DEVELOPMENT OF AUTOGRAFTS OF MUSCLE TISSUE IN ADULT ALBINO RATS AGED 10 MONTHS AND OVER

(UDC 612.6.02 : 612.74-08)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 6, pp. 87-91, June, 1965
Original article submitted January 3, 1964

The investigation of autografts of minced muscle tissue developing in different physiological conditions has shown that their development depends on several specific factors.

Several authors have demonstrated the importance of the nervous system as a factor whose influence determines the end result of development of the autograft [2, 3]. Besides this factor, the role of mechanical, and also of metabolic (functional state of the thyroid gland) conditions in the development and differentiation of myogenic tissue has been studied [6, 10]. The results obtained suggest that in certain specified conditions of transplantation, minced muscle tissue develops and may form a model capable of reproducing functionally and structurally an excised muscle organ. However, these investigations were conducted on young albino rats aged 1.0-1.5 months and weighing 80-120 g.

The object of the present investigation was to carry out experiments on adult rats in order to determine the influence of age on the survival and development of autografts of minced muscle tissue.

EXPERIMENTAL METHOD

In this investigation 37 albino rats, aged at operation between 10 and 12 months and weighing 200-300 g were used. The muscles of the springing group (gastrocnemius, soleus, plantaris) were excised from all the experimental animals on both limbs. The experiments were divided into three series: in series I the springing muscles were completely removed [5, 8]; in series II the muscles were removed but a stump measuring 3-4 mm was left at the proximal end; in series III after removal of the muscles, a stump was left at both proximal and distal ends, 2-3 mm in length.

In all series the muscles removed from the right limb were replaced by autografts of minced muscle tissue, and the left limb had no graft applied. The animals of series I and II (5 in each group) were sacrificed after three intervals—10, 28, and 60 days after the operation, while the rats of series III were sacrificed 60 days after the operation (7 animals).

The material was fixed in Zenker's fluid, embedded in paraffin wax, and stained with Regaud's iron-hematoxylin and Heidenhain's azacarmine.

EXPERIMENTAL RESULTS

Macroscopic Investigation. At autopsy of the animals of series I after 10 and 28 days, the formation of a model of a muscle organ of soft consistency was observed on the right limb, situated in the leg between the knee and ankle joints. After 60 days a firm tendinous band had formed in 3 of the 5 rats at the site of the graft, and in the other 2 animals a model of a muscle organ was observed. In response to stimulation of the sciatic nerve with an induction current, the model muscle gave a contraction in only one animal, and in the other animal no reaction to stimulation was present.

On the left limb, in the region of extirpation of the muscles, in most animals the tibial nerve lay freely, while in a few cases a connective-tissue band was seen alongside it. The formation of a band of this type in similar conditions has previously been observed in young rats [5, 6].

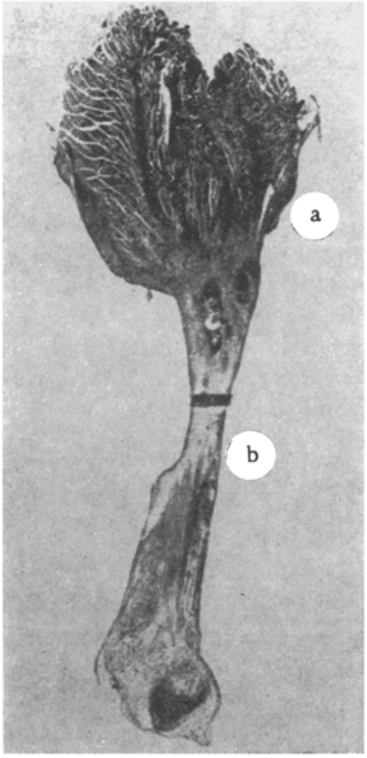

Fig. 1. Left limb; 10 days after operation. a) Stump; b) tendon.

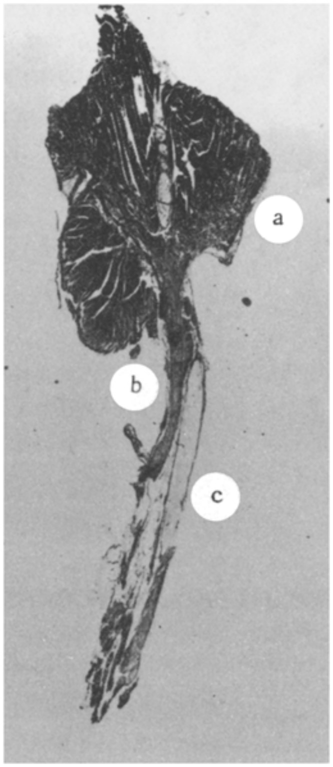

Fig. 2. Left limb; 60 days after operation. a) Stump; b) tendon; c) tibial nerve.

Autopsy of the animals of series II at the first times of investigation (10 and 28 days) showed that the growth of the stump was slightly more marked where minced muscle tissue had been grafted than in the ungrafted limbs.

However, despite this fact, the regeneration process had gone no further than to form a conical stump in both limbs, gradually tapering into a long tendon through which it was attached to the calcaneus (Fig. 1). Later, i.e., 60 days after the operation, only a small increase in the size of the stump by comparison with that on the 10th and 28th days was observed in both the grafted and ungrafted limbs, and there was no attempt at restoration of the whole muscle (Fig. 2). As at the earlier periods of investigation, the stump was nearly always conical in shape and tapered to a tendon which was approximately 1.5 times longer than the stump itself. In contrast to the adult rats, in young animals (80-120 g) in similar experiments grafting of minced muscle tissue led to growth of the residual muscle until it resembled the excised gastrocnemius muscle in appearance and was only slightly smaller in size [7, 8].

The results of series II and III were almost identical. The proximal stump of the muscle showed a slightly greater increase in size after grafting of minced muscle tissue, and in both limbs, as in series II, it was conical in shape, tapering to a long tendon running to the distal stump (Fig. 3). The stump of the distal portion of the muscle showed no increase in size in either limb, whether after grafting or without it, but in fact it was smaller. The proximal and distal stumps were joined by a developing tendon.

The results of the microscopic investigation in the experiments of series I showed that 10 days after the operation the development of the graft in the old animals corresponded to what was observed in young animals. At the site of the graft, besides fragments of minced muscle tissue undergoing degeneration, newly formed elements of muscle tissue could be seen: myoblasts, buds of sarcoplasm, muscle tubes, and solitary, thin muscle fibers. The newly formed muscle elements were situated at the periphery of the graft. As regards the degree of development of the grafts 28 days after the operation, a considerable difference was noted between the old and young animals. In the old rats, side by side with the newly formed muscle tissue in the developing graft, several fragments of minced muscle tissue still persisted. In young animals [5, 6] at this time the fragments of minced muscle tissue had completely disappeared. Furthermore, in the old animals very little newly formed muscle tissue was formed and the

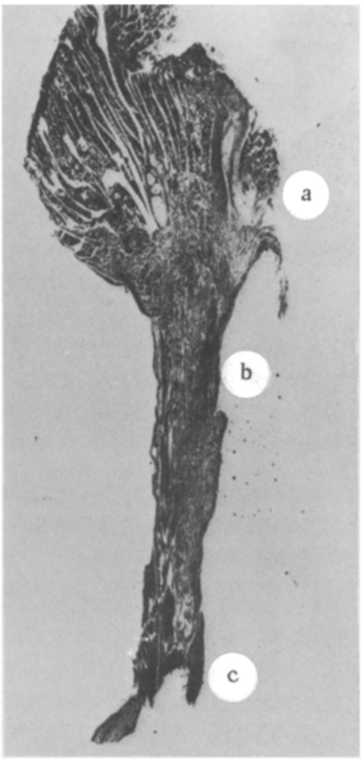

Fig. 3. Right limb; 60 days after operation. a) Stump; b) tendon with a few muscle fibers; c) part of stump.

regenerating muscle consisted mainly of dense connective tissue. The muscle elements in the regenerating structures were at the same stage as in the young animals. Hence, no difference could be observed in the degree of differentiation of the muscle tissue in the adult and young rats.

When the grafts were investigated 60 days after the operation it was found that a model muscle, similar to that developing in the young animals, was formed in only 1 of 5 cases. Microscopic examination of the developing model of the muscle organ showed that it consisted mainly of muscle elements at the stage of the differentiated muscle fiber, and in its microstructure this model was absolutely indistinguishable from that found in young animals. In a second animal the regenerating organ consisted of loose connective tissue with many blood vessels of various caliber. A few solitary, thin muscle fibers could be distinguished among the connective tissues. In the other three animals a dense band of connective tissue had formed at the site of the graft.

Microscopic investigation of the left limb showed that the band formed at the site of the totally excised muscles consisted entirely of connective tissue, differing slightly in character depending on the period of investigation.

Examination of the stump of the left limb in series II, 10 days after the operation, showed all the signs of regeneration of the muscle fiber usually found at this period of development after partial removal of a muscle: pooling of sarcoplasm at the ends of the divided fibers, multinuclear chains in the sarcoplasm and in the fiber itself, myoblasts, and the initial stages of formation of muscle tubes [1, 4, 8].

After 28 days the regeneration processes in the stump were almost complete and the newly formed muscle fibers were chaotically arranged. At the point where the stump changed to tendon, the number of muscle fibers diminished.

Sixty days after the operation the newly formed muscle fibers were at the differentiated stage and the processes of regeneration were finally complete. Where the muscle changed to tendon the stump became narrow and the number of muscle fibers which it contained diminished; in the tendon itself, which at this period of development consisted of dense connective tissue, no muscle fibers could be found.

Microscopic investigation of the right limb revealed a combination of the two processes previously observed: growth and development of the residual stump and development of the grafted tissue. In this case the formation of new muscle elements was more marked, leading to a greater degree of enlargement of the stump than in the stump in the opposite limb; however, the volume of the developing stump did not reach the volume of the control muscle. In this case, too, the stump became conical in shape and was connected by a long, thin tendon to the calcaneus. The only difference marking the development of the stump with the grafted muscle tissue was that the tendon into which the stump changed sometimes contained several muscle fibers.

Investigation of the material from series III, 2 months after the operation, showed that the development of the stump was very similar to that in series II.

As in series II, following transplantation of minced muscle tissue, the stump of the proximal portion of the muscle showed a slightly greater increase in size and was conical in shape, changing into a long tendon by means of which it was joined to the stump of the distal portion.

In the grafted limb, a few muscle fibers were found in the developing tendon, in contrast to their absence in the ungrafted limb. The muscle fibers in the distal stump were mainly arranged regularly, and the chaotic pattern was absent. A chaotic arrangement of muscle fibers is known to be a sign of their regeneration (in late stages).

Hence, the stump of the distal portion of the muscle had no effect on the final result of development of the stump of the proximal portion, and neither in series III nor in series II of the experiments was restoration of the whole muscle observed.

Consequently, in old animals (albino rats weighing 200-300 g) the intensity of the regeneration processes observed in muscle tissue was much lower than in young animals (weighing 80-120 g) used in previous experiments.

In old animals treated by transplantation of muscle tissue, in the earliest periods of development of the graft the development of new muscle elements was observed, although ultimately a connective-tissue band was formed, and only very rarely was a functioning model of a muscle organ produced, in contrast to what is seen in young animals.

Again in contrast to young animals [7, 8], leaving the stumps of the proximal and distal portions of the muscle had little influence in promoting regeneration of the muscle; in no case was enlargement of the stump to the size of the intact organ observed.

It may be concluded from this investigation that the age of the animal is of fundamental importance in determining regeneration of muscle tissue.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**