A series of experiments with transplantation of the embryonic pancreas into the ACE of healthy rats is of great importance for determination of the character and nature of the processes taking place in the implanted gland. In these cases a rather different picture was observed: the developing endocrine tissue of the implant less frequently formed organ structures characteristic of adult animals, in the form of typical islets of Langerhans which, in turn, consisted of a few small endocrinocytes, and were poorly vascularized (Fig. 4). Mitoses were hardly ever found in such implants and acinar-endocrine differentiation did not take place.

This analysis shows that fragments of embryonic pancreas, implanted into ACE of rats, progressively "rid themselves" of the acinar parenchyma of the organ, whereas its endocrine part develops, differentiates, is revascularized, and restores its physiological activity. The fact that the normal blood glucose level is maintained in diabetic rats for a long time [2] can be explained on the grounds that the endocrine tissue in ACE is under immunologic protection. The formation of organ structures by the endocrine cells of the implant, their morphological maturity, and their functional capacity are all evidence that the conditions are created in ACE for morphological and functional reconstruction of the endocrine part of the pancreas.

LITERATURE CITED

1. A. G. Bragin, A. V. Kulikov, Z. N. Zhuravleva, and T. M. Tret'yak, Byull. Éksp. Biol. Med., 95, No. 6, 106 (1983).
2. V. P. Zharkov, V. N. Yarygin, and L. L. Gol'tsman, Byull. Éksp. Biool. Med., 105, No. 2, 216 (1988).
3. V. I. Shumakov, B. I. Shal'nev, V. N Blyumkin, et al., Byull. Éksp. Biol. Med., 89, No. 1, 48 (1980).
4. R. C. McEvoy and P. E. Leung, Diabetes, 32, No. 9, 852 (1983).
5. M. J. Perlow, Brain Res. Bull., 6, No. 2, 171 (1981).
6. H. P. Selawry and K. Whittington, Diabetes, 33, No. 4, 405 (1984).
7. W. J. Tze and J. Tai, Transplantation, 41, No. 4, 531 (1986).
8. C. Weber and R. Weil, Surgery, 77, No. 2, 208 (1975).

EFFECT OF HELIUM-NEON LASER ON POSTRADIATION REPAIR IN SKELETAL MUSCLES OF OLD RATS

N. V. Bulyakova

UDC 616.74-001.28-053.9-085.849.19-036.8-07:616.74-003.93]-092.9

KEY WORDS: regeneration; skeletal muscle; skin; x-rays; helium-neon radiation.

Ionizing radiation has a damaging action on the regenerating capacity of skeletal muscles, which may continue for quite a long time [6, 8, 12]. Intensification of energy and protein metabolism in the postradiation period may reduce the radiation effect. It has been shown that if the time of appearance of radiation damage in a muscle (i.e, the process of regeneration) is delayed, only in young rats, which have a more active metabolism than old rats, is significant recovery of the regenerative capacity of the muscle tissue observed [9]. Recently published data indicate increased activity of metabolic processes in the tissues under the influence of helium-neon laser radiation [4, 7, 11]. It has been shown that red laser radiation increases the intensity of metabolic processes in normal muscle of old rats [1].

Evolutionary Histology Group, A. M. Severtsov Institute of Evoutionary Morphology and Ecology of Animals, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 345-347, March, 1989. Original article submitted May 6, 1988.

0007-4888/89/0003-0382$12.50

The aim of this investigation was to study the stimulating action of helium-neon laser radiation on postradiation repair in irradiated skeletal muscles of old rats, as judged by their ability to regenerate.

EXPERIMENTAL METHOD

Experiments were carried out on 30 rats aged 24-30 months. There were two series of experiments: I) local irradiation of the hind limb in a dose of 20 Gy, total transverse division of the gastrocnemius muscle 1 month later; II) irradiation of the limb in the same dose, laser therapy, division of the gastrocnemius muscle 1 month after x-ray irradiation. The conditions of x-ray irradiation were: RUP-200 apparatus, voltage 190 kV, current 15 mA, dose rate 0.75 Gy/min, filters 0.5 mm Cu + 1 mm Al. Conditions of laser therapy: OKG-12 apparatus, wavelength 632.8 nm, power flux density 2.5-3 mW/cm^2. Irradiation was given for 1 month as six to nine sessions, total duration of exposure 3 min, continuously or pulsed (60 times, each time for 3 sec, action of laser radiation, with intervals of 7 sec between exposures). The laser beam was focused by means of a lens (field diameter 2 cm). The hair on the limb was shaved. The process of regeneration in the gastrocnemius muscle and healing of the skin wound were observed for 1 month. The quantitative ratios between areas occupied by muscle and connective tissues and also by fibrin were determined in percent. The weight of the regenerating tissues also was expressed as a percentage of the animal's body weight. The numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The weight of regenerating tissues in the control series was greater than in the experimental. For instance, in series I the weight of the regenerating tissues 14 days after injury was 0.35 ± 0.05%, falling after 30 days to 0.29 ± 0.03%, whereas the corresponding figures in series II were 0.27 ± 0.02 and 0.23 ± 0.03%. The difference is statistically significant ($p < 0.05$). The reason for this was severe edema in the tissues which persisted for 1 month in muscle regenerating after x-ray irradiation without laser therapy, and this was confirmed by a study of sections at the light-optical level. Histological analysis of the results of series I showed that regeneration of the skeletal muscles was considerably depressed. Both halves of the gastrocnemius muscle 14 days after trauma were edematous and union between them was weak. Resorption of fibrin and necrotic masses was sluggish. There were few macrophages per field of vision. Connective tissue formation was depressed. The divided muscle fibers underwent vacuolar degeneration. Muscle nuclei were small and few in number (Fig. 1). Edema was still present 30 days after division of the muscle. The defect was filled with fibrous and loose connective tissue, poor in cells. Fibrin was still present and leukocytes and macrophages were observed. The distal stump was narrowed due to replacement of many muscle fibers by connective tissue. In series II, 14 days after trauma both muscle stumps were already firmly united, and severe edema was not observed in the tissues. The quantity of fibrin was much reduced. Connective tissue containing fusiform fibroblasts and delicate collagen fibers was formed near the muscle stumps (Fig. 2). Mitoses were frequent. In muscle fibers containing far fewer vacuoles the nuclei were round and large nucleoli appeared in them. However, concentrations of nuclei were not observed at the ends of the injured muscle fibers. Growing myosynctia and muscle tubules contained few regenerating unclei. Fibrin was no longer present in the region of the defect 30 days after muscle damage. The connective tissue which had formed was abundantly vascularized. Growth of muscle tissue in the depth of the defect was slow. In many muscle fibers of the proximal stump, concentrations of two to four muscle were nuclei were observed (Fig. 3).

Morpholmetric analysis of the regenerating tissues is shown in Table 1. Exposure to the beam of helium-laser considerably accelerated fibrin absorption and stimulated growth of connective tissue (significantly, at $p > 0.05$). The quantity of muscle tissue in regenerating tissues of both series of experiments was virtually identical (the difference was not statistically significant). Some differences also were observed in the response of the skin. In the control, on the limb irradiated by x-rays marked inflammation of the skin was observed, and the shaved hair did not grow again. The subsequent operation caused edema of the skin with the formation of purulent scabs. Deep cutaneomuscular ulcers often developed toward the end of 1 month. In the experimental animals exposed to the helium-neon laser, with a continuous schedule of action, erythema of the skin, the formation of weeping scabs, absence of hair growth in the area shaved before the beginning of the sessions of laser therapy were observed in the irradiated limb before undergoing division of the muscle. After the operation, the wound healed slowly and areas of suppuration were seen. Irradiation with the

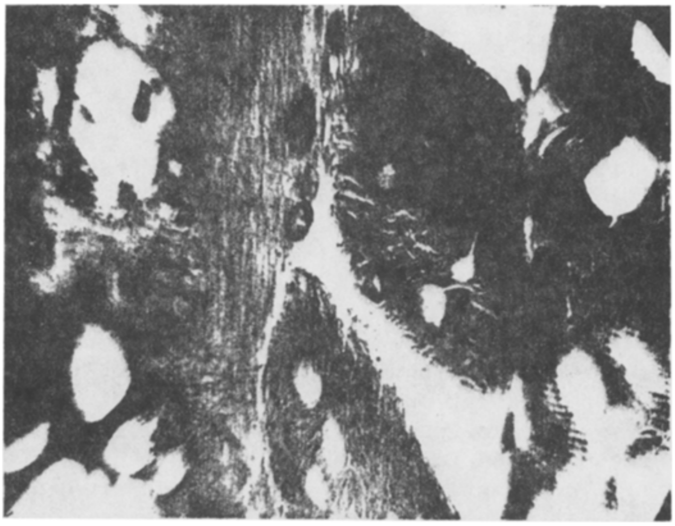

Fig. 1. Gastrocnemius muscle of old rats after irradiation in a dose of 20 Gy and transverse division 1 month later. Few small muscle nuclei and vacuoles in traumatized muscle fibers. Here and in Fig. 2: regeneration for 14 days; 900 ×. Stained with Regaud's iron-hematoxylin and counterstained by Mallory's method.

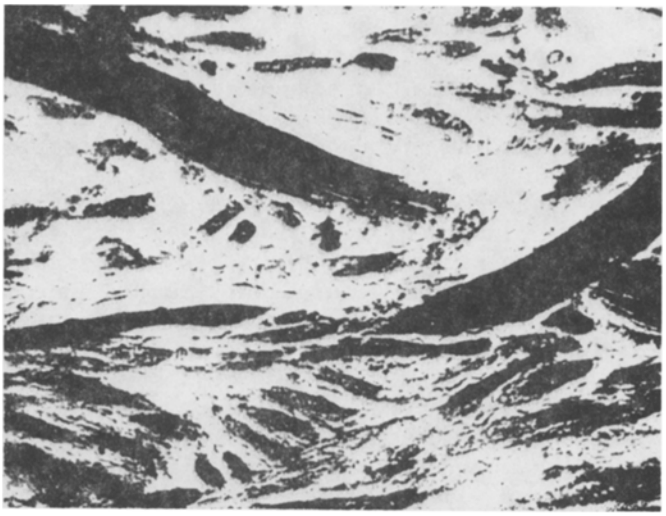

Fig. 2. Connective tissue in regenerating gastrocnemius muscle of old rats after irradiation in a dose of 20 Gy, laser therapy, and transverse division 1 month after irradiation. Fusiform fibroblasts and delicate collagen fibers. Mitosis.

TABLE 1. Relative Proportions (in %) of Muscle, Connective Tissue, and Fibrin in Regenerating Tissues (depending on area occupied by them per section, total area of regenerating tissue taken as 100)

Time of regeneration, days	Series of experiments					
	I			II		
	muscle tissue	connective tissue	fibrin	muscle tissue	connective tissue	fibrin
14	76±6	14±3	10±3	67±3	26±7	7±4
30	70±6	22±4	8±2	65±6	35±6	—

helium-neon laser under pulsed conditions, however, had a more favorable action on the state of the skin: the erythema of the skin caused by x-ray irradiation quickly disappeared, coarse but thin hair grew, and dry scaling of the skin began, whereas after the operation the skin edges were clean and united firmly.

Thus local irradiation of the limbs of old rats in a dose of 20 Gy depresses the regenerating capacity of skeletal muscles, and it is not restored after 1 month. Laser therapy (continuous or pulsed conditions) of the irradiated but intact muscles during this period has a weak effect on their ability to regenerate subsequently. Acceleration of the inflammatory process, intensification of phagocytic activity of macrophages, and stimulation and a much healthier appearance of the connective tissue were observed. The effect on muscle tissue was less marked. Changes in metabolism taking place in the irradiated but mechanically undamaged muscle of old rats under these experimental conditions evidently cannot stimulate the process of postradiation repair in it, and in particular, ability to regenerate. Differences in the response of the connective and muscle tissues to laser radiation may be due to the fact that connective tissue is proliferating, whereas in muscle tissue active processes of proliferation are observed only after trauma [13, 14]. The effect of the laser on postradiation repair in the tissues is evidently effective if it acts on cells which have embarked on a process of proliferation. This conclusion is confirmed by the results of the writer's previous experiments [2, 10] with laser therapy of irradiated muscles after operation, and also by data obtained by other workers on regenerating, nonirradiated tissues [5]. Laser radiation in small doses, i.e., under pulsed conditions, has a more favorable effect on the state of the skin after x-ray irradiation. The results indicate that laser therapy of irradiated tissues is indicated clinically after operation. Laser therapy of irradiated, but mechanically intact tissues is less effective.

LITERATURE CITED

1. M. V. Bitadze, Current Problems in Gerontology and Geriatrics [in Russian], Baku (1984), p. 21.
2. N. V. Bulyakova and M. F. Popova, Byull. Éksp. Biol. Med., No. 4, 481 (1987).
3. S. Wolf, Recovery of Cells from Injury [Russian translation], Moscow (1963), p. 228.
4. S. M. Zubkova, I. B. Laprun, Z. A. Sokolova, and V. I. Popov, Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 4, 24 (1981).
5. D. L. Korytnyi, The Biological Action of Laser Radiation [in Russian], Alma-Ata (1977), p. 84.
6. V. P. Mikhailov, Arkh. Anat., No. 2, 3 (1965).
7. A. M. Moroz, The Use of Methods and Appliances of Laser Technology in Biology and Medicine [in Russian], Kiev (1981), p. 214.
8. M. F. Popova, Radiosensitivity and Stimulating Properties of Regenerating Mammalian Tissues [in Russian], Moscow (1984).
9. M. F. Popova and N. V. Bulyakova, Radiobiologiya, 17, No. 6, 855 (1977).
10. M. F. Popova, N. V. Bulyakova, and V. S. Azarova, Radiobiologiya, 23, No. 1, 50 (1983).
11. M. S. Saulebekova, B. Ya. Bainazarova, A. A. Zelinskaya, et al., The Biological Action of Laser Radiation [in Russian], Alma-Ata (1977), p. 78.
12. G. S. Strelin and S. P. Yarmonenko, Postradiation Repair [in Russian], Moscow (1970), p. 264.
13. N. G. Khrushchov, Functional Cytochemistry of Loose Connective Tissue [in Russian], Moscow (1969).
14. C. P. Leblond, B. Messier, and B. Kopriwa, Lab. Invest., 8, 296 (1959).