FREE AUTOPLASTIC TRANSPLANTATION OF WHOLE MUSCLES

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The free grafting of muscle tissue has for a long time been the subject of study in the practice of restorative surgery and also in experimental morphology [1-4, 6].

In comprehensive works dealing with the transplantation of organs and tissues [7, 11, 12] it is stated that the free grafting of muscle tissue is impossible because a muscle rapidly dies once it has lost its neurovascular connection with the rest of the body. Within 2 hours of the division of its blood vessels, processes of disintegration and dedifferentiation begin to appear in muscle.

Investigations of the conditions of regeneration of muscles have now made it possible for us to envisage the successful solution of the problem of free transplantation of muscles. If the conditions for its normal working activity are disturbed, muscle tissue undergoes profound structural and functional changes, associated with a transition to a special type of metabolism, as a result of which the sensitivity of the muscle to oxygen lack is considerably reduced. These changes characterize the plastic state of muscle tissue [9]. A muscle brought into a plastic state by means of mincing [5, 8, 9] or preliminary trauma [5, 9] readily withstands free autoplastic transplantation.

The disturbance of the normal working activity of muscles by means of denervation also brings muscle tissue into a plastic state, making free autoplastic transplantation possible. The work of A. N. Studitskii and N. N. Bosova [10] has shown that if a piece of denervated muscle is transplanted autoplastically to the site of removal of the middle part of a normal muscle, the grafted tissue, which was in a state of denervational atrophy, survives and develops.

In this paper we describe experiments on the transplantation of a whole muscle, in which a plastic state has been induced by denervation, to the place from which the homonymous muscle of the same animal has been removed.

EXPERIMENTAL METHOD

The transplantation experiments were conducted on white rats and rabbits, and the results obtained from these animals were of the same order. In this communication we describe the results obtained in rats. The experiments were carried out on 48 white rats of both sexes, 5-6 months of age.

The right gastrocnemius muscle was denervated by dividing the tibial nerve at a distance of 1 cm from the point at which it enters the muscle. The proximal end of the divided nerve was buried in the muscles of the thigh and sutured.

Thirty days later the right denervated muscle was transplanted to the site of the excised normal left side. All the adjacent vessels and nerves were preserved; only those directly entering the muscle were divided. At the proximal and distal ends all that was left of the muscle were very small pieces of tendons required for attachment of the grafted muscle. The soleus and plantaris muscles were also removed. Next, very carefully and trying not to injure it, the right, denervated muscle was dissected out and at once transplanted to the bed of the excised muscles of the left lower limb. The muscle was anchored by a silk ligature to the tendo Achillis and to the remains of the upper tendons. The normal tibial nerve was sutured into the grafted muscle. Sutures were applied to the sartorius muscle and the skin.

For control purposes the right normal gastrocnemius muscle was transplanted to the site of the excised normal left gastrocnemius muscle of the same animal. The operation was performed in the same way as in the experimental animals.

At different times after the operation (2, 4, 7, 14, and 21 days, 1 and 6 months) the animals were anesthetized and the restoration of the muscle function was tested by stimulation of the buried nerve with an induction coil. The animals

were then sacrificed, and the muscle was weighed and fixed in Zenker's fluid and a 12% solution of neutral formalin. Sections were cut to a thickness of 6-7μ, stained with azocarmine, and counterstained by Mallory's method and with iron-hematoxylin by Heidenhain's method.

EXPERIMENTAL RESULTS

The results of the investigation showed that in the animals one month after transplantation the muscle appeared to have survived, but destruction and degeneration of the muscle fibers, followed by their replacement with connective tissue, could be seen. After 6 months either there was no trace of the muscle, or a very thin band of connective tissue remained.

In the experiments in which preliminarily denervated muscles were transplanted, the graft survived and its structure and function were subsequently fully restored.

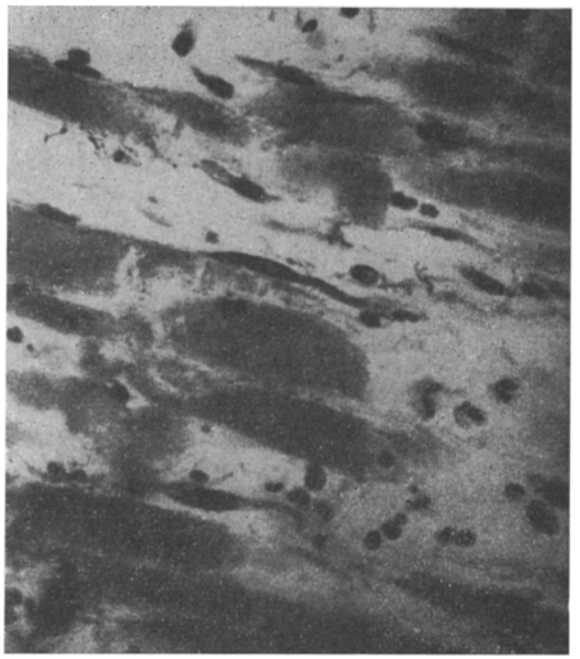

Fig. 1. Formation of myoblasts in the transplant 7 days after grafting. Fixation by Zenker's method. Stained with iron-hematoxylin. Magnification: eye-piece 8 ×, objective 24 ×.

Fig. 2. Nuclear chains in the muscle fibers of the graft 14 days after transplantation. Fixation by Zenker's method. Stained with iron-hematoxylin. Magnification: eye-piece 8 ×, objective 24 ×.

At the time of transplantation (30 days after division of the nerve), the muscle had lost 60% of its weight. It was undergoing atrophy, as shown by thinning of the muscle fibers, and proliferation and fatty degeneration of the connective tissue. At the same time processes directed towards restoration of its morphological and functional state were observed in the atrophying muscle. The nuclei of the muscle fibers were undergoing amitotic division with the formation of nuclear chains; division of the nucleoli was observed in the nuclei. Cleavage of the newly formed muscle fibers was observed. All these processes indicate that the muscle had acquired the signs of a plastic state. In this state it was transplanted to the site of the excised normal muscle. Two days after transplantation the muscle had a slightly edematous appearance. Its connection with the surrounding tissues was very weak. Microscopic examination showed large foci of haemorrhage at the margins of the stump and graft and also at the site of suture of the nerve. Appreciable reparative changes were not yet observed in the muscle tissue of the graft. Infiltration of leukocytes was beginning to take place in the intermuscular tissue. The blood vessels of the muscle itself were in a state of intensive hyperemia.

On the 4th day the graft was firmly attached to the edges of the stump. At the ends of the divided fibers of the stump and graft muscle buds with marge numbers of nuclei could be seen. Myoblasts were beginning to split off them. Processes of development were beginning in the muscle tissue of the graft. In the center of the graft some muscle

fibers were undergoing degeneration and necrosis, while other fibers revealed newly formed myoblasts and the development of myosymplastic structures. The myoblasts were proliferating intensively by mitotic division. The leukocytic infiltration continued. Many macrophages, phagocytes, and mast cells appeared. The formation of new blood vessels proceeded at the periphery of the graft.

Seven days after transplantation the muscle still failed to respond to stimulation of the nerve by the induction current, but processes of repair and development were clearly observed in it. The graft was undergoing profound reorganization. In its center could be seen muscle fibers, disintegrating into fragments, with sarcolemma tubes filled with phagocytes, and fibers with a homogeneous appearance, being without nuclei. The sarcoplasm and nuclei of the disintegrating muscle fibers were taking part in regeneration. From them a massive liberation of myoblasts was taking place not only at the periphery of the graft but also closer to its center (Fig. 1). Processes of formation of muscle tubes were observed. Mitoses could be seen in the myoblasts.

Numerous myoblasts detached themselves from the muscle buds at the edges of the stump and the graft and moved towards each other. The amount of fibrous connective tissue in the muscle was reduced, but the number of leukocytes, phagocytes, and macrophages was still considerable. In addition to the many fully formed vessels penetrating into the whole of the graft, new vessels were still being formed.

On the 14th day after transplantation the denervated muscle responds by contraction to stimulation by an induction current from the sutured nerve. This means that at this time some of its motor end plates have regenerated. Microscopic examination showed that the zone of necrosis in the center of the muscle was very small, and the bulk of the muscle fibers was normal in structure. The nuclei in these fibers were undergoing amitotic division, and chains of 15-30 nuclei were encountered (Fig. 2). Forming muscle fibers were seen, with a central, axial arrangement of their nuclei. New muscle fibers were formed from myoblasts.

In the zone of the suture a small layer of connective tissue could be seen, in which newly formed muscle fibers were situated, with a well marked cross striation. Chains of nuclei could also be seen in these fibers. The line of the distal suture could not be seen. The total amount of fibrous connective tissue was less than in the denervated muscle. A very slight infiltration of leukocytes was observed in the muscle. Hypervascularization of the muscle tissue was conspicuous.

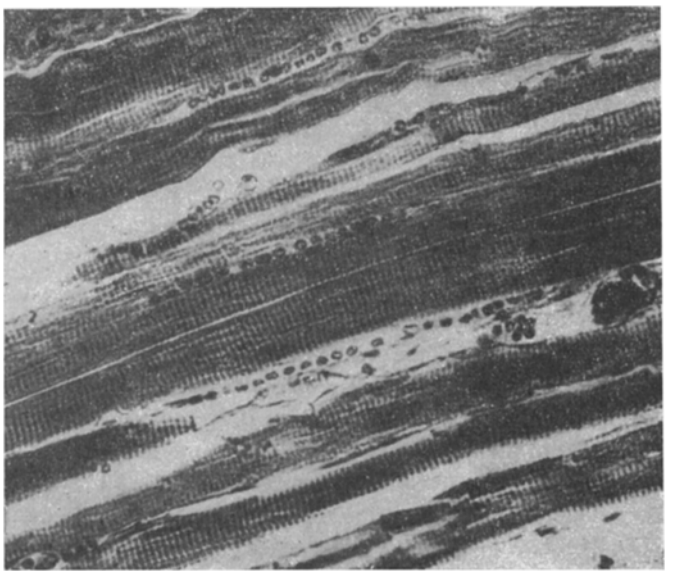

Fig. 3. Differentiated striped muscle fibers in the graft 6 months after transplantation. Fixation by Zenker's method. Stained with iron-hematoxylin. Magnification: eye-piece 8 ×, objective 24 ×.

Twenty one days and 1 month after transplantation the grafted muscle was in fact a well-formed muscular organ. Microscopic examination showed that the muscle consisted mainly of mature muscle fibers, with clearly defined cross striation and nuclei arranged at the edge of the fiber. Meanwhile processes were observed indicating continued

development: young fibers with a central, axial arrangement of their nuclei and nuclear chains, indicating amitotic division, could be seen. The appearances suggested splitting of muscle fibers from those already formed. The suture lines between the graft and the stump could be identified only by a slightly disordered arrangement of the muscle fibers. The blood supply was abundant.

Six months after transplantation, the graft showed little difference from normal in its size and microscopic structure (Fig. 3). However, in spite of its completely differentiated structure, processes of development still continued in the graft: amitotic division of the muscle nuclei in the fibers, and cleavage of muscle fibers. The amount of connective tissue between the fibers was slightly larger than normally.

It may be concluded from these experiments that the free autoplastic transplantation of whole muscles is possible provided that the muscles are brought into a plastic state; this may be done, in particular, by preliminary denervation.

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