SECONDARY DEVELOPMENT OF MINCED AMPHIBIAN MUSCLE TISSUE AFTER TRANSPLANTATION AND DURING CULTURE IN DIFFUSION CHAMBERS

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Skeletal muscle tissue has high regenerative activity, however its working state is disturbed [6]. Research into muscle transplantation in different animals has been in progress for many years. Conditions of regeneration of muscle tissue have been discovered and various methods of treatment of the muscle for both autoplastic and homoplastic transplantation have been worked out [1, 2, 4, 7, 10]. Methods of studying the potential ability of muscle tissue to develop have been devised. The widely used method of tissue culture in diffusion chambers has also been used with muscle tissue [8, 9]. Investigations of invertebrate muscle tissue cultured in diffusion chambers have shown that structural changes in the implanted material end with the formation of organ-like structures [3].

To solve the theoretical problems along the road to discovering the mechanisms of development and growth of muscle tissue under different conditions, some interesting results can be obtained by experiments on representatives of certain lower vertebrates, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Two series of experiments were carried out on frogs (*Rana ridibunda*) weighing 50-80 g. In the experiments of series I the right gastrocnemius muscle was removed, minced, and reimplanted in its old bed. Material was fixed after between 2 and 60 days. Histological sections were stained with Heidenhain's azocarmine and Regaud's iron-hematoxylin. Nerve endings were revealed by impregnation by Bielschowsky's method. In series II a fragment measuring 1•1 mm was excised from the right gastrocnemius muscle and, under a binocular loupe, one muscle fiber was teased from it and placed in a diffusion chamber between two Millipore filters (VVFS, Czechoslovakia, pore diameter 0.1-0.3 μm). The chambers were implanted in the peritoneal cavity of intact frogs of the same species. The chambers were removed after 1, 2, 4, 8, and 14 days and the filters were fixed in alcohol—formol and 2.5% glutaraldehyde. For examination in the light microscope total preparations were stained with Caracci's hematoxylin. The remaining filters were dehydrated, sprayed with gold, and examined in the scanning electron microscope.

EXPERIMENTAL RESULTS

The results of a study of the material from experiments of series I showed that 2-4 days after transplantation the grafts had the appearance of compact and somewhat edematous formations. During the attempt to separate them from their bed they broke up into individual fragments. A study of histological preparations revealed the presence of minced fragments of muscle fibers in a state of disintegration or survival. No trace of development could be observed. The graft was usually infiltrated with neutrophils and other blood cells. In the minced fragments of nerve fibers, the axons and myelin sheath had disintegrated. Blood vessel walls were darkly stained and nuclei of the endothelial and muscular layers were in a state of pycnosis. The graft still remained edematous 14-21 days after transplantation. A connective-tissue capsule was formed around the graft, enabling it to preserve a shape similar to that of the removed muscle. A study of the preparations showed that the capsule consists of fibrous connective tissue. The muscle fibers remained disconnected,

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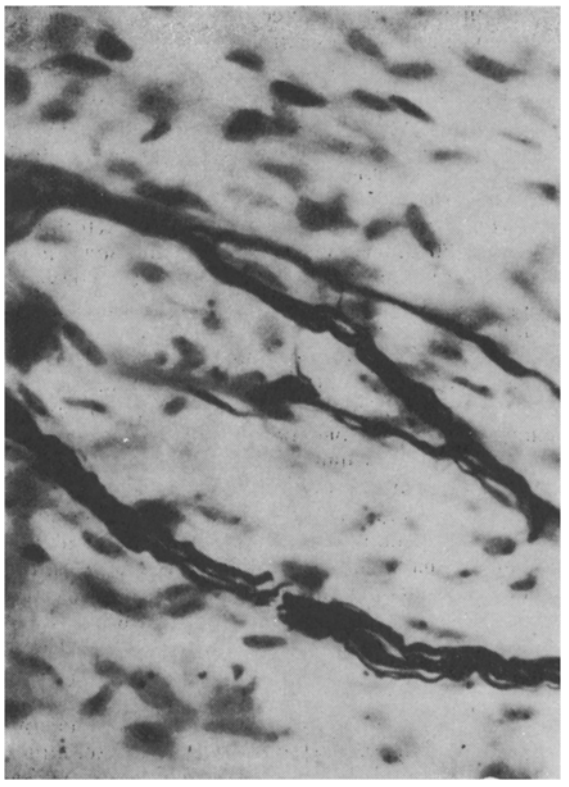

Fig. 1

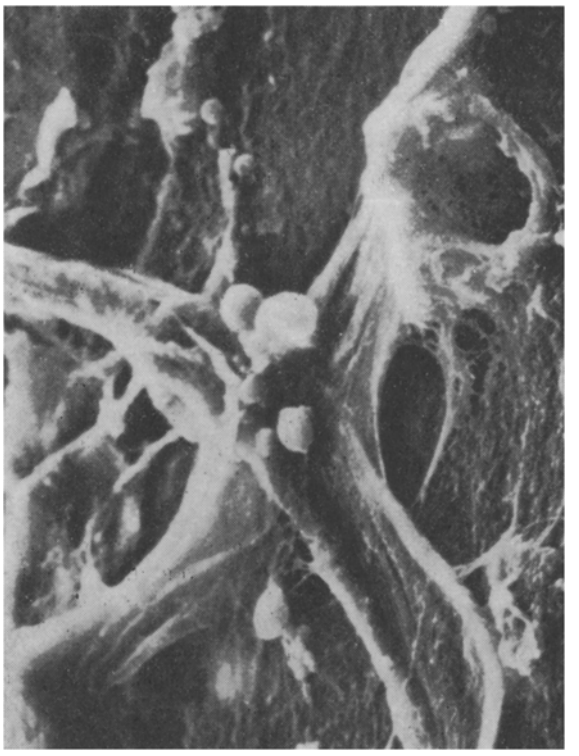

Fig. 2

Fig. 1. Regenerating nerve fibers in autograft 60 days after transplantation. Formalin. Impregnation by Bielschowsky's method, 360 ×.

Fig. 2. Muscle and connective-tissue fibers in culture on 8th day after implantation. Glutaraldehyde. Scanning electron microscope. 1000 ×.

fragmented, and structurally changed. Their outlines were uneven constrictions alternated with expansions, and cross-striation and the nuclei were not always clearly visible. Myoblast-like cells were being set free from the disintegrating muscle fibers, to form myosyncytia. Degenerating regions of the fiber were undergoing phagocytosis. Abundant cellular infiltration still remained.

In external appearance 60 days after transplantation the grafts consisted of formations with the same shape as the removed muscle, but smaller in size. Structural changes continued. The graft contained myogenic cells at different stages of maturity: myoblasts, myosyncytia, muscle tubes, and also differentiated muscle fibers. The latter were arranged mainly in the longitudinal direction, although in some areas the newly formed muscle fibers were haphazardly arranged. In the central part of the graft secondary disintegration of some of the muscle fibers could be observed. Cavities filled with fragments of muscle tissue containing exudate, with cells — mainly dying erythrocytes and other blood cells — could be seen in the same region. The whole graft was penetrated by nerve fibers regenerating from the nerve trunk remaining after the operation, and these spread haphazardly among the myogenic and connective-tissue cells (Fig. 1). In some areas where bundles of differentiated muscle fibers were present, nerve fibers formed primitive motor end-plates. Sensory nerve endings were not seen at this time.

As the light-optical study of the material from the experiments of series II showed, no trace of development could be seen in the implanted muscle tissue 1-2 days after transplantation. Longitudinal and transverse striations continued to be present in the muscle fiber. The nuclei were elongated, with clearly distinguishable chromatin grains and nucleoli, and were arranged at a considerable distance apart beneath the sarcolemma. Deformed dead or dying cells of escaped blood were present in large numbers around the graft. A study of the culture in the electron microscope showed that the muscle fiber was cylindrical in shape. Disks of sarcomeres were clearly visible in the fiber. Alongside the fiber, flat cells without processes were located on the substrate, possible evidence that these cells were in the resting state.

On the 4th day after transplantation the fiber of the muscle implant changed from the resting state into an active state, although its structure differed in different parts. Some areas preserved distinctly visible cross-striation, and pale elongated nuclei lying beneath the sarcolemma. Other regions were fragmented — the sarcoplasm became homogeneous, the nuclei lost their structure and became pycnotic. The lateral surface of the fiber could be destroyed and outgrowths arising from the fiber could be traced in the zone of injury for a considerable distance from the fiber, and they could contain separate nuclei. Meanwhile, in these same areas of disintegrating muscle fibers, viable areas of sarcoplasm with concentrations of nuclei in them were becoming identifiable, with the formation of myoblast-like cells also. A similar picture was observed in the experiments of series I. The number of cells distributed around the implant increased. Many of them were in a state of mitosis. Many neutrophils at different stages of maturity and macrophages were present in the culture. Enlargement of the disintegrating cells also was noted. In the electron microscope the implant had the appearance of a formation consisting of compact and interwoven bands, which could be flat or round in shape. The flat regions evidently corresponded to zones of disintegration. Collections of large spherical cells with a smooth surface (macrophages) and also of smaller, round cells (lymphocytes) could be seen on their surface. Around the implant there were many free-lying cells, arranged singly or in groups.

Observations showed that 8-14 days after implantation the myogenic cells formed previously differentiated as far as the stage of young muscle fibers. These fibers could be arranged beneath the sarcolemma of the mother fiber or could lie independently. The number of fibroblast-like cells, macrophages, and lymphocytes distributed in the immediate vicinity of the muscle fibers remained large as before. Many of them were in a state of mitotic division. The culture consisted of interweaving bands of muscle and collagen fibers, chiefly the latter. A similar picture also was found on electron-microscopic study of implanted muscle tissue (Fig. 2).

A study of the literature shows that during muscle tissue culture *in vitro* differentiated muscle fibers capable of spontaneous contraction are formed [5, 11]. However, in the accessible literature no data could be found on culture of amphibian muscle tissue in diffusion chambers implanted into another animal of the same species.

This investigation showed that in both series of experiments minced adult frog muscle tissue remains viable and preserves its structural activity for a long time. Both after transplantation and during culture the process of muscle fiber formation continues. A previous investigation showed that muscle tissue formation in frogs after transplantation takes place more slowly than in warm-blooded animals [12]. This phenomenon was confirmed by the present experiments. In this connection acceleration of the structural changes taking place in muscle tissue on implantation in diffusion chambers assumes great significance. It can be postulated that under these circumstances histogenetic interactions between tissue components of the graft, especially between connective-tissue cells and myoblasts, proceed under more favorable conditions.

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