A MORPHOLOGICAL STUDY OF THE CARDIAC AND SOMATIC MUSCLE FOLLOWING AUTOTRANSPLANTATION INTO THE MYOCARDIUM OF THE DOG

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G. I. Nepomnyashchikh

Laboratory of Experimental Cytology (Head, Cand. Med. Sci., A. F. Nikiforov),
Department of Experimental Biology (Head, Dr. Med. Sci., M. G. Kolpakov),
Institute of Cytology and Genetics (Director, Corresponding Member AN SSSR, D. K. Belyaev),
Siberian Division, USSR Academy of Sciences, Novosibirsk
(Presented by Active Member AMN SSSR, V. V. Parin)
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Facts have recently been published indicating that somatic muscle tissue of mammals, in certain conditions, possesses high powers of regeneration [8,9]. According to these reports, a so-called plastic state develops in muscle tissue following mechanical trauma, tenotomy, and denervation, and this leads to stimulation of repair processes in the muscle. The author has previously shown [4] that it is possible, in principle, for productive changes to occur in an autograft of minced somatic muscle when transplanted into the myocardium of a dog. Most authors deny that the heart muscle is capable of regeneration. Only isolated reports have been published showing that the heart muscle tissues may undergo reparative changes [6,7]. It has been shown [1,2,5,10] that, after injury to the myocardium in mammals, the administration of certain biological preparations (stimulators of regeneration of the myocardium and inhibitors of scar-formation) leads to the differentiation of muscle fibers and to their formation de novo.

In the present investigation, a morphological study was made of cardiac and somatic muscle following autotransplantation into the myocardium of the dog. Regenerative processes were stimulated by the method suggested by A. N. Studitskii [8], the essence of which is that the transplant (a resected muscle) is transformed into a fine mince, and this is used as plastic material for regeneration of the muscle. Besides the morphological investigation, a histochemical study was made of the autografts of cardiac and somatic muscle.

EXPERIMENTAL METHOD

Altogether 50 experiments were carried out. After left-sided thoractomy, the heart was withdrawn from the pericardium. In the region of the lower part of the anterior wall of the heart, a defect was created in the myocardium by rotary movements of a tube resembling a trocar, consisting of a canal 2-3 cm in length. The tissue remaining in the cavity of the trocar was removed by a plunger. In the experiments of series I (28 animals), the heart muscle tissue removed from the tube was grafted into the canal; in series II (22 animals), pieces of the pectoral muscles from the region of the operation wound were used as autograft. In both cases, a mince was made of these muscles in a watchglass, and this was placed inside the tube of the trocar as it lay in the wall of the heart. The tube was then removed and the graft remained inside the heart muscle. Both openings in the myocardium were sutured. The operative technique described rules out the possible effects of inflammatory changes associated with the suture material on the processes of regeneration. The animals were sacrificed at various times after the operation, from 24 h to 5 months. The material was fixed in 12% neutral formalin. Paraffin sections were stained with hematoxylin-eosin, by Van Gieson's method, and with iron hematoxylin and counterstained with picrofuchsin. Histochemical methods of investigation also were used: staining with methyl green-pyronine by Brachet's method, with toluidine blue using dye solutions of different pH values, with alcian blue, and by the PAS reaction with corresponding control – treatment of the sections with α-amylase, with testicular and bacterial hyaluronidase, by methylation, sulfatation, acetylation, with blocking of aldehyde groups, and with extraction of lipids.

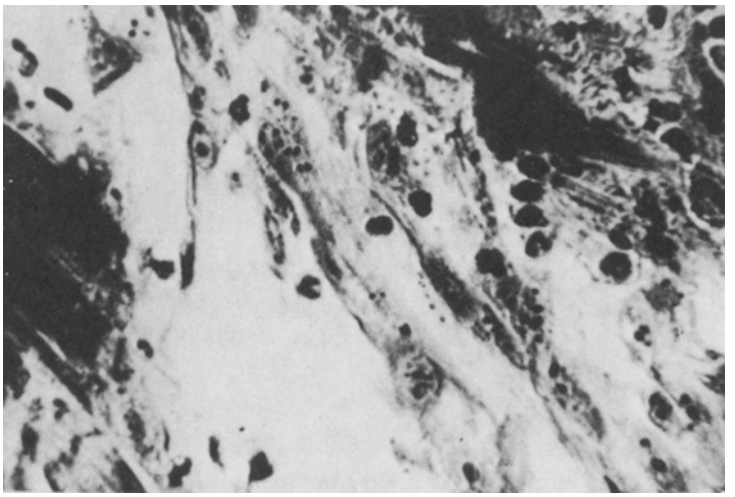

Fig. 1. Separation of myoblasts from fragments of minced heart muscle tissue. 8th day of development of graft. Stained with iron hematoxylin and picrofuchsin. Objective 90×, ocular 7×.

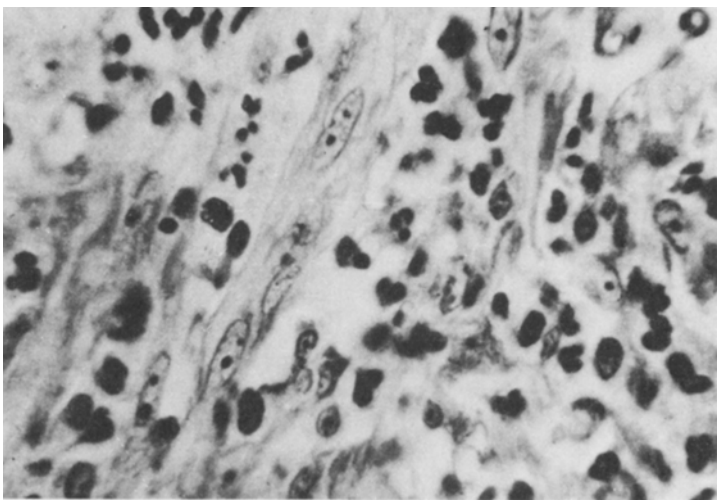

Fig. 2. Fusion of myoblasts into cell bands on the 11th-15th days after transplantation of minced heart muscle into a defect of the myocardium. Stained with iron hematoxylin and counterstained with picrofuchsin. Objective 40×, ocular 7×.

EXPERIMENTAL RESULTS

When minced heart muscle was grafted into the myocardial defect, the transplanted fragments retained their fine structure for the first 24 h. Between the muscle fragments, many erythrocytes and solitary leukocytes were present, in a dense fibrin network. The grafted fragments were PAS-negative, they appeared pale when stained by Brachet's method, they were not stained by alcian blue, and they showed no metachromasia when stained with toluidine blue.

Later, from the second to the 5th day, destructive processes appeared in the implanted fragments: the number of muscle nuclei was reduced, and some of the cross striation was lost. Some of the fragments became homogenized. In the region of the defect, the number of leukocytes, macrophages, and fibroblasts increased.

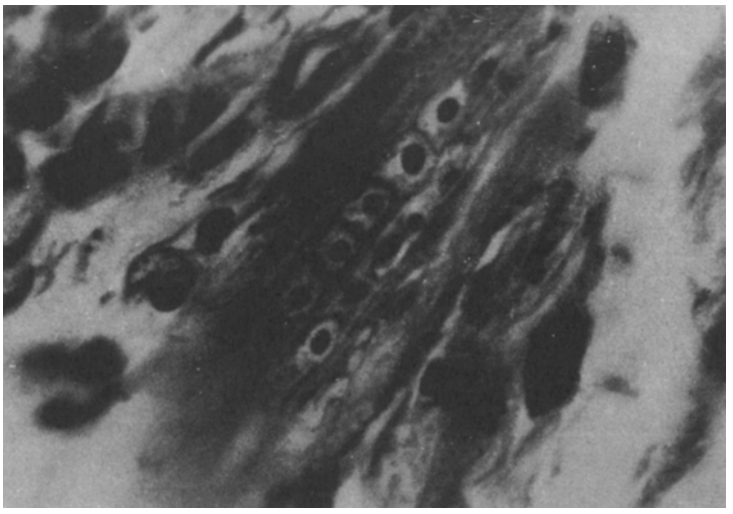

Fig. 3. Formation of muscle tubes after transplantation of a minced somatic muscle into the myocardium of a dog. Graft on 15th day of development. Stained with iron hematoxylin and counterstained with picrofuchsin. Objective 90×, ocular 7×.

The appearance of productive changes was observed on the 8th day after transplantation. Clear areas appeared at the periphery of the implanted fragments from which, in some cases, large cells with basophilic cytoplasm and a large, clear nucleus separated [Fig. 1]. In some places a cytoplasmic connection could be seen between these cells and the cytoplasm of the grafted fragment. These cells gave a PAS-positive reaction in the form of tiny granules. After treatment of the sections of this series with α-amylase, the results of the PAS reaction were negative. Consequently, the PAS-positive material could be identified as glycogen. The cells described did not stain with alcian blue and did not give metachromasia with toluidine blue. A delicate network of collagen fibers developed at the periphery of the transplant.

On the 11th-15th day, the signs of degeneration of the grafted muscles were intensified. Most of the fragments of the implant had disintegrated. The large cells separating from the grafted fragments at the earlier periods now were sometimes joined together into a syncytium, and formed bands (Fig. 2). These bands contained large amounts of RNA; no glycogen or acid mucopolysaccharides could be detected in them. A diffuse PAS-positive reaction was observed, and this was suppressed by acetylation but unaffected by treatment with amylase and extraction of lipids, so that it was evidently associated with the presence of muco- and glycoproteins. The greater part of the defect at this time was filled with developing connective tissue. After the 15th day, signs of degeneration of the newly formed myogenic elements appeared: pycnosis and karyorrhexis of the nuclei, a fine granular degeneration of the cytoplasm, and loss of its basophilic staining. After 20-22 days, the defect was completely filled with connective tissue.

In the transplant of somatic muscle tissue, after the 5th day, muscle buds were seen to form at the end of the grafted fragments. After the 8th day, besides myoblasts and muscle buds, numerous myosymplasts with 3-15 nuclei were seen. The myofibrils developing at the periphery of the myosymplasts converted into a typical muscle tube with nuclei situated along the central axis. In some cases, muscle tubes with a clear cross striation appeared in the myofibrils, indicating a relatively high degree of differentiation (Fig. 3).

When stained with methyl green-pyronine, the cytoplasm of all the myogenic elements developing from the transplant of somatic muscle was strongly basophilic. Glycogen was seen in the myoblasts; no acid mucopolysaccharides were found. On staining with toluidine blue, a diffuse but distinct metachromasia was seen in the cytoplasm of the myosymplasts and muscle tubes. The metachromatic staining of the sarcoplasm was most intensive and distinct when the pH of the dye solution was 4.7-5.0. After incubation of the sections in a solution of testicular hyaluronidase, followed by staining with toluidine blue, no metachromasia was found. The cytoplasm of the myosymplasts and muscle tubes stained a pure bluish-green color with alcian blue. These staining properties were completely suppressed after methylation. This suggests that these myogenic elements contained acid mucopolysaccharides of the type of hyaluronic acid and chondroitin sulfate C. No PAS-positive material was found in these myogenic elements.

After autotransplantation of the somatic muscle, replacement of the defect by connective tissue took place in 20-25 days.

The results of the study of autografts of minced cardiac and somatic muscle tissue, transplanted into the myocardium of the dog, thus showed that, in principle, they could develop myogenic elements. After the 8th day, in the fragments of minced heart muscle,cells became separated which, in some cases, were joined by their cytoplasm with the grafted fragments. These cells later formed bands. In contrast to the graft of somatic muscle, no muscle buds or myosymplasts were formed in the graft of heart muscle tissue. In the fragments of somatic muscle, from the 5th-7th day of development of the graft, muscle buds were formed, together with myoblasts, myosymplasts, and muscle tubes.

Histochemical investigation of the newly formed myogenic elements showed that they contained large amounts of RNA, in agreement with reports in the literature of an increase in protein synthesis in regenerating tissue.

Acid mucopolysaccharides were found in the muscle tubes and myosymplasts. It has been reported in the literature [3] that acid mucopolysaccharides are synthesized in the embryonic muscle tissues of animals at the stage of muscle tubes; in these circumstances, constant synthesis of glycogen takes place in the myogenic elements. In the present investigation, glycogen was seen in the myoblasts but not in the muscle tubes. In this connection, it may be assumed that one of the reasons for the absence of conversion of the muscle tubes into fairly developed muscle fibers is a disturbance of the synthesis of glycogen, a material rich in energy.

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