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INDICATORS OF PERIPHERAL BLOOD AT EXPERIMENTAL COLD EXPOSURE

ABSTRACT

The article presents the results of an experimental study, the purpose of which was to study changes in the cellular composition of the blood of rats depend of their exposure time to cold. The obtained results indicate that the cold affects the activity of cells that provide nonspecific and specific immune responses.

It is shown that the exposure of rats in our experiment for 7 to 30 days had a fairly long-term effect, as it led to suppression of the activity of monocytes and neutrophils. However, we observed an increase in the number of leukocytes on day 14, which may be due to a short-term stimulation of leukocytopoiesis. The number of lymphocytes in our study remained elevated throughout the experiment, maximally increasing by 7 and 30 days. We were also able to demonstrate a decrease in the number of platelets, which was an appropriate reaction of the body in response to cold exposure, as platelets take a direct part in the repair processes observed when the tissue is damaged by cold, and also improve the migration of leukocytes to the focus of inflammation. An increase in hematocrit was also established, which is one of the signs of a reaction to cold stress.

Thus, the present study revealed patterns of changes in the cellular composition of peripheral blood during cold stress in the experiment, which were expressed in the features of the reaction from erythrocytes, platelets and leukocytes.

Keywords: cold stress, cellular blood composition, specific and nonspecific immune response, experiment.

Introduction. In connection with the increased rates of development of the Far North, the question of adapting the human organism to life in northern latitudes, where it is exposed to low natural temperatures, is again becoming relevant [7, 12].

It is known that the human body to the negative impact of various environmental factors corresponds to a violation of the state of regulatory systems, accompanied by a change in the cellular composition of blood [1, 5, 13, 14]. It is shown that leukocytes play an important role in the implementation of the protective reaction of the body. The phagocytic activity of leukocytes is a nonspecific cellular immunity of the body and depends on the effect of any stress factors [10, 13, 14]. Further, this is accompanied by changes in immunological reactivity, a decrease in the adaptive capacity of the organism, the development of transient or persistent forms of secondary immune deficiency [12].

Imbalance of the immune system affects the formation, nature of the course and outcomes of many pathological processes. The highest incidence rates fall on diseases of the respiratory, nervous and sensory organs, and the circulatory system [4, 9, 15].

Thus, the study of the mechanisms of cellular adaptation of the blood system, as well as the response of immune organs, the search for ways to increase the body's resistance, prevention and treatment of immune response disorders in low-temperature conditions is extremely urgent in the Sakha (Yakutia) Republic.

This study is part of a comprehensive work related to the study of the mecha-

nisms of disadaptation in the Arctic and the Subarctic. This work is carried out taking into account the priority areas of the scientific platform "Immunology" of the state program "Strategy for the Development of Medical Science in the Russian Federation 2025".

The aim of the study is to study changes in the cellular composition of blood in rats in the experiment, depending on cold exposure time.

Materials & Methods.

Work has been completed at the Department of Normal and Pathological Anatomy, Operative Surgery with Topographic Anatomy and Forensic Medicine at the M.K. Ammosov North-Eastern Federal University's Medical Institute. 25 male mongrel rats weighing 200-250 g were used as experimental animals, aged 5-6 months. The animals were divided into 4 groups: 1 group as control, which were kept in standard vivarium conditions; groups 2, 3, 4, 5 were animals that were exposed to cold. The cooling was carried out in the climatic chamber "Vestfrost" (Denmark) at $-10 \pm 20^\circ\text{C}$ [2, 7, 8] for 1 hour daily for 7, 14, 21, and 30 days.

The protocol of the experimental part of the studies, used at the stages of animal maintenance, modeling pathological processes and removing them from experience, was consistent with the principles of biological ethics set out in the International Recommendations for Biomedical Research with Animals (1985); The European Convention for the Protection of Vertebrates used for experiments or other scientific purposes (Strasbourg, 1986); Order of the Ministry of Health of the USSR No. 755 of 12.08.1977 "On

Measures to Further Improve Organizational Forms of Work Involving Experimental Animals"; Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003 "On Rules of Laboratory Practice".

The rats were decapitated in accordance with the requirements of humanity in accordance with Appendix No. 4 "On the Procedure for Euthanasia (Killing) of an Animal" to the Rules for carrying out work using experimental animals (annex to the Order of the Ministry of Health of the USSR No. 755 of 12.08.1977). Blood samples and serum were obtained during decapitation of animal. Blood sampling was carried out in glass tubes with anticoagulant heparin in an amount of 5 ml from the abdominal cavity on 7, 14, 21, 30 days. Hematologic examinations were carried out immediately after obtaining samples on the automated hematological analyzer Abacus Junior 30, biochemical studies were made on the Mindray BA-88A biochemical analyzer with ready-made solutions of High Technology. The study of blood and blood serum was performed in the scientific and research clinical diagnostic laboratory for agricultural and domestic animals at the Yakut State Agricultural Academy.

Results & Discussion. As a result of the experiment, it was established that the blood cell counts in the control group of animals kept under optimal temperature conditions were not accompanied by deviations from the physiological norm. While when assessing the condition of the experimental groups of rats, changes in the number of formed elements (from 0.5 to 1.3%) were detected, including a decrease in the level of hemoglobin,

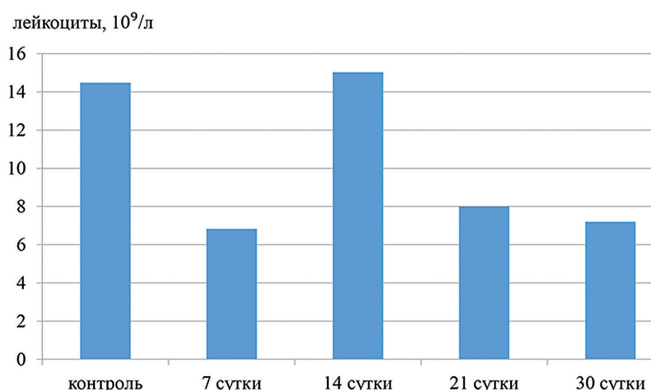


Fig.1. Changes in the total number of leukocytes in experimental animals in comparison with the control group

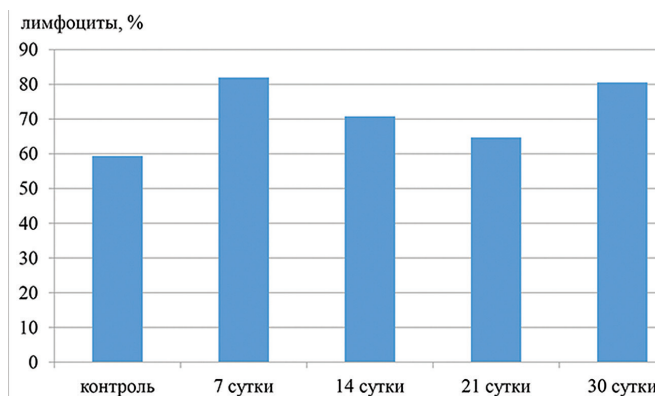


Fig.2. Changes in the relative amount of lymphocytes in the experiment in comparison with the control group

granulocytes, MCHC, an increase in the number of hematocrit and MCV.

The results of the study indicate that cold exposure affects the activity of cells that provide responses to a nonspecific and specific immune response. Thus, the total number of blood leukocytes decreases on days 7, 21 and 30 at 52.9; 44.8; 50.4%, respectively, despite the fact that on the 14th day there was a slight increase (by 3.7%) (Fig. 1).

At the same time, the number of lymphocytes remains elevated throughout the experiment, maximally increasing by 7 and 30 days (by 22.6% and 21.2%, respectively) (Fig. 2).

MID – the indicator reflecting the content of a mixture of monocytes, eosinophils, basophils and immature cells is reduced by 7.1% on day 7; on the 21st day by 45.6%; and 30 days by 62.1%; and on the 14th day there was a slight increase of 2.1%. The number of granulocytes (eosinophils, neutrophils and basophils) also decreases on the 7th, 14th, 30th day of the experiment, and on the 21st day it corresponds to the indicator in the control group (Fig. 3).

A significant decrease in the total number of leukocytes on days 7 and 21 and 30 indicates that cold exposure is indeed a stress factor for warm-blooded animals. This correlates with the studies of E.G. Kostolomova, where it is shown that populations of isolated COCs react differently to the duration of cold exposure [6]. Short-term cooling is a factor that activates the functional activity of monocytes and neutrophils, and prolonged cooling is a depressant. In the studies of V.M. Nikolaev. It was also noted that when adapting rats to hypothermia, the indices of nonspecific cellular immunity associated with phagocytic activity of leukocytes change [7]. Statistically significant decrease in the average number of absorbed particles by

leukocytes in both the first and second groups of experimental animals testifies to the suppression of nonspecific cellular immunity under the influence of negative temperatures. We will assume that the exposure of rats in our experiment for 7 to 30 days, which results in the suppression of leukocyte activity, was quite long. However, the increase in the number of leukocytes on day 14 (by 3.7% to control and 59.6% compared to the index on day 7) is associated with a short-term stimulation of leukocytopoiesis. In the experiments of T.V. Abarashova et al., it was also shown that male Wistar rats subjected to combined (cold) swimming in water at a temperature of + 70C showed an increase in the number of leukocytes, mainly due to a sharp increase in granulocytes. The authors claim that as a result of the action of the cold factor, a specific immune response is stimulated, and reactions of the nonspecific response mediated by leukocytes are suppressed [11].

There was also a significant decrease in the number of platelets after exposure to cold on the body. The observed changes in the hematological status of these

animals are consistent with the literature data, which describes the decrease in the number of platelets, as a reaction to stress [17]. The decrease in the number of platelets seems to be an appropriate reaction of the organism in response to cold exposure, as platelets take a direct part in the repair processes that occur when the tissue is damaged by cold, and also improve the migration of leukocytes to the focus of inflammation. Being a highly active metabolite of arachidonic acid, it is a potent inhibitor of aggregation of the latter. Also, The authors indicate a decrease in the enzymatic and mediator potential of blood cells, as a result of which their ability to form aggregates decreases [16].

The increase in hematocrit and erythrocytes is one of the quantitative characteristics of the physiological "adaptation" of the organism to the new conditions of life. Increased hematocrit and simultaneous enhancement of the synthesis of erythropoietin, stimulating the maturation of red blood cells, may be one of the signs of a reaction to stress, in our case, cold [3].

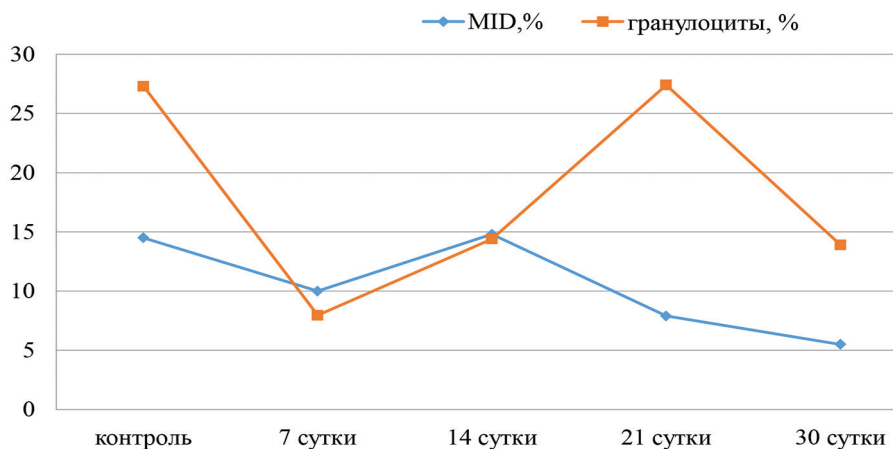


Fig.3. Changes in MID and granulocyte counts during the experiment

Conclusion

Thus, cold exposure is a stress, expressed in the reaction from the whole cellular composition of the blood. As a result of the action of the cold factor, a specific immune response is stimulated, as indicated by an increase in the amount of lymphocytes in the blood, and reactions of the nonspecific response mediated by other types of leukocytes are suppressed. Reducing the number of platelets is also represented as an appropriate response of the body in response to cold exposure, as platelets take a direct part in the repair processes that occur when the tissue is damaged by cold. Increased hematocrit indicates an increase in the proportion of blood cells relative to plasma, and is a reliable criterion for responding to stress.

Further research will concern the study of the processes of proliferation and differentiation of immunocompetent cells of organs and tissues of the lymphoid complex (spleen, thymus, lymph nodes, MALT of hollow organs) under exposure to cold.

References:

1. Agadzhanian N.A. Stress i teoriya adaptatsii [Stress and theory of adaptation] Orenburg, 2005, pp. 60-94.
2. Venkovskaya E.A. Shilo A.V. Babichuk G.A. Izmenenie adaptatsionnykh sposobnostey krysa posle ritmicheskikh holodovykh vozdeystviy [Change in Adaptive Capabilities of Rats After Rhythmic Cold Exposures] Vestnik biologii i mediciny. Vyp. 2, p. 38-42.
3. Vychuzhanova E.A. Vliyaniye khronicheskogo stressa na ostruyu stress-reaktsiyu u kris [The effect of chronic stress on acute stress reaction in rats] Nauka i obrazovanie: problemi, idei, innovatsii: Nauka i obrazovanie. [Science and Education: Problems, Ideas, Innovations: Science and Education], 2015, pp. 9-11.
4. Golderova A.S. Zaharova F.A. Alekseev S.N. Osobennosti nespecificheskoi adaptivnoi reaktsii u bolnykh s ostroi holodovoi travmoi [Features of nonspecific adaptive response in patients with acute cold trauma] Yakutskii meditsinskii zhurnal [Yakut medical journal]. Yakutsk, 2009, №1 (25), pp. 7-9.
5. Kaznacheev V.P. Sovremennye aspekty adaptatsii [Modern aspects of adaptation]. Novosibirsk: Nauka, 1980, 190p.
6. Kostolomova E.G. Sopryazhenost' immunofiziologicheskikh reaktsii makroorganizma i izolirovannykh immunokompetentnykh kletok pri razlichnykh rezhimakh kriovozdeistviya [Conjugacy of immunophysiological reactions of a macroorganism and isolated immunocompetent cells under various regimes of cryo-exposure]: avtoref. dis. ...kand. biol. nauk: 14.01.01]. Moscow, 2011, 171 p.
7. Nikolaev V.M. Izmeneniya prooksidantno-antioksidantnogo ravnovesiya v otvetnykh ekologo-biohemicheskikh reaktsiyah organizma zhivotnykh i cheloveka na deystvie holoda [Changes in prooxidant-antioxidant equilibrium in response to ecological-biochemical reactions of the animal and human organism to cold action]: avtoref. diss. na kand. biolog. n. / V.M. Nikolaev. Yakutsk, 2007, 112 p.
8. Obuhova L.A. Strukturnyye preobrazovaniya v sisteme limfoidnykh organov pri deystvii na organizm ehkstremaalno nizkikh temperatur i v usloviyakh korrektsii adaptivnoy reaktsii polifenolnymi soedineniyami rastitel'nogo proiskhozhdeniya: avtoref. dis. dokt. med. nauk. [Structural Transformations in the System of Lymphoid Organs with the Action on the Organism of Extremely Low Temperatures and in Corrective Conditions for Adaptive Reaction by Polyphenolic Compounds of Plant Origin: author's ABSTRACT. ... Doctor of Medical Sciences]. Novosibirsk, 1998, 43 p.
9. Petrova P.G. [i dr.]. Rol ekspeditsionnykh issledovaniy v izuchenii zdorovya naseleniya Arktiki [The role of expeditionary research in the study of the health of the Arctic population] Vestnik Severo-Vostochnogo federal'nogo universiteta imeni M.K. Ammosova: Seriya Meditsinskii nauki [Bulletin of the M.K. Ammosov North-Eastern Federal University Series Medical sciences], 2017, pp. 28-35.
10. Sapin M.P. Nikityuk D.B. Immunaya sistema, stress i immunodefitsit [Immune system, stress and immunodeficiency], Moscow: APP «Dzhanger», 2000, 84 p.
11. Abrashova T.V. [i dr.]. Spravochnik. Fiziologicheskie, biohemicheskie i biometricheskie pokazateli normy eksperimentalnykh zhivotnykh [Directory. Physiological, biochemical and biometric indicators of the norm of experimental animals]. Saint-Petersburg: LEMA, 2013, 116 p.
12. D.D. Savvinov [i dr.]. Sreda obitaniya i zdorove cheloveka na Severe [Habitat and human health in the North]. Novosibirsk: Nauka, 2005, 100 p.
13. Petrov R.V. Immunologiya [Immunology]. Moscow: Medicina, 1982, 636 p.
14. Haitov R.M. Pinegin B.V. Istamov H.I. Ekologicheskaya immunologiya [Ecological immunology]. Moscow: VNIRO, 1995, 219 p.
15. Chelovek na Severe: sistemnie mehanizmy adaptatsii: sb. tr., posvyaschenniy 20-letiyu NIC «Arktika» DVO RAN pod obschei redaktsiei zasl. deyatelya nauki d.m.n. prof. A.L. Maksimova. [Man in the North: Systemic Mechanisms of Adaptation: Sat. tr., dedicated to the 20th anniversary of the SIC "Arctic" FEB RAS under the general editorship of the hon. the scientist d.m.n. prof. A.L. Maksimov]. Magadan: SVNC DVO RAN, 2011, V.2, 162 p.
16. Shapalov K.G. Mihailichenko M.I. Sizonenko V.A. Funktsionalnoe sostoyaniye trombocitov pri mestnykh holodovykh porazheniyakh [Functional state of thrombocytes in local cold lesions] Kazan. med. zh. [Kazan medical journal]. Kazan, 2008, V.89, №5, pp. 662-665.
17. Shahmatov I.I. Vliyaniye odnokratnoi immobilizatsii razlichnoi intensivnosti na reaktsii sistemi gemostaza [The effect of a single immobilization of different intensity on the response of the hemostatic system]. Byull. SO RAMN [Bulletin of the SB RAMS], 2011, V.31, №4, pp. 33-36.

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