Доклады Национальной академии наук Беларуси

2016 **июль–август** **Том 60 № 4**

UDC 576:577:615.8:536.764

V. I. BAIKOV[1], M. L. LEVIN[1], N. V. GERASIMOVICH[2], A. D. CHORNY[1], A. A. MAKHANEK[1]

SECOND-KIND PHASE TRANSITION IN HUMAN LYMPHOCYTES MEMBRANE STRUCTURES UNDER EXTREME GAS COLD EXPOSURE

(Communicated by Academician S. A. Chizhik)

[1]A. V. Luikov Heat and Mass Transfer Institute of the National Academy of Sciences of Belarus, Minsk, Belarus
baikov@hmti.ac.by; marklvn@belhard.com; anchor@hmti.ac.by; amakh@tut.by
[2]A. D. Sakharov International State Ecological Institute of the Belarusian State University, Minsk, Belarus
nvgerasimovich@mail.ru

A thermodynamic analysis of the after-effects of low temperatures on the structural characteristics of membranes of peripheral blood lymphocytes of human was made. Structure changes in membranes are outlined using the theory of second-kind phase transitions. Based on the analysis made, the trend in raising the immunological status of the body of athletes after a whole-body cryotherapy course is explained from the viewpoint of decreasing a value of Young's modulus of peripheral blood lymphocytes, reducing the microviscosity of annular lipid of peripheral blood and of occurring processes similar to second-kind phase transitions in plasmic membranes of lymphocytes.

Keywords: cryotherapy, lymphocytes membrane, second-kind phase transition.

В. И. БАЙКОВ[1], М. Л. ЛЕВИН[1], Н. В. ГЕРАСИМОВИЧ[2], А. Д. ЧОРНЫЙ[1], А. А. МАХАНЕК[1]

ФАЗОВЫЙ ПЕРЕХОД ВТОРОГО РОДА В СТРУКТУРАХ МЕМБРАН ЛИМФОЦИТОВ ЧЕЛОВЕКА ПРИ ЭКСТРЕМАЛЬНОМ ГАЗОВОМ ХОЛОДОВОМ ВОЗДЕЙСТВИИ

[1]Институт тепло- и массообмена им. А. В. Лыкова НАН Беларуси, Минск, Беларусь
baikov@hmti.ac.by; marklvn@belhard.com; anchor@hmti.ac.by; amakh@tut.by
[2]Международный государственный экологический институт им. А. Д. Сахарова БГУ, Минск, Беларусь
nvgerasimovich@mail.ru

Проведен термодинамический анализ последствия влияния низких температур на структурные характеристики мембран лимфоцитов периферической крови организма человека. Структурные изменения мембран описаны следуя теории о фазовых переходах второго рода. На основе проведенного анализа тенденция повышения иммунологического статуса организма (на примере спортсменов) после прохождения курса общей криотерапии объясняется с точки зрения уменьшения значения модуля Юнга лимфоцитов периферической крови, снижения микровязкости аннулярного липида периферической крови и прохождением в плазматических мембранах лимфоцитов процессов, подобных фазовым переходам второго рода.

Ключевые слова: криотерапия, мембраны лимфоцитов, фазовый переход второго рода.

Introduction. Top-class sports at an elite level exerts an inhibitory effect on the immune system, which leads to the body's adaptation disturbances and, accordingly, to the decrease in sports results [1]. It is found that the frequency of acute and chronic diseases of athletes in top competition form sharply increases [2]. This reduces their athletic potential and the ability to achieve the best sports result. Thus, the immune system is one of the key systems that provide the body's healthy and normal metabolism during intense physical exercises [3]. The study of the mechanisms of the body's stability to the action of various physical factors is of great social and medical significance. Owing to this, of special interest are low-temperature treatment methods [4]. The application of whole-body gas cryotherapy (WBGC) showed that in the first instance, it has a non-specific generally stimulating effect on the human body and allows the polar states of the most important body systems to be corrected [5]. For example, it is adopted to treat allergy and immunodeficiency. In this case, the scheme of procedures in the both cases is the same, but as a result, the activity of the immune system moves to the normal state of the human

body. The decisive role in the effects achieved is played by the receptor reaction and then by the vascular response to cooling [6]. The analysis of the dynamics of immunological indices of a cellular component shows that the body's immunological status raises after a course of whole-body cryotherapy of both men and women; at the same time, the gain of magnitude of their physical efficiency is seen [7; 8]. However the literature lacks for the information about the molecular mechanisms of the WBGC effects.

In this connection, the objective of the present work was to make a thermodynamic analysis of the after-effects of low temperatures on the structural characteristics of membranes of peripheral blood lymphocytes of donors.

Materials and methods. In works [9; 10], the influence of whole-body aerocryotherapy on the structural-functional state of the peripheral blood lymphocytes of elite athletes is analyzed.

Whole-body cryotherapy sessions took place in the Cryospice sauna of the Medizintehnik Firm (FRG). First, elite athletes in groups of 3–4 persons were for 30 sec in the pre-chamber with an air temperature of –60 °C, then they entered the main chamber with a temperature of –110 °C according to the recommendations [11] allowing for the individual characteristics of patients. The whole-body cryotherapy course included 10 procedures. The blood sampling was made prior to a WBGC course, just after it, and 3 weeks later. For tests, blood was sampled in 10 ml plastic tubes (EDTA was used as anticoagulant). Lymphocytes were isolated according to the standard methods [12]. The number of viable cells found from the trypan blue test (0.2 % solution of dye) was at least 96 % in control.

The elastic properties of the cellular membrane of peripheral blood lymphocytes were determined by the atomic-force miscroscopy method and were assessed with the use of Young's modulus *E* that characterizes the ability of a cell to deform (table 1).

T a b l e 1. **Elastic properties of the cellular membrane of peripheral blood lymphocytes**

Index	Prior to WBGC course	After WBGC course	3 weeks later after WBGC course
E, kPa	197.89 ± 23.49	98.59 ± 16.25	150.37 ± 20.73

Many cells of the body respond to changing the environment through involving the internal adaptation mechanisms that have not been fully investigated up to now. Adaptation reactions of cells are either short, or long; in the first case, the adaptation is provided mainly by membrane mechanisms. It is found that the viable cell functions are strongly affected by the phase state of membrane lipids that ensure fluidity, stability, and permeability of biological membranes [13].

The analysis of the structure state of plasmic membranes of peripheral blood lymphocytes showed that the polarity values of annular lipid (PAL) and lipid bilayer (PLB) of lymphocyte membranes underwent cryotherapy practically did not change in comparison with those obtained prior to the low-temperature impact (table 2).

T a b l e 2. **WBGC influence on the indices of the structure state of lymphocyte membranes, rel. unit**

Conditions	Index				
	Polarity		Microviscosity		Quenching degree of protein fluorescence, %
	annular lipid	lipid bilayer	annular lipid	lipid bilayer	
Prior to WBGC course	0.76 ± 0.04	0.96 ± 0.2	0.68 ± 0.02	0.70 ± 0.01	41.38 ± 4.82
After WBGC course	0.74 ± 0.04	0.88 ± 0.08	0.25 ± 0.02*	0.52 ± 0.03*	22.67 ± 3.85*
3 weeks later after WBGC course	0.75 ± 0.01	1.09 ± 0.05	0.20 ± 0.03*	0.54 ± 0.04*	18.86 ± 5.20*

N o t e. * – the values are reliable at $p \leq 0.05$.

The study of the microviscosity of annular lipid of plasma membranes of peripheral blood lymphocytes after cryotherapy revealed a reliable decrease in this index by a factor of more than 2.5 in comparison with its value prior to the temperature impact. It was also found that the degree of quenching tryptophan fluorescence due to pyrene decreased by a factor of approx. 2 in comparison with its value prior to cryotherapy. The similar behavior was observed 3 weeks later after WBGC (table 2).

It is known [13; 14] that in biological membranes the bilayer is micro heterogeneous, and the formation of lipid molecule clusters in this layer contributes to the emergence of such a phenomenon as phase separation in the membrane. Lateral separation of lipid molecules in the bilayer plane is an important feature of the membrane.

It is also shown [14] that a number of biophysical factors help change – increasing or decreasing a phase transition temperature in the membranes of peripheral blood lymphocytes of patients.

Based on the analysis of the experimental data [9; 10], the following conclusions can be made:

1. Significant differences in the indices of Young's modulus (twice) and the annular lipid micro-viscosity (by a factor of more than 2.5) of the cytoplasmic membrane of peripheral blood lymphocytes after a WBGC course in comparison with its value prior to the temperature impact reflect the changes in the molecular structure of the membrane that occur when affected by WBGC.

2. The changes in the structure of the lymphocytic membranes of human peripheral blood occur in the physiologic temperature region and persist for a long time.

Following Landau's theory [15], call such a change in the membrane structure the second-kind phase transition and describe it. The body's state changes at phase transitions. This state can be characterized by a certain internal parameter.

Represent a lymphocyte in the form of an isotropic solid cylinder of length L at elastic deformation conditions when acted upon by a tensile (compressive) external force f. The governing equation of thermodynamics for such a system, written in terms of the Gibbs energy, is of the form:

$$d\Phi(T, p, l) = -SdT + Vdp + fdl,$$

or in the alternative form

$$d\Phi^*(T, p, f) = -SdT + Vdp - ldf,$$

where the analog of the Gibbs energy $\Phi^*(T, p, f) = \Phi(T, p, l) - f(l - l_0)$.

It is obvious that the tensile (compressive) force f acting upon the cylinder can be expressed as follows:

$$f = \tau\Sigma, \tag{1}$$

where Σ is the cross-sectional area of the cylinder and τ is the force per unit cross-sectional area (stress). In practice, the change in body sizes under deformation is usually expressed through a relative elongation:

$$\varepsilon = \frac{l - l_0}{l_0}.$$

Here l_0 and l is the cylinder length in the absence and in the presence of load, respectively.

The equation of state of an elastically deformable solid cylinder, which is widely used in the theory of elasticity, is represented by Hooke's law:

$$\tau = E\varepsilon, \tag{2}$$

where E is the modulus of elastic stresses (Young's modulus). Then for the analog of the Gibbs energy, taking into account formulas (1), (2), the expression is obtained:

$$\Phi^*(T, p, \tau) = \Phi(T, p, \varepsilon) - \tau\varepsilon V_0,$$

where V_0 is the cylinder volume in the absence of load.

Now describe the behavior of lymphocytes. Experience shows that, when the physiological temperature θ decreases, the state of lymphocytes changes continuously, but at some value of θ lymphocytes acquire a qualitatively new property – Young's modulus E for them sharply decreases. Consider a lymphocyte near the temperature θ when the change of Young's modulus is not happening yet. In this case, the relative elongation ε can be considered small and the function $\Phi(T, p, \varepsilon)$ can be expanded in a power series of ε. This series will not contain odd powers since the Gibbs energy Φ cannot depend on the sign of ε: $\varepsilon > 0$ at tension, $\varepsilon < 0$ at compression. Then not limiting to the terms not exceeding ε^4, we have

$$\Phi^*(T,p,\tau)=\Phi_0(T,p)+\frac{1}{2}\alpha(T,p)\varepsilon^2+\frac{1}{4}\beta(T,p)\varepsilon^4-\tau\varepsilon V_0. \quad (3)$$

The conditions of equilibrium and stability of such a thermodynamic system require the first derivative with respect to ε be equal to zero:

$$\frac{\partial\Phi^*}{\partial\varepsilon}=\alpha(T,p)\varepsilon+\beta(T,p)\varepsilon^3-\tau V_0=0, \quad (4)$$

and the second derivative be greater than zero:

$$\frac{\partial^2\Phi^*}{\partial\varepsilon^2}=\alpha(T,p)+3\beta(T,p)\varepsilon^2>0. \quad (5)$$

Lymphocytes not exposed to stress (τ = 0). Analyze the applied field influence on the properties of phase transition of lymphocytes. For lymphocytes not exposed to stress (τ = 0), from equation (4) we have

$$\varepsilon(\alpha+\beta\varepsilon^2)=0.$$

This expression has two roots

$$\varepsilon=0,\quad \varepsilon^2=-\alpha/\beta.$$

If $\alpha(T,p)>0,$ then the state of equilibrium $\varepsilon=0$ is stable since for it

$$\frac{\partial^2\Phi^*}{\partial\varepsilon^2}=\alpha(T,p)>0.$$

Therefore, at $\alpha(T,p)>0$ Young's modulus *E* does not change and phase transition is not observed. The second solution $\varepsilon^2=-\alpha/\beta$ in this case is unstable since for it

$$\frac{\partial^2\Phi^*}{\partial\varepsilon^2}=\alpha-3\alpha=-2\alpha<0.$$

For $\alpha(T,p)<0$ the solution becomes stable

$$\varepsilon=\sqrt{-\alpha/\beta},$$

provided that $\beta(T,p)>0.$ This is what we will assume. Thus, we have a spontaneous change of Young's modulus or the second-kind phase transition.

The equation $\alpha(T,p)=0$ yields the line of the points of the phase transition θ(*p*) on the (*T*, *p*) plane. For temperatures *T* close the phase transition temperature θ, it can be assumed

$$\alpha(T,p)=\alpha'(p)(T-\theta),$$

at that $\alpha'(p)>0.$ The coefficient $\beta(T,p)$ can be replaced by $\beta(p)=\beta(\theta,p).$ Expansion (3) then assumes the form:

$$\Phi^*(T,p,\tau)=\Phi(T,p)+\frac{1}{2}\alpha'(p)(T-\theta)\varepsilon^2+\frac{1}{4}\beta(p)\varepsilon^4-\tau V_0\varepsilon. \quad (6)$$

It is known that second-kind phase transitions are characterized by a specific heat jump. Define this jump in the considered case, i.e., at τ = 0. Then proceeding from equation (6) and considering relation (4), we arrive at:

$$S=-\frac{\partial\Phi^*}{\partial T}=\left(\frac{\partial\Phi^*}{\partial T}\right)+\left(\frac{\partial\Phi^*}{\partial\varepsilon}\right)\left(\frac{d\varepsilon}{dT}\right)=-\frac{\partial\Phi}{\partial T}-\frac{1}{2}\alpha'(p)\varepsilon^2=S_0-\frac{1}{2}\alpha'(p)\varepsilon^2.$$

In the phase where Young's modulus does not change, we have ε = 0 and $S=S_0$ and in the phase where it changes, we obtain:

$$\varepsilon^2=-\frac{\alpha'(p)}{\beta(p)}(T-\theta) \quad (7)$$

and

$$S = S_0 + \frac{\alpha'(p)}{2\beta(p)}(T - \theta). \tag{8}$$

Directly at the phase transition point, expression (8) reduces to $S = S_0$. Now the heat capacity of the both phases at the transition point for p and τ ($\tau = 0$) can be determined as:

$$C_P = T\left(\frac{\partial S}{\partial T}\right)_P.$$

For the phase where Young's modulus changes, upon differentiation of expression (8), we have:

$$C_P = C_{P0} + \frac{\alpha'^2(p)}{2\beta(p)}\theta.$$

For the phase where Young's modulus does not change, $S = S_0$ and hence $C_P = C_{P0}$. Hence, the heat capacity jump is found

$$C_P - C_{P0} = \frac{\alpha'^2(p)}{2\beta(p)}\theta.$$

Since $\alpha'(p) > 0$ and $\beta(p) > 0$, at the transition point $C_P > C_{P0}$, i. e., heat capacity grows.

Lymphocytes exposed to stress ($\tau \neq 0$). Consider how the properties of phase transition of lymphocytes exposed to the action of the tensile (compressive) applied field τ change. In this case, we should proceed from expansion (6) showing that the condition of equilibrium (4) of such a system is of the form:

$$\alpha'(p)(T - \theta)\varepsilon + \beta(p)\varepsilon^3 = \tau V_0. \tag{9}$$

Then, an arbitrarily small force τ leads to the fact that the parameter ε becomes different from zero over the entire temperature range, i.e., the difference between the both phases disappears. Hence, a discrete point of phase transition also disappears – transition 'diffuses'. In particular, instead of the heat capacity jump there appears an anomaly existing within some temperature interval. Assess the order of magnitude of this interval. We will proceed from the requirement $\alpha'(p)(T - \theta)\varepsilon \sim \tau V_0$.

The value of the parameter ε will be defined from formula (7):

$$\varepsilon \sim \sqrt{\frac{\alpha'(p)}{\beta(p)}(T - \theta)}.$$

Then we will have (fig. 1)

$$T - \theta \sim \tau^{2/3}\frac{\beta^{1/3}(p)V_0^{2/3}}{\alpha'(p)}.$$

To analyze the phase transition quantitatively, the conditions of equilibrium (9) and stability (5) must be studied. Define the susceptibility of lymphocytes to the action of the applied field as a derivative

$$\xi = \left(\frac{\partial \varepsilon}{\partial \tau}\right)_{\tau \to 0}.$$

Differentiating relation (9) yields

$$\frac{\partial \varepsilon}{\partial \tau} = \frac{V_0}{\alpha'(p)(T - \theta) + 3\beta(p)\varepsilon^2}.$$

From here, as $\tau \to 0$, for the phase where Young's modulus for lymphocytes does not change, i. e., $\varepsilon = 0$ and $\theta > T$, we obtain

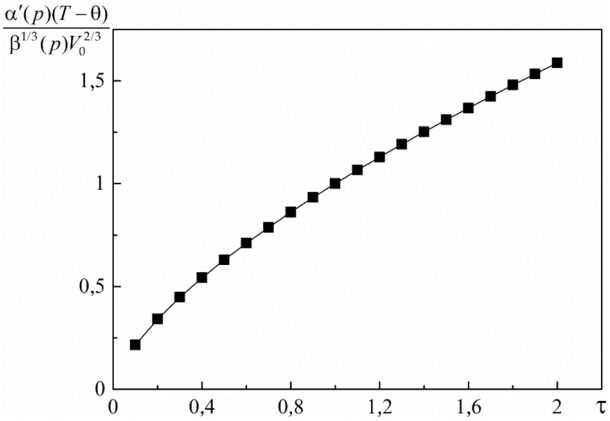

Fig. 1. Anomaly of the temperature range of heat capacity jump vs. the applied field action

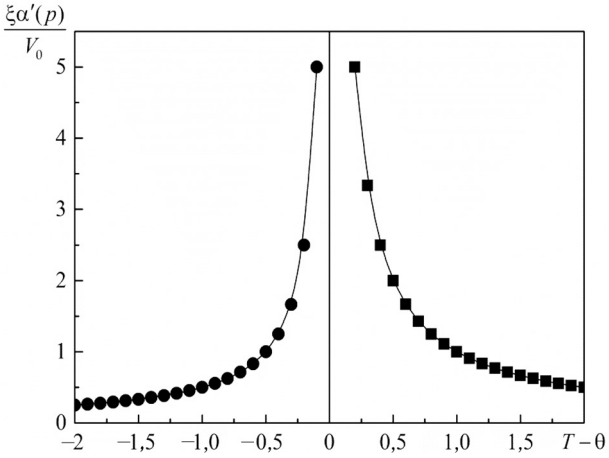

Fig. 2. Lymphocyte susceptibility vs. the difference between the considered temperature and the phase transition temperature

$$\xi = -\frac{V_0}{2\alpha'(p)(T-\theta)}.$$

Hence, the susceptibility of lymphocytes to the action of the applied field is inversely proportional to the difference between the temperature considered and the phase transition temperature (fig. 2).

Thus, it seems possible to follow the trend: raising the immunological status of the body of athletes after a whole-body cryotherapy course → decreasing a value of Young's modulus of peripheral blood lymphocytes → reducing the microviscosity of annular lipid of peripheral blood → second-kind phase transition in plasmic membranes of lymphocytes.

Tables 1 and 2 demonstrate that after the WBGC course, the structural and mechanical properties of membranes gradually return to their initial state. It can be assumed that the thermodynamic state of cell membrane structures under the action of extreme cold exposure to the human body is unstable.

Within the framework of the above-mentioned approach it is assumed to study the dependence of the second-kind phase transition temperature and the change of the heat capacity of structure components of blood cell membranes on the concentration of certain biochemical substances [14] determined on the basis of the common blood analysis and the hormone and immune status of patients undergone the WBGC course.

References

1. *Suzdalnitsky, R. S.* Manifestation of immunodificient states in sports and their corrections / R. S. Suzdalnitsky // Selected Lectures on Sports Medicine. – Moscow: Natyurmort, 2003. – P. 119–134.

2. *Mackinnon, L. T.* Immunity in athletes / L. N. Mackinnon // Int. J. Sports Med. – 1997. – Vol. 18, N l. – P. 62–68.

3. *Taimazov, V. A.* Sports and immunity / V. A. Taimazov, V. N. Tsygan, E. G. Mokeeva. – Saint-Petersburg: Olimp, 2003. – 200 p.

4. *Portnov, V. V.* Whole-body and local air-cryotherapy / V. V. Portnov // 2nd revised, expanded ed. – Moscow, 2008. – 51 p.

5. *Kiriyanova, V. V.* Clinical aspects of use of whole-body cryotherapy / V. V. Kiriyanova // Proc. II Scientific-Practical Conference "Cryotherapy in Russia", 14 May 2009. – Saint-Petersburg, 2009. – P. 127–131.

6. *Belous, A. M.* Cryobiology / A. M. Belous, V. I. Grishchenko. – Kiev: Navukova dumka, 1994. – 432 p.

7. *Dragun, V. L.* Cryotherapy is a new method of increasing the physical performance and adaptative capabilities of the body / V. L. Dragun, M. L. Levin, E. A. Lositsky // Ekolog. Vest. – 2011. – Vol. 17, N 3. – P. 68–74.

8. Whole-body cryotherapy in athletes / G. Banfi [et al.] // Sports Med. – 2010. – P. 509−517.

9. Influence of whole-body aerocryotherapy on the structural and functional state of membranes of peripheral blood lymphocytes of athletes / M. L. Levin [et al.] // VI International Scientific-Practical Conference "Cryotheraphy in Russia", 16 May 2013. – Saint-Petersburg, 2013. – P. 44–50.

10. Physical-chemical characteristics of membranes of peripheral blood lymphocytes upon whole-body cryotherapy / N. V. Gerasimovich [et al.] // Ekolog. Vest. – 2014. – Vol. 28, N 2. – P. 50–53.

11. A way of increasing the physical performance of an athlete (variants) / V. L. Dragun [et al.] // Eurasian Patent No. 019337. – Date of Grant of Patent 28.02.2014 // EAPO Bulletin "Inventions (Eurasian Applications and Patents)". – 2014. – N 2.

12. Lymphocytes. Methods / ed. G. G. Klaus. – Oxford: Oxford University Press, 1990.

13. *Gennis, R. B.* Biomembranes: molecular structure and functions / R. B. Gennis. – New York: Springer-Verlag, 1997.

14. *Kharakoz, D. P.* On a possible role of the "liquid–solid" phase transition in biological membranes / D. P. Kharakoz // Uspekhi Biologicheskoi Khimii. – 2001. – N 41. – P. 333–364.

15. *Landau, L. D.* Statistical physics / L. D. Landau, E. M. Lifshits. – Moscow: Nauka Press, 1976. – 583 p.

Received 30.05.2016