RESTORATIVE PROCESSES IN DOG MUSCLES AFTER FREE AUTO- AND HOMOGRAFTING

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Whole gastrocnemius muscles, denervated 1 month previously by division of the tibial nerve, were autografted and homografted in dogs. The transplanted muscles took at the site of grafting, but some of the transplanted material died and was resorbed; myoblasts were liberated from the viable part of the disintegrating material. As a result of further differentiation of myogenic elements in the autografts the blood supply and innervation were restored in good time and the muscle organ reconstructed. Its contractile activity also was restored. The homografts did not take permanently: The newly formed muscle died because of the incompatibility reaction. Preliminary denervation is thus an effective means of preparing muscle tissue for subsequent autografting.

KEY WORDS: *Transplantation of muscles; denervation of muscles; gastrocnemius muscle.*

Because of the contradictory nature of the resuts, the possibility of free muscle grafting for a long time remained in dispute. Most workers (both clinical and experimental) did not accept it [5, 10, 14, 15].

In 1952 Studitskii [7] published the results of experiments in which for the first time structural and functional restoration of a muscle organ was obtained in birds and mammals from transplanted minced muscle tissue. This method of transplantation was later developed extensively and variously in different species of animals both in the USSR and elsewhere [2-4, 12, 13, 16]. It has also been used in clinical surgery [6]. Good results have been obtained from experimental autografting of large muscle fragments prepared by preliminary trauma or denervation in rats, rabbits, and dogs [8, 9]. In the writer's experiments on rats and rabbits in which preliminarily denervated gastrocnemius muscles were transplanted as free auto- and homografts positive results were obtained [1, 11].

This paper describes an attempt to transplant whole muscles, after preliminary denervation, in dogs.

EXPERIMENTAL METHOD

The right tibial nerve was divided in mongrel puppies of both sexes at the age of 2-3 months under general anesthesia at a distance of 1 cm from the point of its entry into the gastrocnemius muscle. The distal end of the divided nerve was diverted to one side and sutured to the thigh muscles. After 1 month either the denervated gastrocnemius muscle was replaced by autografting or exchange grafting was carried out with muscles of two animals belonging to the same litter (homoplasty). For this purpose, after incision of the skin and covering muscle the denervated muscle was carefully dissected, all vessels entering it were divided, and the isolated muscle was placed in a sterile Koch's dish with solution containing 5000 units penicillin and 30 ml isotonic saline. The end of the previously deviated tibial nerve was dissected free and freshened, the bed of the removed muscle was cleaned

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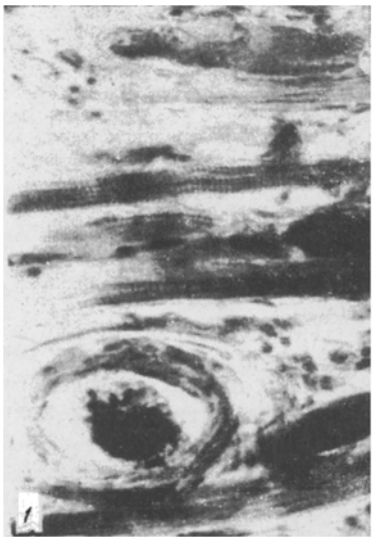

Fig. 1

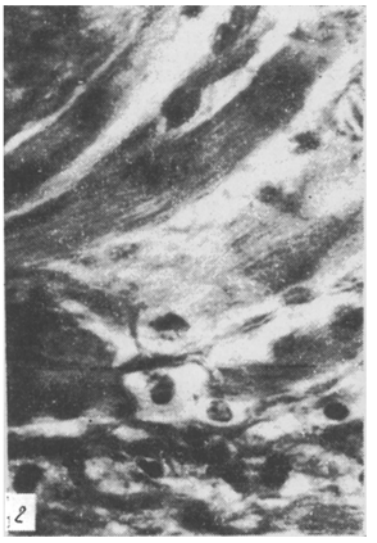

Fig. 2

Fig. 3

Fig. 1. Young muscle fibers in central part of graft 1 month after transplantation. Here and in Figs. 2 and 3, staining with Regaud's iron hematoxylin. Ocular 8×; objective 24× (Figs. 1 and 2), 45× (Fig. 3).

Fig. 2. Muscle tissue in graft 3 months after transplantation.

Fig. 3. Mature muscle fibers and connective tissue in graft 16 months after transplantation.

from blood clots. The muscle was placed in this bed, attached with ligatures to the stumps of the tendons, and the tibial nerve was sutured between the two heads of the muscle. The covering muscle and skin were closed with interrupted sutures. The material was fixed 1, 3, and 16 months later in Zenker's fluid with acetic acid and 12% neutral formalin. The contractile power of the graft was tested before fixation by stimulating the sutured nerve with an induction current. Histological sections were stained with Heidenhain's azocarmine, by Romanovsky's azure–eosin, and by Regaud's iron hematoxylin.

EXPERIMENTAL RESULTS

A study of material prepared for grafting showed that 30 days after transplantation of the tibial nerve simple atrophy developed in the gastrocnemius muscle, as expressed by thinning of the muscle fibers and an increase in the quantity of fibrous connective tissue. Sclerotic changes were observed in the blood vessels. As a result of injury to the walls of the blood vessels, hemorrhagic foci could be seen in the muscles. The effused blood, containing chiefly fragmented and fragmenting erythrocytes, spread for considerable distances from the focus of injury.

The inflammatory reaction thus developing led to disintegration of individual muscle fibers or of small parts of them. One month after transplantation of these denervated muscles the puppies bore weight on the affected limb and did not even limp. The results of autopsy showed that the grafts were slightly hyperemic, close to normal in shape, and a little smaller than the original denervated muscle. The muscles responded by contraction to stimulation of the tibial nerve by an induction current. Pieces of muscle from different parts of the graft were taken for histological examination. The study of these sections showed that the whole peripheral zone was richly supplied with blood vessels and contained samples of mature, differentiated muscle fibers, separated by bands of collagen fibers. Small areas, still in a state of reconstruction, remained in the central part of the graft (Fig. 1). Sarcoplasmic pools with groups of nuclei could be seen at the ends of some fibers; other fibers were breaking up and shedding myoblasts from their viable parts. However, because of the unfavorable conditions in that zone (delayed restoration of the blood and nerve supply) no further differentiation of the myogenic elements took place. Meanwhile the proliferative activity of the connective tissue was increased. The myoblasts were apparently immured in it and subsequently died. Groups of distinctive cavities could be seen

in this zone at the site of the fragmenting bundles of muscle fibers. They consisted of residual sarcolemma with fragments of myofibrils; sometimes fusiform cells of myoblast type could be seen beneath the sarcolemma.

Three months after transplantation the grafts retained their shape: They had a narrow belly consisting of two heads. In the sections the muscle was pinnate in structure. The muscle fibers were attached to the thickened tendon running through the middle. The grafts contained chiefly muscle tissue. Differentiated muscle fibers with clearly defined transverse striation and with elongated nuclei located at the edge of the fiber were arranged in bundles separated by layers of connective tissue (Fig. 2). The muscle had a rich blood supply. The irregularity in the architectonics of the vessels and certain pathological changes such as atrophy of the muscular layer of the large arteries, desquamation and death of the endothelium, and so on continued. This led to loss of elasticity of the vessels, to disturbance of their continuity, and to intramuscular hemorrhages which, in turn, could lead to atrophy or secondary fragmentation of some of the newly formed muscle fibers and to their replacement by fatty and connective tissues. The cavities described above, formed as a result of death of muscle fibers, also were filled with dense fibrous connective tissue.

Sixteen months after transplantation the external appearance of the affected limb was indistinguishable from that of the intact limb in all three dogs investigated at this time. Autopsy showed that the graft was fused with the covering muscle, to form a single muscle organ with it. Careful dissection revealed the graft proper, which was considerably smaller than the intact muscle — it weighed 50% of the weight of the intact muscle. The grafted muscle had a long, narrow belly and a thickened Achilles' tendon. Histological investigation showed that the graft contained mainly muscle tissue. The mosaic structure of the graft still persisted to some extent, chiefly in the central and distal parts, where bundles of muscle fibers intermingled with layers of fibrous connective tissue (Fig. 3). In the peripheral zone a richly developed network of differentiated arteries and veins could be seen. The arrangement and structure of the vessels were not completely normal. Muscle bundles of the peripheral zone contained thick, differentiated muscle fibers with clearly distinguished cross striation and elongated nuclei, distributed at the periphery of the fiber. The very large nucleoli were evidence of continuing synthesis in the graft.

Death of some of the young muscle fibers was observed at the same time. These fibers were becoming homogeneous, their cross striation was no longer visible, their nuclei were swollen, their chromatin fragmented, and their nucleoli indistinguishable. The fibers were broken up and phagocytosed, and their place was taken by loose fibrous connective tissue. Fatty degeneration of the muscle fibers and accumulation of fat between the bundles or between individual muscle fibers were observed. The total quantity of fatty tissue in the graft was greater than normal.

The contractile activity of the grafted muscle was only a little less than that of the intact muscle.

Homotransplantation of the gastrocnemius muscle, prepared by preliminary denervation, in puppies of the same litter gave only a temporary effect and the graft did not take permanently. The muscle took initially where it was grafted but then underwent progressive structural changes. Some of the grafted material died and was resorbed, and myoblasts were liberated from the viable part. As a result of their further multiplication and differentiation a new muscle organ was formed. About a month later, however, because of a well-marked incompatibility reaction (abundant lymphoid infiltration) the newly formed muscle died and was resorbed and connective tissue developed in its place.

It can accordingly be concluded from these results that preliminary denervation is an effective method of preparing whole muscle for autoplasty not only in small laboratory animals but also in dogs. As a result of this operation permanent taking of the graft is achieved, during which most of the total mass of the transplanted muscle survives and is reconstructed.

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