EFFECT OF MINCED MUSCLE IMPLANTATION FOLLOWED BY LASER THERAPY ON GUINEA PIG SKELETAL MUSCLE REGENERATION

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The regenerative power of skeletal muscle tissue in guinea pigs is lower than in some other laboratory animals, such as rats. A considerable quantity of connective tissue is formed in regenerating guinea pig muscles [11, 12]. We have comparatively little information on stimulation of the regenerating power of muscle tissue in these animals. One investigator has shown that implanting minced muscle tissue into a region of injury, namely a perforating wound of the semimembranous muscle of guinea pigs improves the process of regeneration [6]. Other investigators also observed improvement of regeneration of guinea pig skeletal muscles after exposure to laser radiation [7-9]. However, they do not state to what degree the muscle defect was filled with regenerating muscle tissue.

In the investigation described below regeneration of the gastrocnemius muscle of adult guinea pigs was studied under conditions of stimulation by implanting minced muscle tissue and also by a combination of implantation of minced muscle tissue and laser therapy.

EXPERIMENTAL METHOD

Experiments were carried out on 36 adult guinea pigs aged 8-12 months, in three series. 1) Complete transverse division of the gastrocnemius muscle; 2) transverse division of the right gastrocnemius muscle and implantation of a small quantity of minced muscle tissue, taken from the left gastrocnemius muscle of the same animal, into the region of injury; 3) the same operation as in series 2, but followed by laser therapy. The conditions of laser irradiation were: 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 to give a field diameter of 2 cm. The duration of exposure was 5 min daily or every other day, on continuous mode, with 7-10 sessions altogether depending on the time of sacrifice of the animal. Regeneration in the muscle was studied after 14, 30, and 45 days. The quantity of muscle and connective tissue and also of fibrin and of breakdown products in the regenerating tissues was determined from the relative percentages of the total area occupied by them in the sections. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Severe tissue edema was observed in series 1, 14 days after injury to the muscle. The two muscle stumps were weakly connected. Fibrin was quite abundant in the region of transverse section of the muscle. Many macrophages, reabsorbing breakdown products, could be seen. Young connective tissue was forming. Cells dividing by mitosis were observed. Vacuoles appeared at the ends of the injured muscle fibers of the proximal and distal stumps, muscle nuclei accumulated, and myosyncytia and muscle tubules were formed. After 30 days connective tissue was actively

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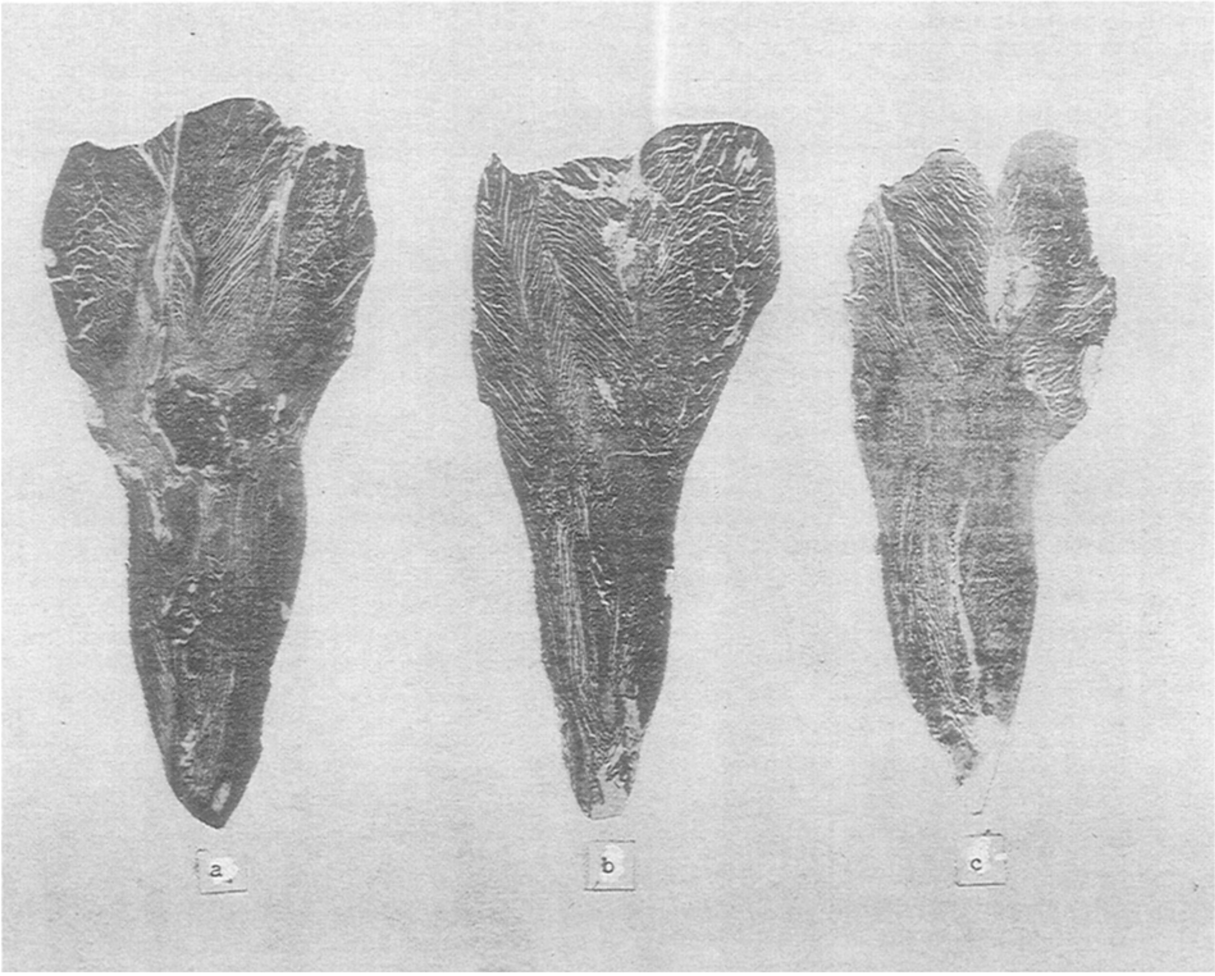

Fig. 1. Histologic sections of regenerating gastrocnemius muscle of adult guinea pigs 30 days after operation: a) fibrin and newly formed connective tissue can be seen in region of injury (complete transverse division of muscle – series 1); b) series 2 (implantation of minced muscle tissue into region of trauma); c) series 3 (implantation of minced muscle tissue into region of trauma, followed by laser therapy): resorption of fibrin and structural regeneration of autografted minced muscle tissue nearing completion in region of muscle injury; regenerating muscle fibers join the two muscle stumps in some places. Stained by Regaud's iron-hematoxylin, counterstained by Mallory's method. 10×.

growing in the region of muscle trauma, in the widened septa and around large blood vessels. In some regenerating tissues remnants of necrotic masses were still found. The inflammatory reaction continued. Regenerating muscle fibers did not penetrate deep into the region of injury and did not join the two muscle stumps (Fig. 1a). The region of trauma 45 days after the operation was composed of fibrous connective tissue.

In series 2, 14 days after transverse division of the muscle, exudative tissue edema was less marked. The two muscle stumps were quite firmly joined together. Some fibrin and unreconstructed muscle fragments were still present in the defect. With removal of the breakdown products, granulation tissue was able to grow. Cells dividing by mitosis were frequently seen. Macrophages and polymorphs could be seen. At the edges of the two muscle stumps vacuoles formed in the damaged muscle fibers, concentrations of large regenerating nuclei appeared, with outgrowths of myosyncytia and muscle tubules. Single narrow myosyncytia and muscle tubules also were formed from the implanted minced muscle tissue. On the 30th day after the operation resorption of fibrin and necrotic masses was complete. However, in many regenerating foci the inflammatory process in the region of trauma still continued: concentrations of macrophages and foci of migration of leukocytes from blood vessels could be seen. Growth of myosyncytia and muscle tubules was observed from the damaged muscle fibers of both muscle stumps. Regenerative reconstruction of the muscle fragments was virtually complete and areas of muscle tissue consisting of narrow muscle

TABLE 1. Content (in %) of Muscle and Connective Tissues and Fibrin in Regenerating Foci (according to area occupied by it on section, taking total area of regeneration as 100%)

Time of regeneration, days	Experiments of								
	series 1: transverse division of muscle			series 2: transverse division of muscle			series 3: transverse division of muscle		
	muscle tissue	connective tissue	fibrin	muscle tissue	connective tissue	fibrin	muscle tissue	connective tissue	fibrin
14	61±5	29±3 n=3	10±2	71±2	24±1 n=3	5±1	72±1	26±2 n=4	2±1
30	62±2	36±1 n=5	2+1	76±1	24±1 n=3	—	77±5	23±5 n=3	—
45	60±2	40±2 n=4	—	74±2	26±2 n=4	—	75±3	25±3 n=7	—

fibers were formed in the defect. The regenerating muscle tissue partly joined the two muscle stumps together (Fig. 1b). After 45 days the region of trauma consisted of interwoven muscle fibers, lying at the edges of the two muscle stumps, and foci of loosely arranged wide and narrow muscle fibers, formed from muscle fragments transplanted into that region. The remaining space was occupied by fibrous connective tissue, in which concentrations of leukocytes were found.

In series 3, the exudative tissue edema was considerably reduced 14 days after trauma and the two muscle stumps had firmly knitted together. Resorption of fibrin and of breakdown products took place more rapidly. In most regenerating tissues reconstruction of the muscle fragments was complete, and their place was occupied by newly formed muscle tubes and narrow muscle fibers. Concentrations of macrophages could be seen among them. Young connective tissue was actively being formed, and there were many mitoses. Pale round nuclei with large nucleoli accumulated at the damaged ends of many muscle fibers of the proximal and distal stumps, and myosyncytia and muscle tubules grew more actively. Resorption of fibrin and regenerative reconstruction of muscle fragments were complete 30 days after the operation. The inflammatory process ceased. Further differentiation of regenerating muscle tissue was observed. At the edges of both muscle stumps active growth of muscle tubules and muscle fibers from the damaged muscle fibers continued. Muscle fibers formed from implanted minced muscle tissue were more compactly arranged than in the experiments of series 2. Groups of macrophages were occasionally visible among them. The newly formed muscle tissue joined the two muscle stumps together in some places (Fig. 1c). Growth and development of young muscle fibers continued 45 days after trauma. Many muscle fibers contained concentrations of pale round nuclei. The connective tissue in the regenerating areas was somewhat condensed.

Morphometric analysis of the regenerating muscle showed (Table 1) that the methods of stimulation used led to an increase in the quantity of muscle tissue in the regenerating muscle compared with the control (significantly, $p < 0.01$). Differences in the content of muscle and connective tissue in regenerating muscles in series 2 and 3 were not statistically significant. There was only a tendency for the quantity of muscle tissue in the regenerating muscle of the experiments of series 3 to increase. Meanwhile laser therapy led to a marked increase in the rate of resorption of fibrin and breakdown products.

Thus implantation of minced muscle tissue into a region of muscle trauma or a combination of this method and subsequent laser therapy led to more complete restoration of the injured muscle in adult guinea pigs. By contrast with the control, the regenerating muscles contained a larger quantity of functionally active muscle tissue, which partly joined the proximal and distal stumps of the transversely divided gastrocnemius muscle together in adult guinea pigs. The muscle tissue consisted of muscle fibers branching out from the damaged muscle fibers of the two muscle stumps, and formed from the implanted minced muscle tissue. However, it must be pointed out that additional exposure to the helium–neon laser beam, while accelerating the course of the inflammatory reaction and connective tissue formation, led to only a very small increase in the quantity of muscle tissue in the regenerating areas compared with the series of experiments in which implantation of minced muscle tissue was the only procedure.

Stimulation of the regenerative capacity of skeletal muscle tissue is determined by its regeneration reserve, itself dependent on the quantity and the proliferative potential of the muscle stem cells, i.e., satellite cells. On implantation of minced muscle tissue into the region of trauma, an additional number of satellite cells, the source of regeneration of muscle tissue, is introduced [13-15]. One manifestation of the stimulating effect of laser radiation is the more intensive proliferative activity of the cells [2, 5]. Since in this investigation laser therapy was carried out within the dose range at which many workers found that laser radiation was most effective in stimulating healing of injured skeletal muscles in guinea pigs [4-6], it can be tentatively suggested that the conditions of laser therapy used were sufficiently effective. In the experiments described above the stimulating effect of laser radiation was limited by the proliferative potential of the satellite cells in the muscle tissue of the guinea pigs. The above hypothesis is indirectly confirmed by the fact that the efficacy of these methods of stimulation in guinea pigs, whose muscle tissue has lower regenerative power, was expressed to a lesser degree than in rats, with significantly higher regenerative power of their muscle tissue [1, 3, 4, 10]. There is evidence in the literature to show that the proliferative potential of satellite cells in the muscle tissue of different species of animals may itself differ [14].

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