cents. The same effect in the CSF of intact donors is less pronounced but also reliable.

Thus, CSF of convalescents activates HRP transport in the cortical neurons both in intact recipients and in animals with brain damage. The CSF of intact donors exhibits the same activity in recipients with a damaged CNS. It is known that an intracisternal injection of convalescent CSF speeds up the restoration of the functions of the contralateral extremities after unilateral removal of the sensorimotor cortex in rats. It may be assumed that the effect of the donor fluid is associated with the mobilization of neuronal connections that are not normally active. The biochemical inductors of this process may be oligopeptide and polypeptide agents which were previously determined to be the factors of postural asymmetry and compensation [2]. The preexistence of oligopeptide factors of postural asymmetry in the CSF of a donor with an intact CNS [4,5] probably accounts for the above-described effect of "intact" fluid.

REFERENCES

1. G. A. Vartanyan and Yu. V. Balabanov, *7th Gagry Meeting: Neurophysiological Principles of Memory* [in Russian], Tbilisi (1979), pp.279.
2. G. A. Vartanyan and B. I. Klement'ev, *Chemical Symmetry and Brain Asymmetry* [in Russian], Leningrad (1991).
3. G. A. Vartanyan, B. T. Moroz, and V. L. Silakov , in: *Theoretical Principles of Pathological States. Theoretical Principles of Diagnosis and Therapy Optimization in Nervous System Diseases* [in Russian], Leningrad (1980), pp.48-51.
4. G. A. Vartanyan, M. V. Neuimina, V. I. Golovkin, et. al., *Vest. Russ. Akad. Med. Nauk,* №3, 34-37 (1992).
5. G. A. Vartanyan, S. V. Shatik, A. V. Tokarev, et al., *Byull. Eksp. Biol. Med.*, **107**, №4, 404-406 (1989).
6. S. N. Ivanova, *Mechanisms of Motor Function Compensation after Lateral Hemisection of the Spinal Cord* [in Russian], Moscow (1980).
7. G. P. Obukhova, E. I. Varlinskaya, and N. V. Krutikova, *Byull. Eksp. Biol. Med.*, **98**, №9, 626-628 (1988).
8. I. B. Ptitsina and M. A. Danilovskii, *Fiziol. Zh. SSSR*, **72**, № 7, 888-892 (1986).
9. S. K. Leong, *Brain Res.*, **265**, 1-9 (1983).
10. M. M. Mesulam, *J. Histochem. Cytochem.*, **161**, №12, 1273-1280 (1976).
11. M. W. Miller, *Exp. Brain Res.*, **67**, 339-351 (1987).

Quantitative Analysis of Rat Myocardial Tissue for General Overheating of the Organism

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A general, even short-lived, overheating of a homoiothermic animal induces pronounced and often irreversible morphofunctional changes in many internal organs and systems [13]. This is the rationale for the use of hyperthermia in oncotherapy, since tumor cells have been shown to exhibit a lower resistance to such treatment [3,14]. However, the induced alterations not only do not disappear after the termination of overheating, but actually may be intensified during the period of postheating restitution [7]. The long-lasting circulation of endogenous toxic metabolites [1] probably causes the destructive changes and the disturbances of circulation in different organs [8,10]. From the first minutes of thermal action the cardiovascular system undergoes functional reorganizations, such as a redi-

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TABLE 1. Results of Morphometric and Stereological Analysis of Rat Myocardium after One-Trial General Overheating ($M \pm m$).

Index	Control	Time after overheating, days	
		3rd	7th
Morphometric assays			
Body weight, g	112.6±5.4	118.0±5.7	98.6±8.8
Heart weight, mg	610.0±22.7	600.0±46.4	466.0±47.6*
Relative heart weight, mg/g	5.60±0.21	5.07±0.19	4.74±0.25*
Stereological assays			
Volume density, mm^3/cm^3:	772.7±18.0	786.7±9.5	796.2±11.8
of cardiomyocytes	17.1±2.4	14.6±1.1	14.1±1.1
of cardiomyocyte nuclei	17.1±2.4	14.6±1.1	14.1±1.1
of capillaries	57.5±1.4	47.3±6.2	39.9±2.5*
of endothelial cells	20.0±2.4	19.9±1.1	18.4±1.8
of connective tissue cells	13.5±1.2	11.6±0.8	13.4±2.4
of connective tissue fibers and matrix	119.2±19.3	119.9±4.4	118.0±9.9
Surface density, m^2/cm^3			
of cardiomyocytes	0.1046±0.0033	0.1154±0.0022	0.1184±0.0021
of cardiomyocyte nuclei	0.0119±0.0019	0.0108±0.0013	0.0112±0.0004
of capillaries	0.0375±0.0045	0.0368±0.0020	0.0305±0.0031
of connective tissue cells	0.0111±0.0006	0.0099±0.0013	0.0108±0.0009S
Surface-volume ratio, m^2/cm^3:			
of cardiomyocytes	0.132±0.005	0.144±0.004	0.146±0.004
of cardiomyocyte nuclei	0.689±0.018	0.737±0.045	0.812±0.099
of capillaries	0.649±0.063	0.795±0.073	0.764±0.058
of connective tissue cells	0.832±0.043	0.847±0.053	0.828±0.070
of capillaries to the cardiomyocytes	0.047±0.004	0.046±0.003	0.038±0.004
Volume ratio, number:			
of capillaries to cardiomyocytes	0.073±0.004	0.059±0.008	0.050±0.003**
of stroma to parenchyma	0.268±0.032	0.248±0.014	0.235±0.019

Note. Asterisks signify reliability of differences. * $p<0.05$, ** $p<0.01$.

stribution of the blood flow and changes of the pulse pressure and cardiac output [4,12,13]. The functional disturbances of the cardiovascular system under heat loads may be so significant that they often become the direct cause of death in human beings and homoiothermic animals [11]. Yet the dynamics and nature of myocardial tissue reorganization remain virtually unstudied, hampering the establishment of morpho functional criteria for the adaptive reorganization of the myocardium after general overheating.

The aim of the present investigation was a qualitative and quantitative assessment of myocardial tissue reorganization in rats after a one-time general overheating within the dynamics of postheating restitution.

MATERIAL AND METHODS

Experiments were carried out on 28 2-month-old male Wistar rats. The animals were exposed to a 45-min overheating in a thermal chamber at a temperature of 43°C. The time of exposure was dictated by the vitality of the animals and corresponded to the time limit after which large-scale mortality occurred. Tissue samples for examination were taken on the 3rd and on the 7th day after the overheating. The control animals of the same age and the experimental animals before and after treatment were held under the usual conditions with standard laboratory food and water ad libitum. After killing, the heart was weighed, tissue samples of the left papillary muscles and of the left ventricle were fixed in 4% paraformaldehyde solution, and both halves of the heart were immersed in 10% neutral formalin. After routine paraffin embedding, sections were obtained and stained with hematoxylin-eosin using the Pearls reaction after van Gieson, with counterstaining of elastic fibers, and the PAS reaction was performed. The slides stained with hematoxylin-eosin were examined under a Docuval polarizing microscope to reveal the state of the cardiomyocyte myofibrils. The heart tissue samples were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, dehydrated routinely, and embedded in Epon-Araldite. The semithin sections (1μ) were prepared with an LKB III ultramicrotome and stained with 1% Azure II. A tissue stereological analysis with the use of a multipurpose test-kit [5] and measurement of the cardiomyocyte diameter with an MOV-1-15 ocular

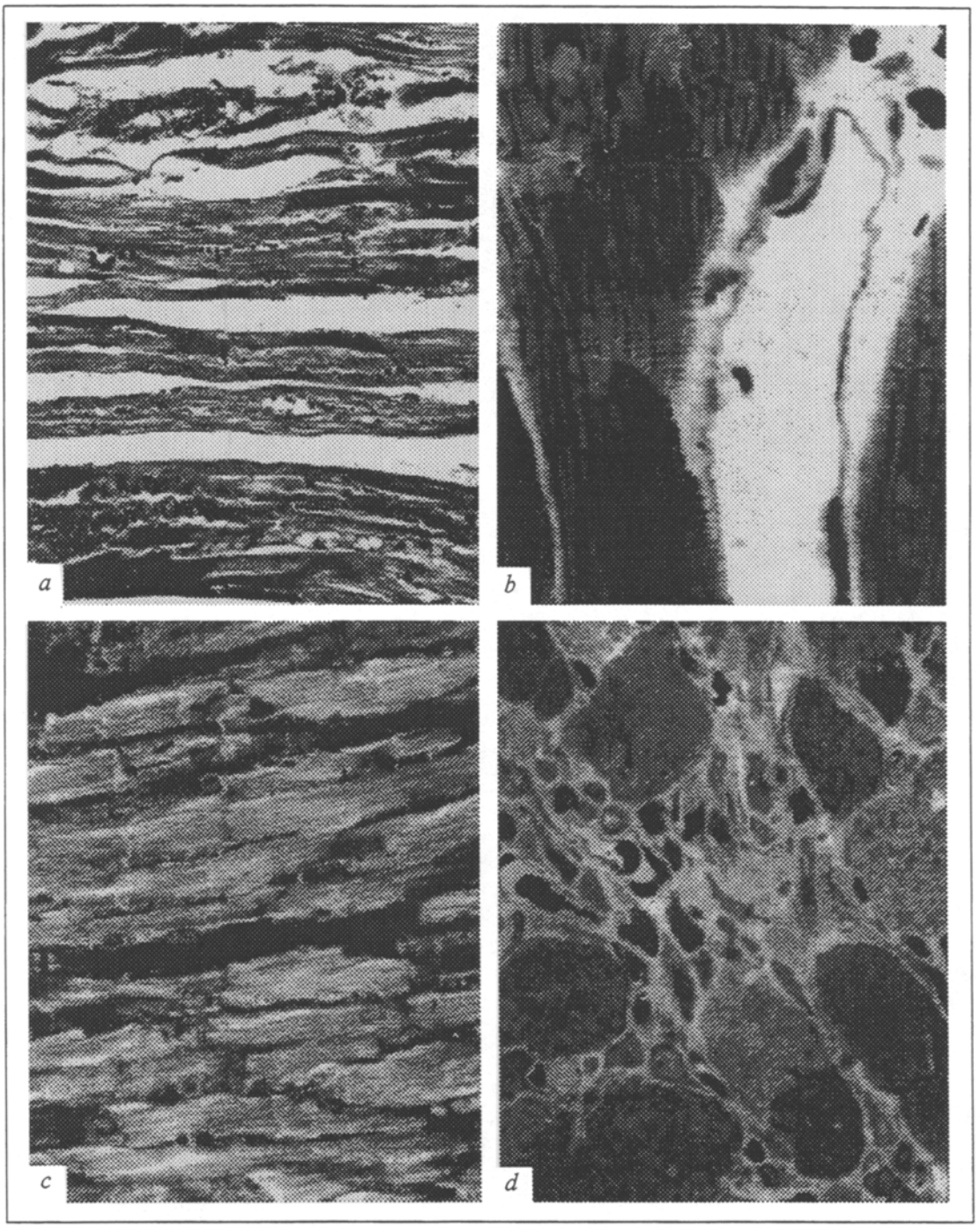

Fig. 1. Morphological changes in Wistar rat myocardium on the 3rd day after one-trial general overheating. *a*) edema of interstitial connective tissue (hematoxylin-eosin staining, ×200); *b*) lymphostasis and contracture damage of individual cardiomyocytes (semithin section, Azure II staining, ×800); *c*) contracture damage of myofibrils in cardiomyocytes (PAS-reaction, ×312); *d*) clasters mononuclear cells around atrophying cardiomyocytes in interstitial connective tissue (semithin section, Azure II staining, ×800).

micrometer (×112) were performed on the semithin sections. The volume density of the cardiomyocytes, their nuclei, capillaries, endothelial cells, connective tissue cells (in sum), matrix, and fibers was assessed. The surface density was determined for the cardiomyocytes, their nuclei, capillaries, and connective tissue cells. On the basis of these primary indexes the secondary stereological parameters were calculated: the surface-volume ratio of the structures, the volume ratio of myocardial stroma to parenchyma, and the volume and surface-volume ratio of capillaries to cardiomyocytes.

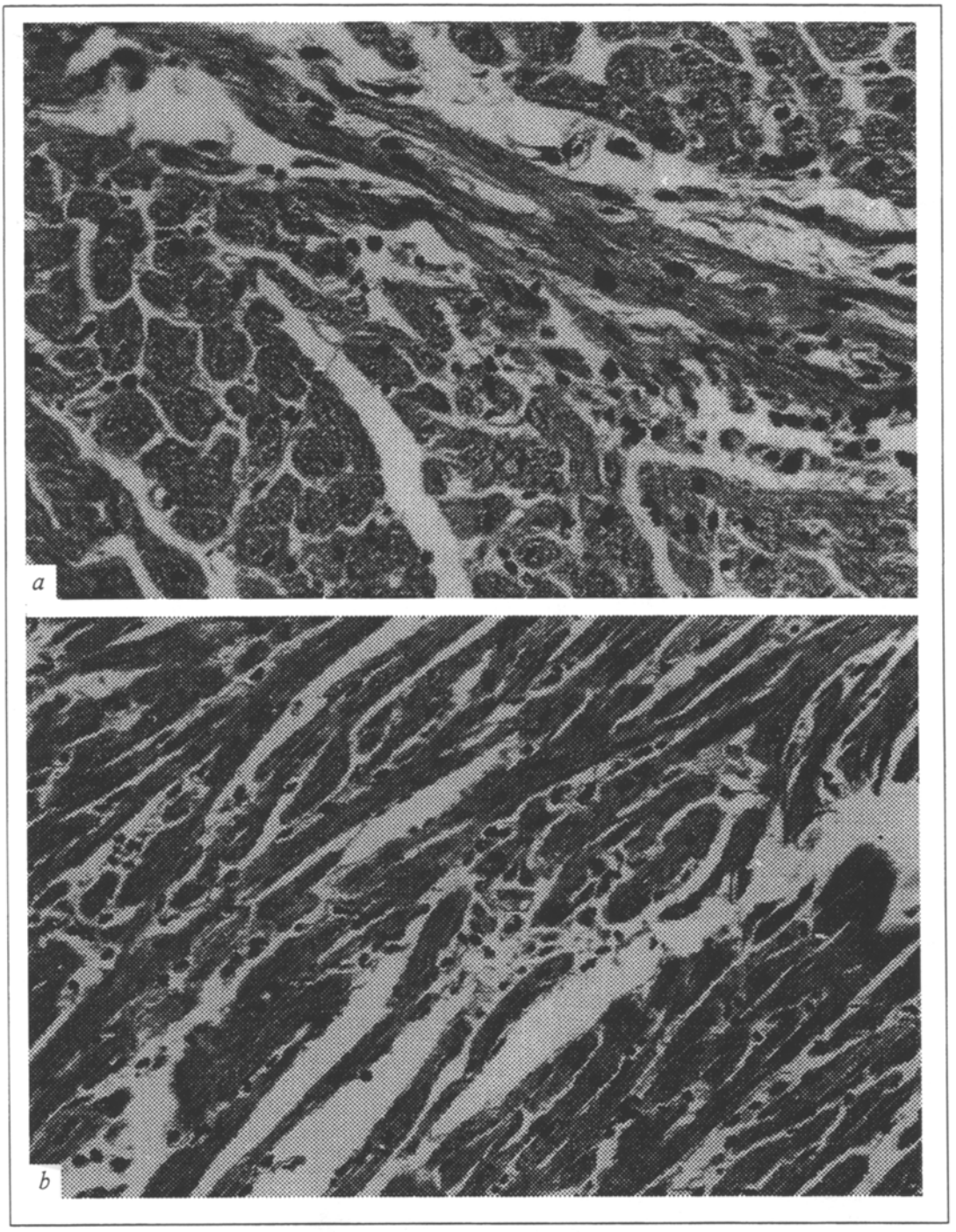

Fig. 2. Morphological changes in Wistar rat myocardium on the 7th day after one-trial general overheating. Hematoxilin-eosin staining. *a*) moderate edema of interstitial connective tissue (×500); *b*) clasters of mononuclear cells in foci of necrosis of cardiomyocytes (×400).

The data were processed statistically using the Student *t* test. The differences between the control and experimental groups were reliable at $p<0.05$.

RESULTS

A one-trial general overheating of the organism (at 43°C) caused a modest (12%) drop of the body weight on the 7th day after treatment (Table 1). At the same time, the weight of the heart toward the end of the experiment reliably (by 24%) decreased (from 610.0±22.7 mg in the control to 466.0±47.6 mg on the 7th day, $p<0.05$). Since the heart weight diminished to a greater degree than the body weight, there was a decrease (by 15 %) in the relative weight of the heart (from 5.06

±0.21 to 4.74±0,25, $p<0.05$). A microscopic examination of the myocardium performed 3 days after the overheating revealed an interstitial edema (Fig. 1, *a*), predominantly in the middle and subendocardial zones, and lymphostasis (Fig. 1, *b*). The blood vessels were markedly plethoric, and focal plasmorrhages were noted. The intramural arteries were mainly in a state of spasm. The changes in the cardiomyocytes mostly related to contracture damage (I, II, and III degree) (Fig. 1, *c*). Most of the damaged cardiomyocytes were in the middle and subendocardial zones. Atrophied cardiomyocytes (Fig. 1, *d*), surrounded by clusters of mononuclear cells, appeared simultaneously in different regions of the myocardium.

On the 7th day after overheating the myocardium still showed features of impaired blood and lymph circulation, such as plethora of vessels, focal plasmorrhages, occasional hemorrahages, and lymphostasis. Moderate edema was observed mainly in the middle myocardial zone (Fig. 2, *a*). Most of the cardiomyocytes stained uniformly with acid stains, there being just a few eosinophilic cells with contracture damage of the myofibrils. There was atrophy of cardiomyocytes and necrosis of individual muscle segments, which were resorbed by mononuclear cells, in some myocardial zones (Fig. 2, *b*).

According to the stereological analysis, the volume and surface density of the cardiomyocytes did not significantly change during the whole experiment, so that the surface-volume ratio of the cardiomyocytes stayed the same. The volume density of the cardiomyocyte nuclei decreased by 17.5% toward the 7th day after a one-trial overheating, whereas the surface density of these structures was scarcely affected. The surface-volume ratio of the cardiomyocyte nuclei rose toward the end of the experiment by 17.8%.

The most significant changes of volume density were in the capillaries: for instance, on the 3rd day this parameter dropped 17.7% and on the 7th day 31% ($p<0.01$). The surface capillary density was less affected, and by the end of the experiment it was decreased by 18.6%. The quantitative indexes of the endothelial cells practically did not change.

The decrease of the volume and surface density of the capillaries during the experiment led to a decrease of the volume and surface-volume ratio of the capillaries to the cardiomyocytes. On the 7th day after treatment these indexes dropped by 32% ($p<0.01$) and 19%, respectively.

The quantitative parameters of the connective tissue components did not significantly change, so that preserves the volume ratio between stroma and parenchyma in the course of the experiment, remained the same.

Thus, a one-time general overheating of the organism resulted in a reliable decrease of the absolute and relative weight of the heart by the 7th day of the experiment. The morphofunctional changes were most pronounced in the myocardium on the 3rd day. At that time there was marked plethora of the vessels, plasmorrhages, moderate edema of the intermuscular tissue, and contracture damages of the heart cells. By the 7th day the histological organization of the myocardium was more or less restored to normal, but atrophic alterations of the cardiomyocytes were noted. Sings of the preceding treatment were also evident from the individual necrotic cardiomyocytes, which were resorbed by the mononuclear cells. In contrast, quantitative changes of the main myocardial components, attesting to spatial reorganization of the myocardium, increased toward the end of the experiment. These changes mostly affected the capillaries and testified to the unbalanced (compared to the cardiomyocytes) decrease of their volume and surface area.

The same features of tissue rearrangement have been described for an array of pathological processes in the heart [6] and reflect a conformity to natural laws of regeneration in parenchymatous and stromal components of the myocardium under conditions of plastic disturbances. It is likely that prolonged circulation of endogenous metabolites, in particular the molecules of a medium weight, that play an imported role in the development of the autotoxic states [2,3], to a large degree depresses the regenerating processes in the endotheliocytes of the capillaries. The subsequent penetration of these metabolites into the intercellular spaces and their cytopathic effect on the cardiomyocytes on the one hand, and the pronounced decrease of the volume and surface area of the capillaries, on the other, determine the disorders in plastic metabolism in the parenchyma cells and their ensuing atrophy [9].

REFERENCES

1. V. V. Vinogradov, N. S. Permitina, and N. F. Vorob'eva, *Byull. Eksp. Biol*, № 5, 616-618 (1988).
2. A. S. Vladyka, E. R. Levitskii, L. P. Poddubnaya, *et al., Anest. i Reanimatol.*, No 2, 34-42 (1988).
3. N. B. Kozlov, *Hyperemia: Biochemical Principles of Pathogenesis, Prophylaxis, and Therapy* [in Russian], Voronezh (1990).
4. A. A. Korchagina, *Pathobiochemistry and Pathohistology of an Overheating* [in Russian], Smolensk (1974), p.54
5. L. M. Nepomnyachshikh, E. L. Lushnikova, L.V. Kolesnikova, *et al, Morphometrical and Stereological Analysis of the Myocardium: Procedure Guidelines* [in Russian], Novosibirsk (1984).

6. L. M. Nepomnyachshikh, *Morphogenesis of the Main Processes in Heart General Pathology* [in Russian], Novosibirsk (1991).
7. T. V. Pavlova, S. V. Kutsevol, and A. I. Kiktenko, *Thermoregulatory System in the Organism's Adaptation to the Environment Factors* [in Russian], Novosibirsk (1990), pp. 238-239.
8. T. V. Pavlova and A. S. Pavlov, *Ibid.*, pp. 229-238.
9. L. A. Semenova, L. M. Nepomnyachshikh, and D. E. Semenov, *Morphology of Cardiomyocyte Plastic Insufficiency* [in Russian], Novosibirsk (1985).
10. M. M. Seredenko, A. A. Moibemko, E. V. Rosova, and L. A. Grabovskii, *Byull. Eksp. Biol.*, № 8, 229-230 (1988).
11. F. F. Sultanov, *Hyperthermia* [in Russian], Ashkhabad (1978).
12. A. Yu. Tilis and M. M. Mirrachimov, in: *Human Ecological Physiology, Part 2, Adaptation of a Man to the Different Climatic and Geographic Conditions* [in Russian], Leningrad (1980), pp. 343-361.
13. Z. T. Tursunov, in: *Animal Ecological Physiology, Part 3. Animal Physiology in Different Physico-Geographic Regions* [in Russian], Leningrad (1982), pp. 153-169.
14. H. S. Reinhold and J. Overgaard, *Europ. J. Cancer*, **26**, № 8, 915-919 (1990).

Effect of Transcardial Galvanization on the Microcirculatory Bed of the Periinfarcted Region in Experimental Myocardial Infarction

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Since the state of the microcirculatory bed (MCB) of cardiac muscle determines in many respects the nature of the adaptive processes and metabolic activity of cardiomyocytes, the mass of necrosis forming in acute myocardial infarction (MI) depends largely on the blood supply of the ischemic region [4], a factor which has to be taken into account in treatment aimed at minimizing ischemic damage to cardiac muscle during disturbed coronary circulation. As previously noted, the use of transcardial galvanization (TCG) in patients with stenocardia of functional class 1-2 improves the microcirculation in the underlying organs, increases fibrinolytic activity, and decreases blood coagulation (enhancing heparin tolerance) [2,3]. All this argues in favor of expanding the uses of TCG, including it in cardiac ischemia therapy, particularly in combined therapy of acute MI.

The aim of this investigation was to study the effects of TCG on MCB state in periinfarcted regions in experimental MI.

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats weighing 280-320 g. A model of experimental MI (EMI) was used. With the animals under nembutal anesthesia (20 mg/kg) and breathing naturally, the chest was opened at intercostal space 4-5, and the anterior descending coronary artery was ligated in its upper third. The operative wound was then tightly closed with sutures. For control an ECG was recorded in 12 standard leads after the initial narcosis and 1 h after MI simulation. There were 3 experimental groups: the first group (n=10) comprised sham-operated rats, the second

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