The results of this investigation thus showed that after pancreatic allografting and in chronic pancreatitis, ultrastructural changes of a compensatory-adaptive character, similar in their morphological features, arise in the enterocytes of the small intestine. They are due to the reaction to antigenic stimulation and are aimed at maintaining the necessary level of functional activity under the conditions of the immune response.

This type of reparative regeneration, namely regeneration "at a distance" [6], evidently not only includes potential powers of compensation of the disturbed function in immunologic trauma, but is at the same time one of the nonspecific components of reactions of immune homeostasis. The results must be taken into account when the clinical manifestations of chronic pancreatitis are assessed and during management of the post-transplantation period after transplantation of the pancreas.

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ROLE OF NEUROMUSCULAR SYNAPSES IN MORPHOGENETIC PROCESSES TAKING PLACE IN TRANSPLANTED AMPHIBIAN MUSCLE

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UDC 616.74-089.843-092.9

KEY WORDS: innervation of muscles; transplantation; denervation.

Dependence of plastic (structural) processes in a skeletal muscle on its contacts with nerve tissue has been examined in amphibians in experiments on regeneration of muscles damaged *in situ* [1, 5, 7 13].

The ability of skeletal muscle tissue of amphibians to undergo morphogenetic changes after autografting of minced muscle [6] was demonstrated previously and subsequently confirmed by investigations on frogs [9, 14] and axolotls [10, 11].

Umnova [8] first performed autografting on a whole muscle in frogs and described the process of transplantation regeneration taking place in a muscle completely separated from the body and reimplanted, with which a nerve was put in contact during the operation.

The object of the present investigation was to compare the dynamics of the morphogenetic process taking place in an autograft of a whole amphibian muscle, deprived of its connections with the nervous system, and on reinnervation of the graft. The results of this investigation have been published previously only in short abstracts [3].

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on 52 pond frogs *(Rana ridibunda)* weighing 50-80 g. The animals were kept at room temperature in an animal house and were fed

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 89, No. 5, pp. 623-625, May, 1980. Original article submitted June 12, 1979.

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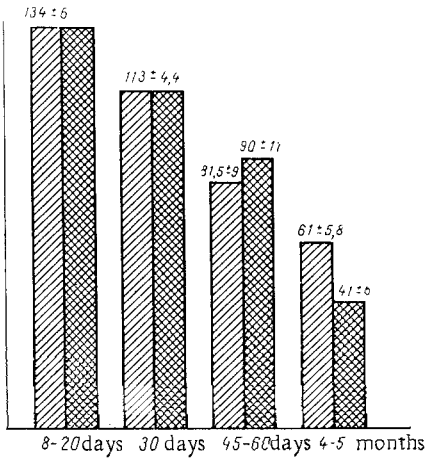

Fig. 1. Weight of innervated (oblique shading) and denervated (cross-hatching) muscle grafts (in percent of weight of symmetrical intact gastrocnemius muscle).

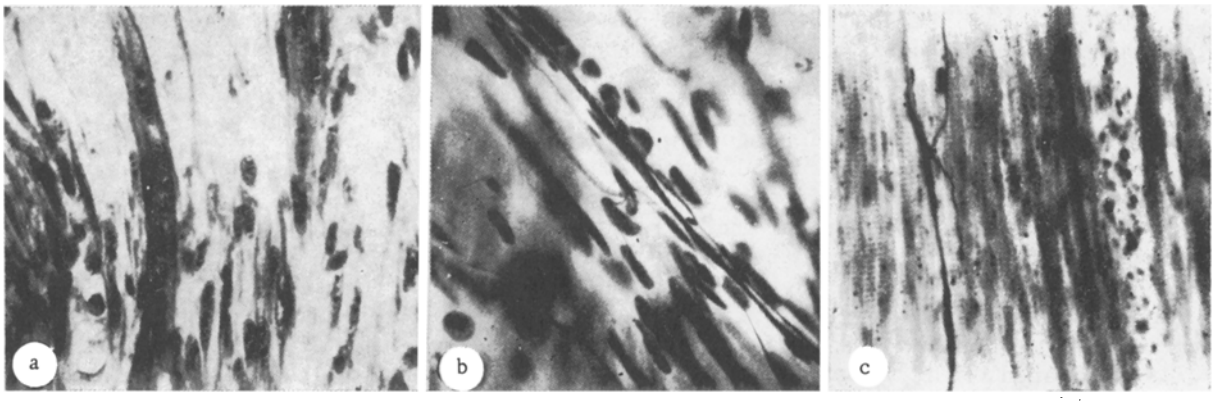

Fig. 2a. Myogenic cells in graft of gastrocnemius muscle one month after transplantation. Zenker. Regaud's iron-hematoxylin, 360 ×.

Fig. 2b. Regenerating nerve fibers in 2-month-old graft with tibial nerve brought up to it. Formalin. Impregnation by Bielschowsky—Gros—Lavrent'ev method, 360 ×.

Fig. 2c. Formation of axo-muscular synapses in reinnervated graft 5 months after transplantation. Formalin. Impregnation by Bielschowsky—Gros—Lavent'ev method, 200 ×.

on butterflies. In the experiments of series I the extirpated intact gastrocnemius muscle was reimplanted in its proper bed in the right limb; the autograft was secured by ligatures to the remains of the tendons, and the tibial nerve, divided during removal of the muscle, was brought up to its central part. In the experiments of series II the gastrocnemius muscle was reimplanted in the same way, but the tibial nerve was led away from the muscle and secured to the thigh muscle at a distance of 1 cm from the graft.

Grafts and intact gastrocnemius muscles from the left limb, acting as the control, were investigated eight days to five months after the operation. The contractile activity of the muscles was monitored by physiological methods, their weight was determined, and the weight of the graft expressed as a percentage of the weight of the intact muscle. Material was fixed in Zenker's fluid and in 12% neutral formalin; histological sections were stained with azocarmine after Heidenhain, with Regaud's iron-hematoxylin, and with asure—eosin, and impregnated with silver by the Bielschowsky—Gros—Lavrent'ev method. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As the physiological control showed, the contractile reaction of the transplanted muscles to direct stimulation by an induction current was observed in the experiments in which the nerve was brought up to the graft 50 days after the operation, and in response to electrical stimulation of the nerve starting from four months; contraction increased in intensity until five months of the investigation. In the experiments in which the nerve was led away from the graft, as a rule the graft gave no contractile response to direct stimulation at any time of investigation. Only in two cases was spontaneous reinnervation of the grafted muscles observed four months after the operation by nerve filaments growing out of the displaced tibial nerve. In these animals contraction of the graft was observed both to direct stimulation and to stimulation of the nerve.

Macroscopically the transplanted muscles appeared hyperemic and edematous in both series of experiments for 1-1.5 months after transplantation. The edema disappeared after 50-60 days in the experiments of series I and the graft assumed the characteristic silver-gray color of the intact muscle. Edema of the limb and erythema and necrosis of the skin were observed in series II and the grafts were greenish-yellow in color. The weight of the grafts for 20 days was considerably greater than the weight of the intact muscles in all experiments (Fig. 1). After one month the weight of the grafts was reduced, but it was still above normal. After 50 days the relative weight of the grafts was a little below normal, but remained higher in the experiments of series II. A significant difference in the relative weight of the innervated and denervated grafts was observed after 4-5 months: The innervated grafts were significantly heavier than the denervated.

A study of the histological preparations showed that transplantation regeneration followed a similar course until 1.5-2 months after the operation in the experiments with both reinnervation and denervation of the graft. After eight days, because of edema of the grafted muscles, the muscle fibers were loosely arranged, intensively stained, and their cross-striation and nuclei could not be detected; on the 20th day some muscle fibers at the periphery of the graft showed degeneration and phagocytosis. Lymphocytes and macrophages were arranged between and within the muscle fibers, individual muscle fibers were pale, and their structure began to become visible. Beneath the sarcolemma of such fibers single small spindle-shaped cells resembling myoblasts could be seen very rarely. After 30 days a narrow peripheral zone of regeneration appeared in the grafts, where in place of the muscle fibers which had undergone degeneration and phagocytosis, spindle-shaped myoblasts arranged in a chain, muscle symplasma with large round nuclei and giant nucleoli and, occassionally, single muscle tubes (Fig. 2a) appeared. The myogenic structures were surrounded by loose connective tissue containing regenerating capillaries and infiltrated with various leukocytes. Starting from the 50th-60th day differences were noted in the course of transplantation regeneration and in the structure of the grafts in the two series of experiments. If the tibial nerve was brought up to the grafts, a few axons regenerating from the nerve trunk could be seen in the proximal end of the graft (Fig. 2b). They ended in sharply pointed terminals or, occasionally, in spherical or oval pools of axoplasm. A sudden change took place in the state of the grafts: The process of transplantation regeneration affected the whole of the transplanted muscle. Myogenic structures in different stages of development were represented in the grafts: myoblasts, muscle symplasms, muscle tubes, and young cross-striated muscle fibers. Meanwhile many old muscle fibers were filled with numerous parallel rows of round nuclei; in some old muscle fibers the structure could not be made out, and some of them were undergoing cloudy swelling and vacuolar degeneration. They were surrounded by loose multicellular connective tissue containing regenerating blood vessels.

In the experiments in which the nerve was led away from the graft, 1.5-2 months after the operation many of the grafted muscles showed total or partial destruction. Abundant leukocytic infiltration of the graft was observed. In the most successful cases the graft contained approximately equal numbers of bundles of old, degenerating and phagocytosed muscle fibers, surrounded by fibrous connective tissue, and newly formed muscle tubes and muscle fibers, some of which were undergoing secondary degeneration.

By the 4th-5th month of the investigation, in experiments in which the nerve was brought up to the graft, their reinnervation showed a considerable advance. Regenerating nerve fibers formed numerous haphazardly arranged plexuses. The formation of axo-muscular synapses was noted: An axon, approaching young cross-striated muscle fibers, branched T-wise into irregularly branching long and short terminals (Fig. 2c).

The grafts were constructed mainly of differentiated muscle tissue and had a feather-like structure. The muscle fibers were of large diameter and contained numerous round nuclei. However, in certain small areas of the graft foci of inflammation, immature myogenic structures, and secondary degeneration of some newly formed muscle fibers could be seen.

Under denervation conditions as a result of intensive secondary degeneration of the regenerating muscle fibers, the grafts in most cases contained old degenerating muscle fibers, distributed among the fibrous connective tissue, and only a few regenerating muscle fibers. Only in two frogs, in which spontaneous restoration of the innervation of the grafts took place, did the grafts contain bundles of newly formed cross-striated muscle fibers as well as connective tissue.

To sum up, it can be concluded that the process of regeneration in an autografted whole frog muscle develops slowly and, in its initial stages, follows a similar course whether the nerve is brought up to the graft or the latter is deprived of the possibility of recovery of its innervation. However, with the beginning of reinnervation of the grafted muscles, differences are observed in the course of transplantation regeneration in the different variants of experiments.

Starting from two months after transplantation, when if the nerve was brought up to the muscle, regenerating axons began to grow into it, many muscle fibers in the denervated grafts died, and by four months after the operation this led to clear differences in the weight and structure of the innervated and denervated grafts. This can be explained by the absence of neurotrophic control of the structural activity of the muscle tissue following denervation of the grafts.

A similar pattern (but with a change in the times of the differences observed) was found after autografting of minced muscle tissue in frogs [12].

It can be concluded from a comparison of the results of experiments with reimplantation of the whole gastrocnemius muscle in frogs and the analogous data obtained in experiments on rats [2] and turtles [4] that neurotrophic regulation of plastic activity of muscle tissue increases in the course of evolution in a comparative series of vertebrates.

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