COURSE OF REGENERATION IN MUSCLES OF THE REIMPLANTED RAT LIMB

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UDC 617.57/.58-089.848-092.9-07:615.747/.748-003.9-07

KEY WORDS: regeneration; reimplantation; neurogenic atrophy; microcirculatory disorders

The operation of reimplantation in cases of severe limb trauma is extensively used in surgical practice although its end results satisfy neither clinicians nor experimental scientists [4, 5]. Completeness of the structural and functional restoration of the reimplanted limb ultimately is decided at the level of tissue and cell interactions, which lie at the basis of repair processes in a complex, multicomponent system such as muscle. Research aimed at studying regeneration of a skeletal muscle after crush injury has demonstrated the role of the various components of muscle tissue in regeneration [1, 2]. The aim of this investigation was to study the course of regeneration in the reimplanted limb.

EXPERIMENTAL METHOD

The operation of reimplantation of the right hind limb was performed on male albino rats weighing 270-320 g. The technique of the operation was described previously [3]. A particular feature of the model used was the absence of any prolonged heat ischemia (during the operation the blood flow along the main vessels was not disturbed) and the effect of traumatic injury to the muscle on the course of repair processes was ruled out, for muscles were investigated below the level of division of the tissues. Material (3 animals at each time) were anesthetized with ether in the early (1 month) and later (8 months) periods after the operation. The volume and mass of the muscles were determined. Pieces of muscle were fixed successively in a cold solution of formol-sucrose and 1% buffered OsO_4 solution, and embedded in Araldite. Ultrathin sections were studied in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

An electron-microscopic investigation 1 month after reimplantation showed that the main mass of the muscle consisted of atrophied fibers with signs of vacuolar and fatty degeneration (Fig. 1b). At the same time, fibers with an intact ultrastructure were identified. Repair processes in the muscle fibers followed a course resembling that of intracellular regeneration, and expressed as the formation of sarcomeres and myofibrils in their damaged regions. However, orientation of the newly formed sarcomeres was irregular.

Most of the intramuscular nerves of varied caliber consisted of empty neural sheaths, filled with collagen fibroblasts and Schwann cells (Fig. 2a). Meanwhile, young growing axons were found in some nerve sheaths (Fig. 2a, b). Some of them reached a motor end plate and formed terminals in an old synaptic zone (Fig. 2c).

The study of the microvessels revealed considerable changes in them. Some destructive changes (rupture of the walls, plasmarrhagia, extravasation, stasis of blood cells and microthrombi) may have been due to the reimplantation operation, disturbing the conditions for drainage of blood in the immobilized limb after degeneration. However, capillaries which had

Laboratory of Experimental Pathomorphology, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 325-328, September, 1990. Original article submitted August 25, 1989.

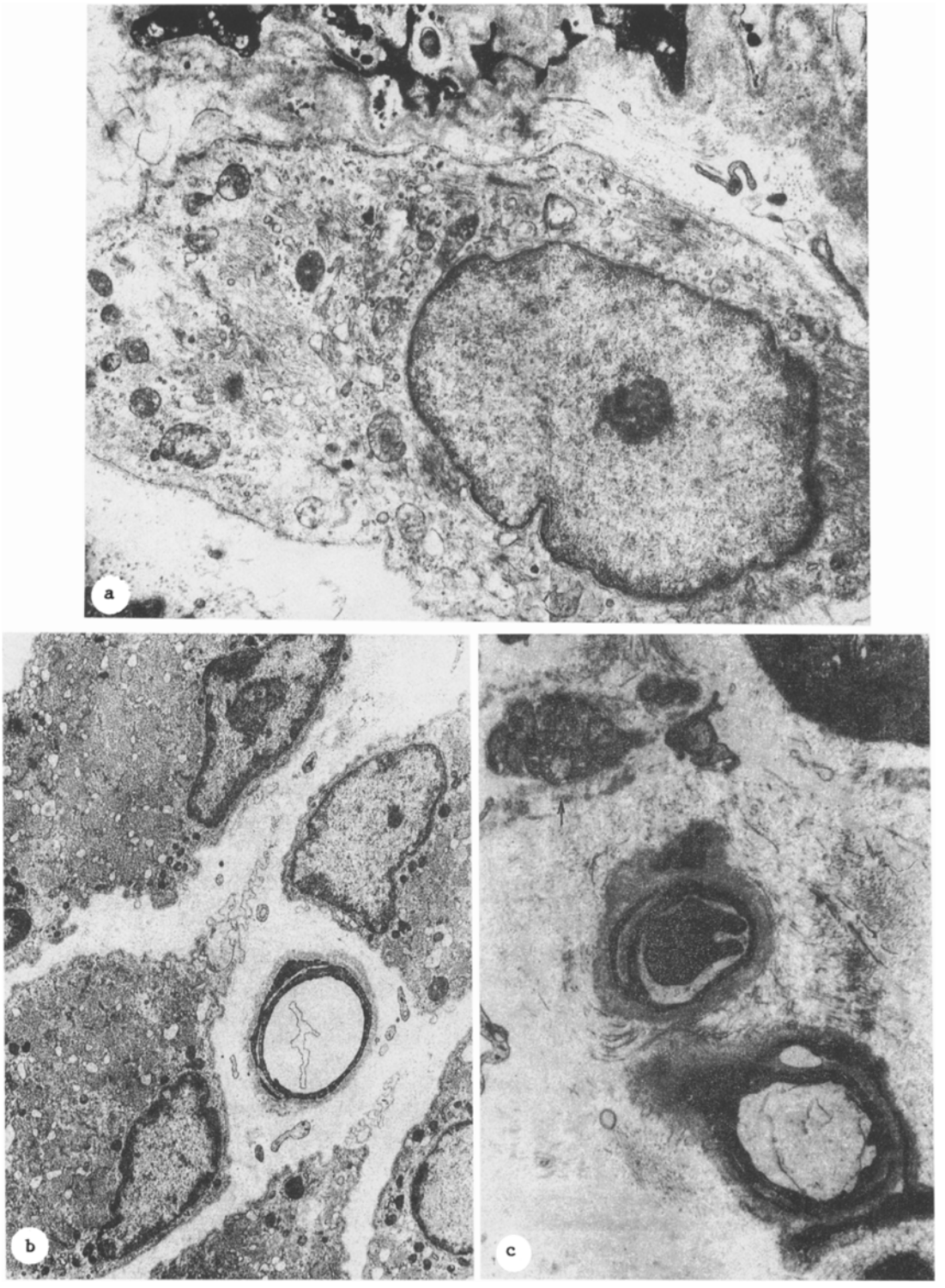

Fig. 1. Destructive and regenerative changes in muscle of reimplanted limb: a) muscle tube (8 months after reimplantation), b) atrophy of muscle fibers (1 month after reimplantation). 3000×, c) hyalinosis of capillary walls (8 months after reimplantation). Arrow indicates pycnotic remnants of endothelial cells of capillaries. 10,000×. Growing axons with signs of degeneration can be seen in top left hand corner. 4000×.

preserved their integrity were formed by electron-dense endothelial cells and had loosely structured and somewhat widened basement membranes (Fig. 1b). Some vessels were found with a desquamated endothelium: acellular capillaries, and ghosts of capillaries also were seen, which could be identified only by the discovery of erythrocytes. Marked condensation of the endotheli-

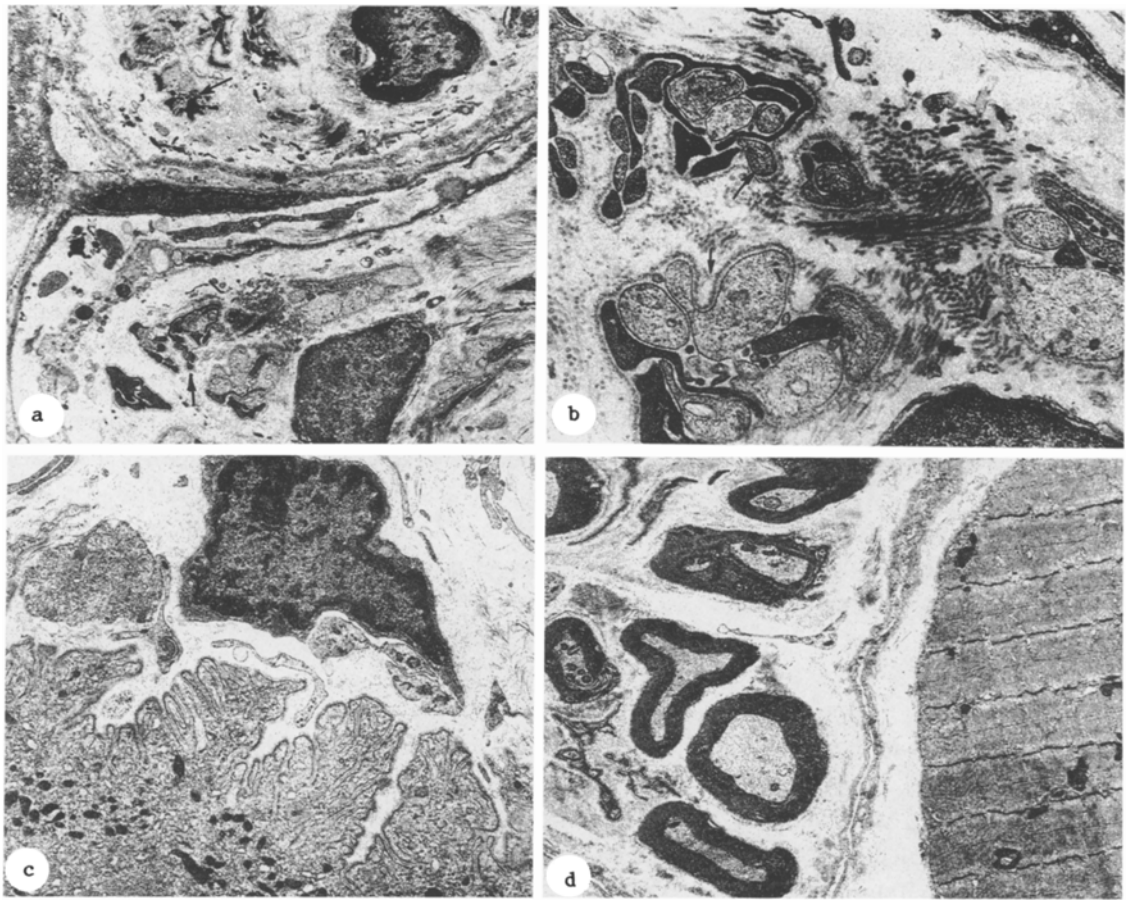

Fig. 2. State of nerve fibers in muscles of reimplanted limb: a-b) growth of single axons in old nerve sheath. a) 3600×, b) 7500×; c) formation of a new terminal in old synaptic zone (8 months after reimplantation), 3000×; d) nerve containing myelinated axon profiles (8 months after reimplantation) 8000×.

al cells and evidence of cytoplasmatosis and desquamation of cells were found in the arterioles. Large vacuoles and caveolae led to the formation of wide ports and channels through the vessel walls. These findings are evidence of destructive changes in the endothelium and a disturbance of vascular permeability. Some vessels, including vessels in foci of atrophy, preserved their regular ultrastructural organization (Fig. 1b).

Thus depending on the character of the ultrastructural changes in the muscles at this time the process may be described as neurogenic atrophy with signs of activation of repair processes in all structural components of the muscle.

In the study of the late stages (8 months) a noteworthy feature was the progressive decrease in mass of the muscles. An ultrastructural investigation revealed aggravation of the dystrophic-atrophic changes, manifested as an increase in the number of fibers with signs of vacuolar and fatty degeneration, with the accumulation of lipofuscin in the cytoplasm and the appearance of sarcolemmal sheaths, filled with pycnotic nuclei. Meanwhile individual stages of regeneration could be identified as far as the formation of muscle tubes (Fig. 1a). These findings demonstrate the active course of the degenerative—regenerative process, as was confirmed also by the active macrophagal response. Predominance of destructive changes led to progressive atrophy of the muscles.

What are the special features of the regenerative process in the muscles of the reimplanted limb that act as an obstacle to the full restoration of its muscle mass, its normal structural organization and, eventually, the perfect functional restoration of the reimplanted limb? The main cause is the imperfection of tissue interaction, especially vaso-neuro-muscular interactions. The results of ultrastructural analysis demonstrate recovery of a large number of nerves containing myelinated axon profiles (Fig. 2b). However, on comparison of the structure of the nerve fibers of comparable diameter, attention is drawn to the decrease in the number of axon profiles in them, and their separation by collagen structures. In these same nerve fibers young growing axons can be observed. The increased electron density of the cytoplasm of the Schwann cells, surrounding the growing axons, is a notewor-

thy feature. Some axon profiles show signs of filamentous destruction or even death of the axons, and all that are left are fragments of electron-dense Schwann cells (Fig. 2b). A significant proportion of the intramuscular nerves consisted of empty sarcolemmal sheaths. The synapses contained small, solitary terminals in the region of an old synaptic zone. Young terminals were overloaded with synaptic vesicles. All these phenomena prevent restoration of adequate neuromuscular connections and they indicate that reinnervation does not take place completely. The basic reason for this may be, first, death of some of the motoneurons and, second, unfavorable conditions for growth and survival of the axons.

One important cause of the development of atrophy in muscles of the reimplanted limb is evidently a combination of structural and functional changes in the microcirculatory. In the late stages destructive changes in the endothelium in the vessels increased, traces of repeated renewal of the endothelial lining were seen in them, together with evidence of hyalinosis and sclerosis (Fig. 1c). The considerable number of emboli and thrombi, obliterating the lumen, at such late periods after reimplantation suggests the presence of an active process, leading to the development of microangiopathies. Although the lumen of most of the capillaries was patent, attention was drawn to a sharp increase in thickness of the endothelium, to plasma seepage through the capillary walls and subsequent hyalinosis and other signs of disturbance of transcapillary exchange. All these factors aggravated the hypoxic disorders, activated collagen synthesis by the fibroblasts, and favored atrophy and sclerosis of the muscles. The possibility cannot be ruled out that besides changes in nerve structures and microvessels, and progression of the destructive changes in the muscles of the reimplanted limb, autoimmune processes may also have played a role, as shown by the discovery of activated plasma cells in the interstitial tissues.

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