insular apparatus are disturbed, with the consequent stimulation of the contra-insular apparatus. Meanwhile the general biological changes revealed at both the light-optical and ultrastructural levels are less marked than in the organs of the digestive system after these procedures. It can accordingly be concluded that the vagus nerves play a less important role in the innervation of the adrenals.

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COMPARATIVE ANALYSIS OF PLASTIC ACTIVITY OF MUSCLE AND NERVE TISSUES AFTER TRAUMA AND TRANSPLANTATION OF MINCED MUSCLE

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UDC 612.6.03:612.74

Repair processes in the body muscle of Varicorhinus capoëta sevangi were studied after removal of part of three muscle segments and after autografting the resulting defect with minced muscle. As a result of trauma a focus of injury developed in the muscle, including the defect and the surrounding zone of degeneration. The inflammatory reaction, resorption of the necrotic masses, and regeneration of the muscle and nerve tissue continued for a long time. The formation of myogenic components did not begin until the 3rd week and single regenerating nerve fibers appeared in the region of injury after 2 months. Filling the defect with minced muscle accelerated regeneration. The transplanted fragments of muscle fibers not only participated themselves in the repair process but also stimulated the plastic activity of the muscle and nerve tissues of the graft bed.

KEY WORDS: regeneration of muscle and nerve tissues; transplantation of minced muscle.

Despite the great interest shown in the regeneration of organs and tissues [4, 6, 7, 9, 14] and, in particular, the problems of regeneration of skeletal muscles [2, 8, 13], the ability of muscle tissue in lower vertebrates to regenerate has received little investigation. For instance, there is brief but contradictory information on the regenerative powers of muscle tissue after minor trauma to muscle in fishes [1, 3, 5, 11, 12]. However, no grafting of muscles whatsoever has been carried out in fishes, nor has the role of the nervous system in muscle regeneration in these animals been investigated. Yet such an investigation is an important stage in the analysis of changes in regenerative activity of the tissues in an evolutionary series of animals.

EXPERIMENTAL METHOD

About 200 specimens of the Lake Sevan khramulya (Varicorhinus capoëta sevangi) 35-40 cm long were used. The experiments were carried out in summer on Lake Sevan. The fish were kept in tanks with running water on the shore of the lake under near-natural conditions. Two series of experiments were carried out: I) removal of part of three muscle segments ($1.5 \times 1 \times 0.5$ cm) on the lateral surface of the body below the dorsal fin; II) filling the defect with minced muscle tissue prepared from the piece of muscle removed. The material was fixed at various times (from 7 days to 3.5 months) in Zenker's and Bouin's fluids and in 10% neutral formalin and treated by various histological methods.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 3, pp. 373-376, March, 1978. Original article submitted July 18, 1977.

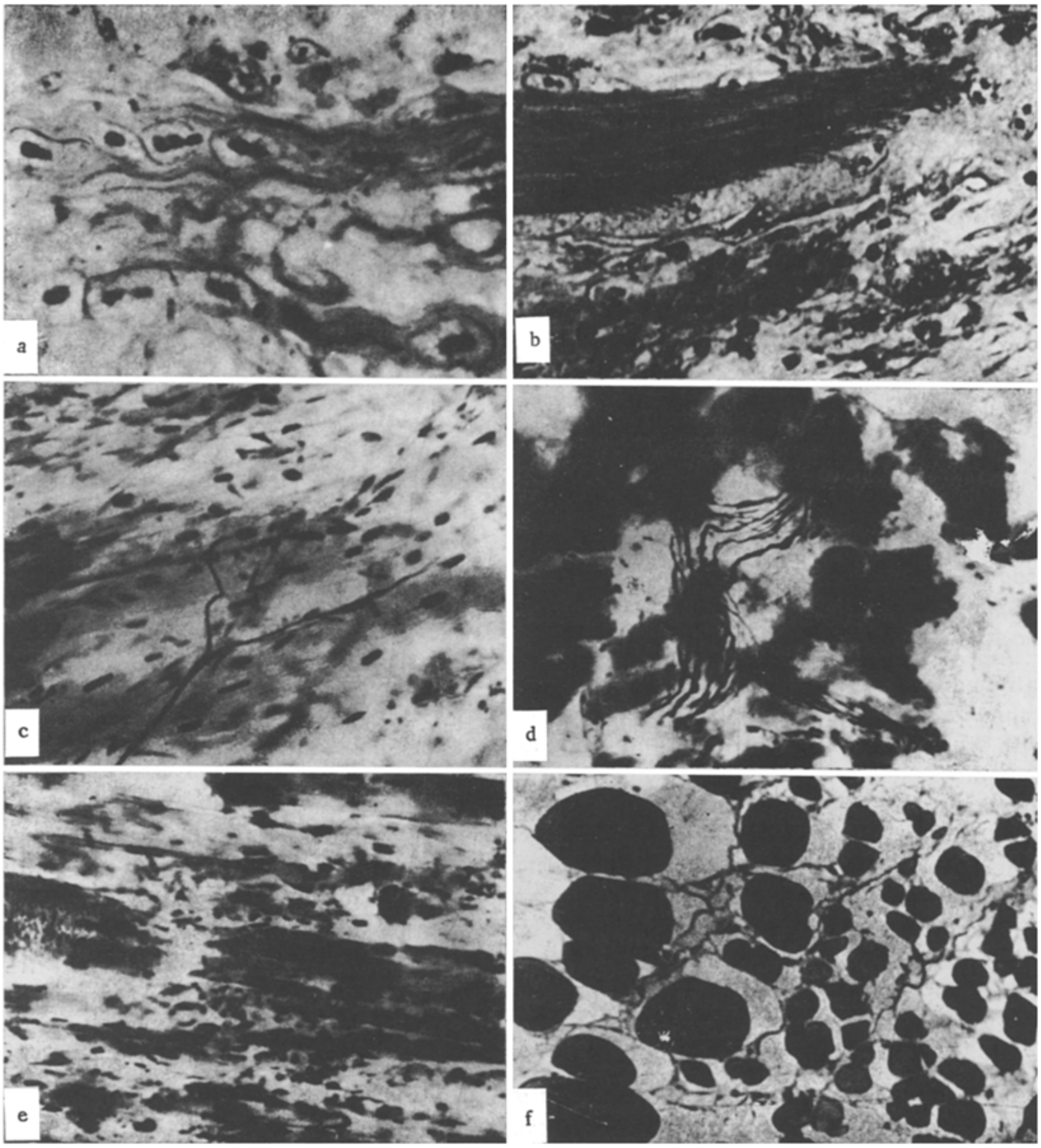

Fig. 1. Various stages of regeneration of muscle and nerve structures in injured and grafted fish muscle: a) muscle tube in regenerating muscle 17 days after injury (iron hematoxylin, 400×); b) muscle fibers with basophilic sarcoplasm in regenerating muscle 17 days after injury (iron hematoxylin, 250×); c) motor nerve ending in zone of regeneration 3.5 months after trauma (impregnation by Bielschowsky–Gros–Lavrent'ev method, 100×); d) nerve trunk with modified axons in graft of minced muscle 13 days after transplantation (impregnation by Bielschowsky–Gros–Lavrent'ev method, 100×); e) formation of myosyncytia from disintegrating muscle fibers in zone surrounding minced muscle graft 1 month after transplantation (impregnation by Bielschowsky–Gros–Lavrent'ev method, 100×); f) transverse section through regenerating muscle fibers in region of minced muscle graft 2.5 months after transplantation (iron hematoxylin, 100×).

EXPERIMENTAL RESULTS

During the first 2 weeks after trauma the defect was filled with blood clots. A region of injury could be seen around the defect in which the muscle fibers were undergoing discoid or granular disintegration and breakdown of the fibers into bundles of myofibrils. The inflammatory reaction, which first became visible during the 1st week after the operation, intensified until the 2nd week, when the whole zone of degeneration was infiltrated by numerous neutrophils, monocytes, cells of the monocytic series, and polyblasts. At the periphery of the region of injury, where blood vessels were numerous, phagocytosis of necrotic areas of muscle fibers began. Some sarcolemma tubes, liberated from the degenerating masses, were filled with phagocytes and polyblasts, among which mitoses were not infrequently found. In the zone of degeneration nerve trunks with their axons intact, some of them with varicosities, were detected by silver impregnation.

The first signs of regeneration of the injured muscle tissue were observed in the 3rd week, when spindle-shaped myoblasts, sometimes forming small chains of 2 or 3 cells, became identifiable on the walls of the sarcolemma tubes remaining after resorption of the destroyed material. Occasionally single myosyncytia and muscle tubes with large pale nuclei, containing large round or rod-shaped nucleoli, could be seen in this zone (Fig. 1a). Muscle fibers with their structure still intact could be seen at the same time in some areas at the periphery of the region of injury. In sections through such fibers bundles of myofibrils surrounded by a continuous layer of granular, often basophilic sarcoplasm, in which round muscle nuclei were distributed, were clearly visible (Fig. 1b). The repair process developed slowly. Even after 1 month the blood clot in the defect was not completely absorbed. Processes of destruction of muscle fibers and phagocytosis of necrotic masses continued. In the zone of injury, just as at the previous time nerve trunks with axons, some with varicosities, were still present. The inflammatory reaction was subsiding but had not ceased completely after 2-2.5 months. At this time the defect was filled with loose connective tissue containing numerous capillaries and pigment cells. Among the cells it was possible to distinguish fibroblasts, macrophages, and small groups of lymphocytes. In the region of injury surrounding the defect remains of old sarcolemma tubes filled with phagocytes could be seen. In some areas regenerating thin muscle fibers, myosyncytia, and bands of myoblasts penetrated into the zone of the defect. They ran in different directions and were separated by wide bands of connective tissue. Only occasionally were single regenerating nerve fibers found in these sections, and they still had no formed endings.

The repair process continued to progress at the subsequent times. After 3.5 months the structure of the region of injury was changed. Loose multicellular connective tissue with blood vessels of different diameters and with stellate and elongated pigment cells now occupied only the central part of the defect. The zone surrounding the central region of the defect contained not only fibrous connective tissue, but also single thin muscle fibers. Further out toward the periphery of the region of injury there were more regenerating muscle fibers which interwove. In the next zone, often reaching as far as the septum separating the muscle segments the thicker muscle fibers were parallel in arrangement, but they differed from the undamaged muscle fibers located further still from the defect (often beyond the septum) in their smaller diameter and greater number of round nuclei. In the zone of uninjured muscle fibers a network of nerve trunks was found; the nerve fibers ended in typical axo-muscular synapses, whereas in the zone of regeneration the nerve fibers were few in number and properly formed motor endings with 1 or 2 nuclei were very rarely seen (Fig. 1c).

During the first 2 weeks after transplantation of minced muscle into the defect fragments of the grafted muscle fibers lost their characteristic structure and became homogeneous or disintegrated with the formation of necrotic masses. They were surrounded by a clot of exuded blood. Infiltration of the degenerating parts of the muscle with leukocytes became distinct 2 weeks after the operation. At this period, by contrast with the experiments of series I, leukocytes had also penetrated into the center of the defect, into the region of the graft surrounding the muscle fragments. Phagocytosis of their disintegrating myofibrillary system and separation of spindle-shaped myoblasts from the periphery of the fragments were observed. In the zone of injury surrounding the graft the muscle fibers were swollen and vacuolated and were surrounded by macrophages. The residual muscle fibers were sharply demarcated from the region of injury. They showed signs of a plastic state: numerous round nuclei and dilated capillaries could be seen in them. Their innervation was completely intact. In the zone of injury and also in the region of the grafted muscle fragments nerve fibers with axons possessing varicosities or fragmented axons could be found (Fig. 1d).

After 1 month solitary fragments of minced muscle with signs of degeneration could be seen in the region of the grafts among numerous leukocytes, fibroblasts, and pigment cells. Mitoses, many dilated capillaries, and thin-walled blood vessels were frequently encountered. Separation of basophilic myoblasts from the muscle fragments, and sometimes short myosyncytia, could be seen. In the region of injury adjacent to the site of transplantation a zone of regenerating muscle structures developing at the site of degenerating parts of the injured muscle fibers could be clearly distinguished. Two or three thin myosyncytia could be seen to leave wide muscle fibers with regions of granular degeneration (Fig. 1e); these myosyncytia contained oval or round nuclei which occupied a centraxial position. The large nuclei formed constriction bands and divided or became fragmented. Single muscle tubes also were seen. Among the myogenic structures there were individual regenerating nerve fibers and small nerve trunks.

At later stages (1.5-2 months) the region of the graft was filled with more differentiated connective and muscle tissues. In the center, among the loose connective tissue, together with myoblasts the structures visible included myosyncytia, muscle tubes, and individual thin striated muscle fibers. In the peripheral zone of the region of transplantation regenerating muscle fibers grew in from the surrounding muscle segments and interwove tightly. They were accompanied by nerve fibers which were beginning to form endings. Irregularly interwoven axon terminals could be seen. In the peripheral zone of the region of injury the thin muscle fibers ran parallel to each other. Fibers remaining uninjured were sharply distinguished from regenerating fibers by their greater diameter, oval nuclei, and regular nerve net.

After 2.5 months the region of the graft under low power was distinguished by its paler staining. It was filled with loose connective tissue which contained thin striated muscle fibers, sometimes single but in places forming bundles and interweaving. The nearer to the periphery of the region of injury, the denser the arrangement of the regenerating muscle fibers accompanied by nerve structures. At this time of investigation also the regenerating muscle fibers were distinguished from the uninjured fibers by their smaller diameter and their round nuclei with very large nucleoli (Fig. 1f).

The striated muscle tissue of the Lake Sevan khramulya was capable of regenerating both after extensive mechanical trauma and after autografting of the minced muscle.

The inflammatory reaction, the process of resorption of the degenerating masses, and the subsequent regeneration took place very slowly and were not complete even 3.5 months later. It must be emphasized that the intramuscular nervous structures of the fishes studied remained for a long time in the zone of degeneration and regenerated very poorly.

Regeneration of the muscle and nerve structures was activated and accelerated if the defect was filled with minced muscle tissue. The material of the grafts not only itself participated in the repair process, but it also stimulated regeneration of the tissues surrounding the defect.

Comparison of the results of the present investigation with those obtained in analogous experiments on mammals [10] leads to the conclusion that even in dogs, whose muscle tissue has low regenerative activity, the process of healing of an open large defect in a muscle takes place much faster than in this chosen representative of the lower vertebrates. When the defect is filled with minced muscle, regeneration of the injured muscle ends with restoration of its normal structure, whereas in the Lake Sevan khramulya even after transplantation of minced muscle into the defect, the regenerating muscle contains a large quantity of connective tissue.

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