ULTRASTRUCTURAL INVESTIGATION OF THE EARLY STAGES OF SKELETAL MUSCLE REGENERATION IN MATURE ANIMALS

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Most investigators who have studied regeneration of skeletal muscles consider that the satellite cells described by Mauro in 1961 [9] are the main source of myoblasts. Many researchers have shown that the regenerating power of the skeletal muscles falls off sharply with age and the number of satellite cells decreases considerably [5, 11, 12]. Meanwhile the view has been expressed that the muscle fibers themselves may be sources of myoblasts [6-8, 12]. Most investigations devoted to this problem, however, have been carried out on embryonic material and in the early stages of postnatal development.

The object of this investigation was to study the sources of regeneration of skeletal muscle after crushing in mature animals.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 300-360 g were used. The medial head of the gastrocnemius muscle was traumatized by Cannon's method [3]. Material was studied on the 1st, 2nd, and 3rd days after trauma. Pieces of the medial head of the gastrocnemius muscle were fixed in cold formol-sucrose solution and post-fixed in a 1% buffered solution of osmium tetroxide, and embedded in Araldite.

Ultrathin sections were examined in the GEM-7A electron microscope.

EXPERIMENTAL RESULTS

On the 1st or 2nd day after crushing the structure of most muscle fibers was considerably altered. Disintegration of myofibrils into separate sarcomeres and lysis and disintegration of thick and thin myofilaments to finely granular material were observed.

Side by side with regions of muscle fibers with relatively small structure, there were empty sarcolemmal sheaths. In the interstitial space and beneath the sarcolemma numerous macrophages, polymorphs, and erythrocytes could be seen in the muscle fibers. Single macrophages, performing active phagocytosis, were simultaneously in a state of mitotic division. Beneath the basement membrane of the preserved or injured muscle fibers mononuclear cells could be seen. Cytoplasm of these cells was very poor in organelles and formed a narrow rim around the nucleus. These cells corresponded in their position and ultrastructure to satellite cells (Fig. 1a).

In severely damaged muscle fibers, in which myofibrils disintegrated to finely granular, moderately electron-dense material, the satellite cells also showed signs of injury. Besides intracellular edema, local regions of liquefaction of the cytoplasm were observed in which, as a rule, large vacuoles, lipid droplets, and myelin-like formations and irregularly widened perinuclear spaces could be distinguished. All these features are signs of irreversible destructive changes in the cell (Fig. 1b).

In the relatively preserved muscle fibers, demarcation of nucleosarcoplasmic regions took place beneath the basement membrane over a wide area. Loosening of the myofibrils with loss of their cross-striation and the formation of narrow layers of fibrillary threads were beginning beneath the nucleus. Gradually a pair of plasma membranes separated by narrow spaces began to form through fusion of individual vesicles

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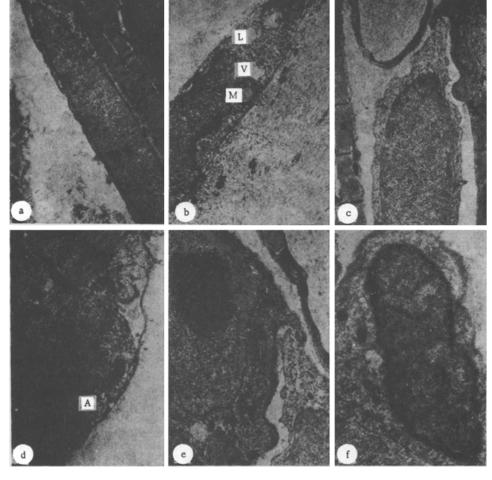


Fig. 1. Electron micrographs of gastrocnemius muscle of rats at different times after injury: a) satellite cell beneath basement membrane of injured muscle fiber, $2800 \times$; b) satellite cell beneath basement membrane of lysed muscle fiber: large vacuoles (V), myelin-like formation (M), and lipid droplets (L) visible in cytoplasm, $7500 \times$; c) isolation of satellite cell from muscle fiber, $8000 \times$; d) unwinding of myofibrils at point of separation of satellite cell, $8000 \times$; e) demarcation of separate nucleosarcoplasmic region, grouping of myofibrils around similar material, $7500 \times$; f) separation of nucleosarcoplasmic region, $8000 \times$.

and slit-like cavities in these regions. As a result a nucleus with a region of dedifferentiated cytoplasm separated, and a mononuclear cell with nucleus elongated along the axis of the fiber was formed beneath the basement membrane. In the remainder of the muscle fiber layers of fibrils, distributed above the nucleus, could be seen (Fig. 1c, f).

In some muscle fibers dedifferentiated regions projecting into the interstitial space could also be found. These regions contained clear signs of myofibril formation. Around the material resembling Z lines myofibrillary threads began to group. Ribosomes, polysomes, and electron-dense small mitochondria appeared in the sarcoplasm (Fig. 1d). Sometimes an independent nucleosarcoplasmic region could be seen (Fig. 1e).

Yet another type of cell, a distinguishing feature of which was the presence of ribosomes and polysomes in the cytoplasm and a rich network of rough endoplasmic reticulum, also was found beneath the basement membrane of the muscle fiber. The nuclei of these cells had a peripheral arrangement of their chromatin. The myofibrillary component of the cytoplasm was unimportant.

In traumatized skeletal muscle, besides destruction, regenerative changes can thus be found as early as during the first 24 h; these changes consist of gradual transition from satellite cells into myoblasts and also the formation of myoblasts from nucleosarcoplasmic regions.

Very occasionally separation of satellite cells from a muscle fiber and the formation of their own basement membrane around them could be distinguished. This process may perhaps take place in the later stages of regeneration.

The second source of myoblast formation in the mature animal is the separation of nucleosarcoplasmic regions of muscle fibers located beneath the basement membrane. By contrast with satellite cells, in which the ultrastructure of the cytoplasm gradually becomes more complex, in nucleosarcoplasmic regions disintegration of the myofibrillary system is the initial process. Later, many ribosomes, polysomes, elements of the rough endoplasmic reticulum, slit-like spaces, vesicles, and myofilaments begin to appear in the perinuclear zone.

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ULTRASTRUCTURAL ORGANIZATION OF TUFT CELLS OF THE SMALL INTESTINAL EPITHELIUM

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KEY WORDS: tuft cells; small intestine; sterile, monocontaminated, and contaminated animals.

Besides undifferentiated, bordered, apical—and basal—granular, and goblet—shaped cells [7–9], the present authors also distinguish in the epithelium of the mucous membrane of the small intestine of mammals and man cells of a special type, similar in structure to the "brush" alveolocytes [1–4, 6] and the tuft cells of the epithelium of the efferent ducts of the liver and pancreas, and the mucous membrane of the gall bladder, stomach, and eye [5, 11]. The study of the ultrastructural features of tuft cells and "brush" alveolocytes led to the suggestion that they are a special type of receptor cell with a specific function in different organs [1–4].

No description of the morphological features of the cells of this type in the epithelium of the small intestine could be found in the literature. It was therefore decided to study the ultrastructure of the tuft cells in different states, in the hope that this would shed light on their role in the activity of the small intestine.

EXPERIMENTAL METHOD

Tuft cells of the epithelium of the villi of the small intestine of rats (sterile germ-free animals, rats monocontaminated with El-Tor cholera vibrios; mature and newborn 3-day-old rats kept under ordinary conditions in the animal house) were studied electron-microscopically. The animals were decapitated in the

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