The results differ from those described by Ol'binskaya and Litvitskii [2]. In our view the reason is differences in the technique of inducing TCS and the time of the reperfusion on period. Further investigations are evidently needed, in which the emphasis laid on the duration of CS will have to be switched to the interval between them, and the reperfusion time and frequency of TCS reduced. Further efforts at improving the experimental model of ischemic myocardial damage will also have to be aimed at creating a remotely controlled TCS with strict monitoring to ensure total or partial reduction of the coronary blood flow and its restoration in order to make the experimental and real conditions as close as possible and, above all, to make anesthesia and artificial ventilation of the lungs unnecessary.

The discovery of damage to the myofibrillary apparatus due to contracture indicates a disturbance of cardiomyocyte function. Metabolites of arachidonic acid and endoperoxidases evidently are involved in the genesis of the disturbance of sarcolemmal function, for the ability of ischemia to activate phospholipase A_2 is well known, and the pathogenic effect of this activation can be realized only if oxygen (i.e., reperfusion) is present.

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EFFECT OF STIMULATION ON REGENERATION OF GASTROCNEMIUS MUSCLE IN OLD RATS AFTER X-RAY IRRADIATION

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KEY WORDS: skeletal muscles; old rats; regeneration; x-ray irradiation; laser therapy

This investigation is a continuation of research into methods of stimulating repair processes in irradiated muscles of old animals. Considerable recovery of regenerative capacity of the irradiated gastrocnemius muscle of young, sexually mature, and old rats was demonstrated previously under the influence both of laser irradiation and of transplantation of minced unirradiated muscle tissue into the region of injury of the gastrocnemius muscle [1, 5]. However, in old animals, areas of dense connective tissue are formed in the region of injury to the gastrocnemius muscle only after treatment with helium–neon radiation, and they limit growth of muscle fibers restored after x-ray irradiation. In the case of transplantation of unirradiated minced muscle tissue the positive effect was reduced in the late stages of regeneration: the number of muscle fibers in the central zone of the defect was reduced compared with the zone located near the muscle stumps. To potentiate the stimulating action on the regenerative capacity of skeletal muscles of old rats, irradiated with ionizing radiation, transplantation of the unirradiated minced muscle tissue was carried out simultaneously with laser therapy.

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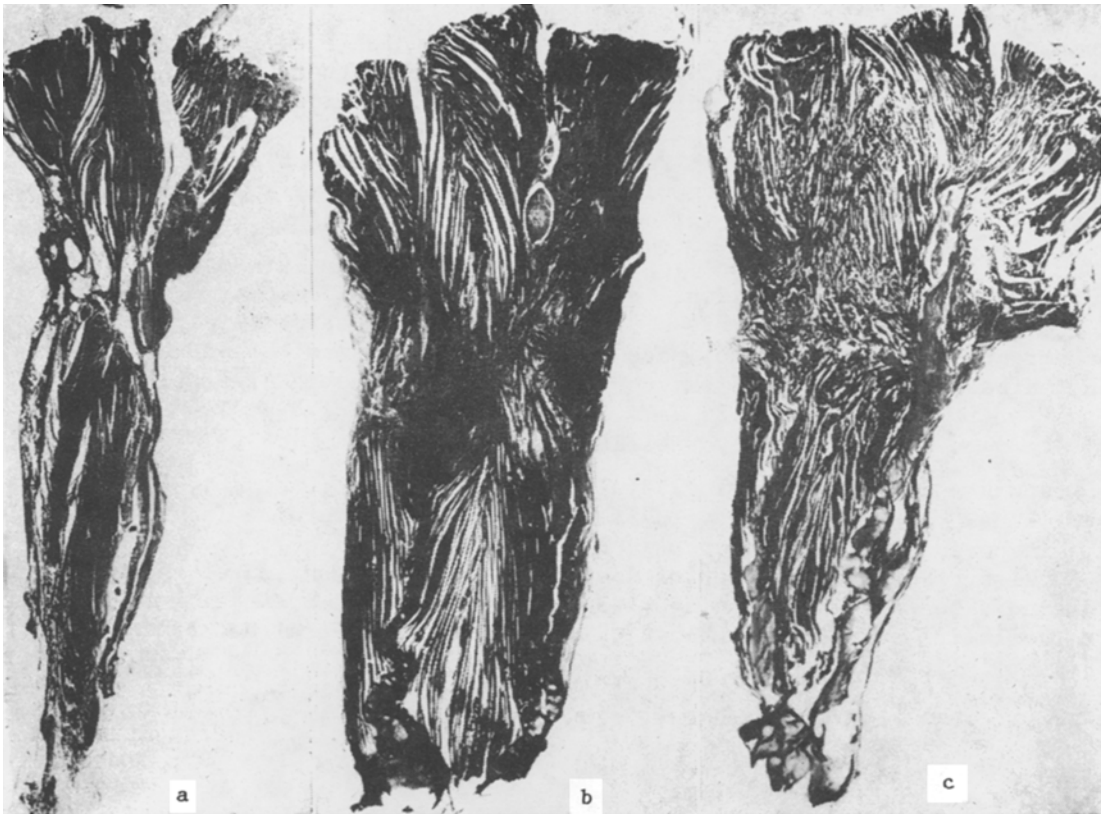

Fig. 1. Histologic sections through regenerating muscles. a) Experiments of series I. Irradiation in a dose of 20 Gy and transverse division of gastrocnemius muscle, stage of regeneration 30 days; b) series II. Irradiation in dose of 20 Gy, transverse division of gastrocnemius muscle, transplantation of unirradiated minced muscle tissue into region of injury, followed by laser therapy; stage of regeneration 30 days; c) the same series of experiments, stage of regeneration 90 days. Stained with Regaud's hematoxylin and counterstained by Mallory's method. 10×.

EXPERIMENTAL METHOD

Experiments were carried out on 36 noninbred albino rats aged 2-2.5 years. There were three series of experiments. In series I the gastrocnemius muscle was divided transversely after local x-ray irradiation in a dose of 20 Gy (RUP-200 apparatus, voltage 190 kV, current 15 mA, dose rate 0.75 Gy/min, filters 0.5 mm Cu + 1 mm Al). In series II, unirradiated minced muscle tissue taken from the left limb was grafted into the region of the defect in the transversely divided right gastrocnemius muscle, irradiated in the same dose, and this was followed by irradiation with a helium–neon laser (OKG-12 apparatus, wavelength 632.8 nm, power flux density 2.5-3 mW/cm^2); the laser beam was defocused by means of a lens with field diameter of 2 cm, and in the course of 22-25 days, 8-9 sessions of laser therapy, each lasting 0.5 min, were given. The process of regeneration was studied for 90 days. It was shown previously by histological methods that blood vessels and nerve fibers actively grow into unirradiated, minced muscle tissue, grafted into the region of transverse division of the irradiated gastrocnemius muscle of young, sexually mature rats [4]. To determine functional activity of the muscle tissue developing from the grafted, minced muscle tissue under conditions of laser therapy, the experiments of series III were undertaken: unirradiated minced gastrocnemius muscle from the left limb of old rats was autografted into the irradiated bed of the right limb, and this was followed by treatment with the helium–neon laser beam with the same conditions of exposure. Contractility of the regenerating muscles was determined 60 days after transplantation. Histologic preparations were stained with Regaud's iron–hematoxylin and counterstained by Mallory's method. The quantitative ratio between areas occupied by muscle tissue, connective tissue, and fibrin was determined on sections through the regenerating muscles in the experiments of series I and II 30 and 90 days after transverse division. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

In the experiments of series I x-ray irradiation inhibited regeneration of the transversely divided gastrocnemius muscle. In the course of 1 month erythema and exudative edema of the skin, the formation of weeping purulent scabs, and the frequent appearance of deep ulcers of skin and muscles were observed. Hair did not grow on the irradiated skin. The inflammatory reaction was sluggish in its course and resorption of fibrin and necrotic masses was considerably delayed. Growth of muscle and connective tissues was inhibited. After 90 days quite large areas of loose connective tissue with fat cells and also wide bundles of dense collagen fibers could be seen in the defect. Many muscle fibers were replaced by connective tissue (Fig. 1a). According to the results of morphometric analysis, these regenerating muscles contained 55 ± 3% of muscle tissue and 45 ± 3% of connective tissue.

In the experiments of series II the methods of stimulation used led to reduction of inflammation of the irradiated skin. The wound edges were firmly joined and on the 10th-14th day the suture line was dry and clean. Growth of the hair was considerably restored. The muscle stumps were firmly connected (Fig. 1b). Histologic analysis of the regenerating muscles showed that 14 days after transverse division no fibrin was present in the region of the defect. Connective tissue was actively formed. As a result of regenerative transformation of the grafted minced muscle tissue, the two muscle stumps were connected by muscle cells, consisting of myosyncytia, muscle tubes, and young muscle fibers, running in different directions. Regenerating muscle cells 30 days after the operation formed a dense plexus, although the diameter of these muscle fibers and muscle tubes was still small compared with the muscle fibers of the stumps. The grafted unirradiated minced muscle tissue also stimulated the regenerative capacity of the irradiated muscle fibers. Along the widened septa of the irradiated proximal stump, growth of myosyncytia containing up to 10-12 muscle nuclei, and also of muscle tubes and young muscle fibers, took place from the intact muscle fibers and ends of the divided muscle fibers. Connective tissue matured more uniformly. It contained many spindle-shaped fibroblasts, delicate collagen fibers, and blood vessels, with frequent mitoses. Inflammatory cells were few, and consisted mainly of macrophages, which were not found throughout the regenerating muscle, but were localized in groups. Areas of loose connective tissue with numerous fat cells were not found. In the region of the defect 90 days after transverse division further growth of muscle fibers in both length and width was observed, and the bands of connective tissue between them were reduced in size (Fig. 1). Morphometric analysis of the histologic preparations showed that the amount of muscle tissue in the regenerating muscles in this series of experiments was 91 ± 1 and 95 ± 3%, and the amount of connective tissue was 9 ± 1 and 5 ± 3% respectively. The results of the experiments of series II differed significantly from those of series I ($p < 0.01$).

The results of the experiments of series III are evidence that helium—neon radiation has a beneficial effect on regeneration of unirradiated minced muscle tissue of old rats in an irradiated bed. Transplanted muscle tissue developed actively. Numerous blood vessels, and many inflammatory cells, chiefly macrophages, invaded the graft and mitoses were frequent. On the 21st day resorption of fibrin and reorganization of the muscle fragments were complete. Myosyncytia, muscle tubes, and young muscle fibers contained large regenerative nuclei. Later the number of muscle fibers increased. Determination of the contractility of the grafts showed that the muscle tissue formed was functionally active after 60 days. Of 12 regenerating muscles only two responded by contraction to direct stimulation, but the remainder contracted in response to stimulation of the tibial nerve.

Transplantation of unirradiated minced muscle tissue into the region of trauma of skeletal muscles irradiated in a dose of 20 Gy in old rats, subsequently receiving laser therapy, thus promotes marked restoration of regenerative activity in them. Simultaneous application of these two methods of stimulation leads to earlier healing of the skin wound, improves the structure of the defect in the irradiated, divided muscle, where the region of injury is virtually completely filled, and there are no dense bundles of collagen fibers or areas of loose connective tissue with fat cells. The effect lasts for quite a long time. The results of morphometric analysis confirm the histologic investigations. The combined action of transplantation of unirradiated, minced muscle tissue and of laser therapy evidently stimulates processes of postradiation repair in irradiated muscles. To restore tissues from radiation damage, it is necessary to stimulate both energy and protein metabolism [7]. Helium—neon radiation has been shown to stimulate tissue metabolism [2, 6]. Transplantation of unirradiated minced muscle tissue also largely helps to restore normal metabolism of the irradiated tissues, enhancing their regenerative powers [3].

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