Effect of Adaptation to Intermittent Normobaric Hypoxia on the Ultrastructure of Cardiac Myocytes in Pregnant Rats

N. P. Lebkova, A. Ya. Chizhov, and L. N. Zeitlenok

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 597-602, June, 1995
Original article submitted October 27, 1994

A comparative ultrastructural study of organelles involved in supplying cardiac myocytes with energy (nucleus, mitochondria, microbodies, and lysosomes) in pregnant rats that had been exposed to intermittent normobaric hypoxia and in rats not so exposed showed no significant differences between these two groups of animals. In both groups, glycogen granules were present in the nucleus, mitochondria, lysosomes, and microbodies (peroxisomes) of cardiac myocytes, and many of their mitochondria had a dense matrix and appeared hyperplastic.

Key Words: *normobaric hypoxia; pregnancy; ultrastructure; cardiac myocytes*

Intermittent normobaric hypoxia (INH) has been increasingly utilized in clinical settings for the prevention and treatment of many diseases. At present, exposure of women to INH during normal pregnancy is also being practiced, and so it is important to evaluate on animal models how such exposure affects the structural homeostasis of the female organism.

Previously, we undertook an ultrastructural study of livers from pregnant and nonpregnant rats after their exposure to INH [4]. In a similar study on rats reported here we examined cardiac myocytes, playing special attention to the intracellular structures (mitochondria, microbodies, lysosomes) implicated in one way or another in cellular energetics, and to the nuclei.

MATERIALS AND METHODS

Pregnant Wistar rats weighing 190±10 g were used. The test group was exposed for 10 days, from day 12 of gestation, to INH in a hermetically sealed 0.6 m^3 chamber. Daily INH sessions each lasted 120 min, during which time the rats alternately breathed atmospheric air for 5 min and a hypoxic gaseous mixture (90% nitrogen and 10% oxygen, HGM-10) also for 5 min, which was delivered at a rate of 60 liters per minute (the gaseous medium in the chamber was monitored with an OA-250 gas analyzer). This regimen of INH using HGM-10 has been shown to mimic the natural biorhythm of Po_2 in the myometrium [7,8]. There were two control groups. Group 1 comprised rats that breathed only atmospheric air in a sealed chamber as described above starting on day 12 of pregnancy, while group 2 consisted of intact nonpregnant rats.

After 10 days, i.e., on day 21 of gestation, the rats were decapitated, their beating hearts were removed, and specimens of myocardial tissue were dissected out from the subendocardial layer of the left ventricle in the upper third of the heart and fixed in paraformaldehyde. The specimens were subjected to a standard treatment procedure and embedded in an Epon-Araldite mixture, after which semithin and ultrathin sections were prepared. The semithin sections were used for cytochemical estimation of fat and glycogen by means of light micros-

Research Center, Russian Medical Academy for Postgraduate Education; Department of Medical Ecology and Naturopathy, Scientific-Education Center, Russian Peoples' Friendship University, Moscow. (Presented by A. V. Smol'yannikov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0006-0574$12.50

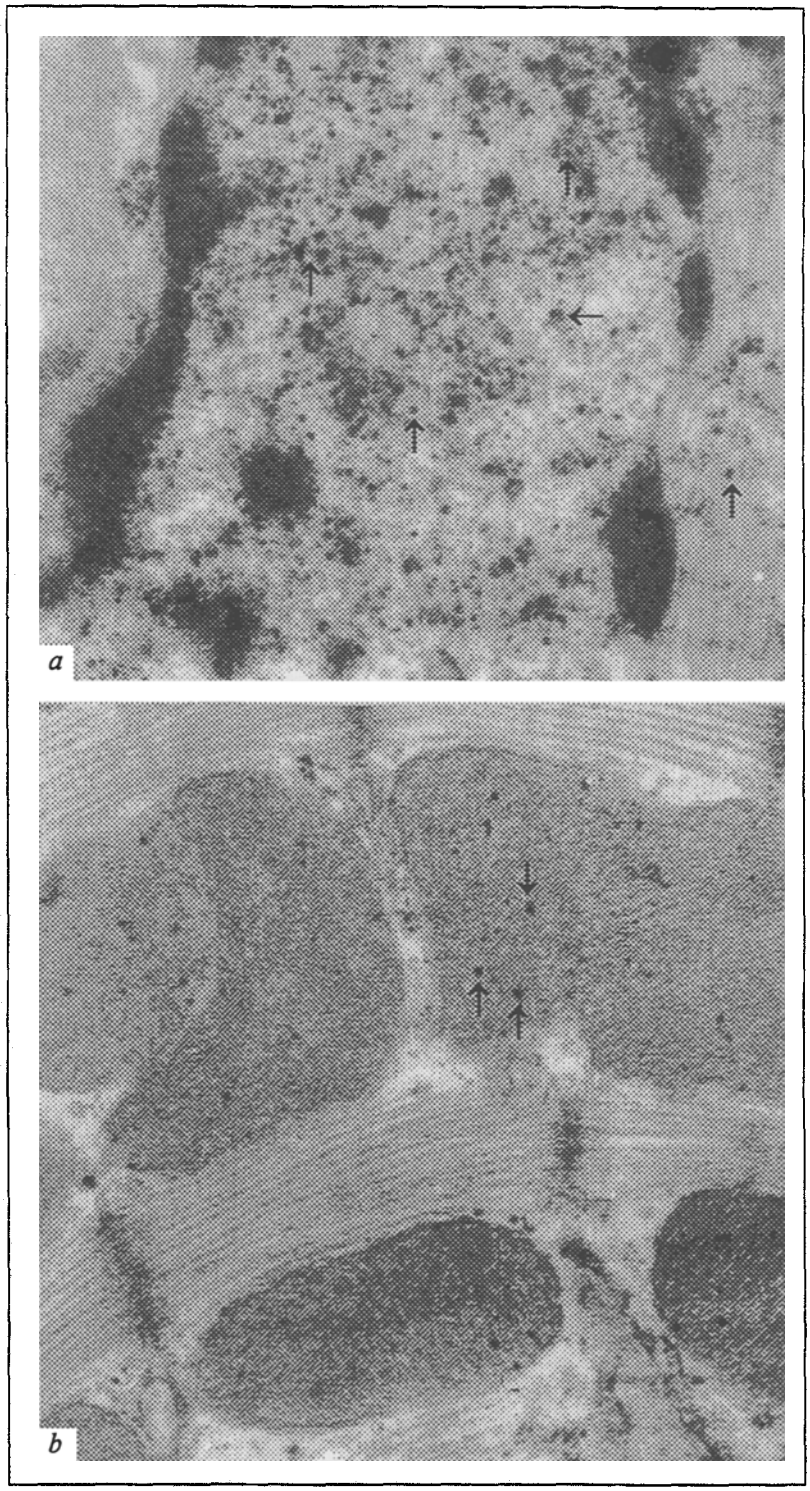

Fig. 1. Glycogen granules (arrowed) in the cardiac myocyte nucleus (*a*) and mitochondria (*b*) of a pregnant rat. ×50,000.

copy. The ultrathin sections were examined under a JEM-7A electron microscope. Procedural details of this part of the work were described earlier [4].

RESULTS

In control group 1, most cardiac myocyte (CMC) nuclei showed signs of high functional activity, such as the predominance of diffuse chromatin, large nucleoli, and numerous pores in the karyolemma. In addition, roundish granules resembling glycogen granules and having a greater electron density than ribosomes were seen (Fig. 1, *a*). Glycogenlike granular material was also contained within mitochondria with a dense matrix (Fig. 1, *b*), lysosomes (Fig. 2), and microbodies (peroxisomes); the last

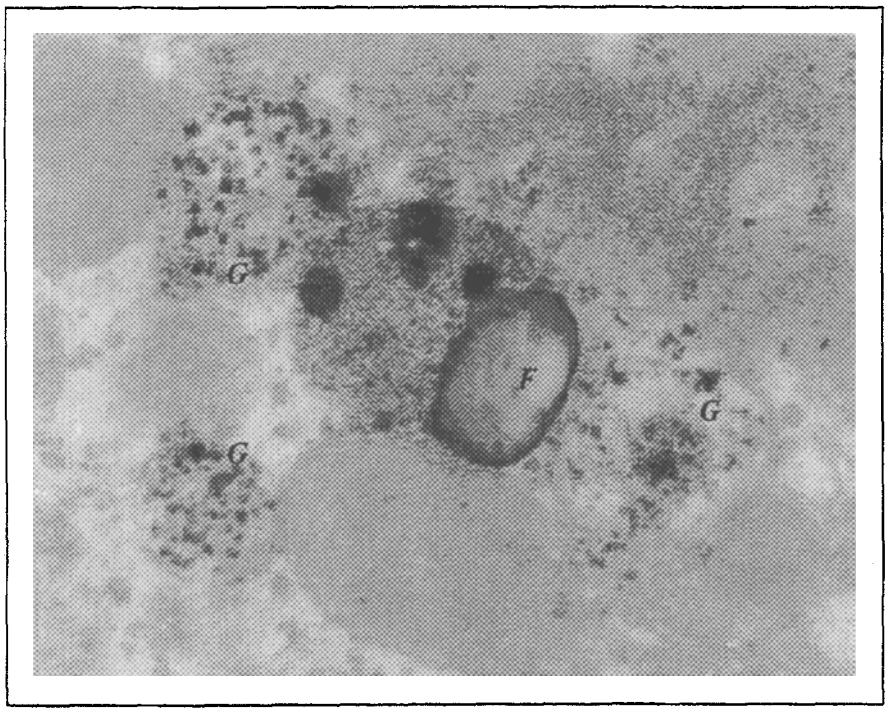

Fig. 2. Glycogen granules (*G*) and fat (*F*) in cardiac myocyte lysosomes of a pregnant rat. ×50,000.

usually closely adhered to the mitochondria (Fig. 3) and tended to have a lowered electron density and to accumulate granules. In the sarcoplasm, glycogen was represented by scattered β-granules or small clusters thereof (Fig. 3, *c*).

Although no specific ultracytochemical assay for glycogen was carried out in this study, the above-mentioned granules, which were encountered in different CMC organelles, could be interpreted with certainty as being those of glycogen, given their close resemblance to the granules whose glycogen nature had been previously ascertained using the marker enzyme glycogen synthase [3].

No typical fat inclusions were found in the CMC sarcoplasm, but small fragments of fat were seen together with glycogen in lysosomal structures (Fig. 2).

In the CMC from pregnant rats that had undergone a course of INH, the energy-producing structures did not differ in morphological appearance from those described above for control group 1 (Fig. 4). The mitochondria were characterized by densely packed cristae and accumulations of glycogenlike inclusions. All nuclei contained electron-dense granules and exhibited signs of the high functional activity noted above for control group 1. Hyperplastic and hypertrophic mitochondria were frequently observed, as were microbodies and small lysosomes with lipid conglomerates and glycogenlike granular material inside. Such material was also regularly seen in the sarcoplasm, despite the periodically repeated exposures to hypoxia. However, morphometric estimation of glycogen in semithin sections did not show significant differences between the test and control groups; the glycogen level in CMC from test rats was not more than 15% higher than in these cells from pregnant controls, and so it is possible to speak only of a trend toward elevation of intracellular glycogen under the action of intermittent hypoxia.

When oxygen is in short supply, the main energy source in the cell is known to be glycogen [6], and the presence of glycogen in the CMC sarcoplasm and organelles is therefore an indication of their heightened resistance to hypoxia.

In the CMC from nonpregnant females (control group 2), glycogen inclusions in mitochondria and microbodies were also encountered, though much less frequently than in CMC from pregnant controls and still less so than in those from their hypoxia-adapted counterparts. It is of interest to note in this context that no intramitochondrial glycogen granules were seen on archival electron micrographs of CMC from intact male rats that did not differ in body weight from the females used in this study and had been maintained under the same conditions in the vivarium over the same period of the year; nor were such granules detectable in the few microbodies or lysosomes that the CMC of those males contained. Presumably, the cardiac muscle of females is better adapted at the subcellular level to withstand oxygen deficiency under physiological conditions. This hypothesis is

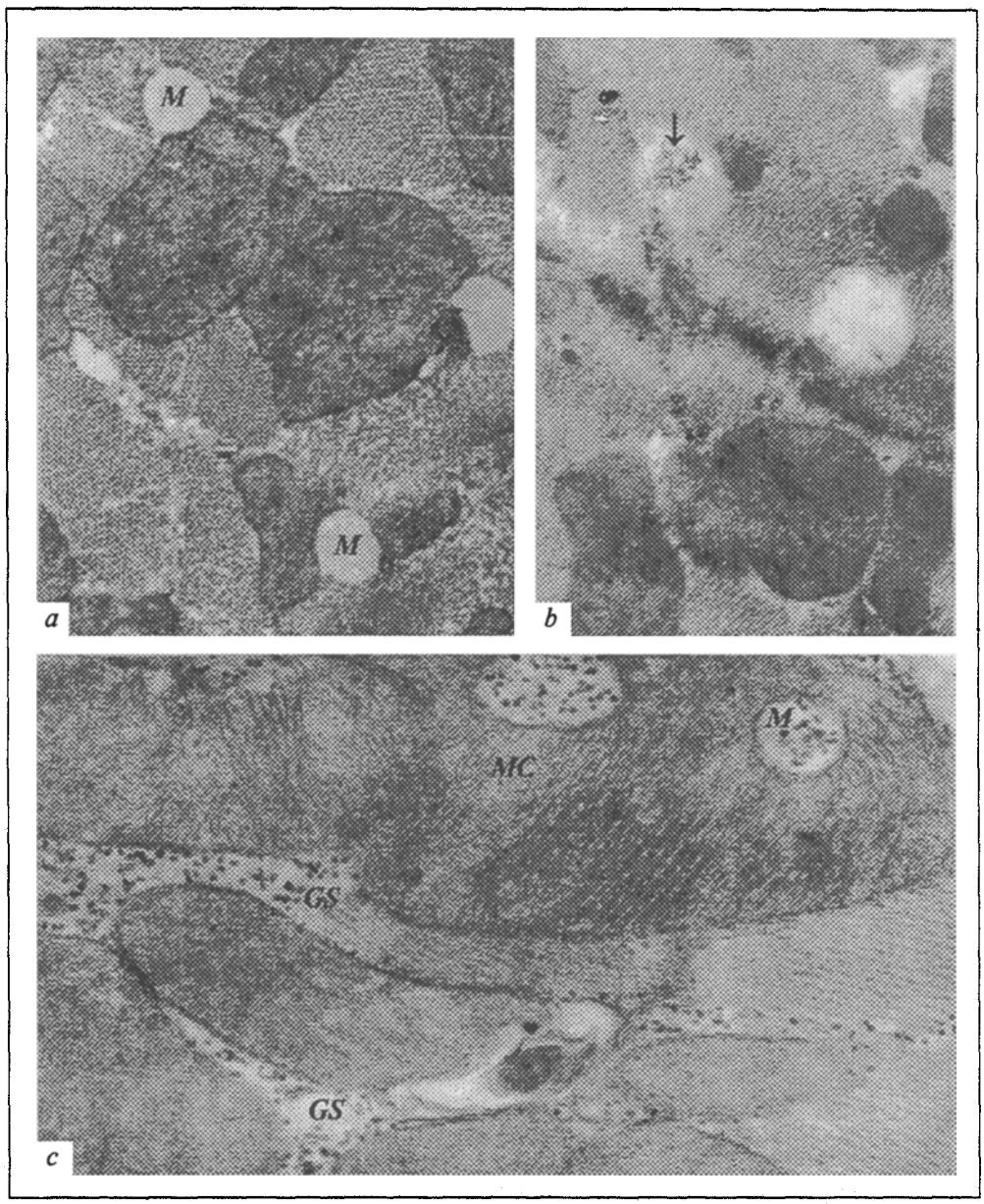

Fig. 3. Glycogen granules in cardiac myocyte microbodies of a pregnant rat. ×30,000. *a*) microbodies (*M*) without glycogen; *b*) microbodies partly filled with forming glycogen (shown by arrow); *c*) microbodies (*M*) with many glycogen granules closely adherent to a hypertrophied mitochondrion (*MC*). *GS*: glycogen in sarcoplasm.

supported by our previously published findings on the contribution of an adaptive biorhythm in females to their increased functional resistance to various insults.

The elevation of intracellular glycogen during pregnancy and the absence of marked differences in ultrastructural energy homeostasis between the CMC conditioned and those not conditioned to INH may attest to the fact that pregnancy with its peculiar periodic states of hypoxia is an endogenous conditioning factor acting to raise the organism's resistance to an additional, exogenously administered hypoxia. If this is so, then, on the one hand, the adaptation of CMC ultrastructure to hypoxia may be thought to reach its highest level already during the course of pregnancy and thus to render the CMC largely insensitive to exogenously produced hypoxia; on the other hand, this suggests that the INH regimen used in our study is a physiological one and does not result in intracellular changes of a pathological nature.

Exposure to HGM-10, like hypoxia of any other kind, increases the contribution of nonesterified fatty acids to the maintenance of energy homeostasis [1]. The presence of glycogen inclusions inside various organelles has been frequently observed in critical or pathological states, but the mechanism of this phenomenon is not clear [3,9,12]. According to our concept [2-4], the energy-related role of fatty acids in hypoxia consists in their conversion into glucose and glycogen so as to provide a carbohydrate substrate for the gly-

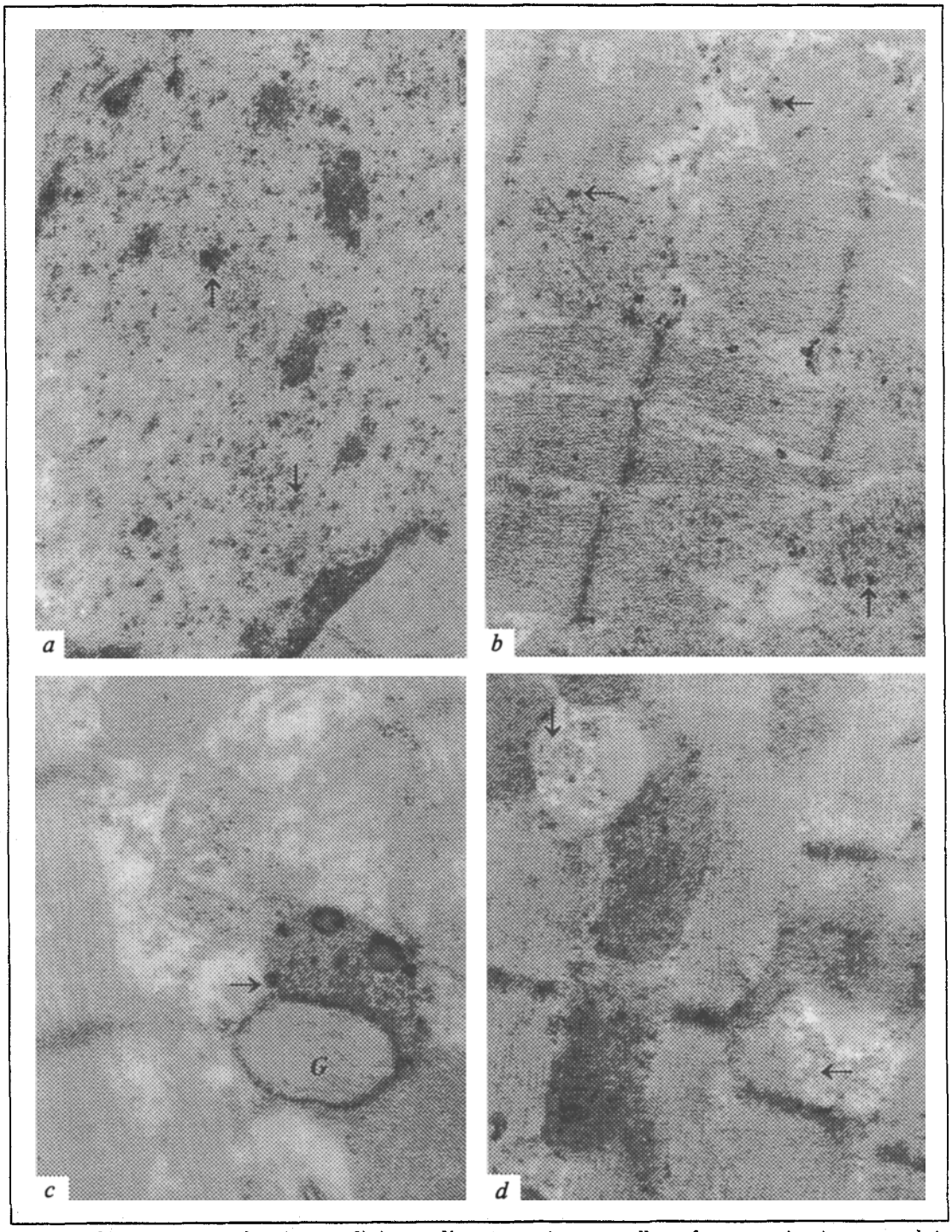

Fig. 4. Glycogen granules (arrowed) in cardiac myocyte organelles of pregnant rats exposed to intermittent normobaric hypoxia. ×30,000. *a*) nucleus; *b*) mitochondria; *c*) lysosome; *d*) microbody and sarcoplasm. A fat inclusion (*F*) near the lysosome, and intramitochondrial inclusions can be seen in *c*.

colytic process. Possible pathways of gluconeogenesis from fatty acids in mammals have been described [10,11,13-15]. It should be emphasized that because glucose-6-phosphatase, which controls glucose synthesis, is not found in cardiac muscle [5], the only possible endogenous carbohydrate substrate for glycolysis in CMC is glycogen.

We believe that fatty acids are utilized in cardiac cells directly for oxidative phosphorylation by mitochondria in the absence of oxygen deficiency, whereas during hypoxic periods they are used in anaerobic glycolytic processes which go on both in the cytoplasm (sarcolemma) and in various organelles, including mitochondria.

REFERENCES

1. Yu. M. Karash, R. B. Strelkov, and A. Ya. Chizhov, *Normobaric Hypoxia in Disease Management and Prevention and in Rehabilitation* [in Russian], Moscow (1988).
2. N. P. Lebkova, *Arkh. Pat.*, **44**, № 6, 68-76 (1982).
3. N. P. Lebkova, *Tsitologiya*, **27**, № 2, 157-162 (1985).
4. N. P. Lebkova and A. Ya. Chizhov, *Byull. Eksp. Biol. Med.*, **113**, № 6, 663-666 (1992).
5. W. C. MacMurray, *Essentials of Human Metabolism*, Harper and Row (1977).
6. P. W. Hochachka and G. N. Somero, *Strategies of Biochemical Adaptation*, Saunders (1973).
7. A. Ya. Chizhov, V. G. Filimonov, Yu. M. Karash, and R. B. Strelkov, *Byull. Eksp. Biol. Med.*, **92**, № 10, 392-394 (1981).
8. A. Ya. Chizhov, *Fiziol. Zh.*, **38**, № 5, 13-17 (1992).
9. A. L. Shabadash, in: *Mitochondria* [in Russian], Moscow (1972), pp. 52-56.
10. J. M. Argieles, *Trends Biochem. Sci.*, № 11, 61-63 (1986).
11. W. L. Davis, R. I. Jones, D. Goodman, and I. L. Mattnewes, *Anat. Rec.*, **214**, № 3, 30A (1986).
12. N. Feroze Chadially, *Ultrastructural Pathology of Cells*, London - Boston (1978), p. 543.
13. C. T. Jones, *Biochem. Biophys. Res. Commun.*, **95**, № 2, 849-856 (1980).
14. P. B. Mortensen, *Biochim. Biophys. Acta*, **620**, 177-185 (1980).
15. F. Wada and M. Usami, *Biochim. Biophys. Acta*, **487**, 261-268 (1977).

Impact of Twelve-Day Combined Exposure to Hypobaric Hypoxia and Physical Exercise on Structural and Metabolic Characteristics of Skeletal Muscle in Rats

T. L. Nemirovskaya, O. S. Tarasova, B. S. Shenkman, and V. B. Koshelev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 602-605, June, 1995
Original article submitted June 2, 1994

This study, in which rats were exposed on 12 successive days to hypoxia in combination with exercise on a treadmill, showed that a reduction in partial oxygen pressure leads to a decrease in the magnitude of the structural component of vascular resistance rather than to improvement in the system of oxygen utilization, and that such combined exposure may cause alterations in protein synthesis and result in early stimulation of capillary growth in muscles, as well as elicit differential changes of enzyme activity in different types of muscle fibers.

Key Words: *hypoxia; physical exercise; muscle fibers; capillaries; enzymes*

It is currently thought that the main strategy of muscle tissue adaptation to reduced partial oxygen pressure is directed at maintaining the concentration of adenosine triphosphoric acid (ATP) at the appropriate level [7]. This can be achieved in two ways: by raising the activity of oxidative enzymes with a resultant increase of metabolic influx into the muscle fibers because of a rise in the concentration gradient of oxygen and substrates [8], or by making oxygen more accessible to the fibers through increased capillarization and the consequent decrease of the diffusion distance for oxygen inside the fibers. It has also been suggested that the local hypoxia arising during physical activity may be one of the major stimuli triggering the development of adaptive changes in skeletal muscle tissue.

Laboratory of Pathophysiology, Department of Fundamental Medicine, Moscow State University; Department of Neurophysiology, Institute of Biomedical Studies, Ministry of Health of the Russian Federation, Moscow. (Presented by A. I. Grigor'ev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0006-0579$12.50