ACTION OF A HELIUM—NEON LASER ON REGENERATIVE POWERS OF ADULT GUINEA PIG SKELETAL MUSCLES

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In relation to intensity of regeneration in skeletal muscle tissue guinea pigs are greatly inferior to other species of laboratory animals, such as rats, rabbits, and dogs [10]. One reason for the low regenerative capacity of muscle tissue in guinea pigs is the low basal metabolism of these animals [9]. Much evidence has now accumulated in experimental biology and medicine of the stimulating effect of low-energy laser radiation on regeneration of tissues and stimulation of metabolism in them [5]. In particular, it has been shown that helium—neon radiation increases the supply and rate of consumption of O_2 in the tissues [2, 6]. Data obtained by other workers point to a positive action of laser radiation on the state and processes of regeneration of guinea pig skeletal muscle tissue [3, 4, 7]. It is not clear, however, whether under these circumstances the damaged muscle fibers undergo regeneration or whether the more rapid formation of a connective-tissue scar takes place in the region of muscle trauma.

The aim of this investigation was to discover whether, by the action of laser radiation, it is possible to obtain sufficiently complete regeneration of injured muscle in guinea pigs in which, by contrast with rats, the low regenerative power of their muscle tissue is determined by the species of the animal. Laser therapy was given under conditions ensuring the greatest positive effect in regenerating rat skeletal muscle [1, 8].

METHODS

Experiments were carried out on 36 guinea pigs aged 8-12 months. In the control, under pentobarbital anesthesia total transverse division of the gastrocnemius muscle of the guinea pigs was carried out in its middle part. The covering muscle and skin were then sutured. In the experimental series the myotomized limbs of the animals were irradiated with a helium—neon laser. The conditions of action of the laser were: 0KG-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 (diameter of field 2-2.5 cm). The duration of exposure was 5 min daily or on alternative days, on continuous action mode, for a total of 3-9 sessions depending on the time of sacrifice of the animal. Regeneration in the muscle was studied for 45 days. The completeness of the process was judged by the ability of the regenerating muscle tissue to fill the region of muscle damage. The relative areas occupied by muscle and connective tissues and also by fibrin were determined quantitatively on histologic sections. The DNA and RNA content in the tissues of the regenerating muscle was determined by a spectrophotometric method, modified by A. S. Spirin. The mass of the regenerating tissues also was measured and expressed as a percentage of body weight. The numerical results were subjected to statistical analysis by Student's test.

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Fig. 1. Gastrocnemius muscle of adult guinea pigs after transverse division. Muscle fibers along edge of proximal stump (on the right) rest against fibrous connective tissue (on left) formed in region of muscle injury, 45th day of regeneration. Here and in Figs. 2 and 3: stained by Regaud's iron-hematoxylin and counterstained by Mallory's method. 400×.

RESULTS

The relative mass of the regenerating tissues 7 and 14 days after injury in the experimental series was less than in the control (0.27 ± 0.02%, 0.24 ± 0.01%, and 0.31 ± 0.02%, 0.26 ± 0.01%, respectively), evidently due to the less-marked tissue edema in the regenerating muscle after laser therapy (difference significant at the $p < 0.01$ level). After 30 days the mass of regenerating tissues in the experimental and control was identical, namely 0.18 ± 0.003 and 0.18 ± 0.01%, respectively. After 45 days the mass of regenerating tissues in the experiment exceeded that in the control (0.19 ± 0.007 and 0.16 ± 0.003%, respectively). The difference was statistically significant at the $p < 0.01$ level.

Exposure to laser radiation stimulates proliferative activity and synthetic processes in regenerating muscle tissues, as shown by the biochemical data. For instance, 14, 30, and 45 days after muscle injury the DNA content in the regenerating tissues was increased by 4, 18, and 7%, and the RNA content by 5, 12, and 7%, respectively, compared with the control. These findings reflect the state of all the regenerating tissues and, in particular, muscle and connective tissue.

Histologic analysis of the regenerating tissues showed that the wound edges and both muscle stumps of the damaged muscle in the control 7 days after the operation were loosely connected, marked exudative edema of the tissues had developed, and an extensive hematoma was present in the defect, together with a large quantity of fibrin and many inflammatory cells. In the region of trauma, along the edges of the muscle stumps, granulation tissue containing fibroblasts, thin collagen fibers, and much ground substance had formed. Mitoses were observed in the mononuclear cells. Sarcoplasmic pools with large, pale nuclei were formed in some muscle fibers of the proximal stump. Many fibers, especially in the distal stump, however, showed vacuolation, degeneration, and phagocytosis. After 14 days the amount of fibrin was somewhat reduced and the defect filled with young connective tissue. Dividing cells were seen. Resorption of breakdown products continued in the damaged muscle fibers. In the zone of injury of the muscle

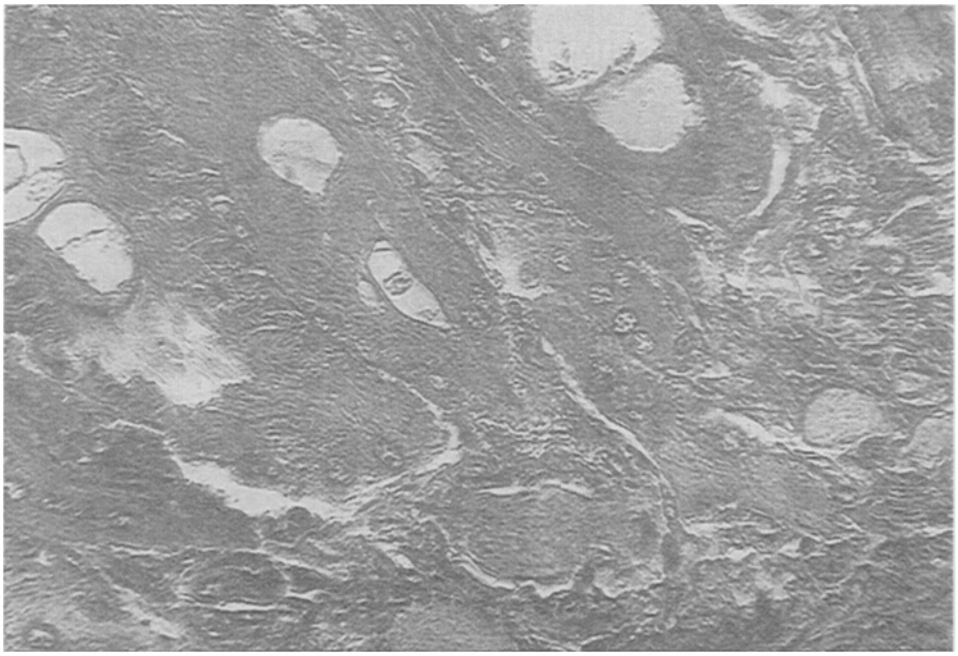

Fig. 2. Gastrocnemius muscle of adult guinea pigs after transverse division and laser therapy. Accumulation of muscle nuclei at ends of damaged muscle fibers along edge of proximal stump, visible in many muscle fibers, 14th day of regeneration.

TABLE 1. Relative Content (in %) of Muscle and Connective Tissue and Fibrin in Regenerating Wounds (based on area occupied by them on section; total area of regenerating wound taken to be 100%)

Time of regnera-tion, days	Series, tissue					
	transverse division of muscle			transverse division of muscle + laser therapy		
	muscle tissue	connective tissue	fibrin	muscle tissue	connective tissue	fibrin
7	63±3	25±5	12±2	62±3	29±2	9±1
14	61±5	29±3	10±2	69±5	25±3	6±2
30	62±2	36±1	2±1	76±1	24±1	—

there were many macrophages. At the ends of the divided muscle fibers which remained viable, muscle nuclei accumulated and myosynctia, were formed. Darkly stained muscle fibers, still remaining ischemic, and fibers containing vacuoles were present in the distal stump. After 30 days the region of the wound and the widened septa were occupied by connective tissue. The latter spread along the blood vessels and between muscle fibers of both stumps. In some regenerating tissues in the region of injury remains of unresorbed breakdown products were still present. Near the blood vessels foci of leukocyte migration could be seen. Cells dividing by mitosis were found. Concentrations of muscle nuclei could be seen at the ends of the muscle fibers. The newly formed myosyncytia rested against dense connective tissue. After 45 days muscle tissues along the edges of the stumps had differentiated into muscle tubes and muscle fibers, but they did not join the muscle stumps together (Fig. 1). The region of transverse division of the muscle was filled with fibrous connective tissue with foci of accumulation of leukocytes, islets of adipose tissue, and hyperemic blood vessels.

In the experimental series 7 days after the operation both muscle stumps and also the skin sutures were firmly united. Edema of the tissues was less marked. In the region of trauma, fibrin and numerous inflammatory cells were

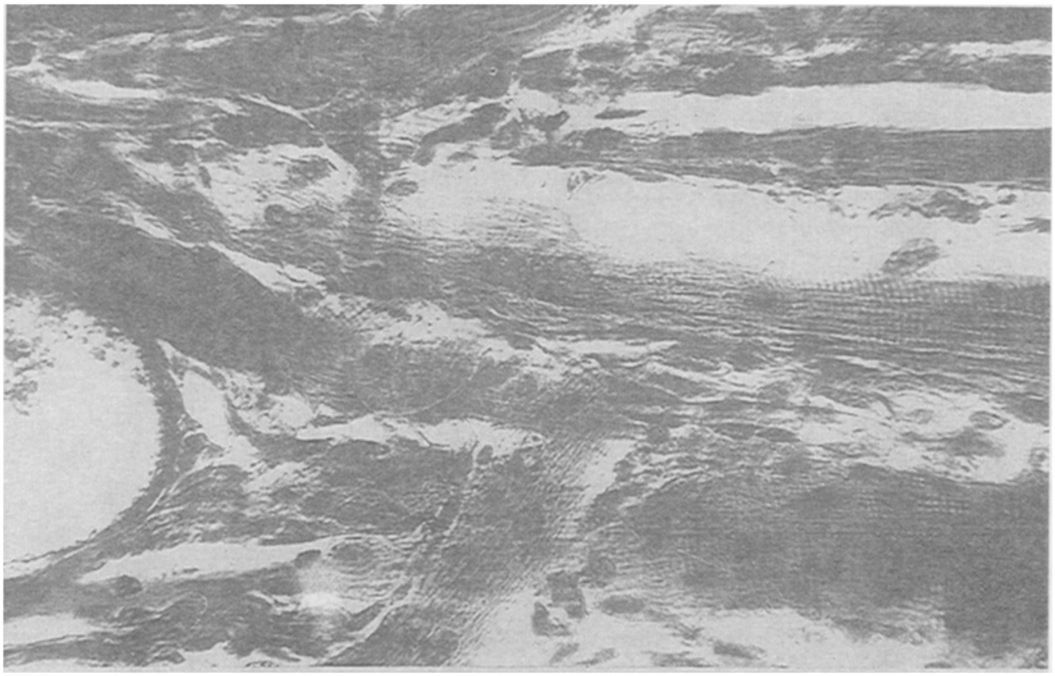

Fig. 3. Gastocnemius muscle of adult guinea pigs after transverse division and laser therapy. Regenerating muscle fibers formed along edge of proximal stump. Some pattern observed also along edge of distal stump, 45th day of regeneration.

visible. Granulation tissue was formed more actively. Many cells were in the stages of mitosis. Some muscle fibers had undergone degeneration, vacuolation, and phagocytosis, but the zone of injury of the muscle fibers, especially in the proximal stump, was much smaller than in the control. Round nuclei with large nucleoli accumulated at the ends of many traumatized muscle fibers, and myosyncytia were formed. After 14 days the quantity of fibrin in the region of trauma was appreciably reduced. The space between the two muscle stumps was occupied by connective tissue, in which there were more fibroblasts and collagen fibers. Along the edge of the stumps active formation of myosyncytia and sarcoplasmic pools, with large pale nuclei, was observed (Fig. 2). Alongside the muscle tissues there were dividing cells which resembled myoblasts. In the distal stump damaged muscle fibers were still present, and phagocytosis of breakdown products continued to take place in them. There were many macrophages with brown pigment. After 30 days resorption of fibrin and necrotic masses had ceased. Accumulations of muscle nuclei were visible at the ends of the divided muscle fibers. Myosyncytia continued to be formed quite actively and the muscle cells formed previously were differentiated. Dividing cells were frequently seen near the myosyncytia. Solitary muscle fibers penetrated into the depth of the defect. However, the regenerating muscle tissue did not join the muscle stumps together. In the region of injury of the muscle a connective-tissue scar formed. After 45 days, narrow muscle tubes and muscle fibers grew from the ends of the muscle fibers of both stumps and along the widened septa (Fig. 3). Along the edge of the proximal stump formation of muscle tissues was more marked than along the edge of the distal stump. Just as in the control, the region of injury consisted of fibrous connective tissue, areas of adipose tissue, and hyperemic vessels resembling lacunae.

Morphometric analysis of the regenerating muscle tissues is illustrated in Table 1. This shows that laser radiation accelerates resorption of fibrin and necrotic masses and facilitates, on the whole, the formation of regenerating tissues with a larger quantity of muscle tissue than in the control, despite the fact that regenerating muscle tissue did not fill the region of injury in the muscle. The difference is statistically significant at $p < 0.01$.

The result showed that helium—neon radiation in the dose used leads to more rapid healing of the region of injury in a guinea pig muscle, accelerates resorption of fibrin and necrotic masses, reduces exudative tissue edema, potentiates DNA and RNA synthesis in the regenerating tissues, and stimulates growth of connective tissue. The regenerating muscle tissues had a greater mass and, on the whole, contained more muscle tissue, due evidently both to a decrease in the degree of degeneration of the muscle fibers following muscle damage, and also to some degree of stimulation of their regenerative activity. However, the regenerating muscle tissue did not fill the defect between the two muscle stumps. A connective-tissue scar formed in the region of trauma.

Consequently, this dose of laser radiation is less effective for guinea pigs than for rats, in which, as was shown previously, the defect in an injured muscle, even after irradiation by x rays in a dose of 20 Gy, is filled with regenerating muscle tissue, either completely [8] or partially [1]. It can be tentatively suggested that the stimulating effect of laser radiation on regenerative processes in the tissues depends to a certain degree on the species of the animal.

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