ULTRASTRUCTURAL INVESTIGATION OF POSTTRAUMATIC REGENERATION OF MUSCLE TISSUE IN MATURE RATS

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The cellular mechanisms lying at the basis of regeneration in muscle tissue are still far from elucidation. Most workers regard the satellite cell as a precursor of the myoblast [2, 3, 6-8]. However, the leading role of the satellite cells still requires further evidence and does not rule out the possibility of other cellular mechanisms.

The writers found previously [1] that signs of regeneration of muscle tissue are clearly visible on the 2nd-3rd day after trauma. Both satellite cells and segregating nucleo-sarcoplasmic areas of the muscle fiber may act as sources of myoblasts after injury.

Data characterizing the subsequent time course of this process are given below.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 300-600 g were investigated. The medial head of the gastrocnemius muscle was injured [1]. Material was investigated on the 8th-10th day after trauma. The muscle was fixed successively in cold formol-sucrose solution and a 1% buffered

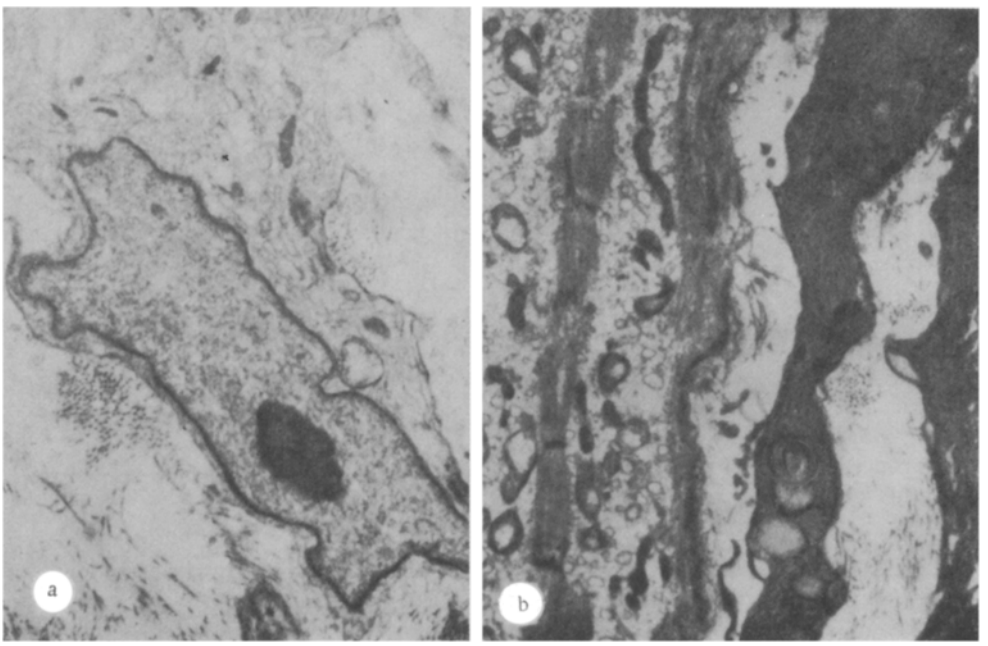

Fig. 1. Pale fibroblast (a) and fragment of dark fibroblast (b). On left side of figure — formation of myofibrils in muscle tubule. 10,000 ×.

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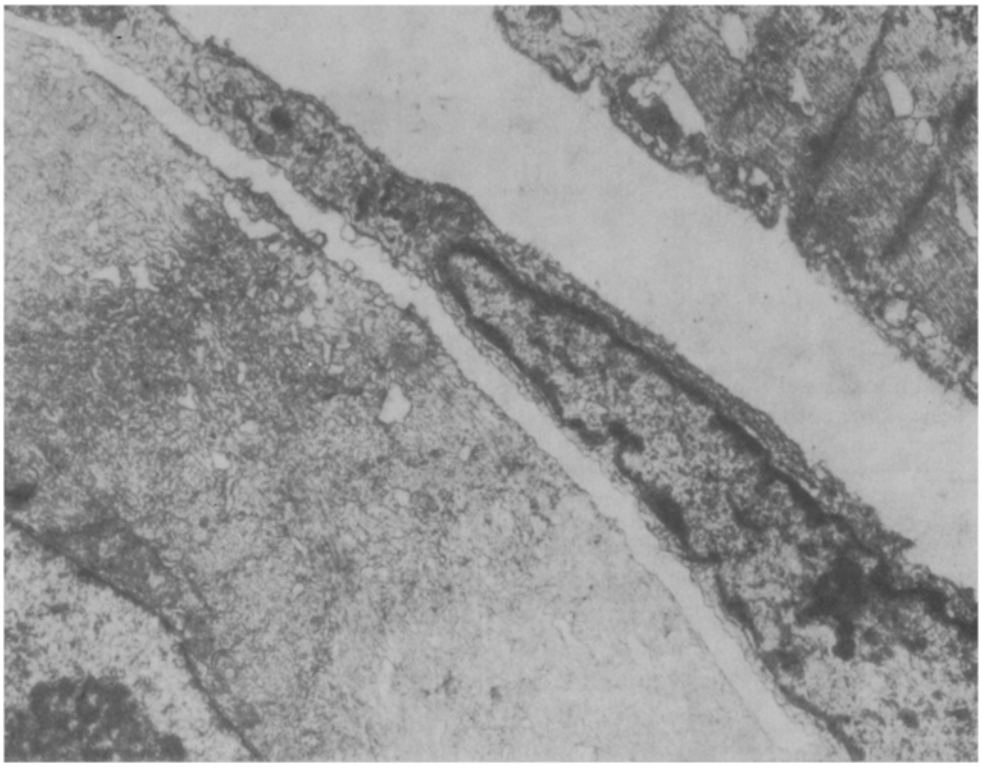

Fig. 2. Muscle tubule. Nucleus located centrally. Cell cytoplasm filled with haphazardly arranged myofilaments. A myoblast can be seen near the muscle tubule. 21,000 ×.

solution of OsO_4 and embedded in Araldite. Ultrathin sections were examined in the IEM-7A electron microscope. Semithin sections, stained with azure-eosin, were studied in a parallel light-microscopic study.

EXPERIMENTAL RESULTS

On the 8th-10th day after trauma resorption of necrotic masses was largely complete. Interstitial spaces were filled with connective-tissue cells. A few macrophages and monocytes were present. Degenerating remnants of muscle fibers were immured by collagen fibers. Two types of fibroblasts predominated among the cells: pale and dark. The pale cells, possibly young forms, were large, with a large multilobular nucleus and hyperosmiophilic nucleolus. Both the rough and the smooth endoplasmic reticulum was well developed in them, with many vesicles, some of which were coated (Fig. 1a). Dark fibroblasts of the same size, with hyperosmiophilic cytoplasm, had many long processes, and most of the cytoplasm was occupied by the rough endoplasmic reticulum, with very densely packed ribosomes. Droplets of fat and myelin-like profiles also were seen. Other organelles were practically invisible because of the high electron density of the cells. Dark fibroblasts were surrounded by areas of collagen fibrils (Fig. 1b).

Destructive fragments of muscle fibers, subsequently undergoing lysis, were separated from normal by plasma membranes. The demarcated viable parts became islands of regeneration. In parts of muscle fibers which remained intact the process of segregation of nucleo-sarcoplasmic areas followed by differentiation and transformation into myoblasts took place more intensively than in the early stages. The ultrastructure of the satellite cells was more complex, with accumulation of ribosomes and polysomes, cisterns of the rough reticulum, electron-dense mitochondria, thin filaments, and vesicles in the cytoplasm. The resting satellite cell, having passed through a series of stages, is thus converted into a myoblast. It must be pointed out in particular that muscle tubules, an undisputed sign of the formation of new muscle fibers, are found at these times. Primary muscle tubules are giant mononuclear cells with a central nucleus, containing one or two nucleoli (Fig. 2). A distinguishing feature of these cells is the presence of thin and thick myofilaments, scattered throughout the cytoplasm. They also contain glycogen granules, small dense mitochondria, ribosomes and polysomes, and also many polymorphic vesicles. Often fusiform cells with a central nucleus lie

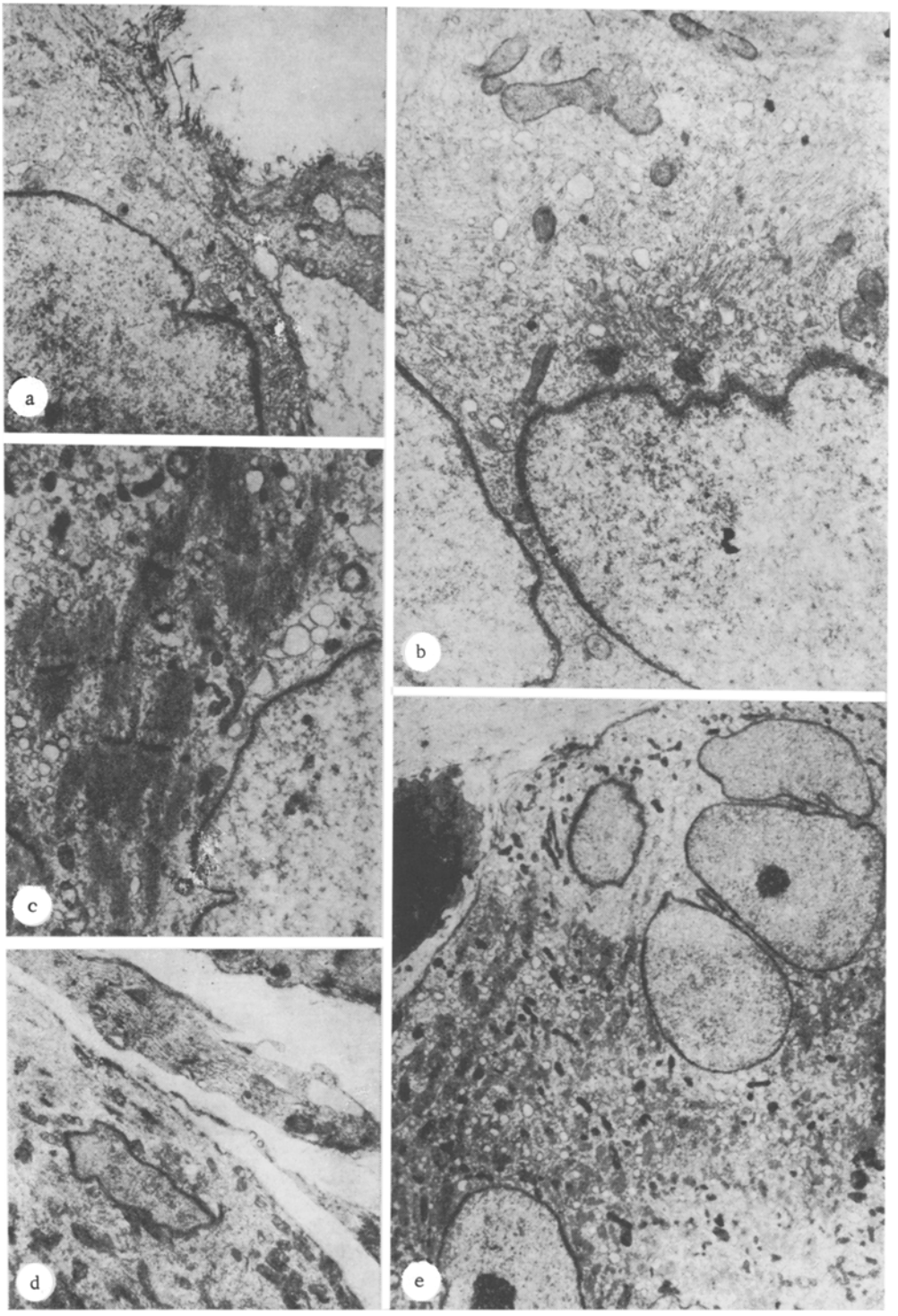

Fig. 3. Fragment of muscle tubule: a) myofilaments visible to cytoplasm and adjacent area of a myoblast can be seen. Arrow indicates site of fusion of myoblast and muscle tubule. 10,000 ×; b) nucleus located centrally. 21,000 ×; c) formation of sacromeres. 10,000 ×; d) muscle fibers of different degrees of maturity, with fragment of fibroblast alongside. 7000 ×; e) condensation of contractile material in cytoplasm. 10,000 ×.

close to the mononuclear muscle tubules — these are myoblasts (Fig. 2, Fig. 3a). Differentiation of the muscle tubules evidently proceeds parallel with maturation of the contractile apparatus of the cell. Mature muscle tubules have a centrally located group of lobular nuclei with finely granular chromatin (Fig. 3b, e). Individual myofilaments are grouped into sarcomeres (Fig. 3c). Sometimes thin myofibrils can be seen under the sarcolemma (Fig. 1b). Muscule tubules are often surrounded by collagen fibers.

Young muscle fibers with different degrees of maturity of their contractile apparatus can be seen at these times. These structures can evidently be formed in two ways: by union of myoblasts and muscle tubules at different stages of differentiation followed by maturation under a common plasma and basement membrane, and by attachment of muscle tubules to a mature muscle fiber and making good the deficient fragment of the muscle fiber (Fig. 3d).

It was concluded from examination of the electron-microscopic material and of the semithin sections that besides the cellular type of regeneration described above, sarcomere formation also takes place within empty basement-membrane sheaths which are still preserved intact, as in intracellular reparative regeneration.

The investigation thus showed that in the mature animal regeneration of muscle fibers can be identified on the 8th-10th day after trauma up to the stage of muscle tubule formation. The process of muscle tissue formation is similar to the formation of muscle fibers in the embryonic period and in early postnatal development [4, 5, 7, 8]. Regeneration is based on satellite cells, but since their number (the cambial reserve) decreases with age, a compensatory mechanism of myoblast formation from segregating nucleo-sarcoplasmic areas of the muscle cell is evidently activated. Seven days after trauma the formation of muscle tubules, in which sarcomere formation is proceeding actively, can be clearly detected. Meanwhile the increase in the number of fibroblasts, and the large areas of collagen fibrils between the muscle fibers must be emphasized. Considerable injury to microvessels and synapses of the muscle apparatus can be observed. The state of these components of muscle tissue largely determines the subsequent course of regeneration.

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