly formed satellite cells. The ultrastructure of the newly formed satellite cells described above was not characteristic of satellite cells of the skeletal muscles of animals in a state of relative rest. The satellite cells of intact animals are distinguished by their scanty cytoplasm, which contains only a few organelles [6, 7].

As was stated above, the mechanism of formation of satellite cells from muscle fibers has been analyzed in detail by electron microscopy in muscle fibers whose circulation is disturbed or during cooling [9] and also after traumatic injury or denervation of muscle [1, 2, 5]. These investigations showed that satellite cells are formed from the muscle fiber by budding of the nucleus and part of the sarcoplasm. The authors cited observed the formation of clefts and cavities between the nucleus of the muscle fiber and the residual mass of the muscle, as also was seen in the present investigation. The ultrastructure of the newly formed satellite cells described by the authors cited and that discovered in the present experiments in the period of fatigue during repeated physical exertion and immediately afterward were very similar. This indicates that the formation of satellite cells from parts of muscle fibers is a frequent phenomenon and that the mechanism of their formation is similar in muscle exposed to widely different conditions. The subsequent fate of the newly formed satellite cells may vary. They may give rise to myoblasts and then to muscle tubes [9], but they may also return into the composition of the muscle fiber. Their fate evidently depends both on the character and extent of the structural changes in the muscle and also on the conditions under which it subsequently functions.

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RNA SYNTHESIS IN RING-LIKE NUCLEOLI OF HEPATOCYTES

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Analysis of serial sections showed that the so-called ring-like nucleoli of hepatocytes consist of cavities with amorphous contents surrounded by fibrillary and granular material. These nucleoli are found only occasionally in normal animals; the number of ring-like nucleoli rises considerably in the chronic pathological process caused by repeated injections of CCl_4. Electron-microscopic autoradiography showed that the ring-like nucleoli can synthesize RNA.

KEY WORDS: *Nucleolus; RNA synthesis; CCl_4; electron-microscopic autoradiography.*

Ring-like nucleoli are constantly found in some normal cells: smooth-muscle and endothelial cells, cells of the sebaceous glands, oocytes, lymphocytes, and plasma cells. They are also found in the cells of some tumors (Ehrlich's ascites carcinoma cells and leukemic lymphoblasts) and in hepatocytes in cases of hepatitis or after administration of inhibitors of RNA synthesis [3, 5, 12]. Many workers consider that cells in which ring-like nucleoli are found are in the final stages of differentiation or in a state of degeneration and that they are characterized by a decrease in RNA synthesis. On this basis they regard a ring-like structure as the morphological expression of low functional activity of the nucleolus and they class such nucleoli among structures virtually not synthesizing RNA [3, 5, 7-10, 12, 12]. However, it must be remembered that an increase in the number of ring-like nucleoli during the action of inhibitors or cell differentiation can be only an indirect indication of probable inhibition of RNA synthesis in just those and not all nucleoli. The direct study of this problem has been undertaken only by light-microscopic autoradiography [10, 13], the resolving power of which is too low, and also with the use of lymphocytes in which the overwhelming majority of nucleoli, irrespective of their structure, are not labeled, as the test object.

In this investigation, in order to resolve the problem of RNA synthesis in ring-like nucleoli, the more accurate method of electron-autoradiography was used to investigate RNA synthesis in hepatocyte nucleoli in the course of a chronic pathological process.

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