EFFECT OF A HELIUM – NEON LASER BEAM ON REGENERATION OF IRRADIATED TRANSPLANTED SKELETAL MUSCLE

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Three series of experiments were carried out on the gastrocnemius muscle of rats. In series I whole muscles were autografted on both limbs. In the experiments of series II, before autografting of the muscles both hind limbs of the animals were irradiated in a dose of 1000 R. In series III, after irradiation in the same dose and autografting of the gastrocnemius muscles, the rats' hind limbs were exposed for 10 days to the action of a laser beam. The process of transplantation regeneration was studied histologically from 1 week to 3 months after these procedures. The results showed that monochromatic coherent polarized red light has a stimulating action on regeneration if totally suppressed by x-ray irradiation. Regeneration takes place not only in the peripheral, but also in the deep zone of the muscle, and leads to the formation of a contractile muscular organ.

KEY WORDS: transplantation of muscles; regeneration; x-ray irradiation; helium–neon laser.

Regeneration of mammalian skeletal muscles after free autografting, first described by Studitskii [10], has subsequently been studied in detail by his collaborators [2, 3, 7-11] and also by researchers outside the USSR [12, 13]. Methods of free grafting of muscles developed in Studitskii's laboratory have found clinical application in the treatment of disturbances caused by diseases of muscles [13]. Transplantation regeneration of skeletal muscle, which under normal conditions ends with the formation of a contractile organ, has been shown to be severely disturbed and depressed after exposure to ionizing radiation in doses of 1000 R or more [7, 11]. The study of the nature of the disturbance of regeneration and the search for methods of restoring the normal course of regeneration in irradiated tissues are of practical as well as theoretical interest.

The object of the present investigation was to study the effect of light from a helium–neon laser on regeneration of the previously irradiated, freely grafted muscle. The work of Inyushin et al. has shown that laser radiation accelerates metabolic processes and thereby improves the course of regeneration, and also that it has a therapeutic effect in certain diseases [5, 6]. It has also been reported that the laser beam accelerates healing of trophic and radiation ulcers [4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats aged 2-3 months. In the experiments of series I both gastrocnemius muscles were completely excised and transplanted in the same bed, and the proximal and distal ends were sutured to the remains of the tendons. In series II, a few hours before transplantation of the muscles one or both hind limbs of the animal were irradiated with x rays in a dose of 1000 R (conditions of irradiation: RUP-200 apparatus, tube voltage 190 kV, current 15 mA, filters 0.75 mm Al + 0.5 mm Cu, dose rate 67 R/min). In the experiments of series III the limbs were irradiated in the same dose, and the gastrocnemius muscles then autografted. Starting with the 2nd day after the operation, in series III the limbs were exposed to a helium–neon laser beam (LG-75, wavelength 6328 Å, for 10 days). Regeneration of the muscles was investigated histologically at various times between 1 week and 3 months later. At each time of fixation five or six animals were used. The muscles were fixed in Carnoy's solution or in 12% neutral formalin. Sections were stained with hematoxylin by Regaud's method, counterstained by Mallory's method, and impregnated with silver by the Bielschowsky–Gros method, with gilding by Lavrent'ev's method. In a few animals the blood vessels were injected with a solution of ink in gelatin. The contractile activity of the regenerating graft was determined in the anesthetized animals before sacrifice. The motor nerve was stimulated by pulses of direct current with a frequency of 60 pulses/min and voltage of between 1 and 3 V.

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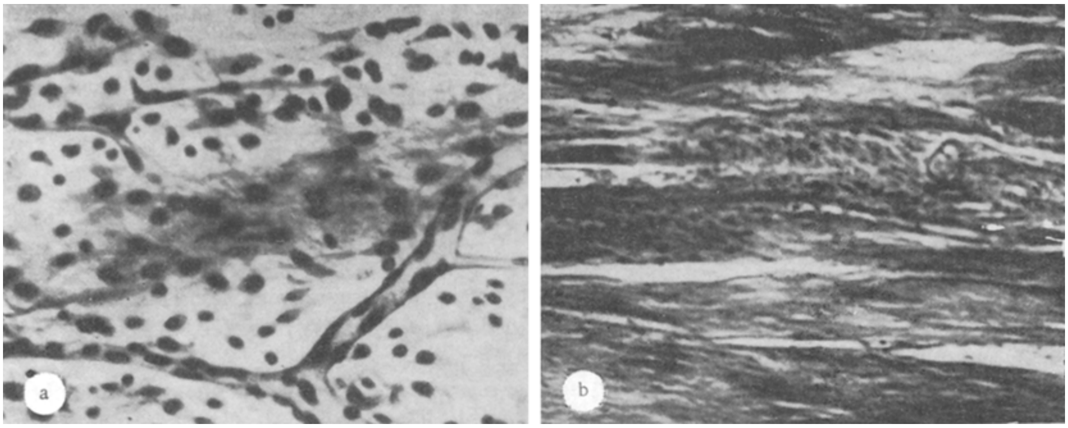

Fig. 1. Connective-tissue structure of autograft of irradiated (1000 R) muscle. Fixation in Carnoy's solution. Iron-hematoxylin, counterstained by Mallory's method; a) 3 weeks after muscle grafting, 600×; b) 3 months after muscle grafting, 100×.

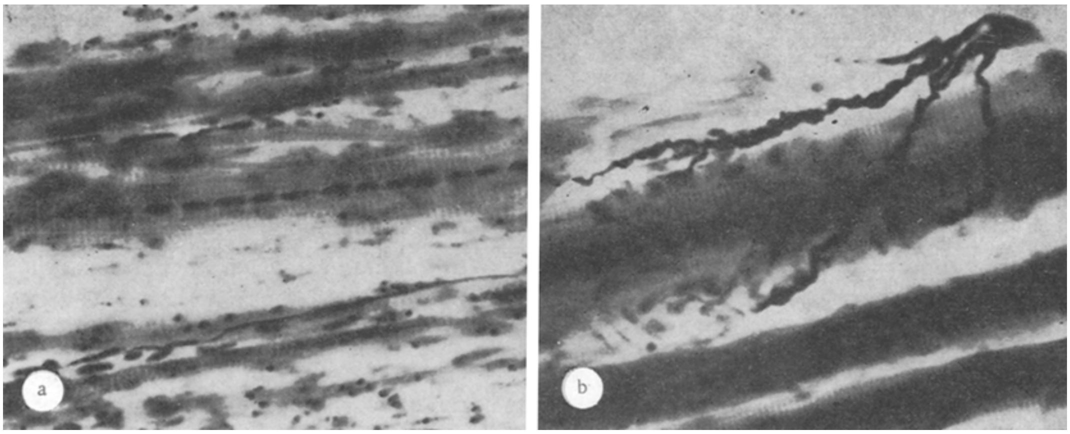

Fig. 2. Muscle tissue in autograft of irradiated (1000 R) muscle treated by laser beam. Impregnation by Bielschowsky–Gros–Lavrent'ev method: a) 3 weeks after muscle grafting. Newly formed and surviving muscle fibers with regenerating axons in center of graft, 400×; b) 3 months after grafting. Differentiated muscle fibers with large neuromuscular plaques, 400×.

EXPERIMENTAL RESULTS

The histogenesis of the muscle tissue after autografting of the whole gastrocnemius muscle took place as follows. The muscle, deprived of its blood supply, innervation, and tension, was completely reconstructed [1]. The process began at the periphery of the graft and gradually extended to all deeper regions. Blood vessels grew into the peripheral zone as early as on the first 2 or 3 days, an active inflammatory reaction developed, and granulation tissue formed. The myofibrillary apparatus and part of the sarcoplasm of the muscle fibers died. Inside the sarcolemma tubes, and also between them, mononuclear cells (myoblasts) appeared; by dividing and joining together these cells formed muscle tubes, which later differentiated into muscle fibers. After 1 week, thin regenerating axons could be seen at the proximal end of the graft. After 3-4 weeks the process of resorption of necrotic masses was largely complete and further differentiation of the newly formed muscle and connective tissue and also of the blood vessels and nerves of the contractile organ took place.

Preliminary irradiation of the limb in a dose of 1000 R almost completely suppressed the ability of the skeletal muscle to undergo transplantation regeneration. The inflammatory process was late in developing and was on a very small scale. The muscle fibers underwent almost total Zenker's necrosis and disintegrated into fragments or, sometimes, into granules. After 1 week, small muscle buds with two to four nuclei were seen only occasionally in the surface layer of the graft and in the region of injury to the proximal stump. There was very little granulation tissue, which was located at the periphery and contained only a few cells. Later it

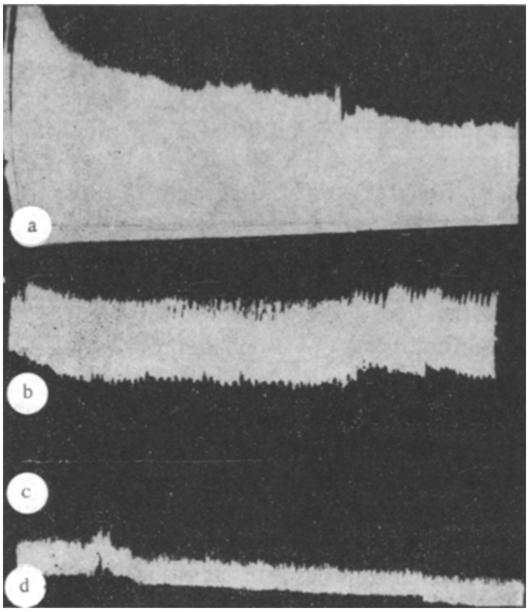

Fig. 3. Kymograms of normal muscle (a), of unirradiated muscle 3 months after autografting (b), or irradiated (1000 R) muscle 3 months after autografting (c), and irradiated (1000 R) muscle 3 months after autografting and treatment with laser beam (d).

became activated and began to occupy regions of dying muscle fibers. After 3 weeks, a central zone of dying muscle fibers and a peripheral zone filled with condensing connective tissue and a network of blood vessels could be distinguished in the graft (Fig. 1a). In some specimens, areas of thin, dystrophic muscle fibers, compressed by connective tissue, could be seen in the peripheral zone. After 2 to 3 months they atrophied, and in place of the graft there remained a connective-tissue formation consisting of dense connective tissue with a sensory innervation (Fig. 1b). The results of experiments in which the irradiated grafted limbs of the animals were exposed to the action of the laser beam differed signifcantly from those of the control experiments. Despite the x-ray irradiation, regeneration took place in the grafted muscles, although after some delay. After 2-3 weeks active reconstruction of the muscle tissue was observed in a relatively wide peripheral zone and also along the connective tissue septa. Newly formed muscle tubes and muscle buds were distributed in the granulation tissue, rich in blood vessels. Here and there thin muscle fibers could be seen. It is important to emphasize that regeneration took place not only at the periphery of the grafted muscle, but also in its deep layers. Many muscle fibers did not die, but survived the unfavorable conditions connected with grafting of the muscle and radiation trauma. Such fibers were of considerable width, and contained many nuclei, located both in the center and at the periphery of the fiber, with large nucleoli and clearly-defined cross-striation (Fig. 2a). They were surrounded by a network of preserved and newly formed capillaries. In the central part of the grafts areas of necrotic tissue were still present. After 3 weeks still more young muscle fibers appeared in the peripheral zone. Regenerating axons grew toward them. After 3 months regeneration was largely complete. The muscle fibers were well vascularized and richly innervated. Neuromuscular plaques were branched and contained up to 10 or more nuclei. Argyrophilic swellings were present along the course of the axons. The small bands of connective tissue formed at the site of resorption of the necrotic tissue gradually became thicker, and areas of fatty tissue appeared in them.

Hence, during regeneration of the irradiated, transplanted muscle treated by the laser beam a muscle which was somewhat smaller than the normal muscle formed. More than half of all the newly formed muscles consisted mainly of functionally active muscle tissue. If the irradiated grafted muscle was not treated with the laser beam, inhibition of transplantation regeneration led to the formation of a connective-tissue structure, incapable of contracting, instead of a muscular organ. The results of investigation of the contractile power of regenerating muscles in all three series of experiments are given in Fig. 3. The results show that the laser beam, if applied under certain conditions to the irradiated limb with an autografted skeletal muscle, diminishes the after-effects of radiation trauma and restores the normal processes of transplantation regeneration of the muscle.

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PROLIFERATIVE ACTIVITY IN SOME TISSUES OF MICE UNILATERALLY NEPHRECTOMIZED AT DIFFERENT TIMES OF DAY

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Unilateral nephrectomy was performed on mice at different times during the morning and evening. Mitotic activity was investigated in the proximal portions of the convoluted tubules of the nephron of the remaining kidney and in the corneal epithelium 48 and 60 h after the operation. If the operation was performed in the morning the mitotic index (MI) was 4.3 times higher than the control and still remained high 60 h after the operation. If the operation was performed in the evening MI was 9.3 times higher than the control and fell after 60 h. It is concluded that the cells of the renal epithelium in animals nephrectomized in the evening divide more synchronously than in mice nephrectomized in the morning. Nephrectomy did not affect the level or rhythm of cellular proliferation in the corneal epithelium.

KEY WORDS: unilateral nephrectomy; mitotic index; diurnal rhythm; cornea.

The presence of a diurnal rhythm of cell division in normal [2] and regenerating organs [3, 4, 8] is a firmly established fact. A problem which has arisen in the course of experiments to study regeneration is whether the time of day at which an organ is resected affects changes in mitotic activity, or whether the mitotic activity of regenerating organs is determined entirely by the time elapsing after the operation, i.e., does not obey the rules of diurnal rhythms. Neither in experiments to study regeneration of the liver [3] nor in experiments to study compensatory hypertrophy of the kidney has an unambiguous answer to this question been obtained. Mitotic activity in the liver after removal of two-thirds of the organ reaches a maximum as a rule in the morning [4], although the time elapsing after the operation is important. Other workers consider that during regeneration of the liver the time elapsing after the operation plays the principal role [6, 9].

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