REACTIVE CHANGES OF MINCED SKELETAL MUSCLE TISSUE AFTER ITS SUBCUTANEOUS TRANSPLANTATION UNDER THE ABDOMINAL SKIN OF RATS

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Notwithstanding the great number of investigations concerning the restoration of muscles as an organ from minced muscle tissue [5] this fact can still not be regarded as firmly established.

A number of authors [3, 7, 8] have shown that minced muscle tissue transplanted at the site of the removed muscle is resorbed, and the stump which remained after the amputation of the muscle serves as the source of regeneration. For this reason, any study which proves or disproves even indirectly the possibility of restoration of whole muscles from minced muscle tissue is of great importance.

From this point of view, the study of O. N. Rumyantseva [4] is of interest: in this study the author made an attempt to prove, by transplantation of minced skeletal muscle tissue under the abdominal skin of rats, the capacity of this tissue to grow and to develop under these conditions, thus confirming her conclusion that minced muscle tissue, transplanted to the site of a completely or partly removed muscle, is not resorbed, but undergoes a number of profound changes and gives rise to the development of a muscle organ. In her experiments this author started from the assumption that there are no preexisting muscle fibers under the abdominal skin of rats which could serve as source of regneration of muscle tissue, and, in consequence, only the minced skeletal muscle transplanted under the abdominal skin of rats can serve as a source of regeneration, should such regeneration occur. This statement would be fully acceptable if the author had referred to man, as the subcutaneous tissue in man consists only of fat tissue. In rats, however, there is a thin layer of the cutaneous muscle, M. panniculus carnosus, under the skin. This muscle originates from the median line of the back. The fiber bundles of this cutaneous muscle are closely connected with the skin and spread, as we were able to establish, far into the abdomen (Fig. 1a). During the transplantation of minced muscle tissue under the skin, these muscle fibers are injured and take part in the reactive processes which develop at the site.

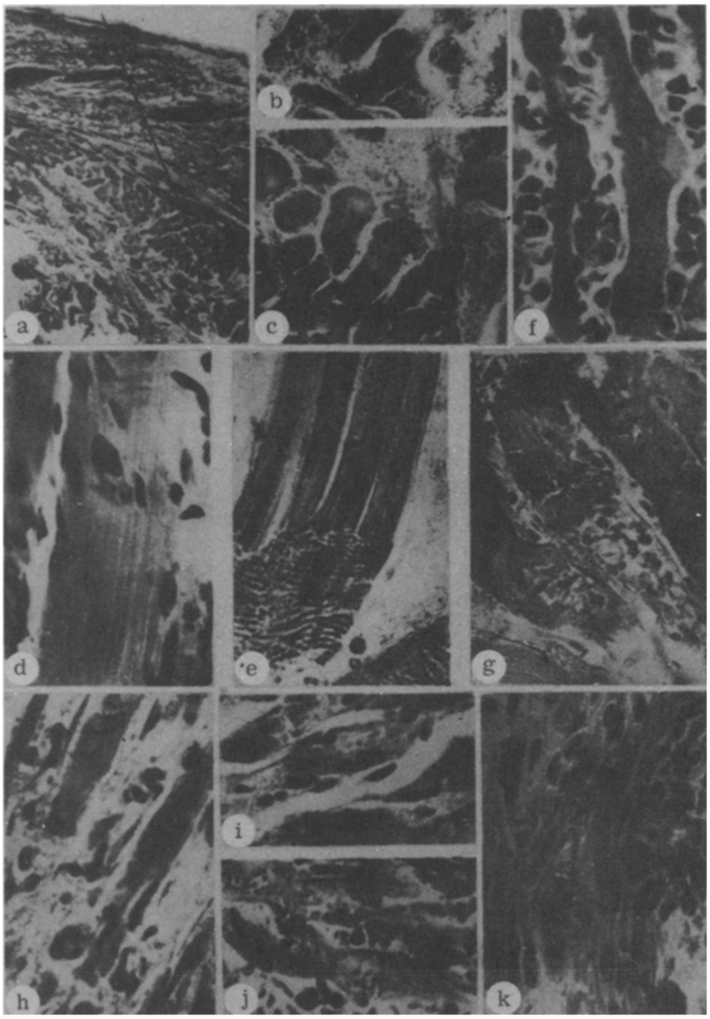
Besides, in the study quoted above [4] the author used only a small number of animals (ten rats) and did not study the early changes in the transplanted muscle tissue.

EXPERIMENTAL METHOD

We carried out experiments on 70 rats, transplanting under aseptic conditions, precisely measured quantities of minced skeletal muscle tissue collected from the M. gastrocnemius of the same animal under the abdominal skin. The operation* was carried out in the following manner: the rat lay on its back; three longitudinal incisions of 5 mm length each were made along the median line of the abdomen between the sternum and the public region at equal distances from each other; a grooved probe was introduced under the skin aiming at the left side at a depth of 0.5 cm. A piece of minced muscle tissue weighing 0.2 g was inserted along the groove of the probe into the pocket which

* O. N. Rumyantseva did not describe her experimental method in detail and this fact rendered the precise repetition of her experiments very difficult.

had formed. The skin was closed by a single suture or by a piece of gauze and collodium.* The muscle tissue was minced in a covered sterile Petri dish at room temperature; in this manner the transplanted material was completely protected against desiccation, overheating or infection.



Reactive changes in minced skeletal muscle tissue after autotransplantation under the abdominal skin of rats. Explanatations in the text. The arrows indicate the M. panniculus carnosus.

Materials for fixation were collected after 10, 20 and 30 min, 1, 3, 6, 12 and 24 hours, and after 2, 3, 5, 7, 9, 11, 16, 28, 35, 45 and 60 days. Paraffin sections of 6-8 m thickness were stained with iron hematoxylin according to Regaud, with azure II-eosin, with Mallory stain and with Caracci-hematoxylin.

EXPERIMENTAL RESULTS

The reactive changes in the transplanted minced muscle tissue and the subcutaneous connective tissue at the site of transplantation depended to a considerable degree on the participation of damaged fibers of the cutaneous muscle (M. panniculus carnosus) in the process. The injuries caused in these fibers could be the result of the direct trauma or of partial destruction caused by the developing inflammatory changes.

Ten to 15 minutes after the transplantation of the minced muscle under the skin of the abdomen, the fragments of the muscle fibers and the undamaged muscle fibers were in a state of strong contraction. The space be-

* Before the operation the abdomen was shaved, the skin was cleaned and painted with a weak iodine solution.

tween the fibers was filled with an exudate, containing numerous red cells, lymphocytes and special leucocytes, numerous fragments of capillaries, endothelial cells, tissue detritus, disintegrating leucocytes and threads of fibrin, forming a network (Fig. 1, b, c). In the connective tissue of the abdomen surrounding the transplanted muscle fibers, dilatation of the blood vessels and infiltration of the tissues with migratory cells could be observed.

Most of the minced muscle pieces underwent waxy degeneration and coagulation necrosis. Part of the fragments, however, preserved its myofibrillary apparatus, and in part, its nuclei for a prolonged period (Fig. 1, d). However, these fragments, too, the myofibrils, underwent partial homogenization and the connection between the fibrils was severed. The muscle fiber fragments lost their nuclei, became swollen, longitudinal spaces formed between the bundles of myofibrils and the muscle fibers themselves disintegrated into long myofibril bundles (Fig. 1, e). All these changes began in the central part of the fiber and gradually spread towards the ends. Later, the fibrils underwent complete necrosis, frequently even before the fiber fragment was fully separated along its longitudinal axis into groups of myofibrils (see Fig. 1, e).

These pictures could be observed until the 12th-15th day as the minced muscle fibers transplanted under the skin perished very slowly.

Beginning from the sixth hour and up to the third day, sometimes even later, a number of muscle fiber fragments which had preserved their nuclei and their myofibrillary apparatus also underwent changes which consisted of an increase in the quantity of undifferentiated sarcoplasma and an increase in the number of nuclei (Fig. 1, f). The latter, however, very rapidly lost their shape and their outlines became irregular; the staining of the nuclei changed. This picture indicates the necrosis of the muscle fibers and is characteristic for changes in the muscle tissue found in various pathological processes, for example, in case of intoxication and circulatory or trophic disorders [1, 2]. Beginning from the first day an energetic resorption of the necrotic transplanted tissues and fibrin threads by means of phagocytes which had entered parts of the muscle fibers could have been mistaken for preserved nuclei. Polyblasts infiltrated the muscle fibers and their fragments which were undergoing waxy degeneration and coagulation necrosis as well as the persisting sarcolemma; these polyblasts turned into macrophages containing the characteristic vacuoles.

The necrotic sarcoplasma of the muscle fibers was gradually resorbed and transformed into typical Waldeyer tubes (Fig. 1, g). We were unable to observe any viable myogenic elements in these tubes which elements could have served as source of subsequent growth of the transplanted muscle fibers.

In view of these findings the following statement of M. G. Shcherbakova [6] seems justified: "the fact that Waldeyer tubes are never formed during the cultivation of explanted muscle tissue also shows that the disintegrated muscle fibers do not contain viable parts from which myophages or myoblasts could develop."

Beginning from the 5th-7th day, various changes take place in the surviving fibers of the transplanted muscle tissue which had preserved their myofibrillary apparatus. In some fibers dedifferentiation can be seen consisting of the loss of the striated structure and the fibrillary character. Initially, relatively few nuclei are formed in these fibers. Later, however, their number increases by amitotic-division. The nuclei are either arranged in chains in several rows or in groups. These nuclei, however, are quite different from the nuclei in the sprouts as they undergo rapid degeneration and gradually "dissolve."

On the 5th-12th day after transplantation the formation of atypical myosymplasts developing can be seen; these formations develop from injured and dedifferentiated fragments of muscle fibers (Fig. 1, k). Formation of muscle tubes, however, could never be observed in these formations even at later stages. These myosymplasts may survive for a prolonged period in the developing granulating tissue but finally they undergo degeneration.

All these findings show that only the initial stages of or attempted regeneration and the regenerative potential of the muscle tissue can be observed; actually, however, no complete regeneration takes place.

On the 8th-25th day, signs of "dedifferentiating dystrophia" [6] can be observed in part of the surviving muscle fibers, which signs become manifest in the fact that the muscle fibers become gradually thinner due to loss of sarcoplasma and due to the disintegration of myofibrils whereas the persisting myofibrils retain their longitudinal and transverse striation (Fig. 1, i). The multiplication of the nuclei becomes more intensive and at the same time the nuclei perish in great numbers. Vacuoles or varying size are formed in the sarcoplasma.

If the first stages of development of the dedifferentiating dystrophia, which develops in the muscle fibers, are not studied in sufficient detail, these formations can be mistaken for regenerated thin young muscle fibers. Study of the pattern of development of these formations, however, enables us to establish that this is not a regnerative process but a process of degeneration, which finally leads to the destruction of the muscle fibers.

Beginning from the 3d day after the transplantation of the minced skeletal muscle tissue under the skin, granulation tissue develops in its surroundings which leads to the formation of a connective tissue capsule. This incapsulated transplantate could easily be separated from the surrounding tissues until the 25th day, but remained nevertheless in close contact with the subcutaneous connective tissue of the abdomen, which tissue took part both in the formation of the capsule and in the infiltration of the transplantate.

All the processes described above, which processes take place after the transplantation of minced muscle tissue under the skin on the abdomen of rats, are frequently rendered even more complicated by the fact that the traumatized fibers of the m. panniculus carnosus infiltrate, in the course of their regeneration, the granulation tissue of the capsule which surrounds the transplanted muscle tissue, in the shape of long myosymplasts, simulating in this way the formation of fully developed myosymplasts, which seem to develop from the fragments of the minced muscle tissue (Fig. 1, k).

The experiments concerning the autotransplantation of minced skeletal muscle tissue under the skin of the abdomen in rats thus show that under these conditions the muscle fibers in the transplantate can survive for a prolonged period; the fibers undergo coagulation necrosis and various forms of waxy (Zenker's) necrosis, they show signs of degeneration (Fig. 1, l) and of dedifferentiating dystrophy. In some fragments of the muscle fibers attempts of regeneration can be observed, which consist in the formation of atypical short myosymplasts, these formations, however, do not develop any further and are finally destroyed.

The formation of fully developed myosymplasts and muscle tubes, which can occasionally be observed in the region of the transplantate, originates from damaged muscle fibers of the subcutaneous muscle (m. panniculus carnosus) which grows in the shape of relatively large muscle bands under the abdominal skin of rats.

We were thus unable to confirm earlier findings [4] concerning the regeneration of a muscle tissue transplantate consisting of minced tissue and placed under the abdominal skin of rats. Even if it appeared, however, that minced muscle tissue, transplanted under the skin, is capable of producing tissue regeneration [4] this would still not confirm the regeneration of a muscle as a whole organ from the minced tissue. It is known that pieces of mammal muscles can grow in tissue cultures; but their capacity to undergo regeneration as an organ has still to be proved.

SUMMARY

Minced muscular tissue obtained from m. gastrocnemius of a rat was transplanted subcutaneously to the abdomen of the same animal. Fragments of the muscular fibers remain viable for a long period of time; only an attempt to regeneration takes place there later they undergo various forms of necrosis, degeneration and dedifferentiation dystrophy. As distinct from the statement made by O. N. Rumyantseva who holds that in subcutaneous transplantation of minced muscular tissue only this tissue may serve as a source of muscular fiber regeneration, it was demonstrated that m. panniculus carnosus gives muscular extensions to the abdominal skin and may serve as a source of muscular tissue regeneration in trauma. The minced muscular tissue transplant degenerates and connective tissue grows into it.

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