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INFLUENCE OF MILLIMETER RANGE ELECTROMAGNETIC WAVES ON HEMOLYSIS KINETICS OF RAT BLOOD ERYTHROCYTES

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The effect of millimeter range electromagnetic waves (MM EMW) on kinetics of acidic hemolysis of rat blood erythrocytes has been studied. It was shown that MM EMW affect the duration of erythrocyte hemolysis and this change depends on MM EMW exposure and frequency. At the irradiation by frequencies 41.8 and 42.2 *GHz* the acidic hemolysis occurs quicker at all durations of the irradiation compared to the control. At the irradiation by 50.3 and 51.8 *GHz* the hemolysis takes place quicker as well, as compared to the control at short durations of the irradiation. At the exposure 60 *min* the hemolysis start is delayed.

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***Keywords*:** millimeter range electromagnetic waves, irradiation, water resonant and non-resonant frequencies, hemolysis kinetics of erythrocytes.

Introduction. It is known that blood in organisms of higher animals, particularly erythrocytes play a key role in organism viability. A relative constancy of relation between young and old erythrocytes in blood is preserved, which has an important role in homeostasis establishment. Homeostasis provides a normal functioning of both erythrocytes and organism in general. When changing physicochemical properties of blood plasma or medium in which the erythrocytes are suspended, hemolysis of the latter is observed both in vivo and in vitro. Factors, changing physicochemical properties, are the chemical composition that induces osmotic pressure alteration; temperature oscillation in wide range; irradiation; electric current; vibration; toxins of biological origin. During hemolysis erythrocyte membrane is broken and hemoglobin comes out. It is possible to study this process spectrophotometrically, measuring the value of optic density (hemolysis is judged by change of light scattering in erythrocyte suspension in time). After hemolysis the erythrocyte shadows practically do not scatter light, but the optic density does not become equal to zero. During lysis process of cells and their turning to shadows, the light transmission increases up to 96–98%, but not to 100%, since the shadows in any case scatter the light a little. The scheme of erythrocyte hemolysis is presented in Fig. 1.

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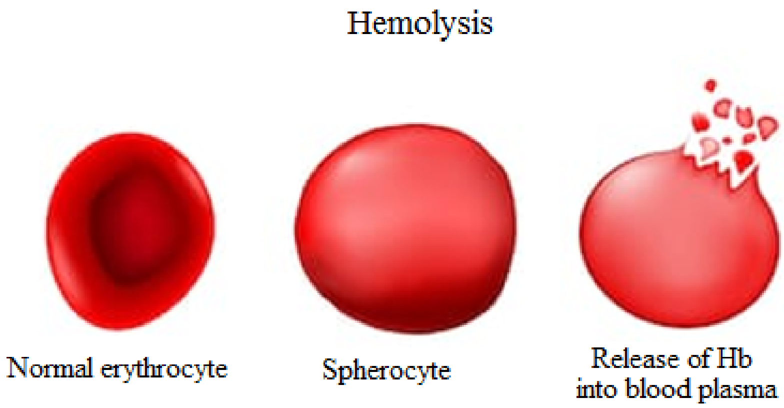

Fig. 1. Scheme of erythrocyte hemolysis.

Among numerous factors of environment, affecting living organisms that are on any level of organization, millimeter range electromagnetic waves (MM EMW) take a special place, their wavelength varies from 1 to 10 *mm*. Influence of MM EMW on biological objects has been studied relatively recently, but there exist many works devoted to the effect of these waves on genes [1–3], proteins and kinetics of enzymatic reactions [4–6]. EMW was shown to have various effects on biological systems that depend on strength, frequency and duration of MM EMW irradiation [7, 8]. Meanwhile, the mechanisms of MM EMW effects still remain an object of discussion [3–5, 9–11]. According to the concept of N.D. Devyatkov, MM EMW act by two modes – through energy of coherent waves and by information mechanism [12, 13]. It should be mentioned that these two mechanisms can overlap (the simultaneous occurrence of both mechanisms is possible). There are numerous works, studying the effect of MM EMW on different biophysical and physico-chemical parameters of whole blood and its components that insist changes invoked by these waves, particularly alterations of surface charge of erythrocytes, permeability of erythrocytes, surface tension of blood plasma etc. [14–16].

In the present work the effect of MM EMW on kinetics of acidic hemolysis of rat blood erythrocytes has been studied.

Materials and Methods. White, inbreed rats with weight 80–100 *g* were used in experiments. 4–5% solution of Na-citrate with volume 1 *mL* was added to animal blood with volume 10 *mL*. Then the blood was centrifuged during 3 *min* with 1500 *g* acceleration. Supernatant was removed and physiological solution was added to erythrocyte sediment, further centrifugation was repeated. Erythrocytes were eluted three-times by physiological solution. Obtained erythrocytes were suspended in physiological solution to receive their suspension with optic density 0.9 at λ=670 *nm*. Suspension was used to study erythrocyte resistance. Hemolysis was carried out using 0.004 *N* HCl. Hemolysis was judged by optic density change of suspension at wavelength 670 *nm*. Optic density measurements were carried out on photocolorimeter KPK-2 at 20°C. Optic density change was registered after each 30 *s* until obtaining of three repeating values. Then kinetic curves of optic density change of erythrocyte suspension were constructed. Source of irradiation of erythrocyte suspension was generator G4-141 with working frequencies 37.5–53.5 *GHz* and power flux density 60 $\mu Wt/cm^2$. Irradiation was carried out during 20, 40 and 60 *min* by frequencies 41.8, 42.2, 50.3 and 51.8 *GHz*. The non-irradiated suspension of rat blood erythrocytes served as a control.

Results and Discussion. Influence of MM EMW with various frequency and duration on hemolysis kinetics of rat blood erythrocytes has been studied. It should be mentioned that at λ=670 *nm*, the optic density change of erythrocyte suspension is due to only light scattering. If a decrease of the optic density of the erythrocyte suspension occurs, a quantity of non-damaged erythrocytes in suspension decreases, because after the hemolysis the formed erythrocyte shadows practically do not scatter light. EMW with frequencies 41.8 and 42.2 *GHz* affect the membrane structures of biological systems. As a result of EMW influence the peroxide oxidation of lipids is stimulated, the viscosity and permeability of membranes change. Kinetic curves of acidic hemolysis of rat blood erythrocytes at MM EMW irradiation of erythrocyte suspension by 41.8 *GHz* frequency are presented in Fig. 2.

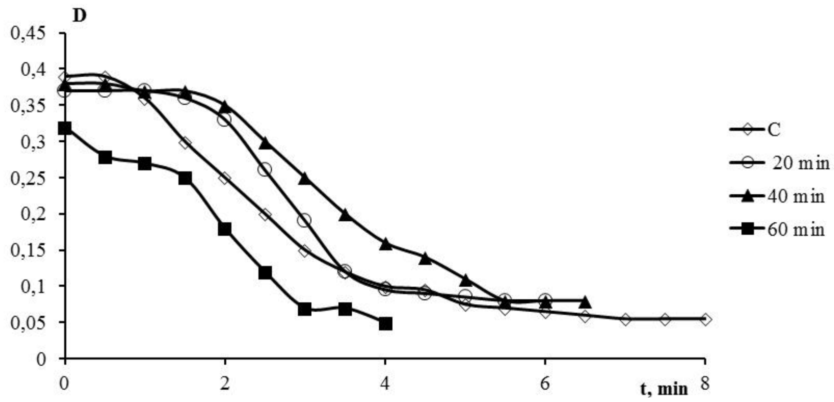

Fig. 2. Kinetic curves of hemolysis of rat blood erythrocytes exposed by MM EMW influence with 41.8 *GHz* frequency.

It is obvious from Fig. 2 that the effect degree and direction of changes, induced by MM EMW, depend on exposition time. Though, in all cases the hemolysis time is shortened. It should be mentioned that with exposition increasing the hemolysis time becomes less. Thus, at the suspension irradiation of erythrocytes with frequency 41.8 *GHz* during 20 *min* the spherulation period is equal to almost 2 *min*, while in control sample is ~1.5 *min*. At the irradiation during 40 *min*, the start of own hemolysis is after 2.3 *min*; during 60 *min* is after 2 *min*. Though, after own hemolysis start the process occurs quicker as compared to control. Consequently, the irradiation contributes to hemolysis of rat blood erythrocytes. The kinetic curves of erythrocyte hemolysis at the MM EMW irradiation with 42.2 *GHz* frequency are presented in Fig. 3.

MM EMW irradiation was shown to result in acidic hemolysis acceleration, moreover, with the increase of irradiation duration the hemolysis rate rises. In this case a delay of the spherulation phase is observed, and then quick hemolysis takes place. If in control sample the spherulation phase is about 1 *min*, at the irradiation during 20, 40 and 60 *min* it is about 2, 1.5 and 1 *min* respectively. It is assumed that the irradiation results in alteration of membrane structures of erythrocytes, particularly, the structures of ionogen groups of proteins or lipids change. These changes lead to the interaction strength alteration (electrostatic, van-der-Waals, etc.) of membrane molecules.

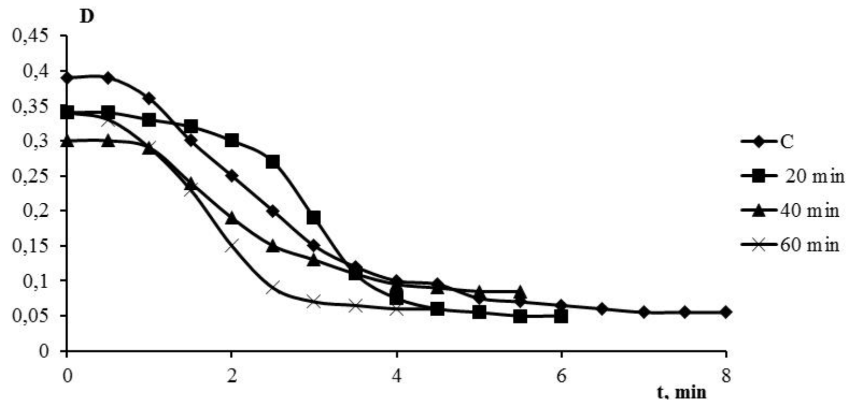

Fig. 3. Kinetic curves of hemolysis of rat blood erythrocytes exposed by MM EMW influence with 42.2 *GHz* frequency.

EMW with frequencies 50.3 and 51.8 *GHz* affect the water structure (resonant frequencies for water), consequently, the water component of biological fluids, including blood plasma (Figs. 4 and 5). The effect of MM EMW is explained by this fact. Irradiation of erythrocyte suspension revealed dependence of plasmatic membrane permeability on medium properties.

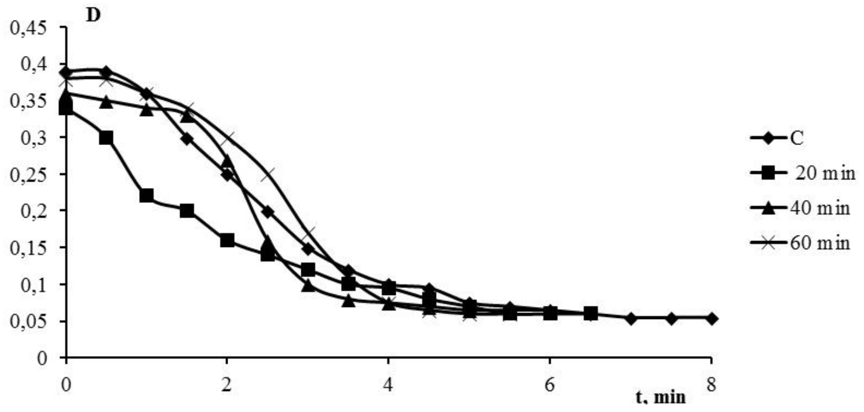

Fig. 4. Kinetic curves of hemolysis of rat blood erythrocytes exposed by MM EMW influence with 50.3 *GHz* frequency.

As it is presented in Figs. 4 and 5, differently directed changes of hemolysis curves take place, meanwhile with the increase of the irradiation duration the erythrocyte hemolysis occurs slower, than at 20- and 40-min exposures. Besides, the hemolysis duration increases at the irradiation with 51.8 *GHz* frequency and 60 *min* duration attaining to almost the same parameter of the control sample.

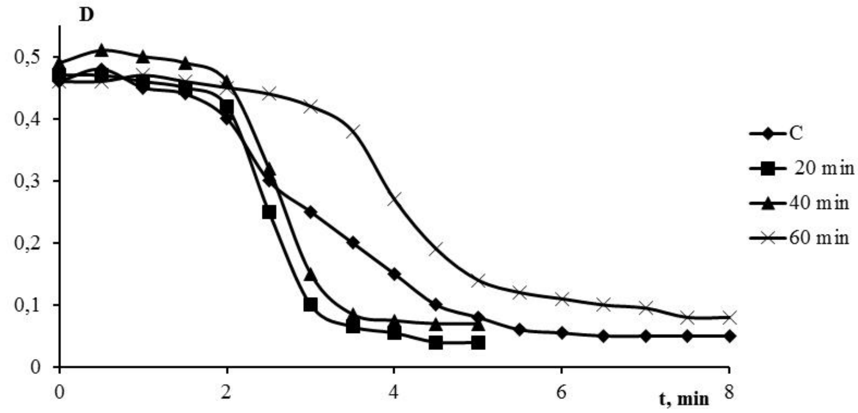

Fig. 5. Kinetic curves of hemolysis of rat blood erythrocytes exposed by MM EMW influence with 51.8 *GHz* frequency.

In the case of the irradiation by water resonant frequencies, the spherulation phase extension is longer, than in control samples. Most probably, MM EMW effect by water resonant frequencies and long duration changes the water structure, which in turn induces alteration of dimensional structure of membrane proteins, raises viscosity and decreases permeability of erythrocyte membranes in spherulation phase, which leads to delayed start of own hemolysis. We have suggested earlier that under the effect of MM EMW the erythrocyte membrane permeability increases, which is insisted by the obtained erythrograms [17, 18]. Based on this one can assume that MM EMW irradiation with 51.8 *GHz* frequency and 60 *min* duration leads to such changes in erythrocyte membranes, particularly in membrane structural groups that the resistance of erythrocytes enhances. At short durations of MM EMW irradiation, the protective mechanisms are not triggered and the acidic hemolysis is accelerated.

Conclusion. Taking into account that the experiments have been carried out in vitro, one can conclude that changes of duration and form of the hemolysis kinetic curves are induced by MM EMW irradiation. Value and direction of induced changes depend on exposure duration. At the irradiation by frequencies 41.8 and 42.2 *GHz* the acidic hemolysis occurs quicker at all durations of the irradiation compared to the control. At irradiation by resonant frequencies for water (50.3 and 51.8 *GHz*) the hemolysis takes place quicker as compared to the control at short durations of the irradiation. At the exposure 60 *min* the changes of plasmatic membrane properties of erythrocytes lead to the enhancement of duration, spherulation and delaying of own hemolysis start. MM EMW effect on erythrocyte membrane, most probably, occurs in directed or mediated ways.

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ВЛИЯНИЕ ЭЛЕКТРОМАГНИТНЫХ ВОЛН МИЛЛИМЕТРОВОГО ДИАПАЗОНА НА КИНЕТИКУ ГЕМОЛИЗА ЭРИТРОЦИТОВ КРОВИ КРЫС

Исследовано влияние электромагнитных волн миллиметрового диапазона (ММ ЭМВ) на кинетику кислотного гемолиза эритроцитов крови крыс. Показано, что ММ ЭМВ воздействуют на продолжительность гемолиза и это изменение зависит от экспозиции и частоты ММ ЭМВ. По сравнению с контролем, при облучении частотами 41,8 и 42,2 *ГГц* кислотный гемолиз происходит быстрее при всех продолжительностях облучения. При облучении частотами 50,3 и 51,8 *ГГц* гемолиз также происходит быстрее при коротких экспозициях облучения по сравнению с контролем. При экспозиции 60 *мин* начало гемолиза задерживается.

Մ. Ա. ՇԱՀԻՆՅԱՆ, Մ. Ս. ՄԻՔԱԵԼՅԱՆ, Ա. Վ. ՆԵՐԿԱՐԱՐՅԱՆ

ՄԻԼԻՄԵՏՐԱՅԻՆ ՏԻՐՈՒՅԹԻ ԷԼԵԿՏՐԱՄԱԳՆԻՍԱԿԱՆ ԱԼԻՔՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԱՌՆԵՏՆԵՐԻ ԱՐՅԱՆ ԷՐԻԹՐՈՑԻՏՆԵՐԻ ՀԵՄՈԼԻԶԻ ԿԻՆԵՏԻԿԱՅԻ ՎՐԱ

Ուսումնասիրվել է միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների ազդեցությունը (ՄՄ ԷՄԱ) առնետների արյան էրիթրոցիտների թթվային հեմոլիզի կինետիկայի վրա: Ցույց է տրվել, որ ՄՄ ԷՄԱ ազդում են էրիթրոցիտների հեմոլիզի տևողության վրա և այդ փոփոխությունը կախված է ՄՄ ԷՄԱ տևողությունից ու հաճախությունից: 41,8 և 42,2 *ԳՀց* հաճախություններով ճառագայթահարման դեպքում ստուգիչի համեմատ թթվային հեմոլիզն ավելի արագ է ընթանում ճառագայթահարման բոլոր տևողությունների ժամանակ: 50,3 և 51,8 *ԳՀց* հաճախություններով ճառագայթահարման դեպքում հեմոլիզը ստուգիչի համեմատ նորից ավելի արագ է տեղի ունենում կարճատև ճառագայթահարումների ժամանակ: 60 *րոպե* տևողությամբ ճառագայթահարման դեպքում հեմոլիզի սկիզբը ուշանում է: