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STUDY OF ASPIRIN EFFECT ON STABILITY OF MEMBRANES OF RAT BLOOD ERYTHROCYTES BY ACIDIC HEMOLYSIS METHOD

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In this work the effect of aspirin on stability of rat blood erythrocyte membranes was studied, using acidic hemolysis method. Hemolysis rate of erythrocytes was shown to depend on preservation time of rat erythrocyte suspension. With increasing of preservation time the original value of optic density of rat erythrocyte suspension decreases and hemolysis time reduces. It was also shown that in the presence of aspirin the membrane stability of low-stable erythrocytes increases, moreover, hemolysis duration enhances, while aspirin leads to decreasing of membrane stability of both high-stable and enhanced-stable erythrocytes of rats.

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***Keywords*:** erythrocyte membrane, acetylsalicylic acid, erythrocyte stability, hemolysis duration.

Introduction. Enhanced interest of researchers to erythrocyte occurs by its participation in processes, connected to attaining of homeostasis on the level of whole organism. Being included in pathological process, erythrocyte changes its structure and function, depending on disease genesis.

Physicochemical properties of blood are characterized by relative constancy, which is necessary for contribution in optimal occurrence of physiological functions. Specific density is equal to 1050–1060 ($g \cdot L^{-1}$) for blood, 1090 for erythrocytes, 1030 for plasma. Blood viscosity is higher, than water viscosity by 4–5 times, than plasma by 1.7–2.2 times (water viscosity is equal to 1 conditional unit). At the increasing of protein and erythrocyte content in blood, the viscosity of the latter can increase up to 7–8 conditional units. Blood viscosity enhancement results in increasing of blood current resistance through vessels, which becomes a reason of blood pressure enhancement. Erythrocyte quantity, as other blood cells, is relatively constant for the certain species of animals, although it depends on age, physiological state of organism and environment conditions. Among multiple factors, determining erythrocyte shape, there divided into: system of membrane proteins (cytoskeleton); membrane lipid component, chemical composition and possible heterogeneity along membrane; concentration of ions; ATP, PO_2; electrostatic factors (membrane surface

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charge and ionization state of cytoskeleton proteins); hemoglobin molecule state, intracellular structures [1]. Security problem of drug measures becomes one from actual topics of healthcare in the world. It is induced by appearing of many drug preparations with high biological activity, enhanced by people, sensible to chemical and biological compounds, non-rational application of drugs, interaction of preparations with each other and with biologically active additions, using malignant preparations. As a result, many patients have heavy, irreversible complications, number of hospitalizations and lethal outcomes increase, induced by drug therapy.

Recent pharmaco-epidemiological studies showed that the complication problems of drug therapy are more serious, than it was assumed earlier [2, 3]. Recently carried out studies permit characterizing blood erythrocytes, erythropoiesis, regulation of differentiation processes of erythroid cell-precursors and regulation of hematopoiesis on systematic, molecular and cell-cell levels, in physiologically comfortable conditions and at changed states of organism [4]. Attaining of shape and deformability of erythrocytes depend on structural and functional integration of membrane proteins [5].

Aspirin is the first anti-aggregate preparation, which today is more known and widely applied preparation in clinical practices. Aspirin is a base for prophylactics and curing of atherothrombosis, as well as serious complications connected to it. Aspirin is observed as “golden” standard for testing of new anti-aggregate preparations. Aspirin acting compound is an acetylsalicylic acid (ASA) [6]. It was shown that aspirin affects physical properties of blood plasma [7]. The presented work is aimed at studying of ASA effect on hemolysis of rat erythrocytes.

Materials and Methods. The study was carried out on white rat-males with mass 100 *g* and their blood was used (1–2 *mL*). Blood was taken before feeding. The whole blood was centrifuged with acceleration 1500 *g* by 90-1 ELECTRIC CENTRIFUGE CAPACITY during 15 *min*. After centrifugation erythrocytes were placed in physiological solution. It is obtained a suspension of erythrocytes with optic density 0.66. In this solution ASA was added with amount 0.15 *mg*/*mL*. Added dose of ASA corresponded to moderate overdose. Erythrocyte solution without ASA with optic density 0.66 served as a control. Then hemolysis curves and erythrograms were obtained. Erythrocyte resistance study toward acidic hemolysis is sufficiently simple and spread method for estimation of their physicochemical properties, which is appeared in their persistence in relation to damaging effect. That is why erythrocyte resistance criterion can be used as a characteristic of its state [8].

Method of acidic erythrograms permits differentiating erythrocytes by chemical persistence. Depending on stability to acid action there divide into low-persistent, middle-persistent and high-persistent erythrocytes. Erythrogram shift can indicate about both breaking of bone marrow hematopoiesis and presence of such compounds in blood that change erythrocyte persistence. Erythrocyte membrane is more successful biological model for studying of breaking dynamics, taking place in organism at pathology development. That is why among many criteria, characterizing erythrocyte properties, the most important one its resistance – stability to breaking effects of different factors, being an integral criterion, allowing to judge about functional state of erythrocytes [9, 10].

For optic density measurement of erythrocytes the solution is diluted up to standard concentration by physiological solution, controlling the dilution process by photo-colorimeter. After that the solution with volume 2 *mL* with erythrocytes with standard concentration is added to working cuvettes. Solution of 0.004 *N* HCl with volume 2 *mL* is gathered by pipette, moved to working cuvette, quickly mixed in the cuvette by glassy stick and a second-meter is turned on. After each 30 *s* determination of solution optic density with erythrocytes was carried out that are exposed to breaking as a result of hemolysis under the effect of HCl. Broken erythrocytes settle and a gradual decrease of optic density of the solution was observed.

If during two intervals between 30 *s* the value of optic density on digital table of photocolorimeter does not change, it indicates the hemolysis termination. Distribution of erythrocytes by persistence is presented in percentage. Percent distribution of erythrocytes by their persistence is drawn graphically in percent difference of optic density:

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

where E is a percent distribution of erythrocytes by their persistence, ΔD_i is a difference between current and precursor values of optic density; $\Delta D = D_0 - D_n$ – value, equal to difference between initial value of optic density and that after finishing of hemolysis [11].

Results and Discussion. It should be mentioned that at carrying out of drug therapy, different complications emerge frequently, conditioned by unfavorable effects of drug preparation on patient organism. Such unfavorable effects are signed by term of "side effect of drug preparation" [3, 12]. Erythrocyte membrane is sufficiently successful biological model for investigation of dynamics of breaks, taking place in organism at pathology development. That is why among many criteria, characterizing erythrocyte properties, their resistance – stability to breaking effects of various factors that are integral criterion, is the most important, which permits judging about functional state of erythrocytes. Effect of drug preparation on hemolytic stability of erythrocytes was observed in conditions *in vitro* at addition of hemolytic agents into erythrocyte suspension, as compared to analogous criteria of probes without testing preparation.

For investigation of drug preparation effect on hemolytic properties of blood, ASA was chosen as a drug. As it was aforementioned, to estimate a drug preparation effect on erythrocytes a method of hemolytic stability determination was chosen. The experiment data were obtained, permitting constructing hemolysis curves and erythrograms of erythrocytes in the presence of drug preparation.

It was known that hemolysis of human erythrocytes, induced by 0.004 *N* solution of HCl, occurs from 6 to 7 *min* in norm.

Portion of cellular populations, breaking in intervals 0–1.5 *min* (low-persistent), 1.5–3 *min* (mid-persistent), 3–4.5 *min* (high-persistent), and from 4.5 up to hemolysis end (enhanced-persistent). The results are presented in Figs. 1–4.

As it is shown from Fig. 1, hemolysis rate of erythrocytes depends on suspension preservation time of rat erythrocytes. With increasing of preservation time the original value of suspension optic density of rat erythrocytes decreases and hemolysis time reduces. Quickly after preparation of erythrocyte suspension the

optic density was equal to 0.66; after 20, 40, 60 *min* of suspension preservation the optic density respectively decreases by 17.4, 53, 62%, respectively, which indicates the presence of spontaneous hemolysis during preservation period. Number of fully non-damaged erythrocytes decreases, due to which the suspension optic density decreases as well. The same dynamics of change is observed during hemolysis, particularly, if hemolysis is fresher, then the prepared erythrocyte suspension is equal to 5.5 *min*, hemolysis duration decreases up to 2.5 *min* due to preservation (up to 60 *min* of preservation). The aforementioned data indicate the spontaneous hemolysis, which in turn, indicates the decrease of membrane stability.

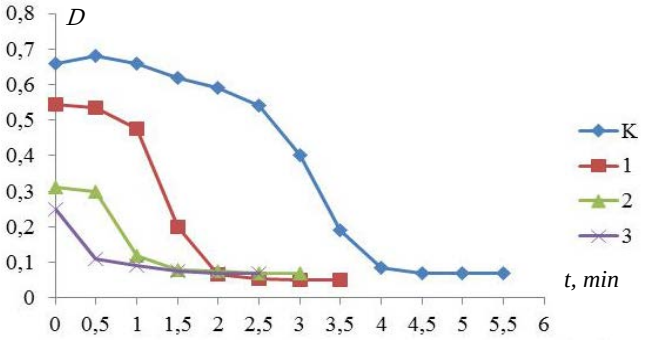

Fig. 1. Hemolysis curves of erythrocytes in suspension.
K – hemolysis curve after suspension preparation;
1 – hemolysis curve after 20 *min* from suspension preparation;
2 – after 40 *min*;
3 – after 60 *min*.

Analysis of curves in Fig. 2 showed, that in presence of ASA the membrane stability enhances, particularly, if after 40 *min* of erythrocyte preservation the optic density becomes equal to 0.31, at ASA effect during 40 *min* the suspension optic density composes 0.54, at the erythrocyte preservation during 60 *min* without ASA the original optic density is equal to 0.25, but at the effect of ASA it enhances up to 0.45.

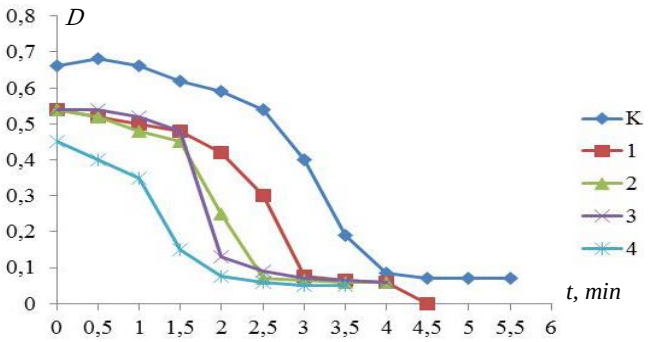

Fig. 2. Hemolysis curves of erythrocytes.
K – control;
1 – hemolysis curve after ASA addition;
2 – hemolysis curve after 20 *min* from addition of ASA;
3 – after 40 *min*;
4 – after 60 *min*.

The obtained data permit assuming that in the presence of ASA the spontaneous hemolysis is suppressed, due to which the suspension optic density does not sharply change, particularly, after 40 *min* from ASA addition it changes by 18% and after 60 *min* – by 31% and the hemolysis duration increases up to 3.5 *min* (to 60 *min* of preservation).

Analysis of data permits suggesting that in the presence of ASA the membrane stability of erythrocytes increases and the spontaneous hemolysis is suppressed apparently.

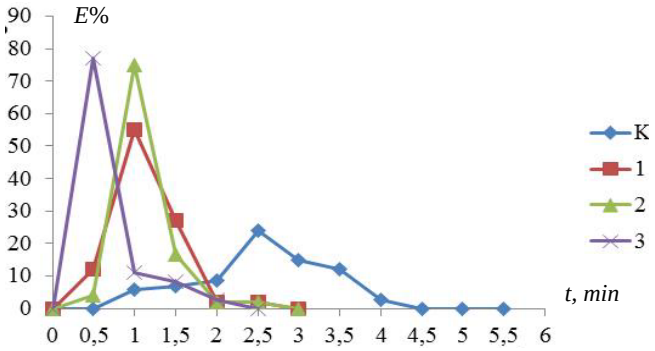

Fig. 3. Erythrograms of acidic resistance.
K – control, hemolysis of erythrograms in fresh suspension;
1 – erythrogram after 20 *min* from suspension preparation;
2 – after 40 *min*;
3 – after 60 *min*.

It was shown that the control erythrogram has a peak, appearing in 2.5 *min*. In the case of erythrocyte suspension preservation the change of curve shape is observed, which indicates the decrease of unstable erythrocyte number, while the other erythrocytes were subjected to structural changes.

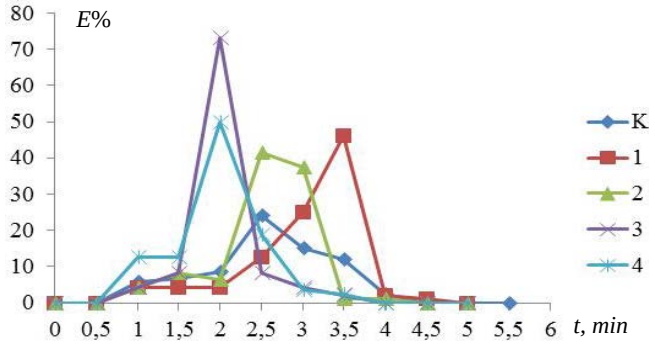

Fig. 4. Erythrograms of erythrocyte hemolysis.
K – erythrogram of hemolysis of fresh suspension;
1 – erythrogram after 20 *min* from addition of ASA;
2 – after 40 *min*;
3 – after 60 *min*.

Therefore, erythrocyte hemolysis at suspension preservation after 60 *min* takes place more intensively, than in control. Control erythrogram has a peak, appearing after 2.5 *min*. During time under the effect of ASA, the erythrogram peak after 20 and 40 *min* of ASA addition is shifted to right. One can assume that in membranes of enhanced-stability erythrocytes, structural changes occur that result in hemolysis, but one can mention that under the effect of ASA the resistance of low-stable and mid-stable erythrocytes increases.

Conclusion. Thus, analyzing the above mentioned data, one can assume that at moderate overdose of ASA an increase of membrane persistence to breaking effect is observed and the spontaneous hemolysis is suppressed. Solution of HCl stabilizes low-persistent and mid-persistent erythrocytes and destabilizes high-persistent and enhanced-persistent erythrocytes.

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ԱՌՆԵՏՆԵՐԻ ԱՐՅԱՆ ԷՐԻԹՐՈՑԻՏՆԵՐԻ ԹԱՂԱՆԹՆԵՐԻ ԿԱՅՈՒՆՈՒԹՅԱՆ ՎՐԱ ԱՍՊԻՐԻՆԻ ԱԶԴԵՑՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ ԹԹՎԱՅԻՆ ՀԵՄՈԼԻԶԻ ՄԵԹՈԴՈՎ

Աշխատանքում ուսումնասիրվել է ասպիրինի ազդեցությունը առնետների արյան էրիթրոցիտների թաղանթների կայունության վրա՝ կիրառելով թթվային հեմոլիզի մեթոդը: Ցույց է տրվել, որ էրիթրոցիտների հեմոլիզի արագությունը կախված է առնետների էրիթրոցիտների կախույթի պահպանման ժամանակից: Պահպանման ժամանակի աճի հետ նվազում է առնետների էրիթրոցիտների կախույթի օպտիկական խտության սկզբնական արժեքը և կրճատվում է հեմոլիզի ժամանակը: Ցույց է տրվել նաև, որ ասպիրինի առկայությամբ աճում է ցածր կայունությամբ էրիթրոցիտների թաղանթների կայունությունը, ընդ որում մեծանում է հեմոլիզի տևողությունը այն դեպքում, երբ ասպիրինը հանգեցնում է առնետների բարձր և գերբարձր կայունությամբ էրիթրոցիտների կայունության նվազմանը:

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ АСПИРИНА НА СТАБИЛЬНОСТЬ МЕМБРАН КРОВИ ЭРИТРОЦИТОВ КРЫС ПО МЕТОДУ ГЕМОЛИЗА

В работе исследовано влияние аспирина на стабильность мембран эритроцитов крови крыс с использованием метода кислотного гемолиза. Показано, что скорость гемолиза эритроцитов зависит от времени хранения суспензии эритроцитов. С увеличением времени хранения уменьшается исходное значение оптической плотности суспензии и сокращается время гемолиза. Показано также, что в присутствии аспирина повышается стабильность мембран низкостойких эритроцитов и при этом увеличивается продолжительность гемолиза, в то время как аспирин приводит к уменьшению стабильности мембран у высокостойких и с повышенной стойкостью эритроцитов крыс.