RESPONSE OF UNDAMAGED SKELETAL MUSCLE TO MINCED MUSCLE TISSUE GRAFTING

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Grafting of minced muscle, which was first performed by Studitskii [7], has subsequently become extensively used in the study of some aspects of myohistogenesis [2, 8, 10]. Intensive regeneration taking place in minced muscle has been shown to effect regeneration of a muscle in contact with it [1, 2, 8, 9]. Minced muscle tissue, introduced into a defect in a muscle irradiated with x-rays actively stimulates regeneration, when inhibited by irradiation [4, 5].

The question arises how undamaged muscle reacts to grafting of minced muscle tissues. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male albino rats weighing 120-140 g. The left gastrocnemius was removed from the animals and part of it minced and grafted on the surface of the right gastrocnemius muscle. The gastrocnemius muscle together with the graft was fixed in Carnoy's fluid and 12% neutral formalin after 7, 10, 14, and 21 days. Sections were stained with Reguad's hematoxylin and counterstained by Mallory's method, stained with azure–eosin by Romanovsky's method, and impregnated by the Bielschowsky–Gros–Lavrent'ev technique. Pieces of gastrocnemius muscle, taken from a zone bordering the graft, were fixed for electron-microscopic examination in 2% OsO_4 solution by Palade's method. Ultrathin sections were stained with uranyl acetate and lead citrate. The following procedures were carried out for the control: The gastrocnemius muscle was removed from the left limb, an incision made through the skin and enveloping muscle, as in the experimental series, but no minced muscle was grafted. The right gastrocnemius muscle was fixed after 7 days and processed by the same method as the experimental muscle.

EXPERIMENTAL RESULTS

The graft of minced muscle tissue began to become encapsulated 7 days after the operation. The graft consisted mainly of loose multicellular connective tissue, in which threre were disintegrating fragments of minced muscle fibers and pieces of tendons and nerve fibers with degenerating axons. At the periphery of the graft dilated capillaries were growing into it. In this wide peripheral zone muscle fragments were completely or partially broken into segments containing nuclei, surrounded by sarcoplasm with marked basophilia. Multiple basophilic myoblasts and thin basophilic myosyncytia with chains and clusters of nuclei could be seen. Mitoses were observed in the cells. Fragments of minced muscle tissue in the center of the grafts showed no signs of structural change but stained intensely; neither cross-striation nor nuclei could be detected.

The undamaged gastrocnemius muscle had a mainly normal structure. Gross striation was clearly visible in the muscle fibers and the nuclei were oval or elongated in shape, and in their usual position. The innervation and vascularization were within normal limits. Motor end-plates had no evidence of stimulation. In some areas, however, in the zone bordering grafts, the connective-tissue membrane of the muscle was reduced in thickness or quite invisible. Some muscle fibers in this region had bright basophilia of their sarcoplasm (Fig. 1).

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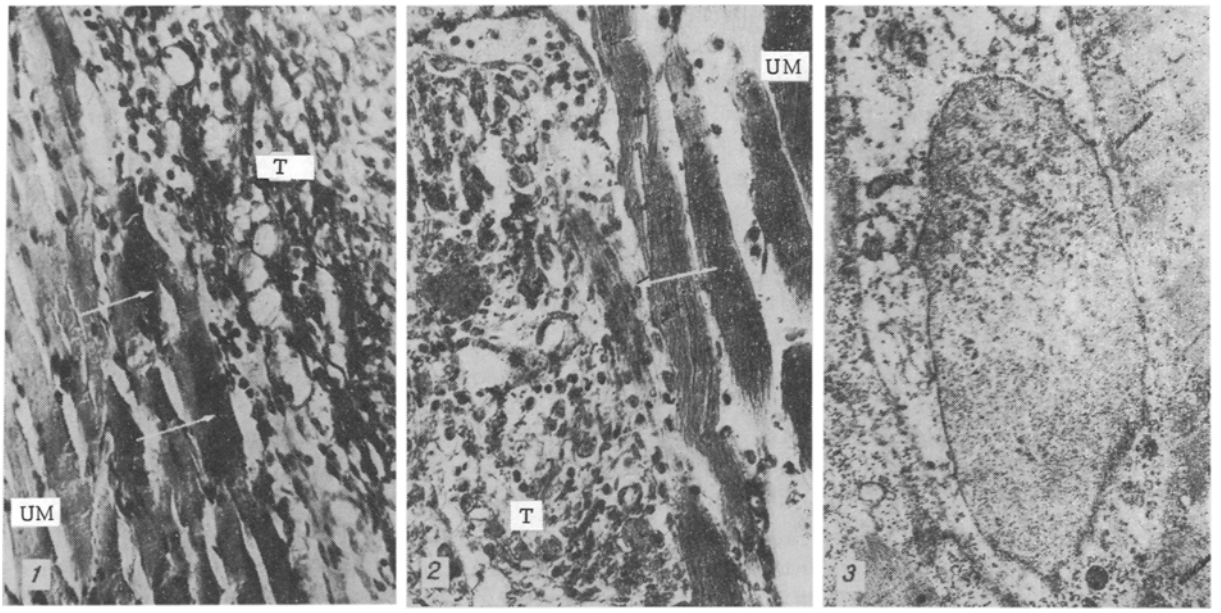

Fig. 1. Fig. 2. Fig. 3.

Fig. 1. Basophilia of sarcoplasm of muscle fibers (arrow) in zone of undamaged muscle bordering graft (7 days after transplantation of minced muscle). T) Transplant, UM) undamaged muscle. Stained with azure-eosin by Romanovsky's method. 500 ×.

Fig. 2. Muscle fiber with concentration of nuclei (arrow) in undamaged muscle on border with graft (7 days after transplantation of minced muscle). Stained with Reguad's iron-hematoxylin and counterstained by Mallory's method, 400 ×. Legend as to Fig. 1.

Fig. 3. Satellite cell in zone of graft bordering undamaged muscle, 16,500 ×.

Cross-striation was indistinct. The muscle nuclei were enlarged and were often round in shape. Small groups of nuclei or short chains were found (Fig. 2). Sometimes cells — leukocytes, fibroblasts and perhaps, myoblasts — could be seen among the muscle fibers. The vessels here were often dilated and congested with blood. Invasion of axons from a nearby nerve trunk could be seen. Electron-microscopic examination of the minced muscle 7 days after transplantation often revealed satellite cells in the zone of undamaged gastrocnemius muscle bordering the graft (Fig. 3). Their cytoplasm contained many polysomes, mitochondria, and wide tubules of the rough endoplasmic reticulum. Centrioles could be seen in the endothelium of the blood vessels, indirect proof of division of the endothelial cells. Short tubules of the rough endoplasmic reticulum, free ribosomes and polysomes, a Golgi complex, and multivesicular bodies were visible in the muscle fibers, and occasionally the perinuclear space was widened. All this is evidence of the metabolic activity of the muscle fibers in the undamaged muscle.

Grafted minced muscle could not be found in all cases studied 10 days after transplantation. Where the graft was preserved it was surrounded by connective tissue. Among loose connective tissue in the graft, infiltrated by numerous cells, bundles and bands of myosyncytia, muscle tubes, and myoblasts were running in different directions. The myogenic cells preserved their basophilia. Discrete fragments of degenerating muscle fibers were found in the center of the graft.

In a narrow zone of the undamaged muscle bordering the graft signs of a plastic state of the muscle tissue were observed, as at the previous time. Regions of muscle fibers had pale sarcoplasm. The muscle nuclei were located in groups and chains, they were round, and had distinct nucleoli. Sometimes basophilia of the sarcoplasm was observed around the nuclei. Hyperemia was distinctly visible in this zone: The vessels were dilated and congested with blood. The intramuscular innervation (nerve trunks, motor end-plate, neuromuscular spindles) throughout the muscle retained its typical structure. In some cases, however, near a zone bordering the graft proliferation of the terminals of the motor end-plate could be observed: They had a sperical pool of axoplasm on their ends, and so-called "growth bulbs," evidence of a state

of irritation of the axomuscular synapses.

After 14 days the muscle cells in the graft were arranged in the form of separate islands. They consisted of myosyncytia, thin muscle tubes, and single muscle fibers. Some syncytia contained large concentrations of pale and dark nuclei. Basophilia was discovered in this case, not throughout the sarcoplasm, but only around the nuclei.

In the undamaged muscle, in the region bordering the graft, a connective-tissue membrane was not present on the muscle in some areas. In these areas small concentrations and short chains of nuclei were occasionally found in the muscle fibers. Myosyncytia containing round nuclei came right up to the intact muscle from the graft. No signs of hyperemia were observed at this time of the investigation also. In the boundary zone single regenerating axons were found.

After 21 days the minced muscle graft was almost completely absorbed in all the cases studied. Its remnants consisted of a small area of connective tissue containing blood cells. Muscle fibers and the innervation in the undamaged muscle around the whole of the peripheral zone preserved their typical structure. Only very rarely were small groups of nuclei seen in single muscle fibers.

In the control series of experiments, after mock operation signs of slight damage were found in a very limited area of only one muscle of five studied. In the remaining four cases the muscles and their connective-tissue membrane were undamaged after the operation. No signs of a plastic state could be found in the muscle fibers. In the single case of damage to the muscle after operation in the control, the character of its response differed from that after transplantation of minced muscle tissue: Inflammatory changes were found in the connective-tissue membrane and myogenic cells were distributed not only superficially, but also in the deeper parts of the muscle, and they were similar to those observed after muscle trauma. In the experiments with transplantation of minced muscle foci of inflammation were not found in the muscle membrane in a single case. At sites of contact with the graft the membranes appeared to have dissolved. In the undamaged muscle only single superficial muscle fibers showed signs of a plastic state. In deeper parts of the muscle they could no longer be seen These observations show that after grafting of minced tissue to the intact gastrocnemius muscle, the initial stages of secondary development, similar to embryonic histogenesis, which have been adequately studied in several investigations, took place but, by contrast with transplantation into the bed of the excised muscle, the process did not end with the formation of a muscular organ. The material of the graft began to be absorbed after 2 weeks, and by the end of the 3rd week it had almost completely disappeared. Similar results were obtained after subcutaneous transplantation of minced muscle in the abdominal wall [6, 9]. The authors cited explain this result by the absence of conditions essential for development of muscle fibers, such as tension, connection with the nervous system, and function. It must be emphasized that the signs of a plastic state in the undamaged muscle described above were found in the period of most intensive regeneration in the graft. As regeneration subsided, the response of the muscle fibers of the intact muscle also disappeared.

The results are thus evidence in support of the view that a minced muscle graft has some influence on the structure of undamaged muscle. It can be tentatively suggested that the phenomenon observed is the result of the presence of intertissue interactions, which, as is generally considered, take place through transmission of specific substances, activating the reparative reaction, from regenerating tissue to intact tissue.

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GLYCEROL MODELS OF CILIATED EPITHELIUM OF THE BRONCHIAL MUCOSA AND THEIR USE FOR THE DIAGNOSIS OF CHRONIC NONSPECIFIC LUNG DISEASES

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By treating cells with glycerol to render their membrane permeable and by carefully extracting the cell contents it is possible to obtain what are called cell models, namely cells from which the soluble components have been removed, but whose contractile apparatus remains intact. If the method of extraction is suitable, the contractile apparatus of the extracted cells retains the power of techanical movement of the same kind as *in vivo*, in the presence of exogenous ATP and appropriate inonic conditions. The first model of this kind was obtained from muscle fibers by extraction with a solution of low ionic strength, containing glycerol [9, 10]. Later, models were obtained by glycerol extraction from a whole range of different cell objects [2] and a method of obtaining glycerol models of the ciliated epithelium of some vertebrates was devised [1].

The aim of this investigation was to determine the characteristics of models of ciliated epithelial cells of the human bronchial mucosa and to discover whether these models may be used to evaluate the functional state of the ciliated epithelium in various forms of bronchopulmonary pathology.

EXPERIMENTAL METHOD

Brush biopsy specimens of the bronchial mucosa, obtained during diagnostic bronchoscopy on adults and children with chronic bronchopulmonary diseases were used for investigation. Cell models of the human ciliated bronchial epithelium were obtained by the method in [1] with some modifications. The sample of epithelium of the bronchial mucosa was kept in 45% glycerol containing 20 mM sodium-phosphate buffer, pH 7.0, and 120 mM KCl and incubated for 24 h at 4°C to render the epithelial cell membranes permeable. The permeable preparation was transferred to a slide and throughly washed at room temperature with buffer containing 20 mM sodium phosphate buffer, pH 7.0, 120 mM KCL, and 5 mM $MgCl_2$ to remove the glycerol and soluble cell components. Testicular hyaluronidase also was added to the buffer in a concentration of 2 μg/ml to produce hydrolysis of viscous mucopolysaccharides, which could prevent beating of the cilia, The models were reactivated by addition of ATP solution to a final concentration of 5 mM. The observations were made by phase-contrast and luminescence microscopy. Preparations for luminescence microscopy were stained with a 0.1 mM solution of acridine orange in 0.9% NaCl.

EXPERIMENTAL RESULTS

The results of phase-contrast microscopy of models of ciliated epithelial cells from the human bronchial mucosa, washed free from glycerol and viscous mucopolysaccharides, are illustrated in Fig. 1a, b. The specimen of the models consisted of a suspension of cell groups, single ciliated cells, and cell fragments. The cilia were nonmotile. After staining with acridine orange, the cytoplasm of the models fluoresced brightly, a characteristic feature of nonviable cells.

On addition of 5 mM ATP the cilia of most cells began to beat. The frequency of beating

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