Twenty days after the start of Levosin treatment, the granulation tissue was characterized by a further increase in the number of fibroblasts, on average twofold as compared to the former time, and 2-12 (in one observation 21) times over the initial numbers (*i.e.*, before treatment). The vascularization proceeded more gradually. The number of macrophages drastically fell, and the microbe count of the burn wound also decreased. Fibroblasts became the predominant cellular elements in the wound. The presence of multiple collagen fibers, interspersed with fibroblasts and vessels, reflected the maturity of the granulation tissue. The increase in the number of proliferating fibroblasts correlated with the increase of vascularization at all assayed periods. This is consistent with the hypothesis earlier suggested by D.S. Sarkisov et al. [5] regarding the histogenetic relationship between fibroblasts and small vessels of granulation tissue.

One should stress the difference between the courses of the wound process in various body areas of the same patient. Analysis of biopsy material obtained from three different injured sites of patient Ch. (upper arm, forearm, thigh) 20 days after the start of treatment with the ointment, revealed a marked drop in all studied cellular parameters at one particular site (upper arm). Bacteriological analysis also revealed only a slight decrease in microbial dissemination in this region (only by one order of magnitude). Thus, in this particular wound region a delay in healing was registered. This leads us to conclude that the regeneration process in extensive burn wounds is of a heterogeneous character.

Thus, electron-autoradiographic analysis has shown that topical application of Levosin ointment activates the healing of burn injuries, as demonstrated by an increase in the total quantity of fibroblasts and vessels of the granulation tissue, as well as activation of the functional and proliferative capacities of fibroblasts. In addition, the destruction of bacterial bodies and a decrease in their number take place.

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Changed Permeability of Cardiomyocyte Sarcolemma after Short-Term Total Ischemia

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Changes in the membrane system of myocardial cells [2,4] contribute much to the pathogenesis of postischemic involvement of the myocardium developing after heart and respiratory arrest and resulting in impairment of its contractility [3]. Sarcolemma structural integrity is known to determine to a great extent such characteristics of the myocardium as excitability, conductivity, and contractility, and it is of crucial importance for recovery of the structure and function of the ischemic cells [4,6,7]. Recent research has revealed the possibility of injury to the cell membranes under conditions of reperfusion and reoxygenation [2,4,9]. The status of the myocardial membranous system after total ischemia of the whole

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body developing in sudden or induced (in cardiosurgery) heart and respiratory arrest is still to be studied.

The aim of the present research was to elucidate the relationship between ischemic cardiac arrest and subsequent reperfusion, on the other hand, and cardiomyocyte membrane permeability and ultrastructure, on the other.

MATERIAL AND METHODS

Experiments were carried out with 20 mature white rats weighting 180-210 g. 4 of which were controls. All the experiments were carried out under ether anesthesia. Ten-minute total ischemia was induced by pinching the vascular bundle at the base of the heart [1]. Resuscitation was carried out by indirect massage of the heart and artificial lung ventilation. The left ventricular myocardium was dissected for electron-microscopic examination at the 10th minute of clinical death, and 1.5, 6, and 24 h after reanimation. The material was fixed in 3% glutaraldehyde in tris buffer with 1% lanthanum nitrate (ionic La - pH 7.4) or 1% La hydroxide (colloid La - pH 7.8). Individual fixation permitted a differentiated assessment of the status of the cardiomyocyte glycocalyx and plasmalemma [9]. The material was additionally fixed with osmium tetroxide, dehydrated, and then embedded in epon-araldite. Ultrathin sections were examined after contrast staining or without it (for precise identification of the La precipitate in the cells) under an EMB-100B electron microscope (the examination was carried out by V.A.Akulinin, postgraduate student in histology).

RESULTS

Examination of the myocardium of intact animals revealed that La, both ionic and colloid, did not penetrate the cardiomyocyte sarcolemma and was detectable only in the intercellular space, in the T-system canaliculi, and between the intercalated disk membrane . Negligible amounts of tracer particles were detected in the endotheliocyte cytoplasm of some capillaries, this indicating a certain permeability of the endothelium for these particles.

No significant morphologic shifts were detected in the cardiomyocytes at the 10th minute of clinical death. The structural integrity of the sarcolemma was intact, which was proved by the absence of lanthanum (in either of its forms) in the myocardial cells. The microvascular wall permeability was increased. La precipitation was detected in high volumes in the endotheliocyte cytoplasm between numerous pinocytotic vesicles; in a number of cases the precipitation penetrated into the vascular lumen. Intensive transcapillary exchange associated with an increase of endotheliocyte permeability appears to be one of the adaptation reactions of the myocardium to reversible total ischemia.

Dyskinetic disturbances, expressed as sub-segmentary and segmentary myofibril contractures, sometimes combined with foci of myofibril relaxation, were found in cardiomyocytes 1.5 h after reanimation. The structure of the majority of the mitochondria was intact, but in some of them the crests were concentrically located or disoriented. Chromatin condensation and margination were seen in many nuclei, this reflecting the oxidation of the myocardial cell internal medium. The sarcotubular system canaliculi were dilated in some areas. Marked disseminated degenerative changes in the microvessel endothelium were found, manifested, first, by an increased electron density of individual endotheliocytes, paralleled by the appearance of numerous cytoplasmic growths and, second, by a marked swelling of the endotheliocytes, associated with manifest destruction of their organelles, leading to dilatation of the vessel lumen. Such endothelial changes seem to result in significant disorders of the nutrient and oxygen transport to the cardiomyocytes.

The study of sarcolemma permeability showed that ionic lanthanum diffused into the cytoplasm of many cardiomyocytes and was detected mainly round the mitochondria, although it did not penetrate the mitochondrial membranes. Colloid lanthanum cells generally failed to penetrate the muscle cell sarcolemma, this evidencing the intactness of the plasma membrane 1.5 h after ischemia. Only seldom were electron-dense precipitations detected at the boundary with the mitochondria, presumably due to the presence of ionic La admixture in colloid lanthanum, and this admixture diffused into the cells [8]. Such a position of the tracer near the mitochondria is the most characteristic of ionic lanthanum, which starts penetrating into the cardiomyocytes when the sarcolemma glycocalyx is disorganized [10]. Lanthanum was found in the cells when the sarcolemma was visually intact and its presence was often paralleled by a rather intensive intracellular edema, resulting in separation of the organelles.

Changes involving, first of all, the cardiomyocyte contractile system develop during the course of the following several hours. Six hours after reanimation, myofibrin relaxation with the appearance of H-zones in the center of the sarcomers was observed in many muscle cells, this indicating that their optimal length was surpassed and the maximal contractility strength reduced. In addition, myocytes with myofibril contractures of various intensity and extension were detected. In some mitochondria focal destruction of the cristae or their archlike arrangement were seen, although the energy production process seemed to be virtually unchanged.

Sarcolemma permeability for ionic lanthanum remained high. Electron-dense precipitations were

still detectable round the mitochondria, which pointed to basal membrane defects. Colloid lanthanum remained on the external surface of the sarcolemma in the majority of myocytes. However, in a few of these cells finely dispersed La precipitation was observed, located between the protofibrils but not penetrating through the mitochondrial membranes.

Twenty-four hours after heart work restoration, the sarcolemma of many cardiomyocytes was as permeable for ionic lanthanum similarly as after 6 h. Colloid lanthanum could still penetrate into just isolated myocytes, but this marker was already detectable not only in the myofibrils, but in the matrix of some mitochondria as well. This fact was indicative of mitochondrial membrane destabilization and of abnormally increased permeability which led to the destruction of the mitochondria. Significant changes in contractility are usually observed in such cells, expressed as myofibril contractures and lysis, accompanied by pronounced edema. The development of destructive changes in the cardiomyocytes containing colloid lanthanum precipitate corresponded to the succession of its penetration into the cell organelles during the course of resuscitation after total ischemia, which fact indicated a relationship between these phenomena. Similar changes in cardiomyocyte ultrastructure, directly related to the formation of perforated defects in the plasmalemma, seem to be irreversible.

The studies have demonstrated a disorganization of the basal membrane glycocalyx in the cardiomyocytes during the course of the postreanimation period; the basal membrane function as a diffusion barrier, this resulting in an increase of sarcolemma permeability as a whole. These changes seem to develop over the period of deep ischemia, and recovery of the heart contractions and coronary blood flow ("reperfusion") are directly responsible for the formation of the defects in the glycocalyx. These defects may be attributed to conformational changes in the basal membrane structural proteins that are related to glycoproteins containing sialic acid. Disintegration of the acids during reperfusion and loss of the negative charge by it are directly responsible for the penetration of La ions via the intact plasma membrane into the cardiomyocyte sarcolemma [9,10]. Ca ion capture and retention by membrane electroactive sites being one of the most important functions of the glycocalyx [5], loss of this ability because of the sialic acid negative charge loss may result in uncontrollable calcium flow into the cell. This is one of the causes of pathologic shifts in cardiomyocytes and, first of all, in the contractile system that was observed over 24 h of the recovery period.

The absence in the majority of the myocytes of electroneutral particles of colloid lanthanum easily penetrating via the glycocalyx but retained by the intact plasmalemma [9] is evidence of the structural integrity of the plasmalemma or at least quite negligible (less than 2 nm) defects in it. The few cardiomyocytes in whose sarcoplasma colloid lanthanum was detected were unable to maintain the required transsarcolemmal ion gradient, this resulting in intensive cellular edema, a calcium overload, and, eventually, pronounced and irreversible injury to the organelles. Thus, myocytic plasma membrane intactness is one of the prerequistites for the reversibility of reperfusion damage to the myocardium and for the maintenance of its contractility after total ischemia.

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