The fate of the population of diploid hepatocytes found in individual animals in vanishingly small numbers even after eight stimulations of growth also is interesting. There are two alternative explanations of this phenomenon: replenishment of diploid cells on account of self-support [14] and long preservation of these cells in the tissue in the undividing state [5]. The available results do not enable any decisions to be made regarding the mechanisms which lie at the basis of preservation of extremely small numbers of diploid cells during intensive reparative growth of the liver.

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FUNCTIONAL MORPHOLOGY OF THE MYOCARDIAL ULTRASTRUCTURE DURING LONG-TERM ADAPTATION TO PRESSURE CHAMBER HYPOXIA

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KEY WORDS: myocardium; ultrastructure; pressure chamber hypoxia.

During adaptation of man and animals to high-altitude hypoxia a combination of compensatory and adaptive processes develops in the heart, in the form of hypertrophy of the muscle, an increase in the intensity of functioning of the intracellular structures, and an increase in the capacity of the coronary circulation [2]. However, insufficient attention has been paid in the literature to the morphofunctional aspects of myocardial adaptation to the prolonged, intermittent action of pressure chamber hypoxia. It was therefore decided to undertake the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 108 adult noninbred male albino rats weighing 200-250 g. Daily for 6 h the animals were "raised" in a pressure chamber successively to altitudes of 2000, 3000, 4000, and 5000 m, after which for 2.5 months they were raised to an altitude of 6000 m. The period of the experiments was calculated from the number of days which the

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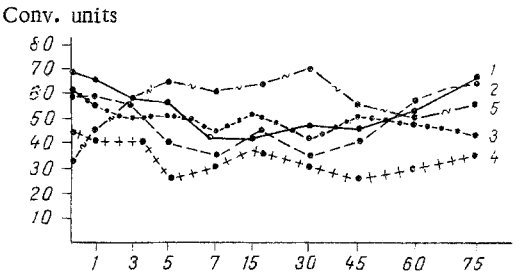

Fig. 1. Activity of myocardial enzymes of rats during adaptation to pressure chamber hypoxia. 1) SDH; 2) CCO, 3) ATPase, 4) NADH-diaphorase, 5) LDH. Abscissa, days of experiment; ordinate, enzyme activity (in conventional cytophotometric units).

rats spent in the pressure chamber at an altitude of 6000 m. The rats were decapitated on the 1st, 2nd, 3rd, 7th, 15th, 30th, 45th, 60th, and 75th days of the experiment. Twelve rats killed in the same way in the city of Frunze (altitude 760 m above sea level) served as the control.

Morphometric, histochemical, and electron-microscopic methods of investigation were used. The body weight of the animals was determined, the heart was weighed separately by Müller's method in Il'in's modification, and the area of cross section of the cardiomyocytes was studied by direct microplanimetry. For light microscopy the myocardial tissue was fixed in 10% neutral formalin and in Carnoy's fluid and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, with iron-hematoxylin, and by Van Gieson's method; argyrophilic fibers were identified by Foot's method. Neutral lipids were stained with Sudan III and Sudan black and glycogen was detected by the PAS reaction with diastase control. Activity of the following enzymes was determined in frozen sections: cytochrome oxidase (CCO), succinate dehydrogenase (SDH) by Nachlas' method, lactate dehydrogenase (LDH) and NADH-diaphorase by Gomori's method, and adenosine triphosphatase (ATPase) by the method of Wachstein and Meisel. Enzyme activity was determined quantitatively by cytospectrophotometry.

Pieces of myocardium for electron microscopy were fixed in glutaraldehyde solution and a 1% solution of OsO_4 in phosphate buffer, pH 7.4. The material was embedded in a mixture of Epon and Araldite. Ultrathin sections were stained by Reynolds' method and studied in the LEM-100B electron microscopy.

EXPERIMENTAL RESULTS

On the basis of the results two periods were distinguished in adaptation of the rat myocardium to the conditions of pressure chamber hypoxia.

In the first period (1st-30th days of the experiment) mainly destructive changes predominated in the rat myocardium. The harmful action of hypoxia on the myocardium was due primarily to its effect on oxidative phosphorylation. Quantitative analysis of enzyme activity is illustrated in Fig. 1. In the rat myocardium SDH activity was reduced by 38.1% ($P < 0.001$), CCO activity by 29% ($P < 0.001$), NADH-diaphorase activity by 20% ($P < 0.05$), and ATPase activity by 22.9% ($P < 0.05$) compared with the control group of animals. As a compensatory reaction of the body to maintain the energy balance of the heart the intensity of glycolysis was increased, as shown by an increase of 43.6% ($P < 0.001$) in LDH activity and a decrease in the glycogen content. The first glycogen to be utilized was the large granules from the middle layers of the myocardium. Glycogen granules were irregularly distributed along the length of the muscle fiber, and most frequently were oriented beneath the sarcolemma. Groups of muscle fibers not containing glycogen were found in various parts of the heart. The earlier disappearance of the large glycogen granules than of the small granules in different pathological states of the myocardium has also been reported by other workers [3, 8, 9]. This is evidence that glyocogen in the form of large granules is a more labile substance and is utilized in the initial stages of the hypoxic state of the heart.

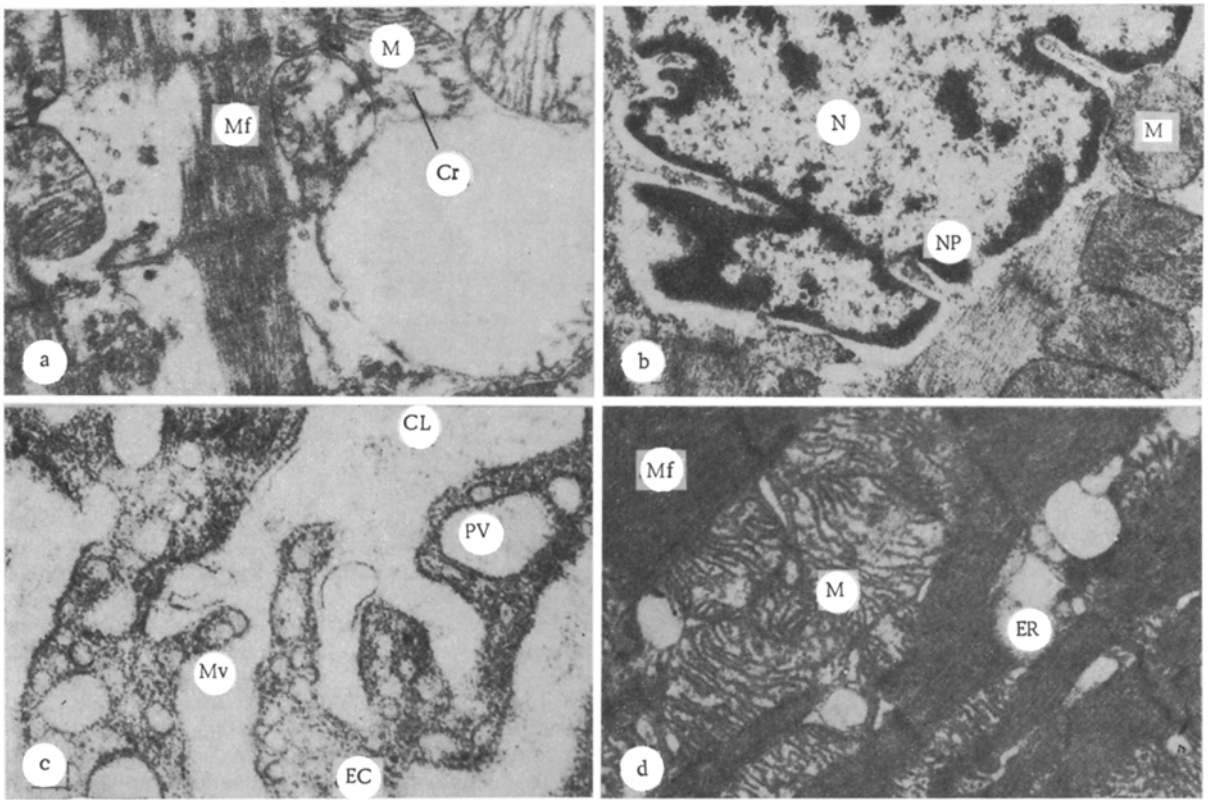

Fig. 2. Changes in myocardial ultrastructure of rats at different times of adaptation to pressure chamber hypoxia. a) Swelling and vacuolation of mitochondria (M) and lysis of their cristae (Cr), and destruction of myofibrils (Mf) (7th day of experiment, 15,000×). b) Nucleus of myocyte (N): tortuosity of nuclear membrane, widening of nuclear pores (NP), aggregation of chromatin, invagination of mitochondria (M) into nucleus (7th day of experiment, 25,000×); c) dilatation of capillary lumen (CL), swelling of endothelial cells (EC) with formation of numerous microvilli (Mv). The latter contain many pinocytotic vesicles (PV) (15th day of experiment, 35,000×); d) increase in size and number of mitochrondria (M), arranged in several rows along myofibrils (Mf); widening of cisterns of endoplasmic reticulum (ER) (75th day of experiment, 12,000×).

Electron-microscopic investigation showed that a single exposure to hypoxia led to changes in the ultrastructures of the cardiomyocytes and, in particular, to changes in the mitochondria. Most preserved their usual structure. However, individual mitochondria swelled, their matrix became translucent, and the intercristal spaces widened. Intercellular and intracellular edema and a reduction in size of the intracellular granules were observed. During more prolonged exposure to hypoxia degenerative changes in the ultrastructures in the heart muscle cells increased.

On the 3rd-7th days of the experiment the mitochondria in many cardiomyocytes were greatly swollen, their matrix translucent, and the intercristal spaces irregularly widened. The cristae of many mitochondria were arranged haphazardly, they were shortened and fragmented, and they could be detected only near the inner cell membrane. Destruction of cristae and "washing out" of the matrix gave the mitochondria an appearance of a vesicle (Fig. 2a). A characteristic feature of the changes mentioned above was that they differed in severity both in different parts of the same cell and also in nearby cells. Besides sharply changed mitochondria there were others which were less severely injured, and sometimes some which were completely intact.

By the 15th day of the experiment marked edema of the sarcoplasm had developed. The number of intracellular granules was reduced proportionally to the degree of edema of the sarcoplasm. Marked dilatation of the channels was observed as the experiment proceeded, evidence of disturbance of intracellular conduction and of changes in synthetic processes in the muscle cell [10, 13]. Changes in the structure of the mitochondria and sarcoplasmic

reticulum were followed immediately by changes in the ultrastructural organization of the myofibrils, the contractile elements of muscle fibers. The myofibrils and myofilaments composing them were loosely packed and disconnected, their outlines were blurred, and their characteristic cross striation indistinct. Changes in the ultrastructural organization of the nuclei were expressed as tortuosity of the nuclear membrane, enlargement of the pores, and widening of the perinuclear space (Fig. 2b). According to some workers, these changes are evidence of the intensification of nucleo-cytoplasmic exchange associated with disturbances of cell function [5, 7].

The lumen of many capillaries was already dilated after the first days of hypoxia. The endothelial cells of the blood vessels were swollen and of unequal electron density: pale endothelial cells alternated with dark. Many pinocytotic vesicles were seen in the cytoplasm of the endothelial cells. In some blood vessels microvilli were enlarged (Fig. 2c). In consequence of the accumulation of edema fluid a capillary was often shifted toward one muscle cell away from another. These observations agree with Mul'diyarov's views and they explain to some extent the "mosaic" pattern of changes in the various myocardial cells, because the uneven interstitial edema changes the oxygen supply differently to different cells. Histological investigation of the myocardium revealed focal changes in muscle fibers, expressed as homogenization, eosinophilia, and picrinophilia of the sarcoplasm and disappearance of the cross striation.

The second period of adaptation (30th-75th days) to the action of hypoxia was characterized by a more distinct manifestation of compensatory and adaptive changes in the myocardium of the rats. The glycogen content increased gradually, activity of oxidoreductases increased, whereas there was a small decrease in LDH activity (Fig. 1).

Electron-microscopic investigation revealed a sharp increase in the number of mitochondria in the cardiomyocytes of both ventricles. They were arranged in groups, sometimes in several rows, in the perinuclear zone between the myofibrils. The internal septa in most mitochondria were densely packed and were parallel to one another. In the right ventricle, besides hyperplasia in the cardiomyocytes, large circular or long oval mitochondria, 3 to 4 sarcomeres in length, were seen (Fig. 2d). In different parts of the myocardium the cristae had unequal electron density, both in different mitochondria and also within the same mitochondrion, evidence of variation in their functional activity [6, 7, 12]. Lipid granules were seen among the mitochondria.

Simultaneously with an increase in the number and size of the mitochondria the myofibrils became thickened, especially in the right ventricle. Dilatation of the tubules of the sarcoplasmic reticulum was a constant feature. These data agree with the results of investigations by other workers who studied the ultrastructure of heart muscle in various hypoxic states [1, 2, 4, 5, 7, 11, 12].

Morphometric investigation of the heart of rats killed on the 75th day of the experiment showed that the weight of the right ventricle was 123 ± 7.5 mg/100 g body weight ($P < 0.001$) compared with 76 ± 7.6 mg in the control, whereas the weight of the left ventricle was 150.2 ± 5.2 mg ($P < 0.05$) compared with 136.8 ± 6.28 mg in the control. The area of cross section of the cardiomyocytes in the right ventricle increased by 13.3%, and that of the left by 10.9% compared with its area in the control animals. The developing hypertrophy of the myocardium, mainly of the right ventricle, was the result of hypertension in the pulmonary circulation.

It can thus be concluded from the results of these investigations that two types of structural and functional changes develop simultaneously in the myocardial cells at different periods of adaptation to prolonged hypoxia, accompanied by an increase in the intensity of the functional strain on the heart: destructive changes and compensatory-adaptive changes.

In hypoxic hypoxia changes in the complex and closely interconnected processes of respiration and glycolysis are reflected primarily in the energetically most active intracellular organelles of the myocardial cells, namely the mitochondria, destruction and death of which are the morphological reflection of the functional disturbances. In long-term exposure to hypoxia in a pressure chamber, gradual recovery of the fine architectonics of the cardiomyocytes takes place as a result of processes of adaptation, on the basis of intracellular regeneration taking place through intensification of protein synthesis, activation of metabolic enzymes, and the formation of new mitochondria and myofibrils. These intra-

cellular structural and functional transformations are the basis of the abundant adaptive powers of the cells whose aim is to preserve homeostasis relative to external and internal environmental factors.

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HISTOMORPHOLOGICAL EVALUATION OF EFFECTIVENESS OF ANESTHESIA DURING PREPARATION FOR CROWNING TEETH

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KEY WORDS: anesthesia; preparation of teeth; histomorphological changes.

Pain is a subjective sensation and it may be difficult [2] or almost impossible [3] for an objective assessment of pain to be given. The genesis and conduction of a nociceptive impulse and the formation of a sensation of pain are impossible without the material substrate, consisting of receptors, sensory nerve fibers, and nerve cells [1, 4, 5]. Hence the great scientific and practical importance of histomorphological assessment of the effectiveness of anesthesia with procaine, trimecaine, celnovocain and lidocaine during the preparation of teeth for crowning.

EXPERIMENTAL METHOD

Experiments were carried out on 16 mongrel dogs with an intact maxillodental system, aged from 10 months to 20 years, which were divided into four groups, with four dogs in each group. Nerve-block anesthesia was carried out on the animals of group 1 with 10 ml of 2% procaine solution, on the animals of group 2 with 2% celnovocain solution, in the dogs of group 3 with 2% trimecaine solution, and in the animals of group 4 with 2% lidocaine solution. With an electric drill the tip of which revolved at a speed of 5000 rpm, three teeth of the lower jaw of each dog were prepared for complete metal crowns. When the hard tissues of the teeth were polished, the same conditions were observed as during preparation, and the tooth meanwhile was cooled with water. The animals were killed under morphine-thiopental anesthesia by exsanguination through the femoral artery. The gasserian and

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