# Development of Aseptic Inflammation during the Adaptation of the Organism to High Altitude Hypoxia

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UDC 612.017.2:616-002

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 9, pp. 243-246, September, 1994
Original article submitted December 29, 1993

The dynamics of lipid peroxidation and of the aseptic inflammatory process is studied in rats adapted to high altitude hypoxia. A greater activity and shorter duration of the acute period of inflammation are found in these animals, while in the reparative period stimulation of the proliferative processes and slowed formation of the fibroblast capsule are noted.

**Key Words**: *hypoxia adaptation; inflammation; lipid peroxidation*

Previous experiments established that the inflammatory process is regularly followed by a stress reaction, which markedly affects its course [4]. It may be assumed that limitation of this stress reaction would considerably modify the dynamics of the inflammatory process. One of the most effective ways of boosting the power of stress-limiting systems is the adaptation of the organism to environmental factors, including high altitude hypoxia (HAH), which greatly enhances the organism's resistance to ischemia, ionizing radiation, toxic chemicals, etc. [6]. However, there are still no data on the possibility of optimizing the course of inflammation using such adaptation, i.e., of mitigating the pathogenic aspects of this process. On the basis of the above the aim of the present investigation was to study the effect of HAH adaptation on the dynamics of the inflammatory process.

## MATERIALS AND METHODS

Experiments were carried out on 100 male albino rats weighing 140-160 g, 40 of which were controls, while 60 rats underwent adaptation to HAH in a KU-7 pressure chamber during 6 weeks (5 days a week for 6 hours each day at an "altitude" of 5500 m) [6]. Aseptic inflammation was simulated 24 hours after the adaptation was completed using a 1×5 mm celloidin plate implanted in the subcutaneous connective tissue of the shank. For assessment of the activity of lipid peroxidation (LPO) processes and of the antioxidant systems of the organism the content of lipid LPO-hydroperoxide products and malonic dialdehyde and the antioxidative activity (AOA) was determined in the serum [2,3]. Vascular and cell reactions in the inflammatory focus were assessed morphometrically on histological preparations [1,5] 0.5, 1, 2, 3, 5, 10, and 25 days after the initiation of inflammation. The functional activity of mast cells was determined using the mean index of degranulation [8].

## RESULTS

It was found that the acute period of inflammation in HAH adapted animals proceeds more vigorously and finishes earlier than in control animals. For example, in the leukocytic phase (0.5 day of inflammation) control animals at first show activation only of capillaries (mean area of one vessel $S=265\pm55$ $\mu^2$) and of arterioles and venules only toward the end of the 2nd day ($S=500\pm100$ $\mu^2$). The activation of microvessels of all calibers ($S=503\pm72.2$ $\mu^2$, $p<0.05$) was already noted in test

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0007-4888/94/0009-0940$12.50 

animals during the first 12 hours of the inflammatory process, indicating an earlier and more intensive vascular reaction in HAH adapted rats. It should be stressed that whereas in test animals the vascular reaction markedly diminishes toward the end of the first day, it is increased in controls. The more pronounced plethora of microvessels noted in adapted animals may be due to a high concentration of histamine in the inflammatory focus, because the number of mast cells surrounding the foreign body is 4.4 times greater as compared to the control (35±1.3/mm² and 8±2/mm², $p<0.001$, respectively). The active vascular reaction in test rats is accompanied by a greater density of the leukocytic infiltrate (53.9±5.1 macrophages and 14.7±0.57 neutrophils per 5000 μ²) as compared to the control (21.5±1.5 macrophages and 5.1±2.5 neutrophils, $p<0.001$). The thickness of the leukocyte rampart surrounding the celloidin plate is regularly increased under the test conditions (79.2±5.1 *vs.* 54.5±2.4 μ, $p<0.001$). This rampart consists 72-74% of neutrophils in animals of both groups (17.1±7.6 per 1000 m² in the test and 22.1±1.8 in the control).

Toward the end of the first day the density of leukocyte infiltration on the periphery of the inflammatory focus declines in test animals and approximates the control level. The thickness of the leukocyte rampart remains as before, while in the control animals it increases almost twofold and constitutes 102.3±14.6 μ. Mass death of neutrophils is noted in the leukocyte rampart at this time, the losses being markedly more pronounced in test animals, resulting in a drop in the concentration of these cells to 8.6±0.3 per 1000 μ² in the test and to 15.7±0.2 in the control ($p<0.001$).

The cell rampart surrounding the foreign body is formed at the macrophagic stage of inflammation, i.e., toward the 2nd day, and contains more macrophages in test animals (70±3.8% in the test and 56.7±3.5% in the control, $p<0.05$). Toward the 3rd day the macrophages completely clear the inflammatory focus of detritus. The more intensive course of the acute period may be due in large measure to the early activation of LPO processes (Fig. 1). The maximal content of LPO products in test animals is reached 12 h after the onset of inflammation, while the same indexes in the control group peak only after 3 days of inflammation. The rapid curtailment of the acute period of inflammation in HAH adapted rats is probably related to the drastic limitation of lipid hyperperoxidation induced by the early activation of the antioxidant systems, as is confirmed by an increase of the blood AOA (Fig. 2).

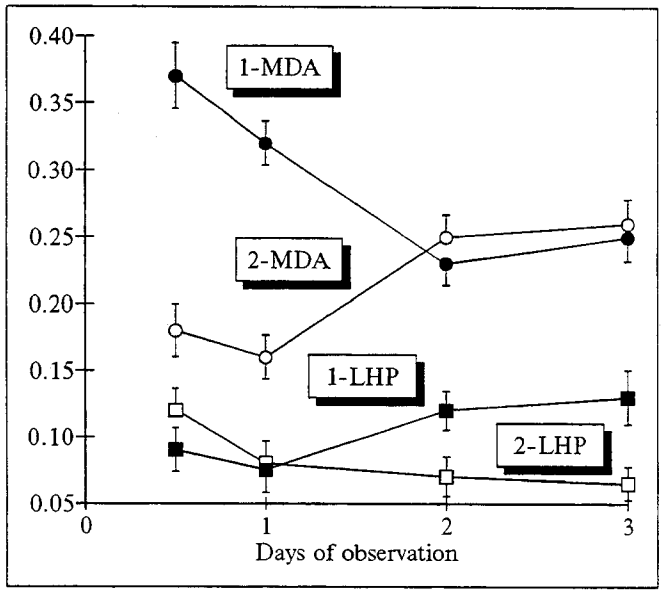


**Fig. 1.** Content of LPO products in control (*1*) and test (*2*) groups in the dynamics of inflammation. Ordinate: level of lipid hydroperoxides (*LHP*) and malonic dialdehyde (*MDA*), rel. units.

It may be assumed that the lability of the processes responsible for the activation and inhibition of LPO processes increases during adaptation to hypoxia. On the one hand, rapid activation of LPO results in the stimulation of cell and vascular reactions in the inflammatory focus due to changing properties of the cell membranes, and, on the other hand, the antioxidant systems, boosted due to the HAH adaptation, "kick in" quickly in response to LPO activation [6]. This prevents excessive LPO activation, lowers cell activity, and limits the development of secondary alteration of tissue.

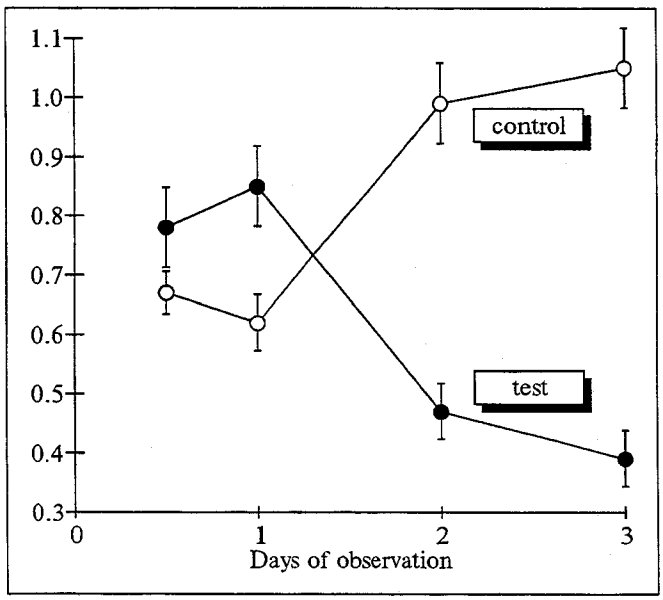


**Fig. 2.** Serum AOA in control and test groups in the dynamics of inflammation. Ordinate: level of AOA, rel. units

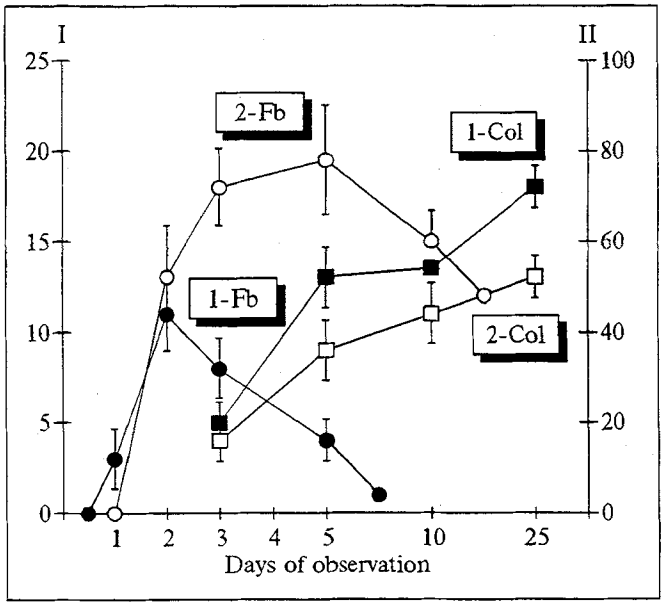


Fig. 3. Concentration of little-differentiated fibroblasts (*Fb*) in the inflammatory focus and of collagen (*Col*) in the fibroblast capsule in the process of development of inflammation in control (*1*) and test (*2*) animals. Ordinate: *I*) number of Fb (per 5000 $\mu^2$); *II*) area of fibroblast capsule occupied by Col, %.

Toward the beginnig of the reparative period the proliferation of fibroblasts, which peaks 2 days after the onset of inflammation, is terminated in control animals. In contrast, the proliferative processes in test animals are still further stimulated and continue till the 10th day of the inflammation process (Fig. 3).

In a discussion of the findings, several groups of factors stimulating the proliferation of fibroblasts may be isolated: 1) serum factors [7,9]; 2) factors released by neutrophils [10], 3) the macrophagal growth factor [7]. Our results suggest that the concentration of the above factors is greatly increased in the inflammatory focus. The more vigorous reaction of blood vessels, neutrophils, and macrophages in HAH adapted animals may indirectly point to this.

Despite the more active and prolonged proliferation of fibroblasts under the test conditions, the differentiation of these cells begins simultaneously and proceeds at the same rate in animals of both groups till the 5th day of inflammation. But the subsequent process of formation of the fibroblast capsule is somewhat inhibited in the test animals, and after 10 days there are 9.5±0.3 layers of fibroblasts *vs.* 15.2±0.9 in the control group ($p<0.001$). The synthetic function of fibroblasts is also more active in animals of the control group (Fig. 3). As early as the 5th day of inflammation the collagen content in the fibroblast capsule accounts for 51.7±3.2% in the control, while in the test animals it comprises 37.4±4.1% ($p<0.05$), and later on it remains lower in test animals than in controls. Thus, the capsule, fully formed by the 25th day of inflammation, is younger and contains more fibroblasts (19.4±1.0 *vs.* 11.9±0.9 cells per 1000 $\mu^2$ in the control) and less collagen as compared to the control (Fig. 3), while the thickness of the capsule is the same in both groups.

In our view, the decreased activity of the collagen-production process may be due to the following causes. Firstly, the shorter activation of LPO as well as of the vascular and cell reactions in the acute period of inflammation in HAH adapted rats may determine the low level of production of collagenogenesis stimulators in the reparative period. Secondly, the increased power of the antioxidant systems results in a change of some properties of the fibroblast membranes, which is responsible for their somewhat lesser activity. Thirdly, the more intensive degranulation of mast cells noted in the reparative period (Table 1) probably leads to a rise in the concentration of histamine (known to inhibit collagenogenesis) in the inflammatory focus [7].

Thus, a more active and shorter course of the acute period of inflammation as well as a rise in the proliferative activity of fibroblasts, together with a slight lowering of the rate of collagenogenesis in the reparative period, are noted HAH adapted animals.

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TABLE 1. Mean Index of Degranulation of Mast Cells in Dynamics of Inflammation in Control and HAH Adapted Rats (*M*±*m*; arb. units)

| Group of animals | Days of observation | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 3 | 5 | 10 |
| Control | 0.64±0.07 | 0.49±0.06 | 0.7±0.08 | 0.96±0.1 | 0.72±0.1 | 0.8±0.1 |
| HAH adaptation | 0.67±0.08 | 0.85±0.09* | 1.05±0.1* | 1.6±0.3* | 1.6±0.1* | 1.4±0.05* |

Note. Asterisk denotes a reliable difference from the index of the control group in the same observation period, $p<0.05$; $n=6$.

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# Changes in the Coronary Vessel Tone Resulting from Immobilization StressA

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UDC 616.45-001.1/.3.616.132.2-005

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 9, pp. 246-249, September, 1994
Original article submitted December 26, 1993

Coronary perfusion pressure at increasing flow of Krebs-Henseleit buffer in the hearts of animals exposed to stress is decreased 23%, a decrease which is eliminated by administering the NO-synthase blocker $N^G$-monomethyl-L-arginine. Stimulated vasodilation of coronary vessels (administration of sodium nitroprusside and acetylcholine) decreases markedly in stressed animals; the concentrations of sodium nitroprusside and acetylcholine inducing a half-maximum coronary response increase.

**Key Words:** *coronary blood flow; endothelium; nitrogen oxide; stress*

Immobilization stress causes pronounced alterations in the coronary circulation [1]: the volume of coronary blood flow increases, whereas intraventricular pressure decreases. A similar disparity between the demand for and the supply of the myocardium with blood is largely associated with a reduced tone of coronary vessels, which may ensue from alterations both of the contractile function of coronary vessels and of the functional activity of erythrocytes. The baseline activity of the nitrogen oxide system in coronary endotheliocytes has an important role in the regulation of coronary tone [7]. The amount of constantly released NO, which reduces coronary tone, is determined by the activity of the constitutional NO-synthase [5]. The role of this system in stress-induced changes in coronary blood flow is unknown. In this study we investigated the role of the NO-synthase system of rat coronary vessels in the post-stress alterations in coronary tone. For this purpose we studied: first, the effect of the specific competitive NO-synthase blocker $N^G$-monomethyl-L-arginine ($N^G$-MMLA) on stress-induced alterations in the tone of coronary vessels; second, the response of coronary vessels to administration of the exogenous NO source sodium nitroprusside; and, third, the acetylcholine-stimulated, endothelium-dependent dilatation of coronary vessels.

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## MATERIALS AND METHODS

Experiments were performed on isolated hearts of female rats weighing 180-230 g. The control and experimental series included 96 and 65 animals, respectively. Hearts were excised under urethane anesthesia (1 g/kg), and Langendorff perfusion was performed at a constant flow of carbogen-aerated (5% $CO_2$/95% $O_2$) Krebs-Henseleit buffer in a thermostatically controlled chamber. When the dependence between coronary perfusion pressure (PP)

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