# CROSSED TRANSPLANTATION OF WHOLE MUSCLES IN RATS OF THE SAME LITTER

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After preliminary denervation, the whole gastrocnemius muscle was transplanted in noninbred albino rats to the place of the extirpated homonemous muscle of another animal. The homografts were found to take and to undergo structural changes and dedifferentiation. The formation of the new muscle as an organ took place through subsequent differentiation of the myogenic elements. Differentiation of the newly formed muscles went parallel with the restoration of their innervation.

The problem of morphological changes in homografts of various organs and tissues has been widely discussed in the literature [3, 15, 11, 13, 1, 10]. Too little attention has been paid to grafts of muscle tissue because it has been considered that its power of regeneration is limited [5, 14, 8].

A number of examples have been developed in the writer's laboratory of the good results which have been obtained by the use of autografts and homografts of muscle tissue. Homografting of fragments of preliminarily denervated muscles and minced muscle tissue is accompanied by profound structural reorganization, as a result of which functioning muscular organs are formed from the transplanted muscles [6, 7]. A preliminary observation [2] demonstrated that lymphoid infiltration of the grafted muscle tissue takes place during homografting. Lymphoid infiltration is known to express a response to the introduction of foreign bodies, against which the recipient develops transplantation immunity [4, 9, 12].

The object of the present investigation was to study structural reorganization in homografts of whole muscles in animals of the same litter.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 50-70 g. The gastrocnemius muscle was denervated, and 30 days later the muscle was transplanted to the site of the extirpated gastrocnemius muscle of another animal of the same litter. The tibial nerve was sutured to the graft. The muscles were fixed in Zenker's fluid and in 12% neutral formalin after periods varying from 3 days to 8 months. The specimens were stained with azan by Heidenhain's method and with iron hematoxylin by the methods of Regaud and Giemsa. Nerve endings were demonstrated by the Bielschowsky–Gros method followed by gilding by Lavrent'ev's method.

## EXPERIMENTAL RESULTS

After transplantation of the denervated muscles, the grafts could develop in two directions. Most transplanted muscles died within the first few days of grafting and were subsequently absorbed. A smaller group of muscles showed progressive development. After 3-4 days the graft was loosely adherent to the edges of the stump. The sutured nerve was hyaline. Edema and considerable hemorrhage could be observed in the muscle. Most of the fibers retained their normal structure. The muscle fibers were loosely

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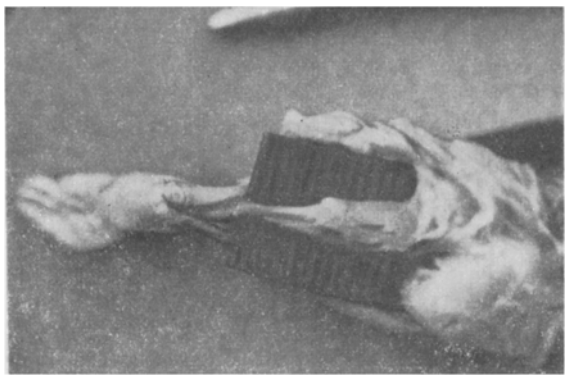

Fig. 1. General appearance of a graft 1 month after transplantation.

Fig. 2. Motor end-plate in a graft 8 months after transplantation. Silver impregnation by Bielschowsky–Gros method, 320×.

arranged, and along the course of the large blood vessels bands of connective tissue could be seen. Some areas of the muscles showed intensive leukocytic infiltration. At the periphery of the graft, new blood vessels were beginnining to form. The muscle fibers were changed: some were disintegrating with liberation of myoblasts.

Six days after grafting the edema of the muscle was slightly reduced. Two zones were clearly visible: a marginal zone of reorganization and development and a central zone of unchanged muscle fibers. The marginal zone had an abundant blood supply. Muscle fibers breaking up into fragments could be seen, and their degenerating portions were subsequently absorbed. Meanwhile dedifferentiation of the viable portions of the muscle fibers was observed; the myoblasts were liberated from them and either remained inside the old sarcolemmal membrane or lay freely between the disintegrating muscle fibers, stretched out in chains, after which they merged to formed myosyncytia and differentiated into a new muscle fiber. Many cells in the graft were in a state of mitotic division.

The central parts of the graft contained surviving muscle fibers with clearly defined cross striation. No nuclei could be seen in most fibers. Many leukocytes and connective-tissue cells were present between the myogenic elements and muscle fibers.

On the 9th day after transplantation the marginal zone was considerably widened and contained muscle tubes with central nuclei and cross-striation at the periphery. Collections of undifferentiated myogenic elements could be seen in this zone, many of them in a state of mitotic division. Where young muscle fibers were present, groups of lymphocytes were visible. Where myoblasts and connective-tissue cells were predominant, only a few lymphocytes were present. Nearer to the center of the muscle, the muscle fibers were thick, darkly stained, and homogeneous or cross-striated. As a rule no nuclei could be seen. Some fibers were in a state of disintegration. In certain places in the central part of the muscle, collections of neutrophils and lymphocytes were observed.

Two weeks after transplantation the muscle was small in size. The reorganization continued to proceed. The marginal zone consisted of closely packed differentiated muscle fibers of small diameter. The nearer the center of the graft, the younger the muscle fibers; they were smaller in diameter, and their nuclei often occupied the whole of the cross section and were arranged in chains. The nucleoli were frequently enlarged. Individual dying fibers could be seen among the young newly formed muscle fibers. The central part of some grafts contained no muscle fibers and consisted of connective and adipose tissues.

From 21 days to 1 month after transplantation the graft consisted of a fully formed organ, although it was smaller than the original muscle (Fig. 1). It consisted of closely packed differentiated muscle fibers. Their diameter was small but their cross striation was clearly defined. Bands of fibrous connective tissue and collections of lymphocytes were found between individual muscle fibers and also between the bundles of fibers. The muscles had an abundant blood supply.

In sections impregnated with silver, the reinnervation of the grafts was studied. Restoration of innervation began on the 14th day. At this time, axons growing from the sutured nerve were visible in the proximal part of the muscle, chaotically arranged and with varicose expansions. Short branches left the growing nerves and terminated in primitive nerve endings consisting of "boutons" or loops on the young

muscle fibers. Later they formed motor end-plates with several nuclei in each plate. The functional recovery of the muscle began 2 months after grafting. By this time some of the muscles were beginning to contract in response to stimulation of the nerve by an induction current.

After 4 months the muscle consisted mainly of mature muscle fibers. The number of nuclei was within normal limits. Many blood vessels were present in the muscle. Near the large blood vessels there were collections of small lymphocytes, and a very few of these cells could be seen here and there between fibers. In the later stages, as well as the continuing process of development, there was a further increase in volume of the muscle and further restoration of its working capacity.

Eight months after grafting most of the newly formed muscles had attained the size of the original denervated muscle, and consisted of a perfectly formed organ capable of responding by contraction to stimulation of the nerve by an induction current. It consisted of differentiated muscle fibers, abundantly supplied with blood vessels and nerves. Many motor nerve endings, of different levels of maturity, were present in the muscle: besides more mature motor end-plates (Fig. 2), there were others of primitive structure. Around the blood vessels in certain muscles lymphocytes could be seen. In most newly formed muscles, however, there were no lymphocytes.

After grafting of whole, preliminarily denervated muscles, the muscle homografts do not therefore simply take, but undergo complex reorganization with liberation of myoblasts and the subsequent formation of new differentiated muscle fibers. Nerves growing into the graft bring about its further differentiation. As a result, in the place of the completely excised muscle, a new muscle organ is formed. This organ has the same shape, structure, and function as the removed muscle, evidence of the mutual adaptation of the donor's and recipient's tissues.

## LITERATURE CITED


1. M. M. Averbakh, N. I. Gerasimenko, G. V. Latsis, et al., Arkh. Pat., No. 12, 20 (1968).
2. N. N. Bosova, in: Transplantation of Organs and Tissues [in Russian], Moscow (1966), p. 128.
3. N. A. Bykova, in: Problems in Tolerance and Homotransplantation [in Russian], Moscow (1966), p. 38.
4. M. Gashek, Pat. Fiziol., No. 6, 2 (1960).
5. A. A. Nemilov, Fundamentals of the Theory and Practice of Transplantation of Tissues and Organs [in Russian], Leningrad (1940).
6. A. N. Studitskii and N. N. Bosova, Arkh. Anat., No. 12, 18 (1960).
7. A. N. Studitskii and O. N. Rumyantseva, Dokl. Akad. Nauk SSSR, 155, No. 6, 1435 (1964).
8. A. N. Filatov, Yu. V. Beringer, G. V. Golovin, et al., Transplantation and Replacement of Tissues and Organs [in Russian], Leningrad (1960).
9. A. Ya. Fridenshtein, Uspekhi Sovr. Biol., 52, No. 3, 291 (1961).
10. Y. Idezuki, F. C. Goetz, and R. C. Lillehei, Surgery, 65, 485 (1969).
11. J. Leandri, Thorax, 22, 397 (1967).
12. P. B. Medawar, Proc. Roy. Soc., 149, 145 (1958).
13. P. Mikaeloff, Bull. Soc. Internat. Chir., 26, 489 (1967).
14. L. A. Peer, Transplantation of Tissues, Vol. 1, Baltimore (1955).
15. A. G. R. Sheil, G. I. Dammin, R. M. Mitchell, et al., Brit. J. Surg., 54, 1013 (1967).