fibroblasts on the 3rd day in the experimental subgroup and on the 7th day in the control subgroup was equal. Does this not reflect the stimulating action of potassium orotate? Wound healing is accelerated: on the 3rd day, during the action of potassium orotate the fibroblast functions just as it does on the 7th day in the control. Our investigation thus suggests that the action of potassium orotate as a biological stimulator is manifested as activation of the proliferative ability of wound fibroblasts and not as stimulation of the protein-synthesizing function of each single cell.

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EFFECT OF AN INCREASED FUNCTIONAL LOAD ON STRIATED MUSCLE ULTRASTRUCTURE OF THE RAT ESOPHAGUS

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The morphological and functional properties of esophageal striated muscle (ESM) have been inadequately studied [1, 5, 7]. Their ultrastructural features, innervation, and plastic transformations in response to an increased functional load require further investigation.

The object of this investigation was a microscopic and ultramicroscopic study of ESM in control animals and during the development of experimental hypertrophy.

EXPERIMENTAL METHOD

Experiments were carried out on 30 albino rats. Under aseptic conditions and ether anesthesia the abdominal portion of the esophagus was enclosed in a longitudinally cut elastic tube 10 mm long with an internal diameter of 3.5 mm, which led to disturbance of patency. The experiments lasted 1, 3, 7, 10, and 20 days. For morphological investigation the animals were

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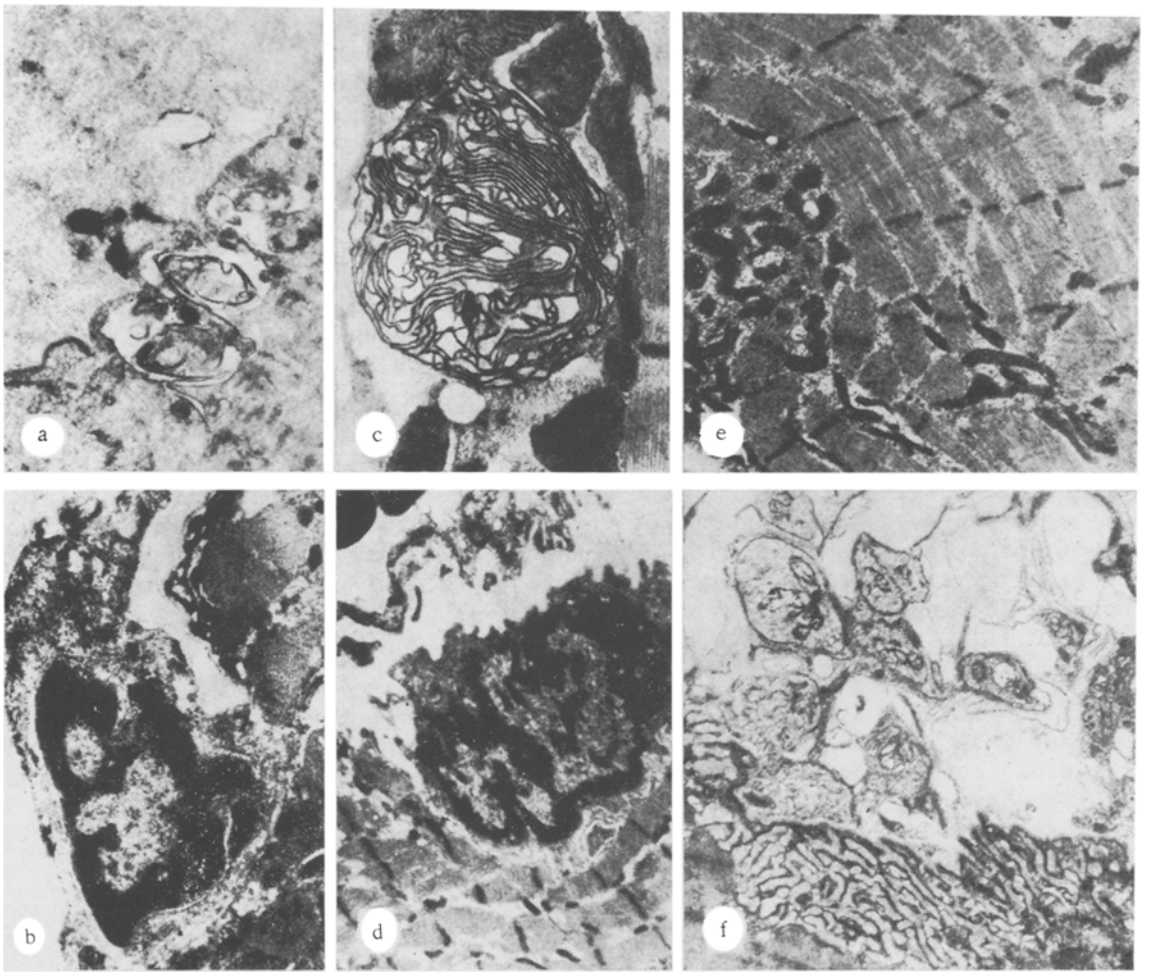

Fig. 1. Electron micrograph of rat esophageal muscle at different stages of the experiment. a) Destruction of myofibrils in injured muscle fibers (experiment, 1st day): 4000 ×; b) myelinated formations in subsarcolemmal region of muscle fiber (experiment, 3rd day): 15,000 ×; c) satellite cell in muscle fiber (experiment, 7th day): 8000 ×; d) segregation of nucleosarcoplasmic fragment of muscle fiber (experiment 10th day): 4000 ×; e) region of muscle fiber on 20th day of experiment: 6000 ×; f) neuromuscular synapase on 20th day of experiment: 4000 ×.

anesthetized with ether and a segment of the muscular coat located above the point of constriction of the esophagus was removed. In fresh frozen sections succinate dehydrogenase (SDH) activity was demonstrated by Nachlas' method [6], after which the area of cross-section of the muscle fibers (ACSMF) was determined planimetrically. The numerical results were subjected to statistical analysis. Material for electron-microscopic study was fixed in Karnovsky's fixative, washed in 0.1 M phosphate buffer, and postfixed in 1% OsO_4 solution. Ultrathin sections were stained with uranyl acetate and lead citrate. Ultrathin sections were examined and photographed in the ÍEM-7A microscope.

EXPERIMENTAL RESULTS

Transverse sections through muscle fibers of the esophagus of the control rats were round or oval in shape, surrounded by thin bands of connective tissue. ACSMF varied from 150 to 750 μ^2 (on average 425.6 ± 10.5 μ^2). Muscle fiber units (myons) with an area of cross section of 250-450 μ^2 predominated (67%). The study of the SDH activity showed that in the muscular coat of the esophagus, by contrast with somatic muscles, there is only one population of muscle fibers which have average enzyme activity, and the typical white and red myons are absent.

Ultrastructural investigation showed that myofibrils in esophageal muscle fibers differ in thickness, their A- and I-disks are prominant, and their Z-bands and M-lines are clearly

defined. Myons of the esophagus are characterized by properties of both red and white muscle fibers simultaneously. They resemble red myons in the large number of mitochondria distributed in the form of clusters (most frequently beneath the sarcolemma and in the perinuclear zone), the weak development of the sarcoplasmic reticulum and the presence of wide Z-bands. At the same time they have features characteristic of white muscle fibers: numerous grains of glycogen are present in the space between the myofibrils, but lipid inclusions are poorly developed.

The motor innervation, unlike muscles of the locomotor system, is effected by unmyelinated nerve fibers, gathered into bundles. All components of neuromuscular junctions can be found in the neuromuscular synapses of the esophagus: an axon terminal with mitochondria and synaptic vesicles scattered in it, a presynaptic membrane, a synaptic space with moderately electron-dense substance, and a postsynaptic membrane forming many folds.

Abrupt widening (up to 7 mm in diameter) of the esophagus was observed one day after the beginning of the experiment and was accompanied by a decrease in thickness of its wall (the average ACSMF fell to $344.7 \pm 8.0\ \mu^2$). SDH activity decreased, the formazan granules became larger and were distributed around the periphery of the muscle fiber. The ultramicroscopic changes were expressed as loosening of the fibers of the myofilaments, disturbance of the structure of the Z-bands, and their disorientation. In some fibers the myofibrils disintegrated to finely granular electron-dense material (Fig. 1a). Starting from the 1st day, large mitochondria forming myelin figures (Fig. 1b), resembling those described previously in skeletal muscle [4], appeared in the subsarcolemmal region of the relatively undamaged muscle fibers.

Later (3rd-10th days) the widening of the proximal part of the esophagus was not reduced. A gradual increase in ACSMF was observed morphometrically, and on the 7th day the mean values reached the control levels, but by the 10th day they exceeded them statistically significantly (ACSMF on the 10th day was $549.7 \pm 13.1\ \mu^2$). SDH activity also returned to the control level. By the 10th day all muscle fibers of the esophagus showed a moderate reaction for SDH. Just as in the control, subdivision into red and white myons was not present.

Changes were observed in the structure of the myofibrillary apparatus. The distance between the myofibrils increased, and many glycogen granules were visible in this region also. In some cases areas of destruction of the myofibrils were found, and under these circumstances the myofilaments disappeared completely whereas the Z-bands remained. The ultrastructure of the nuclei also changed. Often nuclei with condensed chromatin arranged around the periphery were observed. At these same times mononuclear cells with a narrow rim of cytoplasm could be seen in the muscle fibers, adjacent to the sarcolemma and having a common glycolemma with the myon (Fig. 1c). They could be classed as myosatellitocytes. At the same time, segregation of nucleo-sarcoplasmic fragments, i.e., myoblast formation, could be observed in certain muscle fibers (Fig. 1d). In experiments lasting 20 days, longitudinal splitting of the muscle fiber could be observed.

By the 20th day after the beginning of the experiment, signs of hypertrophy of the ESM were quite conspicuous. The number of myons with an area of cross section of between 450 and 750 μ^2 was considerably increased and the mean SCSMF was increased to $594.5 \pm 13.0\ \mu^2$. Ultramicroscopic study also revealed hypertrophied muscle fibers (Fig. 1e). The irregular course of the myofibrils will be noted. Their thickness was about equal, the A- and I-disks and Z-bands and M-lines were clearly defined. The spaces between the myofibrils were enlarged and filled with glycogen granules. Many mitochondria were seen, forming quite large subsarcolemmal concentrations. Changes in the pre- and postsynaptic parts of the neuromuscular synapse reflected their structural transformation (Fig. 1f). Fibers which can be identified as newly formed were found in the muscular coat of the esophagus. They were thinner than the remaining myons, and they had densely packed myofibrils with distinct M-lines and Z-bands. A few mitochondria and many polysomes and glycogen granules could be seen between the myofibrils.

The striated muscle fibers of the rat esophagus thus have ultrastructural features characteristic of both red and white myons of muscles of the locomotor system. Creation of an experimental functional load on the esophagus leads to structural changes in its muscle. Signs of injury are replaced by the development of muscular hypertrophy. The ESM are able to form myosatellites, to take part in segregation of nucleo-sarcoplasmic areas, and in some cases, their muscle fibers can split longitudinally. These processes, leading to an increase in weight of the muscle and repeatedly observed in skeletal muscles [2, 3], were discovered here for the first time in striated muscle of the esophagus.

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