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EXPERIMENTAL AND BASIC RESEARCH

Levels of Protein and the Main Protein Fractions in Human Blood during a Year-Long Exposure to Hypobaric Hypoxia, Hypokinesia, and Isolation

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Absract—Venous blood samples were collected for biochemical analyses from 16 members of a year-long expedition to the Vostok research station in Antarctica. The physiologically important factors were hypobaric hypoxia ($P = 460$ mm Hg) due to the station location (the ice dome of Central Antarctica), hypokinesia due to prolonged stay indoor because of the extremely low temperatures outside the station, physical and social isolation from the outer world. The participants were divided into two groups: the experimental one, who periodically received a vitamin-amino acid complex for the prevention and correction of asthenia, and the control one, who received a placebo. Biochemical analysis revealed undulating blood levels of protein and main protein fractions within the physiological norm. Starting from month 4 of the expedition, concentrations of γ- and β-globulins showed a statistically significant increase. This increase persisted for 2 months after the expedition, therefore, there are sufficient body reserves. There were no differences in concentrations of the studied parameters between the experimental and control groups. It is speculated that hypobaric hypoxia plays a positive role in maintaining non-specific body resistance in the year-long period of life in isolation and hypokinesia.

Keywords: total protein, albumin, globulins, chronic hypoxia, hypokinesia, isolation

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The inland Vostok station located in Central Antarctica (78°27′51″ S; 106°50′14″ E), from the point of view of physiologists, is a research laboratory with the most extreme conditions of human life. Extremely low temperatures (down to –89.2°), annual isolation from the outside world, autonomy (inaccessibility) for 10 months make it one of the most suitable places for studying the mechanisms of adaptation of the human body to a complex of extreme factors.

The station is located on an ice dome at an altitude of 3488 m above sea level, however, due to the low air temperature in the station area, the barometric pressure there is 460 mmHg, i.e., similar to an altitude of 4000 m, therefore, the oxygen content in the atmosphere in the station area is equivalent to that at an altitude of 4000 m (95–100 mmHg). Due to low air temperatures, the polar explorers of the Vostok station leave the station for a short time, which inevitably leads to the fact that the annual wintering is accompanied by a long limitation of motor activity. This is another additional unfavorable factor of wintering with a minimal effect of low temperatures on the body.

Interest in studying the mechanisms of adaptation of a healthy person to life in such harsh conditions is dictated by the need to identify and prevent medical risks for both polar explorers and cosmonauts facing a similar set of adverse factors [1]. A program of integrated biomedical studies was developed at the Institute of Biomedical Problems. It was carried out during several annual winterings at the Vostok station and included the study of certain biochemical blood parameters. The main blood protein fractions are important for the maintaining homeostasis, including the nonspecific resistance of the body. Therefore, it was considered appropriate to study their condition during a yearlong stay in extreme conditions.

METHODS

Sixteen apparently healthy men aged 30 to 40 years who were participants of the annual wintering at the Vostok station in 1968 were included in the study. All had different professions. All of them, in accordance with the objectives of the scientific program, were divided into two groups close in age and occupation: control and experimental, each of which included eight subjects. During the wintering period, no one had any infectious or other diseases, as well as changes in body weight. The participants of the experimental group received two courses of pharmacological cor-

Table 1. Mean values of some biochemical parameters of the blood of polar explorers for the entire cycle of studies during the annual wintering at the Vostok Antarctic station

Parameters	Norm	Control group	Experimental group
Total protein, g/L	65–85	75.16 ± 0.91	72.68 ± 1.24
Albumins, g/dL	3.5–5.0	4.60 ± 0.07	4.53 ± 0.09
Gamma-globulins, g/dL	0.7–1.6	1.37 ± 0.04	1.20 ± 0.03
Alpha-1-globulins, g/dL	0.1–0.3	0.21 ± 0.02	0.20 ± 0.02
Alpha-2-globulins, g/dL	0.5–1.0	0.49 ± 0.02	0.44 ± 0.02
Beta-globulins, g/dL	0.7–1.2	0.88 ± 0.03	0.81 ± 0.03
Properdin, units/mL	20–80	19.75 ± 1.91	24.02 ± 1.91

rection during months 2 and 8 of wintering. Men were treated with preventive drug complexes daily for two and three weeks, respectively. The first complex was aimed at accelerating the restoration of central nervous system functions after the end of the acute period of adaptation to wintering conditions and included ascorbic acid with glucose, calcium glycerophosphate, glutamic acid and the tranquilizer Elenium. The second complex consisted of vitamins, minerals and amino acids. It was taken by the participants of the experimental group on month 8 of wintering for the prevention and correction of asthenia. The control group received the placebo in tablet form, at the same time and with the same duration. The scheme of using pharmacological drugs and the results were described in detail earlier [2].

Blood for subsequent biochemical studies was taken from each member of the expedition in the following months from the beginning of the annual wintering: analysis I in month 2 (February), analysis II on in month 4 (April), analysis III in month 6 (June), analysis IV in month 9 (September), and analysis V in month 12 (December). The sixth blood sample was taken 2 months after the end of wintering (analysis VI).

Samples were obtained by venipuncture under aseptic conditions, by puncturing the antecubital vein with blood collection into tubes to obtain serum. The samples were kept at a low temperature and delivered frozen to the laboratory (Moscow), where all samples were analyzed simultaneously.

Total protein was determined by refractometry. The main protein fractions were studied by cellulose acetate electrophoresis. Properdin was determined by the zymosan method based on the adsorption of properdin on zymosan at a low temperature, followed by fractionation on DEAE cellulose.

The studies were carried out in compliance with the requirements of biomedical ethics. Informed consents for the participation of polar explorers of the Vostok station in the research were obtained.

For data processing, one-way analysis of variance was used using the Statistica 10.0 scientific professional statistical package from StatSoft.

RESULTS AND DISCUSSION

As follows from Table 1, the content of total protein and the main blood protein fractions stayed within the physiological norm in all analyzed groups during the annual stay of polar explorers at the Vostok Antarctic station. The values of the studied parameters changed in wave-like manner in different periods of wintering.

A periodic increase in the albumin fraction in both groups of subjects indicated, in our opinion, a possible hemoconcentration due to a change in water balance caused by an increase in extrarenal moisture loss due to low relative humidity in the station room (14–26%) and low barometric pressure. This was manifested clinically by dry skin, nasal mucosa, and oral cavity.

The content of protein fractions was normalized to albumin during further analysis in order to consider the possible effect of changes in the body's water balance on the content of the main blood protein fractions of the subjects. Due to the absence of significant differences in the studied parameters between both groups of subjects, the analysis was carried out according to the mean normalized values for all polar explorers in each study period.

γ-Globulins

As follows from the data presented in Fig. 1a, the mean values of γ-globulins normalized to albumin significantly increased ($p < 0.05$) from the 4th month of wintering (analysis II) compared with the initial period and remained increased until the end of wintering. It is known that γ-globulins in the blood are represented by the main classes of antibodies A, G, M, D, E and their level characterizes the state of the humoral link of nonspecific resistance of the body. The data obtained on the values of the blood γ-globulins fraction indicate that the humoral factors of resistance were activated in the subjects at the beginning of the polar night (April) and remained at this level during the subsequent periods of wintering.

In the absence of acute or chronic pathology in polar explorers of the Vostok station, a slight increase in the levels of γ-globulins in the blood cannot be considered hypergammaglobulinemia.

α_1-Globulins

It is known that the fraction of α_1-globulins determined by electrophoresis, is complex and includes the following main components: α_1-antitrypsin, α_1-antichymotrypsin, orosomucoid, α-fetoprotein (AFP), high density lipoproteins. α_1-Antitrypsin (an α_1-proteinase inhibitor) is a glycoprotein produced in the liver, it is an inhibitor of proteinases (trypsin, chymotrypsin, kallikrein, plasmin) and accounts for 92–94% of the total antiproteolytic activity of the blood. Its concentration makes the main contribution to the level of the entire faction. The second most important in the fraction is α_1-antichymotrypsin, a glycoprotein that is one of the first reactant proteins of the acute phase (its level in blood serum can double within a few hours). However, it is, although specific, but a weak inhibitor of chymotrypsin. During the yearlong stay at the Vostok station, the level of the α_1-globulin fraction in the blood mainly fluctuated within the normal range but had greater variability in the control group. In this group, only in two subjects, the concentration of the α_1-globulin fraction slightly increased beyond the normal range of values. In the group with pharmacological correction, the concentration levels of the α_1-globulin fraction were higher than normal values in 4 participants at separate periods of stay at the station, and in 2, significantly reduced. The dynamics of the mean normalized values (see Fig. 1b) nevertheless showed that there was a tendency to an increase in the level of α_1-globulin fraction in polar explorers blood in the ninth month of wintering (analysis IV) and during the recovery period after its end (analysis VI). It is believed that the contribution of the α_1-globulin fraction to the blood proteinogram changes dramatically in certain diseases accompanied by destructive and inflammatory processes, in traumatic tissue damage, allergies, and in stressful situations. The changes noted by us obviously have no clinical basis, since they are insignificant and, most likely, are associated with general arousal caused by the appearance of the sun after a four-month polar night and the early end of wintering.

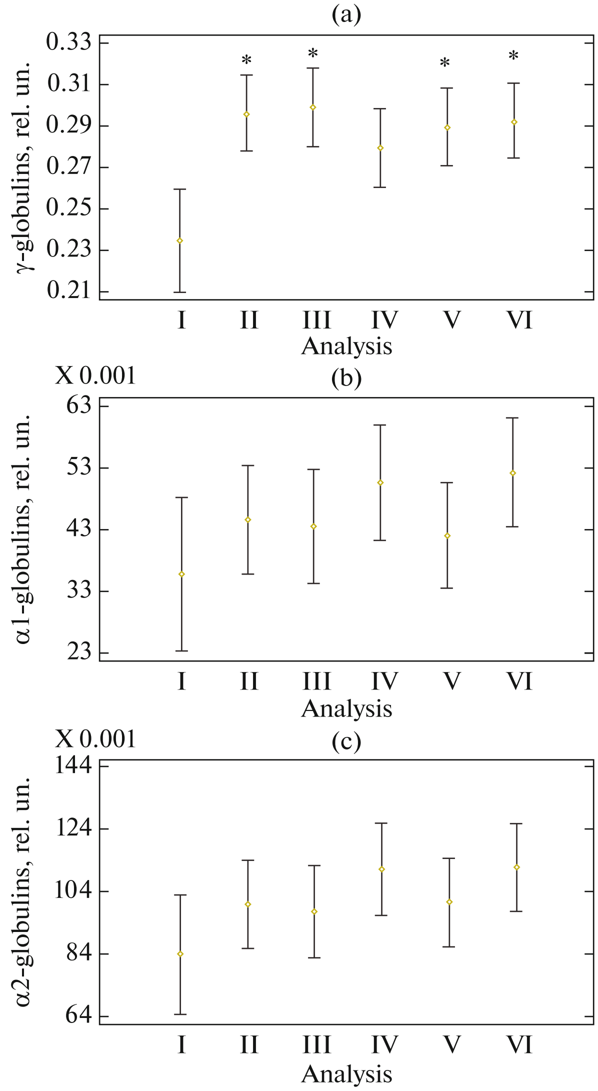

Fig. 1. Dynamics of the mean albumin-normalized values of the protein fraction in the blood: (a) γ-globulins; (b) α_1-globulins; (c) α_2-globulins. Here and in Fig. 2, * indicates significant differences in values in comparison with the results of the analysis I, $p < 0.05$.

α_2-Globulins

The fraction of α_2-globulins is also combinatorial, it includes acute phase proteins (their concentrations prevail in the group, and therefore they are considered to be basic). The fraction includes α_2-macroglobulin (the main protein of this group), which is involved in immune reactions when infectious agents enter the body and develop inflammatory processes. Another component of the glycoprotein fraction is haptoglobulin, which forms a complex compound with hemoglobin released from erythrocytes. Haptoglobin protects iron from loss by the body during its natural excretion, serves as a transport protein for vitamin B12. The fraction of α_2-globulins also includes ceruloplasmin, a metalloglycoprotein that specifically binds (up to 96%) and transfers copper. It has antioxidant capacity as well as oxidase activity against vitamin C, serotonin, and norepinephrine. This fraction also contains apolipoprotein B, a carrier of low-density lipoproteins and a transport protein of vitamin D, which delivers it to target tissues.

The results of the conducted studies indicate that during the one year stay at the station, the levels of the α_2-globulin fraction in many of the subjects were either at the lower limit or slightly below the physiological norm. In the experimental group, a tendency towards an increase in the levels of the α_2-globulin fraction in the blood was observed in the second half

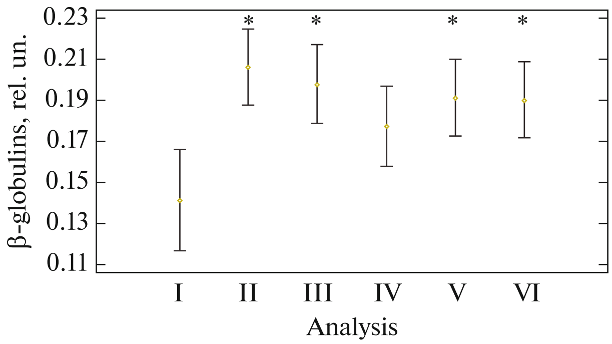

Fig. 2. Dynamics of the mean albumin-normalized values of the β-globulin fraction in the blood of polar explorers.

of wintering, apparently, due to the intake of pharmaceuticals.

This tendency was reflected in the dynamics of the mean albumin-normalized values of the α_2-globulin fraction in the blood for the entire aggregate group of polar explorers. Figure 1c shows that after the end of the acute period of adaptation to the conditions of the Vostok station, a tendency to an increase in the levels of α_2-globulins in the blood was observed.

β-Globulins

The levels of the β-globulin fraction during the annual wintering period only rarely deviated slightly from the limits of the normal range, and most often it was in the direction of increasing concentration. The dynamics of the mean albumin-normalized values of the β-globulin fraction in the subjects' blood is shown in Fig. 2, from which it follows that starting from the 4th month of wintering (analysis II), the levels of β-globulins in the blood increased ($p < 0.05$). It is reasonable to associate changes in the concentration of this fraction exclusively with transferrin. This protein is an important member of a large group of iron regulatory proteins that control iron homeostasis. Transferrin transports iron to the reticuloendothelial system, to the parenchymal cells of the liver and to all proliferating cells of the body, where it leads to the absorption of iron by the cells through receptor-mediated endocytosis [3]. A change in its synthesis is considered a sign of an acute phase reaction. The acute phase reaction is the main physiological protective reaction of the body to tissue/cellular damage, aimed at eliminating the consequences of damage and restoring homeostasis, including that caused by hypobaric hypoxia. This response is mediated by several signaling cascades and leads to an increase in plasma levels of a number of "positive" acute phase proteins, including blood coagulation proteins, transport proteins, antiproteases and complement factors, with a parallel decrease in "negative" proteins such as albumin and transferrin [4]. There is also a decrease in serum iron levels in an acute phase reaction [5].

Since iron in the body is an important cofactor for the transport of oxygen, heme and non-heme iron-containing proteins, electron transfer, synthesis of neurotransmitters, energy metabolism and mitochondrial function in organs [6], it is possible to assume a change in the level of the transferrin-containing fraction to be the result of the tension in the oxygen transport system. Indeed, significant hypobaric hypoxia, which is one of the unfavorable wintering factors at Vostok station, should naturally have led to an increase in the level of transferrin and stimulation of erythropoiesis in the process of adaptation to it.

Properdin

Properdin is a protein of β- and γ-globulin fractions involved in an alternative pathway for complement activation. The properdin system has an antibacterial effect against many pathogenic and opportunistic microorganisms, inactivates herpes and influenza viruses. The content of properdin in the blood to a certain extent reflects the state of nonspecific resistance of the body.

It should be noted that the properdin concentration in the blood of most polar explorers at all stages of research was at the lower limit of the norm. The dynamics of this indicator in both groups is shown in Fig. 3. There is a tendency, on the verge of significance, towards an increase in the properdin concentration in the experimental group, especially since in some periods of research (I, II, IV) the content of properdin in the blood significantly differed between the groups. However, it is impossible to associate this difference with the intake of pharmacological preparations, since even in study I conducted before the start of pharmacological correction, the properdin concentration in the blood of the subjects of the experimental group was significantly higher than in the control group. Most likely, this reflects the individual characteristics of persons who fell into different groups.

Analysis of the results of studies of the total protein content and the main blood protein fractions indicates that during a person's yearlong stay under the effects of hypobaric hypoxia, hypokinesia, and isolation, the values of the studied parameters did not go beyond normal values, although for such parameters as γ-globulins and β-globulins, from the fourth month of wintering (analysis II), statistically significant fluctuations in the levels of their content were observed, mainly toward the increasing. Similar data were obtained in an experiment with 370-day stay of the subjects under conditions of antiorthostatic hypokinesia (HDBR experiment) [7]. In this experiment, no statistically significant changes were found in the subjects and in the content of immunoglobulins [8].

All these data indicate a fairly high level of reserve capabilities of the body, in particular, about its nonspecific humoral resistance in extreme conditions. Indeed, under the conditions of an annual wintering at the Vostok station and in the 370-day HDBR experiment, neither infectious nor any clinically significant diseases were observed. It is possible that in the experiment with HDBR, the factor supporting nonspecific resistance was physical activity, as in the group with more intense physical activity the level of resistance was higher [8]. It can be assumed that, under wintering conditions at the Vostok station, the mechanisms supporting nonspecific resistance and immunoreactivity of the body were based on reactions induced by hypobaric hypoxia.

It is known that chronic hypobaric hypoxia leads to a number of physiological adaptation reactions to maintain O_2 homeostasis, including metabolic reprogramming of cells [9]. The reorganization of the metabolic mechanism, with the predominant use of glucose as the main energy substrate, is necessary to reduce oxygen consumption and maintain redox homeostasis during prolonged oxygen deficiency. Many physiological reactions in long-term chronic hypoxia are mediated at the transcriptional level, stimulating multiple effects at the cellular and systemic levels [10].

Changes in the functions of the main physiological systems under hypoxic conditions are also reflected in the many times described biochemical signs: deviations in the concentration of hemoglobin [11, 12], lactate, and free fatty acids in blood [13].

Hypoxia modulation of the functions of immunocompetent cells in their natural niches has been confirmed by several works [14, 15]. At the Concordia French–Italian Antarctic station, located at an altitude of 3233 m, the reactions of immunomodulation in the human body were studied under conditions of hypobaric hypoxia. Ex vivo tests for receptor-dependent and receptor-independent functional innate and adaptive immune responses indicated dynamic immune activation and the implementation of a two-stage escalation/activation scheme. The early phase was characterized by moderately sensitized global immune responses, while after three to four months of wintering at the Concordia station, immune responses were sharply increased. These functional observations were reflected at the level of gene transcription, in particular, in modulating hypoxia-induced pathways. Therefore, the adaptation mechanism of the immune system under these conditions was epigenetic [16, 17]. These data agree with results of our research, indicating a certain stimulating effect of hypobaric hypoxia on the state of humoral factors of nonspecific resistance of the body.

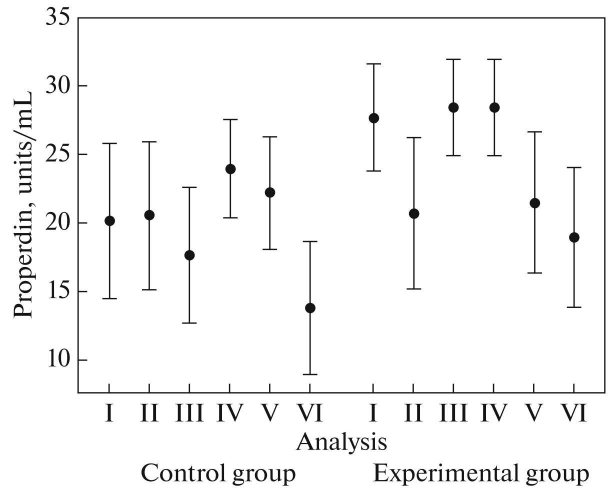

Fig. 3. Dynamics of changes in the content of properdin in the examined control and experimental groups during wintering at the Vostok station.

CONCLUSIONS

(1) The body's regulatory systems ensured the maintenance of normal physiological levels of protein and the main protein fractions of the blood throughout the year of a person's stay in conditions of hypobaric hypoxia, hypokinesia, and isolation.

(2) Starting from the 4th month of wintering, there was a statistically significant increase in the content of γ- and β-blood globulins (within the standard values), which remained until the second month of the readaptation period.

(3) Probably, there is the positive effect of hypobaric hypoxia on the humoral factors of nonspecific resistance of the body in conditions of prolonged limitation of motor activity and isolation.

(4) There were no statistically significant differences in research results between the control and experimental groups of subjects. The vitamin and amino acid complex taken to prevent and correct the psychophysiological status of polar explorers did not have any significant effect on the content of total protein and the main protein fractions of blood during wintering at the Vostok station.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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