RESTORATION OF THE BLOOD SUPPLY OF AUTOGRAFTS OF WHOLE RAT GASTROCNEMIUS MUSCLES AFTER X-RAY IRRADIATION

M. M. Umnova

UDC 616.748.53-089.843-092.9-036.864

Restoration of the blood supply to autografts of gastrocnemius muscles irradiated in a dose of 2500 R, developing in an unirradiated graft bed, and grafts of unirradiated muscles developing in a graft bed irradiated in the same dose, was investigated. The more rapid recovery of the blood supply of grafts of unirradiated muscles can be explained, in the author's opinion, both by the participation of surviving vessels in vascularization of the grafts and by the fact that the unirradiated muscle stimulates regeneration of blood vessels of the irradiated bed.

If attached muscles (as part of a reimplanted limb [11]) are transplanted, the graft is for a short time in a state of ischemia. After suture of the main vessels the blood flow in the organ is largely restored via the old blood stream. In the case of free muscle grafts, the technique of which was first developed in Studitskii's laboratory [4, 7, 8], the divided vessels are not sutured and the blood supply to the grafts is restored gradually. Using a method of injecting the blood vessels with ink to study the vascularization of grafts of whole and minced muscles, Zhenevskaya [3] showed that the restoration of the circulation in the transplanted muscles begins from the periphery and takes place through the ingrowth of vessels from the surrounding tissues of the bed (neighboring muscles, tendons, nerve). Zhenevskaya [3] and Carlson [14] describe coincidence of the zones of restored blood supply and of reorganization of the material of the grafted muscles. As the writer showed earlier in experiments in which muscle grafts were wrapped in cellophane film [9], the blood vessels located in the surface zone beneath the connective-tissue fascia resemble in shape, structure, and character of branching the pattern of the vascular network in the peripheral zone of grafts not wrapped in cellophane, and also of the intact rat gastrocnemius muscles. This observation suggests that some surviving vessels of the surface zone participate in restoration of the vascular network of autografts of whole muscles. A similar hypothesis was expressed previously by Zhenevskaya [3]. The blood supply in grafted muscles, as in grafts of other organs [12, 15], evidently begins to be restored through the formation of anastomoses between the vessels of the bed and individual vessels of the grafts.

The problem of vascularization of the transplanted muscles after x-ray irradiation, inducing considerable disturbances of regeneration, has not been discussed in the literature.

In the investigation described below, recovery of the blood supply in grafts of whole skeletal muscles was studied after exposure of the grafted muscle or of the graft bed to x-ray irradiation.

EXPERIMENTAL METHOD

The test object consisted of the gastrocnemius muscles of noninbred albino rats weighing 120 g. In 40 animals, one of the hind limbs was irradiated with x-rays in a single dose of 2500 R. The gastrocnemius muscles were then grafted reciprocally. The irradiated gastrocnemius muscle was placed in the unirradi-

Laboratory of Evolutionary Histology, A. N. Severtsov Institute of Evolutionary 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. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 3, pp. 41-44, March, 1974. Original article submitted June 14, 1973.

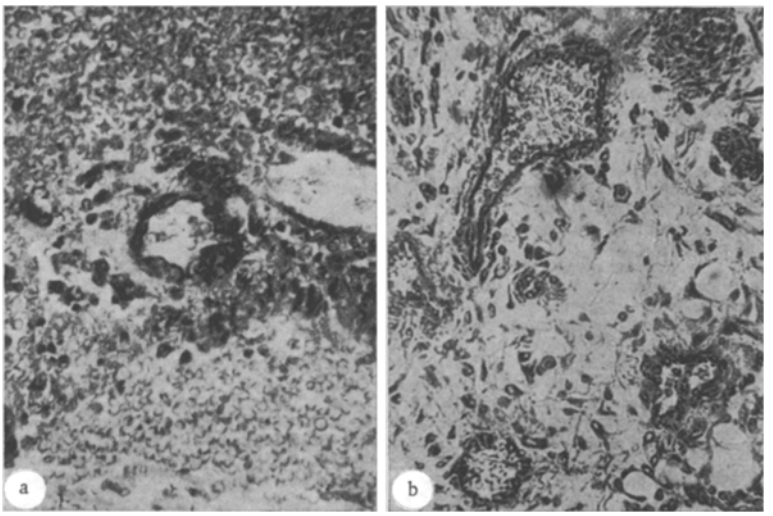

Fig. 1. Blood vessels in autograft of irradiated gastrocnemius muscle in unirradiated bed (azure-eosin): a) third day after grafting (320×); b) seventh day after grafting (160×).

ated graft bed of the opposite limb and the unirradiated muscle was placed in the irradiated graft bed. To study the dynamics of recovery of the blood supply in the autografted muscles, the blood vessels were injected with a solution of ink in gelatin. Grafts also were investigated by the use of the ordinary histological methods in the period from 2 days to 4 months after grafting.

EXPERIMENTAL RESULTS

Toward the end of the second day the blood supply was beginning to be restored at the sites of contact of the grafts with the tissues of the bed both in the irradiated gastrocnemius muscles in the unirradiated bed and in the unirradiated muscles in the irradiated bed. On the third day the circulation of blood was restored in the most superficial part of the grafts, largely, presumably, on account of the survival of some vessels in the graft itself. The endothelium of the surviving superficial vessels in the grafts of unirradiated muscles became basophilic on the second-third day after grafting, the cells increased in size, and mitoses were found in some endothelial cells. The proliferative reaction of the endothelium of the surviving vessels in the grafts of irradiated muscles was much weaker. Solitary mitoses in these muscles were found only on the 3rd-4th day, and many endothelial cells were vacuolated or desquamated. In vessels invading the grafts of irradiated muscles from the unirradiated bed, sometimes excessive proliferation of the endothelium was observed, leading to the formation of endothelial "plaques" on their walls (Fig. 1a).

Since only a few superficial vessels are preserved in freely grafted whole muscles and blood vessels located in the deep zones of the grafts undergo thrombosis and are destroyed, the vascular network of the grafts must largely be reconstructed afresh. A leading role in its formation is played by vessels growing in from the bed tissues. In longitudinal sections the densest network of blood vessels is found in the areas of attachment of the muscles where close contact exists between the tissues of the bed and graft. In the autografted muscles, blood vessels were seen to be formed by budding. This method has been described in investigations of regeneration of blood vessels in various tissues and organs [6, 10, 11]. The endothelium of the regenerating vessels was distinguished by the basophilia of its cytoplasm and its large size. The cells projected into the lumen of the vessels, forming bead-like structures (Fig. 1b). On the seventh day after transplantation of the muscles the zones of restored blood supply in the grafts were much more extensive. However, the network of blood vessels in the grafts of the irradiated muscles was less well developed (Fig. 2a) than in the grafts of unirradiated muscles (Fig. 2b). As the muscles formed, they continued to differentiate. In 6-7-day grafts, many vessels consisted of two layers of cells: the inner layer was composed of endothelial cells and the outer layer of large cells with basophilic cytoplasm, evidently differentiating into smooth-muscle cells. During the 7-15 days after transplantation, many mitoses were found in the endothelium of the growing vessels, but later their number decreased. In subsequent periods the rate of vascularization of the grafts of the irradiated gastrocnemius muscles as before was slower than in grafts of unirradiated muscles. For instance, the blood supply in irradiated gastrocnemius muscles grafted into an unirradiated bed was restored only 2 months after the operation, whereas grafts of unirradiated gastro-

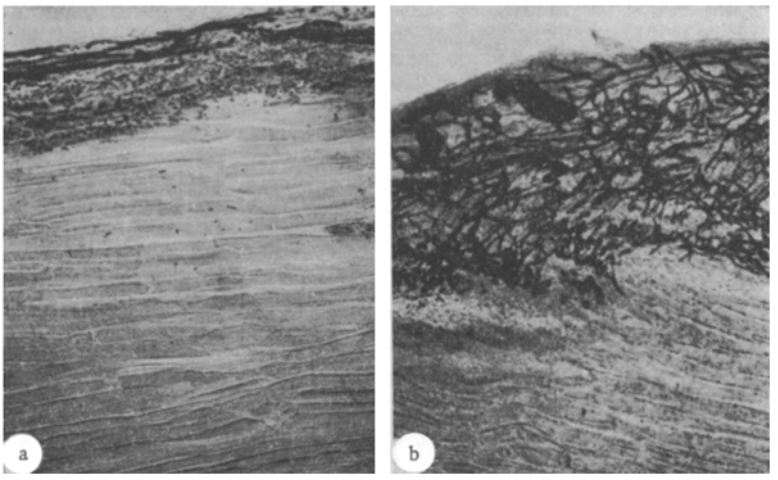

Fig. 2. Vascularization of peripheral zone of autografts of whole rat gastrocnemius muscles on seventh day after transplantation: a) graft of irradiated muscle in unirradiated bed; b) graft of unirradiated muscle in irradiated bed. Vessels injected with ink. Stained with eosin (72×).

cnemius muscles in an irradiated bed were almost completely vascularized 1 month after the grafting operation. After 2-4 months the autografts possessed the characteristic angioarchitectonics. The grafts of irradiated gastrocnemius muscles consisting chiefly of dense connective tissue received a poor blood supply. Capillary anastomoses were few in number and the spaces inside the capillary network were extensive. After grafting of unirradiated gastrocnemius muscles into an irradiated bed muscle organs composed of muscular and, in part, of connective tissue were formed. These grafts differed from the intact rat gastrocnemius muscles by the greater density of their capillary network, the spaces within which were small and irregular in shape.

The results show that after irradiation of muscles in a dose of 2500 R the process of transplantation regeneration is inhibited and, in particular, regeneration of the blood vessels is slowed and vascularization of the grafts disturbed. After irradiation of the graft bed in the same dose the process of vascularization of transplanted unirradiated muscles is inhibited to a lesser degree, and the blood supply of the grafts is restored more completely. The differences discovered in the character and rate of restoration of the blood supply in the grafts of irradiated and unirradiated muscles can be explained, it is considered, on the one hand, by the harmful action of x-ray irradiation on the endothelium of the blood vessels of the grafts themselves. This conclusion is supported by observations of many workers who found changes in the endothelium of the blood vessels after exposure to ionizing radiation [1, 5, 13]. On the other hand, the more rapid vascularization of the unirradiated muscles grafted into a bed irradiated with a relatively large dose, considerably depressing the plastic activity of the vessel [2], can be explained by assuming that grafts of unirradiated muscles have a stimulant effect on regeneration of the blood vessels of the irradiated bed.

LITERATURE CITED

1. E. V. Dmitrieva, Arkh. Anat., No. 11, 11 (1960).
2. N. V. Zhdanova, Arkh. Anat., No. 7, 61 (1968).
3. R. P. Zhenevskaya, Éksper. Khir., No. 2, 9 (1971).
4. R. P. Zhenevskaya, O. N. Rumyantseva, I. L. Novoselova, et al., Zh. Obshch. Biol., No. 5, 569 (1965).
5. Yu. V. Ivanov, Radiobiologiya, No. 2, 310 (1969).
6. V. V. Kupriyanov and É. I. Simagin, in: The Morphological Basis of the Microcirculation [in Russian], No. 2, Moscow (1967), p. 75.
7. A. N. Studitskii, Dokl. Akad. Nauk SSSR, 84, 389 (1952).
8. A. N. Studitskii and N. N. Bosova, Arkh. Anat., No. 12, 18 (1960).
9. M. M. Umnova, Dokl. Akad. Nauk SSSR, 203, 248 (1972).
10. N. G. Khlopin, Arkh. Anat., No. 1, 13 (1958).
11. A. D. Khristich, Transplantation of the Limbs [in Russian], Kiev (1968).

12. V. F. Tsel', Byull. Éksperim. Biol. i Med., No. 2, 121 (1959).
13. N. A. Shevchenko, Proceedings of the All-Union Conference on Medical Radiology [in Russian], Vol. 1, Moscow (1957), p. 140.
14. B. M. Carlson, J. Morph., 125, 447 (1968).
15. T. Clemmesen, Acta Chir. Scand., 124, 11 (1962).