AUTOGRAFTING OF PREVIOUSLY DENERVATED MUSCLES IN RABBITS

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Whole gastrocnemius muscles of rabbits were grafted after preliminary denervation. At the time of grafting (60 days after denervation) the muscles were in a state of advanced atrophy, accompanied by degenerative changes. The autografts survived at the site of transplantation and underwent structural changes which led to the progressive development of the muscle tissue preserved in the graft, the spreading out of the tissue, and the formation of definitive muscle fibers supplied with nerve endings. Meanwhile, after a certain time secondary degeneration was observed in the muscle, leading to its replacement by connective tissue. The results confirm the writer's earlier hypothesis that free grafting of previously denervated whole muscles is possible. However, advanced denervation atrophy is evidently reflected in the late results of transplantation.

KEY WORDS: grafting of muscles; denervation; degenerative changes.

The method of muscle tissue grafting in which vascular and nervous connections with the rest of the body are preserved, is widely used both experimentally and for various purposes in plastic surgery [1, 6, 7]. Studitskii [9] first reported free grafting of minced muscle tissue followed by structural and functional restoration of muscles in birds and mammals. This method of grafting was subsequently developed experimentally in animals of different species both in the USSR [4, 5, 8] and elsewhere [10-14]. The present writer has performed free grafting of whole muscles, prepared by preliminary denervation, in experiments on rats and dogs with favorable results [2, 3].

It was accordingly decided to study the potential regenerative power of muscle tissue when in a state of advanced denervation atrophy, accompanied by degenerative changes, under autografting conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 50 chinchilla rabbits of both sexes weighing from 700 g to 1 kg. The whole gastrocnemius muscle was transplanted when in a state of atrophy 60 days after denervation. Denervation was carried out by dividing the trunk of the tibial nerve 1 cm from its point of entry into the muscle. To avoid spontaneous reinnervation, the central end of the divided muscle was transposed to the thigh muscles. The right, denervated gastrocnemius muscle was transplanted 60 days later to the site of the previously removed intact left gastrocnemius muscle. The tibial nerve was sutured to the graft. Material was fixed between 3 days and 6 months later. Before fixation the contractile activity of the autografts was tested by stimulation of the tibial nerve with an induction current. Histological sections were stained with azocarmine by Heidenhain's method, with Regaud's iron–hematoxylin, and with azure–eosin by Romanovsky's method. Nerve endings were revealed by impregnation by Bielschowsky's method.

EXPERIMENTAL RESULTS

A study of material prepared for grafting showed that denervation atrophy in rabbit muscles follows a very rapid course and leads to degenerative changes after 60 days: the muscle fibers were loosely arranged, reduced in thickness, and most of them in a state of disintegration. The muscle was profusely infiltrated by connective-tissue and blood cells, most of which were neutrophils. The quantity of fibrous connective tissue was sharply increased. Proliferation of fatty areolar tissue was observed. The circulation was disturbed.

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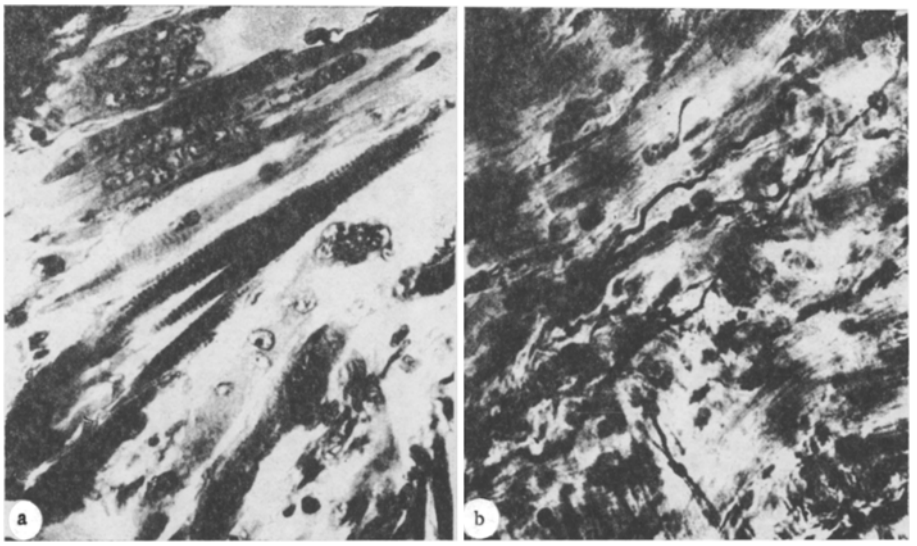

Fig. 1. Formation of muscles and growth of nerve fibers in graft: a) myosymplasts in graft (Regaud's iron-hematoxylin, 360×); b) spread of nerve fibers from sutured nerve in proximal part of graft (impregnation with silver, 360×).

The walls of the large blood vessels were thickened because of proliferation of connective tissue. Venous stasis led to hemorrhages and thrombosis.

During the first few days after transplantation about 30% of the grafted muscles had died: a dense barrier of leukocytes formed around the grafts, followed by a connective-tissue capsule. The muscle tissue beneath the capsule was fragmented and became resorbed. At the site of the graft a connective-tissue band could be seen after 2-3 weeks. The remaining grafts survived at the site of transplantation and underwent structural changes.

Three days after grafting the muscles were weakly adherent to the edges of the stumps: the zones of the sutures were filled with escaping blood. The state of the muscle was substantially unchanged, but slight hyperemia and edema were observed. The integrity of the muscle fibers was disturbed and the cross-striation and nuclei could no longer be detected in them. Because of disturbance of tension the muscle fibers were twisted in shape. The whole graft was profusely infiltrated with blood and connective-tissue cells. Because of the weak macrophagal reaction and disturbance of the blood supply and lymphatic drainage, the necrotic masses were slow to be removed and large quantities of breakdown products were found in the graft.

Fifteen days after grafting the muscles were firmly adherent to the stumps and the region of the suture was filled with scar tissue, in which chaotically arranged young muscle fibers could be seen. The graft was in a state of active reconstruction. Peripheral and central zones were sharply distinguished. The whole peripheral zone was permeated by blood vessels either invading it from the surrounding tissues or newly formed in the graft itself. This zone contained myogenic elements at different stages of maturity: myoblasts, myosymplasts, muscle tubes, and dense bundles of young muscle fibers (Fig. 1). The central zone of the graft, like a foreign body, was surrounded by a dense connective-tissue capsule. The muscle tissue enclosed within it was fragmented and undergoing resorption, the blood vessels were dilated, and their lumen was empty or filled with decomposing blood.

At the proximal end of the graft a chaotic arrangement of nerve fibers could be seen spreading from the sutured tibial nerve. Thin, straight, unmyelinated nerve fibers could be traced in the muscle for a considerable distance, to end blindly or as club-shaped swellings (Fig. 1b). The muscles did not respond by contraction to stimulation of the sutured nerve with an induction current; this is evidence that no regeneration of neuromuscular synapses had taken place. In the course of 1-3 months the structural changes became largely complete and outwardly the graft appeared as a reduced model of the transplanted muscle. Differentiation of myogenic elements as far as the muscle fibers stage took place in the graft. Dense bundles of thin, sometimes haphazardly arranged, young muscle fibers with clearly defined cross striation and with large, pale nuclei could be traced along the peripheral zone of the graft (Fig. 2). The muscle bundles were sometimes separated by layers of collagen fibers, with fibroblasts immured in them. Traces of continuing structural change were found

Fig. 2

Fig. 3

Fig. 2. Young muscle fibers in peripheral zone of graft. Regaud's iron–hematoxylin; 360×.

Fig. 3. Grafts 6 months after transplantation (total preparation).

in the zone nearer to the center of the graft. Myosymplasts with clusters of nuclei in them, and also disintegrating muscle fibers were visible there. The whole zone of reconstruction was richly vascularized. Resorption of the dying central part of the muscle continued. The muscles responded by contraction to stimulation of the sutured nerve with an induction current, evidence of restoration of their motor innervation. Nerve endings of various stages of maturity, including motor end-plates could be seen in the sections.

Between 4 and 6 months after transplantation the grafts were outwardly indistinguishable in appearance from those at the previous times: they had the same shape and size as the original denervated muscle (Fig. 3). However, this was the critical period for the developing graft. Histological study of the sections showed the development of degeneration in the newly formed muscles, resembling fatty degeneration and connective-tissue degeneration in type. The blood supply to the graft was disturbed. The walls of the large blood vessels were sclerotic and the perivascular spaces were invaded by connective tissue. Disturbance of the integrity of the blood vessel walls led to intramuscular hemorrhages and to partial destruction of the muscle tissue in that region. Thrombus formation was observed in the vessels and in some cases the lumen of the vessel was completely occupied. These changes in the blood supply in the newly formed muscle led to a disturbance of its metabolism and to degenerative changes. However, even in such cases the muscle tissue of the graft showed some signs of regenerative activity: collections of myosymplasts could be seen in the graft but, because of the unfavorable conditions, they could not undergo further differentiation and the newly formed myosymplasts died as a result of secondary degeneration of the muscle tissue. Meanwhile the fatty areolar tissue proliferated and fat accumulated both in the muscle fibers and between them. The young muscle fibers compressed by fat underwent atrophy. Proliferative activity of the connective tissue, mainly fibroblasts, increased in intensity. The contractile activity of the graft was disturbed. As a result, 6 months after transplantation an organ with the same shape and size as the extirpated muscle was found at the site of the operation, but it contained mainly fatty and connective tissue, with thin interlayers of mature muscle tissue capable of contracting.

The results of experiments with transplantation of muscles in a state of advanced denervation atrophy showed that the grafts possessed well-marked regenerative activity, capable of ensuring the progressive development of muscle tissue preserved in the graft, proliferation of that tissue, and the formation of definitive muscle fibers supplied with nerve endings.

The hypothesis that free grafting of previously denervated muscles is possible, based on a previous investigation, was thus confirmed. However, the advanced degeneration atrophy evidently was reflected in the late results of transplantation. The elucidation of the causes of this phenomenon will be a task for future research.

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CHARACTERISTICS OF ESTRADIOL RECEPTOR SYSTEM OF THE ANTERIOR HYPOTHALAMUS AND ADENOHYPOPHYSIS IN GUINEA PIGS

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The presence of a specific estradiol-receptor system (E_2-R) with limited capacity and with a high degree of strength of formation of the E_2-R complex was demonstrated in the cytosol of the adenohypophysis and anterior hypothalamus of guinea pigs in experiments in vivo and in vitro. The physicochemical properties of the E_2-R system of the adenohypophysis and anterior hypothalamus differ in certain parameters. The E_2-R complexes of the cytosols of the adenohypophysis and anterior hypothalamus formed at different temperatures are not identical.

KEY WORDS: adenohypophysis; hypothalamus; physicochemical characteristics; receptor system.

The study of the estrogen-sensitive receptor system of structures of the CNS is one way of elucidating the mechanisms of regulation of the reproductive function.

There is extensive evidence of the presence of specific cytoplasmic and nuclear protein receptors for estradiol in the anterior hypothalamic and adenohypophyseal structures of the CNS [2, 5, 7-11]. However, because of the diversity of the experimental models and methods used, there are still no clear ideas on the properties of the estradiol-receptor (E_2-R) system of the CNS. It was therefore decided to study the basic parameters of steroid-receptor interaction in the anterior hypothalamic region and the adenohypophysis of guinea pigs under different experimental conditions.

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