REGENERATION OF SKELETAL MUSCLE TISSUE OF WHITE MICE AFTER INJURY BY THE ELECTRIC CURRENT

V. S. Nikitin

Department of Biology (Head—Prof. G. M. Litver), I. P. Pavlov First Leningrad Medical Institute
(Presented by Active Member AMN SSSR A. V. Lebedinskii)
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Research during recent years has demonstrated the high regenerative capacity of the skeletal muscle tissue of a number of mammals [5, 6]. The influence of factors of the external and internal environments on the course of the regeneration of skeletal muscle tissue has been studied [3, 4]. In spite of the high regenerative capacity of muscle fibers, muscle as a whole usually fails to regain its normal structure after injury. Khlopin [7], who investigated tissue cultures, concluded that one of the factors which prevented the complete recovery of muscle fibers is the abundant development of connective tissue in the wound. This was subsequently shown in experiments on muscle regeneration in the intact animal [6].

The object of the present research was to study the factors influencing the relative proportions of muscle fibers and connective tissue in the wound and, in particular, to ascertain how this reaction was related to the specific features of the injury inflicted.

EXPERIMENTAL METHOD

Investigations were carried out on sexually mature male white mice aged from 3 to $3\frac{1}{2}$ months. The gastrocnemius muscle was injured in aseptic conditions by means of an alternating electric current of 127 v. Electrodes were placed directly on to the exposed muscle at its distal and proximal ends. Trauma was inflicted in the form of two or three electric shocks (the current was passed for 0.4-0.6 seconds). A 100 W lamp was connected in series in the circuit. The edges of the skin wound were kept clear of the electrodes. After trauma had been applied, the skin edges were approximated by one or two sutures. The operations were performed during the autumn and winter. The animals were kept at room temperature on an ordinary diet. The material for histological examination was fixed with Zenker's formol or neutral formalin at various stages from 10-15 minutes to 25 days after injury. Serial paraffin-wax sections were stained with hematoxylin-eosin, iron-hematoxylin, azure II-eosin, and by Mallory's method.

EXPERIMENTAL RESULTS

From 10 to 15 minutes after trauma, gross destruction of the muscle was found. Many muscle fibers were strongly contracted. This contraction was accompanied by irreversible changes in the myofibrillar apparatus, and led to amyloid degeneration of this apparatus. Some muscle fibers were ruptured in many places. Most of these tears were within the sarcolemma, i. e. they involved mainly the myofibrillary apparatus. The changes in the middle part of the muscle fibers between the points of application of the electrodes showed an exceptionally marked mosaic pattern. Most fibers were speckled with numerous waves of persistent contraction of the myofibrillary apparatus, of various shapes. In several cases the longitudinal fibrillary structure and the cross striation of the muscle fibers were replaced by a chaotic distribution of the myofibrils. In the deep layers of the wound the muscle fibers showed granular degeneration, with the formation of masses of large granules, which sometimes escaped through the tears in the sarcolemma during contraction of the muscle and were situated between the muscle fibers. In other fibers granules were found in the form of chains along the individual myofibrils. In these cases it was possible to show that the granules were products of the disintegration of the myofibrillary apparatus. In certain muscle fibers the cross striation had almost disappeared. The disks of individual myofibrils were displaced in relation to each other, and their contours became merged. Such fibers subsequently underwent disintegration into large and small granules. At the points of application of the electrodes at the distal and proximal ends of the wound, as a result of the thermal action of the electric current the muscle fibers showed signs of necrosis, with the formation of necrotic masses very resistant to phagocytosis and autolysis. Besides the forms of destruction of muscle fibers described, various transitional forms were encountered. In this respect our observations were in agreement with the findings of Dmitriena [1], who investigated the relationship between various forms of destruction of muscle.

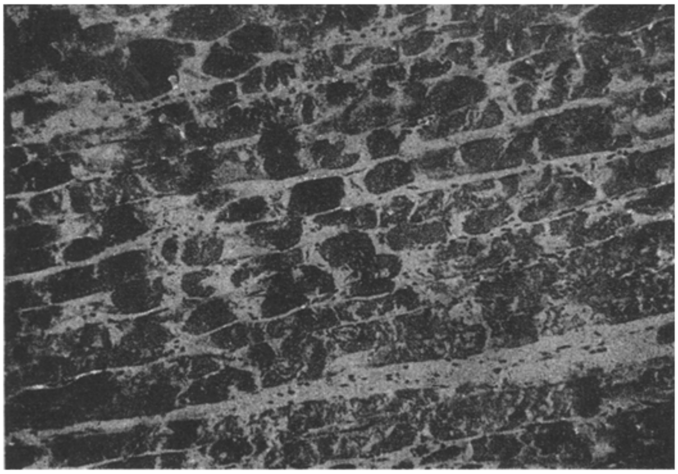

Fig. 1. Necrosis of traumatized muscle fibers 24 hours after injury. Stained with iron-hematoxylin. Magnification: objective 8 x, ocular 15 x.

One hour after injury, the pattern of necrosis of the muscle was essentially altered. Within the sarcolemmic tubes that remained, separate masses of the substance of the muscle fibers could be seen, in the form of disks, spirals, and so on. The spaces between the masses were filled with small amounts of finely granular material, resulting from the autolytic destruction of the muscle substance between areas of contracture of the myofibrillar apparatus. At this stage and later, until three days after trauma, many muscle fibers underwent gradually developing, finely granular degeneration. The granular mass thus formed differed, however, in its staining properties and the polymorphism of its granules, from the granular pattern described 10-15 minutes after injury. The source of the granules in this case was both the myofibrillary apparatus and the sarcoplasm. Subsequent changes in the necrotic masses took the form of swelling, crumbling, and conversion into detritus. The general picture of the degenerative phenomena on the first day after trauma was characterized by disintegration into large and small granules, with the arrangement of the necrotic masses in rows along the course of the former muscle fibers (Fig. 1). Among the necrotic muscle fibers, and within the fibers themselves, phagocytes were seen, although on the first and second, and even on the third day they were relatively few in number, and the disposal of the products of disintegration was mainly effected by autolytic processes. The spaces between the necrotic muscle fibers were permeated by connective tissue cells and invaded by capillaries containing blood. These surrounded the rows of granules and created a characteristic framework at the site of the disintegrating muscle fibers, thereby facilitating the preservation of the structural integrity of the pattern of the injured muscle (Fig. 2). On the fourth day the resorption of the necrotic masses in the greater part of the wound was almost complete.

The processes of regeneration in the muscle tissue developed on account of the surviving and partially injured muscle fibers in the deeper layers of the wound. Many muscle fibers, which preserved their normal structure,

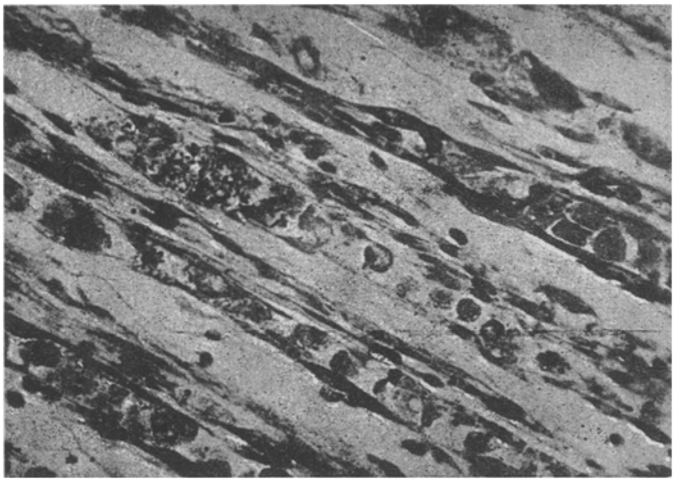

Fig. 2. Resorption of the necrotic masses and growth of the connective-tissue cells in the wound. Third day after trauma. Stained with azure II-eosin. Magnification: objective 40 ×, ocular 10 ×.

subsequently underwent partial disintegration or considerable reorganization. These fibers were the main source of regeneration of the muscle. The initial signs of regeneration were reasonably uniform, and were characterized by the accumulation of a basophilic sarcoplasm around the muscle nuclei. Spindle-shaped mono- and multinucleated sarcompasmic complexes were formed, which fused together along the length of the fiber and laid the foundations of the basophilic nucleated sarcoplasmic bands, occupying both a central and a peripheral position in relation to the axis of the muscle fiber. In the more severely damaged muscle fibers this process did not go far, for signs of degeneration developed rapidly in these fibers, affecting the old myofibrillary apparatus in the first place. Some of the multinucleated sarcoplasmic complexes which remained after destruction of the muscle fiber continued to develop into myosymplasts, and mitotic division of the nuclei sometimes took place in these structures.

In the less severely injured muscle fibers, the process of formation of the nucleated sarcoplasmic bands went further, and a large amount of sarcomplasm accumulated and the nuclei divided intensively. There could be several such bands in a muscle fiber. Those which lay immediately beneath the sarcolemma could become detached from the muscle fiber and become converted into free myosymplasts. When several nucleated sarcoplasmic bands were present in the muscle fiber, the latter could break up at once into a whole bundle of myosymplasts. After strong stimulation with the electric current many muscle fibers, although remaining outwardly little changed, could not exist for long without reorganization of the whole internal system, so that their differentiation and conversion into structures of the myosymplast type, capable of development, must be regarded as progressive phenomena.

The splitting of muscle fibers in the depth of the wound was also important for the filling up of the more superficial layers of the wound defect with myosymplasts. Here, however, we often found fine myosymplasts, accompanied by spindle-cells with basophilic cytoplasm, growing along the rows of necrotic masses. From the investigation of the relationship between these cells and the myosymplasts mentioned above, it may be supposed that after injury to a muscle by the electric current, the myoblasts play some part in regeneration.

A characteristic feature of the regeneration of muscle after injury by the electric current was that in almost every case the myosymplasts grew parallel to each other. To a large extent this could be explained by the splitting of the myosymplasts from the old muscle fibers in the depth of the wound. The principal cause of the favorable course of regeneration, however, was absence of a wound cavity and of mechanical displacement of the tissues as a result of the trauma, and also the partial preservation of the fibrous structures of the endomysium. A new connective-tissue framework, with myosymplasts growing into it, was formed from the remnants of the old framework. Favorable conditions of tension were created in the wound, and this encouraged regeneration of the muscle [6]. The myosymplasts as a rule grew between the dying fibers and did not utilize the old sarcolemmic

Fig. 3. Differentiation of young muscle fibers. Twelfth day after trauma. Stained with iron-hematoxylin. Magnification: objective 40 ×, ocular 7 ×.

sheaths, as was described by Zenker [8], who investigated the regeneration of muscles in typhoid fever. A certain part of the fibrous structures of the old sarcolemmas evidently entered into the composition of the connective-tissue foundation of the new muscle, but on the whole the sarcolemmas of the regenerating muscle fibers were formed anew.

In most cases by the end of the fourth day and on the fifth day after trauma a considerable number of myosymplasts had accumulated in the wound. Their orderly arrangement facilitated their rapid growth and differentiation. On the sixth day a myofibrillary apparatus had formed in the myosymplasts, and these were converted into muscle tubes. Growth and differentiation of the latter also took place quickly. The number of their nuclei diminished, and on the 10th-12th day young muscle fibers were formed (Fig. 3). On the 15th day they had attained a considerable thickness, and only the presence of small areas with an incorrect arrangement of fibers indicated that we were dealing not with old muscle fibers undergoing reactive changes, but with young tissue. On the 25th day the number of nuclei in the muscle fibers was very small; most nuclei were situated beneath the sarcolemma, and fibers were rarely found with short chains of nuclei. The general character of the arrangement of the muscle fibers had become much nearer to normal.

Regeneration of the muscle was complicated only by the presence of peculiar necrotic masses resistant to both phagocytosis and autolysis. These were found in the distal and proximal parts of the wound, at the points of application of the electrodes. Their number was related to the degree of the thermal action of the current. These necrotic masses were only destroyed by the 10th-12th day, when myosymplasts from the middle parts of the wound enveloped them. The differentiation of the muscle fibers in this region was considerably delayed. Nevertheless, the main property of the regenerating muscle fibers—their orderly arrangement—remained unchanged. Although our observations ended on the 25th day of the course of regeneration, there is, therefore, every reason to suppose that subsequently the regenerated muscle remained a perfect structure, in both morphological and physiological respects.

Although injury by the electric current was accompanied by severe destructive processes in the muscle, its normal structure was thus almost completely restored. One of the more important reasons for this was the presence of a supporting framework, directing the growth and movement of the connective-tissue elements and the myosymplasts.

SUMMARY

Regeneration of gastrocnemius muscle was studied in white mice after an injury inflicted by electric current (127 v.). Electric lesions of the muscle result in complete destruction of a considerable number of its fibers. Degeneration of the muscle fibers is varied and mosaic in character. Small lump, large lump, and granu-

lar forms of degeneration are prevalent. The absence of wound cavity, rapid resorption of necrotic masses, preservation of some fiber structures in the injured muscle endomysium create favorable conditions for regular growth and free movement of young connective tissue and myosymplasts. In such conditions connective tissue does not prevent regeneration of the muscle, the normal structure of which is restored almost completely.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-over English translations appears at the back of this issue.