MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Hypogeomagnetic Field on Tissue and Intracellular Reorganization of Mouse Myocardium

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Structural reorganization of the myocardium is observed in CBA mice under conditions of hypogeomagnetic field (reduction by 10^5 times). Changes in tissue architectonics manifest themselves in a reduced capillary/cardiomyocyte volume ratio against the background of hemodynamic disturbances. Intracellular disturbances result from impaired regeneration processes.

Key Words: *hypogeomagnetic field; myocardium; cardiomyocytes; ultrastructure; stereology*

Numerous studies of the effect of magnetic fields on animals and men show that hypogeomagnetic medium is a biologically active factor [3,4,10-12,14]. Biological effect of hypogeomagnetic fields (HGMF) becomes now a more and more important problem in view of the modern tendency to isolate man from the geomagnetic field (space flights, concrete constructions, etc.) [2]. Of special interest is the effect of HGMF on the cardiovascular system, in particular, on the myocardium. It should be noted that adaptive rearrangements of the cardiovascular system under conditions of abnormal magnetic field remain practically unstudied.

The aim of the present study to evaluate the effect of HGMF on structural reorganization of the myocardium in inbred mice under conditions of medium latitudes.

MATERIALS AND METHODS

Hypogeomagnetic conditions were modeled by means of a ferromagnetic screen developed at the Institute of General Human Pathology and Ecology, Siberian Division of the Russian Academy of Medical Sciences and used in biophysical experiments. The screen was constructed from 2 sections consisting of six permalloy plates (1.5-mm thick) spaced with copper plates. Permalloy plates shielded biological objects from direct and alternating low-frequency magnetic fields, while copper plates shielded from industrial electromagnetic fields. This screen reduced the Earth's magnetic field strength from 50×10^{-6} to 50×10^{-11} T.

Experiments were carried out on 52 CBA male mice (26 experimental and 26 control animals). The animals were placed for 30 min, 1, 3, 6, and 24 h into a hypomagnetic chamber with a reduction coefficient of 10^5. The control animals were kept in wood cases for the same times. Control and experimental animals were decapitated simultaneously. The heart was weighted, and specimens of the left papillary muscles and left ventricle were placed in 4% paraformaldehyde, the heart was fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin with Perls reaction, by the method of Van Gieson, and by PAS reaction.

For electron microscopy heart specimens were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and then processed routinely. Semithin sections (1-μ)

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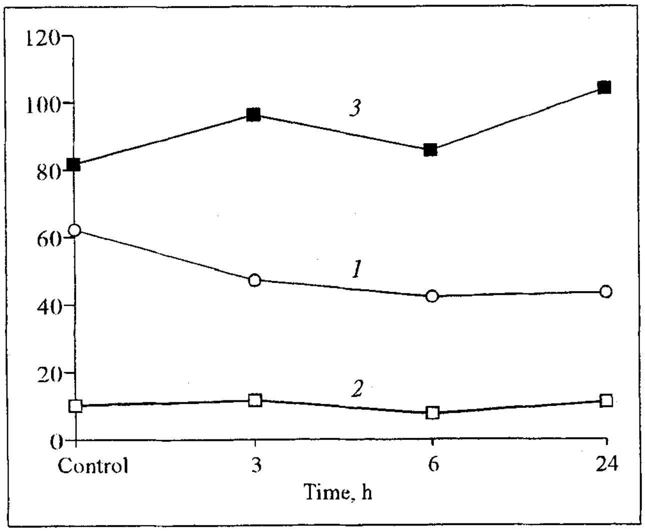

Fig. 1. Changes in volume density (mm³/cm³) of stromal components in myocardium of CBA mice subjected to hypogeomagnetic field. *1*) capillaries; *2*) endotheliocytes; *3*) intercellular substance and fibers of connective tissue.

and ultrathin sections were prepared in an LKB III ultratome. Semithin sections were stained with 1% azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-100C electron microscope at an accelerating voltage of 80 kV.

Tissue organization and ultrastructure were analyzed by stereological methods developed for anisotropic structures [8]. Tissue stereological analysis was performed on semithin sections at a final magnification of 1060, while ultrastructural analysis was done using cardiomyocyte microphotographs at a final magnification of 18,000. Volume and surface density of the main tissue components and organelles and volume and surface-volume ratios of these structures were evaluated. The data were processed statistically using the Student's *t* test.

RESULTS

Marked disturbances of blood and lymph circulation in mouse myocardium were observed starting from the 3rd hour of HGMF exposure. Most common features were venous and capillary plethora, interstitial edema, and lymphostasis; spasm of intramural artery and sludge of erythrocyte were also seen. We found a mosaic pattern of cardiomyocyte damage: contractures and lysis were observed simultaneously. Necrobiotic changes in some cardiomyocytes and their resorption by mononuclear cells were noted by the end of experiment.

Stereological analysis showed that the surface and volume density of cardiomyocytes and their nuclei remained practically unchanged throughout the observation period. Hypogeomagnetic conditions considerably reduced the volume density of capillaries: it decreased by 24, 32, and 30% after 3-, 6-, and 24-h exposure, respectively (Fig. 1). The volume ratio of capillaries to cardiomyocytes decreased by 24% after 3 h, by 35% after 6 h, and by 31% after 24 h. The decrease in the surface density was less pronounced and was observed after the animals spent more than 6 h in the hypogeomagnetic chamber. This resulted in a slight decrease in the surface-volume ratio of capillaries to the cardiomyocytes.

The volume and surface densities of connective tissue cells were unchanged, whereas the volume density of the intercellular component increased by 18 and 27%, after a 3- and 24-h exposure to HGMF, respectively (Fig. 1). Nevertheless, the volume density of the stroma to the parenchyma remained practically unchanged due to a considerable reduction in the volume density of capillaries.

No substantial shifts in the cardiomyocyte ultrastructure arose during a 3-h stay in the HGMF. Moderate dilation of sarcoplasmic vesicles and minor loosening of myofibrils were the only phenomena observed. The most marked ultrastructural changes in cardiomyocytes seen after a 6-h stay in the hypogeomagnetic chamber persisted to the end of the experiment. In the majority of cardiomyocytes we found lysis of sarcoplasmic matrix and myofibrils exposing well-defined cellular structure of the sarcoplasmic reticulum (Fig. 2, *a*).

Considerable changes were observed in fine structure of the mitochondria: focal lysis of mitochondrial matrix and crista destruction (Fig. 2, *b*); in some cases we observed global lysis of mitochondrial cristae. Destruction of mitochondria accompanied by the formation of myelin-like structures was noted in the subsarcolemmal zone near the intercalated disks and sometimes in the intermyofibrillar spaces (Fig. 2, *c*). In some cases, huge vacuoles containing membrane fragments and glycogen particles arose in the focuses of degradation. Marked dilation of vesicles of the granular and agranular sarcoplasmic reticulum is a characteristic feature of ultrastructural rearrangements in cardiomyocytes under hypogeomagnetic conditions (Fig. 2, *d*).

Three-hour stay in the hypogeomagnetic chamber induced a 13% rise in the volume density of myofibrils followed by a 10% decrease in this parameter by the 6th hour (Fig. 3). The surface density of myofibrils markedly increased after 6- and 24-h stay in the hypogeomagnetic chamber (by 34 and 21%, respectively), leading to a considerable (by 49%) rise of the surface-volume ratio after 6 h in HGMF.

After a 24-h stay in the hypogeomagnetic chamber, the volume density of mitochondria significantly

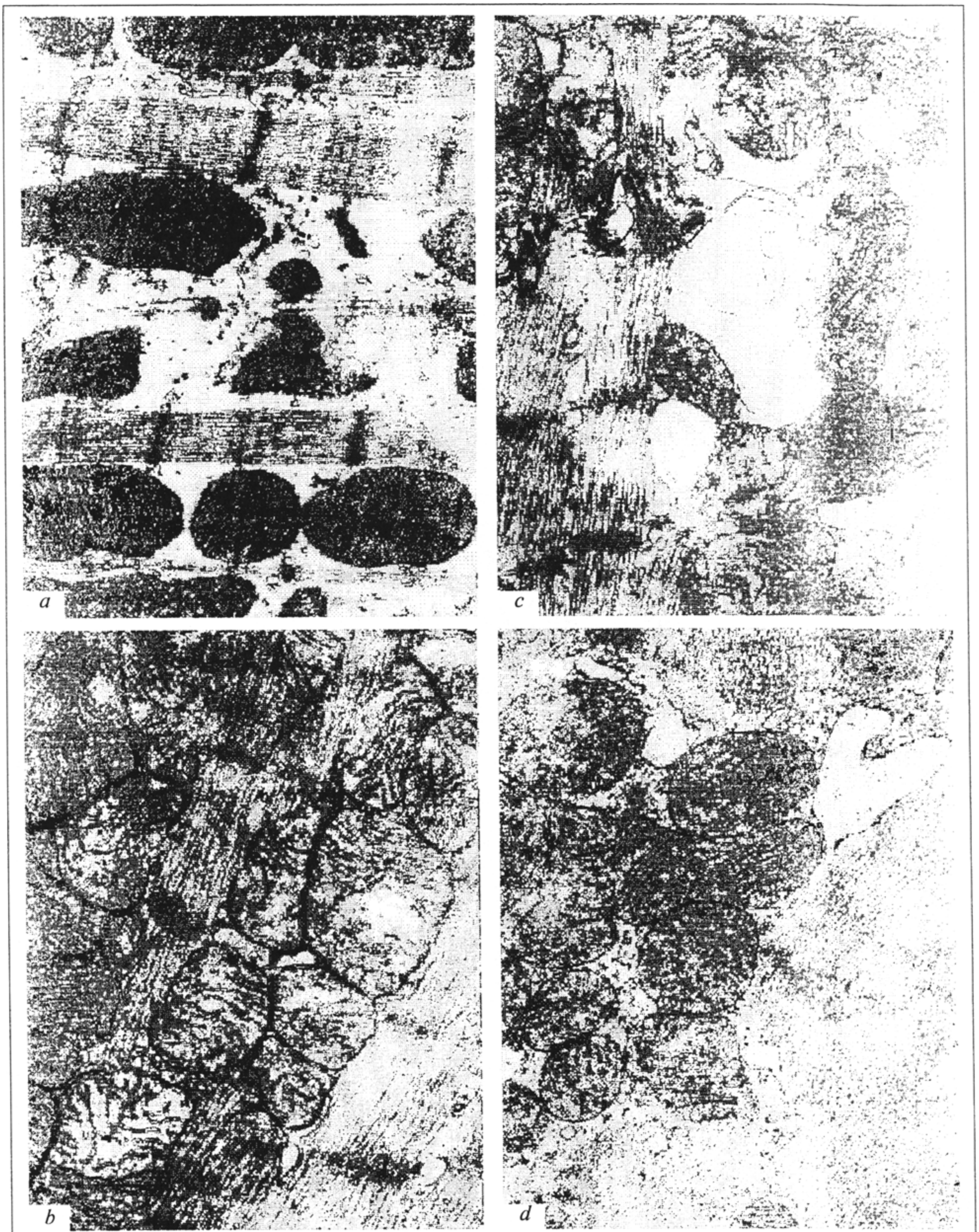

Fig. 2. Ultrastructural changes in cardiomyocytes of CBA mice after a 6-h stay in hypogeomagnetic chamber. *a*) thinning of myofibrils and lysis of sarcoplasmic matrix, ×8300; *b*) focal destruction of mitochondrial cristae, ×13,000; *c*) myelin-like structures near intercalated disks, ×10,000; *d*) dilated vesicles of granular and agranular sarcoplasm; ×13,000.

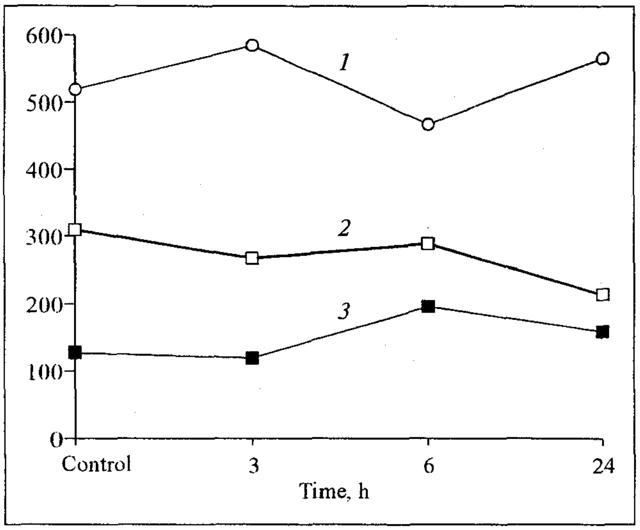

Fig. 3. Changes in volume density (mm³/cm³) of main cardiomyocyte compartments in CBA mice subjected to hypogeomagnetic field. *1*) myofibrils; *2*) mitochondria, *3*) sarcoplasmic matrix.

decreased (by 31%, Fig. 3), while the decrease in the surface density of these structures was most pronounced after 6 hours in HGMF (23%). This coincided with a 18% reduction of the surface-volume ratio indicating the prevalence of large organelles. In contrast, the increase in this parameter (by 31%) by the end of the experiment attested to a reduction of the mean size of mitochondria.

The quantitative parameters of the agranular sarcoplasmic reticulum (ASR) and T system underwent wave-like changes. After 3 h of the experiment, the volume density of ASR and T system decreased by 32 and 39%, respectively; by the 6th hour the volume density of ASR increased by 24%, while this parameter for T system decreased by 34%. After a 24-h stay in HGMF, the volume density of ASR and T system increased by 29 and 57%, respectively. Similar shifts were noted for the surface density of ASR and T system, so that the surface-volume ratio of these compartments remained unchanged. It should be noted that 6-h stay in the hypogeomagnetic chamber led to a considerable rise of the volume density of the sarcoplasmic matrix (by 54%, $p<0.05$, Fig. 3), which reflects aggravation of destructive and lytic processes in cardiomyocytes.

In general, after 3- and 24-h stay in the hypogeomagnetic chamber the volume ratio of the main cytoplasmic organelles to myofibrils decreased by 24 and 28%, respectively, and was primarily due to a reduced volume ratio of mitochondria.

The observed ultrastructural changes in cardiomyocytes, namely, focal lysis and thinning of myofibrils, destruction of mitochondria, focal degradation of the sarcoplasm, stimulation of phagocytosis, and marked reduction in the number of β-glycogen granules, result from disturbed biosynthesis of structural proteins in geomagnetic abnormalities and correspond to those observed in regeneratory and plastic insufficiency of cardiomyocytes [9,13]. Similar changes in cardiomyocytes were induced by disturbances or suppression of protein synthesis (at both the transcriptional and translational levels) [9], as well as under the action of extreme ecological factors [5-7]. Stereotype pattern of ultrastructural changes in cardiomyocytes implies universal molecular and cellular mechanisms of their regeneratory and plastic insufficiency. In light of this the observed complex of ultrastructural changes in cardiomyocytes can be regarded as a nonspecific reorganization under conditions of plastic and energy metabolic disturbances of various origins.

Similar changes in cardiomyocytes induced by long-term influences result in cell atrophy and their resorption by macrophages. Terminal stages of the morphofunctional rearrangement are similar to the phenomenon of programmed cell death, apoptosis [1,15]. However, unlike apoptosis, atrophic (involutive) changes in cardiomyocytes are reversible (to certain stages). In view of possible cell death in the absence of necrosis, these morphofunctional shifts can presumably be specified as apoptoid states.

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