

ERYTHROCYTES AS A MODEL FOR STUDYING THE EFFECT OF TEMPERATURE ON THE RESISTENCE OF CELLULAR MEMBRANES

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In this work the effect of different temperatures on erythrocyte resistance has been studied. It was also examined the influence of various temperatures on optic density change of erythrocyte suspension. It was shown that there are no sharp changes of optic density values of erythrocyte suspension, based on which we assume that under the effect of the given temperatures the significant alterations are not observed, but the state of erythrocytes changes, particularly the structure of erythrocyte membranes changes. It was also revealed that the structural changes of erythrocyte membranes occur under the impact of environmental factors, including anthropogenic ones.

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Introduction. Temperature is one of the important abiotic medium factors and the adaptation of living organisms to it is realized throughout physiological-biochemical mechanisms [1].

The studies of the effect of high temperatures on the erythrocyte properties started many years ago. The first studies were devoted to the examination of high temperature effect on physicochemical properties of red blood cells. Nowadays to protect the organism from the traumatic damages, to recovery the functions of organs and tissues (especially, heart and brain) after ischemic-reperfusion, to correct and cure various diseases of animals and human, hypothermia is applied successfully [2]. Decreasing of the rat body temperature up to 20°C induces the state of, so called, cool anesthesia with sharp suppression of mobility, metabolism intensity and disappearing of electric activity of brain [3].

Thus, it was implemented a study of high temperature effect on osmotic properties of erythrocytes. It was shown that the temperature increase of the samples of entire blood during 20 min up to 40°C, 45°C, 50°C and 55°C leads to changing of osmotic resistance of erythrocytes, which was estimated by lysis-test. When the temperature gets to 50°C, the differences in erythrocyte thermo-sensibility were

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established. The cells of “middle ages” were the least stable to high temperature action, i.e. those are cells that should have lived in hem-circulation system during two months in minimum [4]. The osmotic resistance change of erythrocytes in conditions of thermo-induction also was mentioned by other researchers [5].

In later study of Baar S. and Arrowsmith D.J. it was cleared out the thermo-sensibility of erythrocytes of various ages. The samples of whole blood were exposed to heating up to 49°C and 50°C in conditions of the same time exposition. Erythrocytes were divided by morphological changes into old and young ones, though the young erythrocytes demonstrated low thermo-resistance. It was shown that at the action of high temperatures the erythrocyte membrane elasticity changes. According to the opinion of the authors, the change of erythrocyte membrane elasticity was due to the membrane protein denaturation, meanwhile, the temperature diapason of irreversible transitions was determined between 46°C and 50°C [6].

Luo J.Z. et al. [7] showed that the heating of erythrocytes of healthy human blood during 20 *min* induces deformation of erythrocytes and the relevant change of cell form change and hemolysis took place in temperature diapason from 48°C up to 50°C.

Erythrocyte membrane proteins demonstrated various thermosensibility. Thus, denaturation of some integral proteins of erythrocyte membranes, particularly, Na⁺-K⁺ ATPase, occurred at temperatures higher than 54°C.

Temperature enhancement affected not only on deformation ability, but also on reversibility of erythrocyte aggregation at shift tension [8].

Comparatively recently there has been developed a new direction: the study of effects of chemical and physical factors on erythrocytes in conditions of thermo-induction. Thus, it was shown that the deformation-ability of erythrocytes at their heating up to 48°C decreased.

Joint action of hyperthermia and lanthan led to decreasing of erythrocyte aggregation *in vitro* [9]. Addition of sucrose into the incubation medium decreased thermo-induced lysis of erythrocytes [10]. Tarasova et al. [11] showed that the exposition of erythrocytes during 30 *min* at 50°C resulted in strengthening of saponin-mediated hemolysis, which according to the authors, indicated the participation of cytoskeleton proteins in this process.

It was shown that at the action of high temperatures the binding of erythrocytes to chemical compounds, particularly to bilirubin, changes. According to researchers, the observed effect is connected to the change of topography of erythrocyte membranes [12]. Leyko et al. established that the environment temperature enhancement in the interval 39–49°C increased the intensity of lipid peroxide oxidation in erythrocyte membrane [13].

The study of molecular mechanisms, participating in realization of the thermal damage of erythrocytes, was carried out. Thus, Gershfeld & Murayama expressed an opinion that the thermo-induced hemolysis of erythrocytes is mediated by the lipid bilayer state of membranes [14].

Erythrocyte is a flexible, elastic structure, which changes its form passing through the capillary. By the electronic microphotographs it was shown that erythrocytes are like homogenous structures or small-grained electronically dense

forms, covered by the layer with 6–12 *nm* thickness. Nowadays, it was shown that the erythrocyte membrane is heterogeneous in its different parts [15].

Erythrocyte important part is its plasmatic membrane. It functions as mechanical layer with regulating physical properties and simultaneously plays a role of coordinator for cell viability, depending on physical and chemical signals, thus implementing a key role in homeostasis determination and cell functional capacity. To retain its shape, erythrocyte has a protein skeleton of membrane, particularly it takes place through enzymatic phosphorylation of spectrin as well as formation of spectrin-actin complex (possibly other supra-molecular structures as well). Flexibility and fluidity of erythrocyte membrane are due to the presence of lipid bilayer, which functions as a liquid crystal in native membrane [16].

Erythrocyte membrane is a composite structure; its basis is composed of lipid bilayer with asymmetrically inserted proteins. Membrane proteins are capable of influencing the lipids, changing their molecular order and limiting the mobility of annular lipids, inducing the alteration of low-frequency oscillations of lipid phase, stimulating division of phases and contributing to asymmetric distribution of lipids [17]. Membrane lipids regulate the mobility and activity of intra-membrane proteins, providing a selective permeability of cell and normal functioning of membrane enzymes and receptors [18].

The work is aimed at the study of different temperatures on human erythrocyte resistance.

Materials and Methods. Erythrocyte resistance characterizes its ability to survive up to some limits of the effect of mechanical, chemical, osmotic, temperature factors, as well as to resist the damaging action of ultraviolet, ionizing and X-ray radiations. The given ability of blood cells depends on their age and decreases along with their aging.

Erythrocyte suspension was taken from the Blood Bank (Hematological Institute after Yolyan, Armenia). After it the suspension was diluted in physiological solution; the solution with 0.9 optic density and 100 *mL* volume was obtained. This solution was divided into 5 parts 20 *mL* each. The first solution was put in room temperature (25°C) as control. The second solution was put in thermostat at 35°C, the third one – 45°C, the fourth one – 15°C, the fifth one – 5°C. They were incubated during 30 *min*. After incubation period the erythrocyte resistance was studied through acidic hemolysis. This method was sufficiently simple and spread one for assessment of physicochemical properties of erythrocyte membranes that appeared in their stability toward the damaging effects. That is why the criterion of erythrocyte resistance can be used as characteristics of its state [19].

Erythrocyte membrane is the most successful model to investigate the dynamics of deviations, occurring in organism at pathology development. That is why among numerous criteria, characterizing the properties of erythrocytes, their resistance is the most important one – stability toward destroying effect of different factors, being an integral criterion and permitting judging about functional state of erythrocytes [20]. To determine the resistance of erythrocytes, the optic density of erythrocyte suspension is attained to 0.7 by physiological solution, at the same time controlling the dilution via photoelectrocolorimeter. After that the suspension with 2 *mL* volume was added to working cuvette, settled in photoelectrocolorimeter cell. Solution of 0.004 *N* HCl was added to working cuvette, rapidly stirring the content

of cuvette by a glassy stick and the timer was switched on. Optic density of erythrocyte suspensions, subjected to destruction resulted from acidic hemolysis, was measured after each 30 s. Destroyed erythrocytes settled down and a gradual decrease of the optic density (D) of erythrocyte suspension was observed.

If during two upcoming 30-second intervals the value of D on digital tabloid does not change, it indicates the end of hemolysis.

Percentage of destroyed erythrocytes in each moment of time was calculated according to the following formula:

$$E\% = \frac{\Delta D_i}{\Delta D},$$

where ΔD_i is the difference between current and previous values of optic density, $\Delta D = D_0 - D_n$ is the value, equal to difference between initial and terminal values of optic density during hemolysis process.

Based on the calculation data, the curve of the change of $E\%$ in time – erythrogram was constructed. We obtained the kinetics of erythrocyte hemolysis by this way as well. Hemolysis measure was a change of transmission light throughout erythrocyte suspension or optic density change. As a result, a number of values of optic density was obtained that decrease and each of them responses for the hemolysis degree in the measurement moment. Optic density decrease in time characterizes the decrease of erythrocyte amount under the effect of hemolytic compound [21].

Results and Discussion. The important function of erythrocyte membrane is barrier creation, allowing light transmission and realizing selective transport. High barrier properties were determine by membrane lipid bilayer. The main criterion of erythrocyte membrane stability is their resistance, stability toward the action of various factors. At extreme effects their characteristics change. Resistance characterizes structural-functional state of erythrocyte membrane; this fact has a permanent diagnostic value and is connected to the dissolution of one of the important topics of blood system physiology and pathology – study of qualitative composition of functioning erythrocytes. Values of the optic density of the solutions after 30 min incubation were presented in Fig. 1.

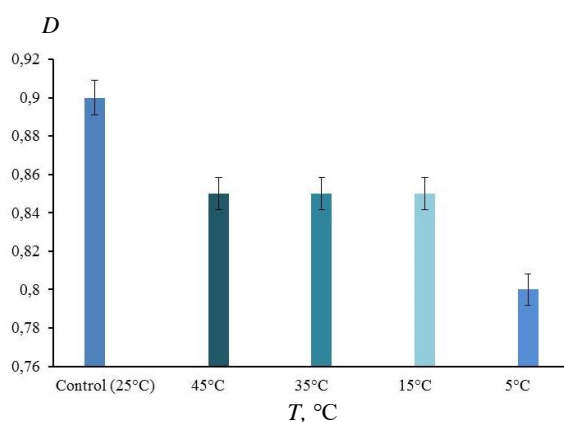


Fig. 1. Optic density of erythrocyte suspension after 30 min of incubation.

Analyzing the data, we have concluded that after 30 min incubation at different temperatures the optic density of the solutions does not change significantly.

At 35°C, 45°C and 15°C the optic density of the solutions changed by 5.5% as compared to the control and by 11.1% at 5°C. After incubation we carried out erythrocyte hemolysis. At decreasing of erythrocyte resistance up to minimum the hemolysis process started.

Despite the fact that after 30 *min* incubation we did not observe a sharp change in solution optic densities, the hemolysis process altered. Hemolysis curves at 25°C, 35°C and 45°C were presented in Fig. 2.

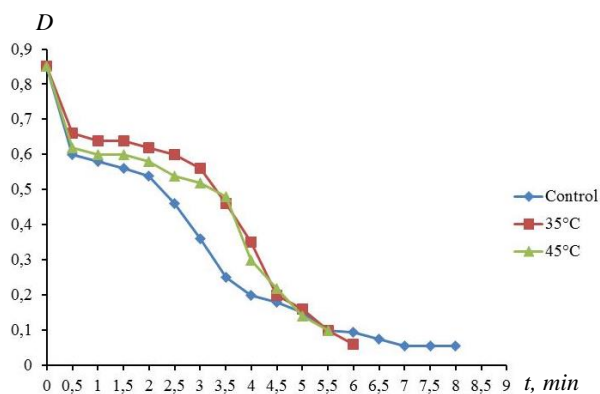


Fig. 2. Hemolysis curves at 25°C, 35°C and 45°C.

It is obvious from Fig. 2, that hemolysis duration is different – at 25°C it composes 8 *min*, 35°C – 6 *min* and at 45°C – 5.5 *min*. Most apparently, hemolytic acceleration process was connected to structural-functional changes of membranes under the impact of temperature. It also was not excluded that stressor influences result in shifts in membrane functional activities that are accompanied by conformational reconstructions in the structure of fatty-acidic content.

It was shown that the higher is non-saturated fatty acid composition in membranes, the more stable it is, moreover, fatty acids in phospholipid molecules are the most rapidly recovering components and their synthesis is under genetic control and depends on environment impacts [22].

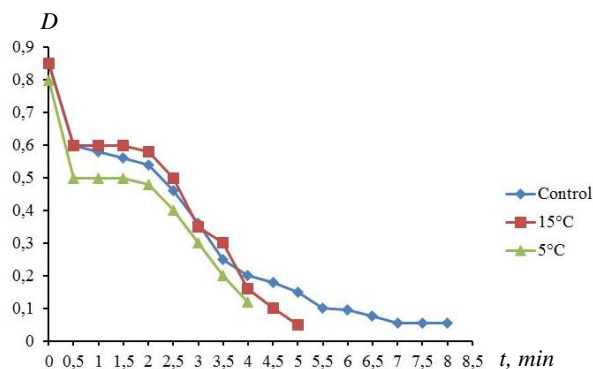


Fig. 3. Hemolysis curves at 25°C, 15°C and 5°C.

The results, obtained in this work, showed that at hyperthermia the erythrocyte resistance alters. It is obvious from Fig. 3, that hemolysis durations differ – at 15°C it is equal to 5 *min*, at 5°C – 4 *min*.

Strengthening of hemolysis indicated the erythrocyte membrane destabilization at temperature lowering, though, alteration in erythrocyte membranes at 5°C is more significant, than at 15°C. Gulevskii et al. showed that at preliminary low-temperature impact, the following effect was observed: Na⁺, Ca²⁺, Mg²⁺ ions, adsorbing on membranes, change its permeability for water and ions as well as mechanical properties [23]. Ions K⁺ and Ca²⁺ decrease the membrane stability.

Erythrogram peak of the control sample at 25°C was equal to 3 min. In this point about 20% of erythrocytes were exposed to hemolysis (erythrocytes with minimal stability in interval 2–3 min) (Fig. 4). At 35°C the erythrogram peak appeared from 3.5 min; erythrocytes with middle stability composed 24.5% in the interval 3.5–4.5 min. At 45°C the erythrogram peak appeared from 3 min. At this point about 32% of erythrocytes were exposed to hemolysis (erythrocytes with minimal stability in the interval 2–3 min). At 45°C we also observed a little peak in the region of highly-stable erythrocytes.

At 15°C the erythrogram peak appeared from 2 min. In this point about 26% of erythrocytes were exposed to hemolysis (erythrocytes with minimal stability in the interval 2–3 min). At 5°C the erythrogram peak was obtained after 2.5 min. In this point about 20.8% of erythrocytes were exposed to hemolysis (erythrocytes with minimal stability in 2–3 min).

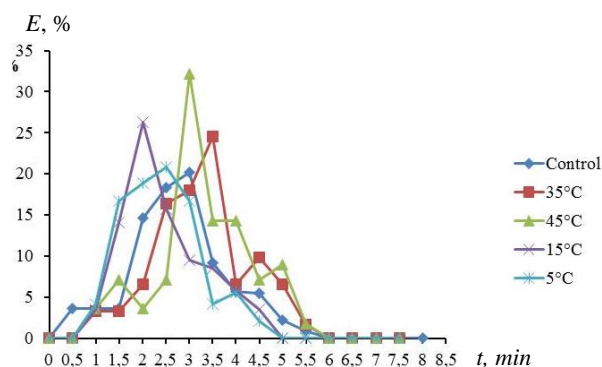


Fig. 4. Erythrograms obtained at acidic resistance at the temperatures 25°C, 35°C, 45°C, 15°C and 5°C.

Analysis of kinetics of acidic hemolysis curves and erythrograms permit determining the effect of different temperatures on distribution character of erythrocytes by their stability degree. Most apparently, the mentioned changes were connected to both the structural reconstructions of erythrocyte membranes, due to deviation of cellular metabolism and the decrease of stability of a number of erythrocytes; though it should be mentioned that at given temperatures (45°C, 15°C and 5°C) the part of erythrocytes with minimal stability enhances, but at 35°C the part of erythrocytes with middle stability enhances as well, but it should be mentioned that at 45°C we observed a little peak in the region of highly-stable erythrocytes.

Conclusion. It is concluded from the aforementioned that the erythrocyte state plays a role of a sensitive indicator of changes of physiological, biochemical and biophysical processes, resulted from the effect of environmental factors, including anthropogenic one. Measurement of acidic fragility of erythrocytes is an important method for investigations and diagnosis in medicine and is used to study the

mechanism of pathological processes and the action of several drugs and biologically active compounds.

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ԷՐԻԹՐՈՑԻՏՆԵՐԸ ՈՐՊԵՍ ՄՈՂԵԼ ԲՁՋԱԹԱՂԱՆԹՆԵՐԻ
ԴԻՄԱՑԿՈՒՄԻՆՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅԱՆ ՀԱՄԱՐ
ՉԵՐՄԱՍՏԻՃԱՆԻ ԱԶԴԵՑՈՒԹՅԱՆ ՆԵՐՔՈ

Աշխատանքում հետազոտվել է տարբեր ջերմաստիճանների ազդեցությունը էրիթրոցիտների դիմացկունության վրա: Ուսումնասիրվել է նաև տարբեր ջերմաստիճանների ազդեցությունը էրիթրոցիտների կախույթի օպտիկական խտության վրա: Ցույց է տրվել, որ էրիթրոցիտների կախույթի օպտիկական խտությունը կտրուկ չի փոխվում, սակայն տարբեր ջերմաստիճանները ազդում են էրիթրոցիտների դիմացկունության վրա, ինչից ելնելով, մենք ենթադրում ենք, որ տարբեր ջերմաստիճանների ազդեցության ներքո տեսանելի փոփոխություններ տեղի չեն ունենում, սակայն էրիթրոցիտների վիճակը փոխվում է, մասնավորապես, փոխվում է էրիթրոցիտների թաղանթի կայունությունը: Ցույց է տրվել նաև, որ էրիթրոցիտների թաղանթների կառուցվածքային փոփոխությունները տեղի են ունենում արտաքին միջավայրի գործոնների ազդեցության ներքո, այդ թվում նաև մարդածին գործոնների:

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ЭРИТРОЦИТЫ КАК МОДЕЛЬ ДЛЯ ИЗУЧЕНИЯ ВЛИЯНИЯ
ТЕМПЕРАТУРЫ НА РЕЗИСТЕНТНОСТЬ МЕМБРАН КЛЕТОК

В работе исследовано влияние разных температур на резистентность эритроцитов. Исследовано также влияние разных температур на изменение показателя оптической плотности суспензии эритроцитов. Показано, что резких изменений оптической плотности суспензии эритроцитов не происходит, однако, температура влияет на резистентность эритроцитов, из чего мы полагаем, что под влиянием разных температур видимых изменений не наблюдается, но меняется состояние эритроцитов, в частности, меняется структура их мембран. Показано также, что структурные изменения мембран эритроцитов происходят под влиянием факторов внешней среды, в том числе и антропогенных.