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AGGREGATIVE PROPERTIES OF ERYTHROCYTE UNDER THE INFLUENCE OF MILLIMETER RANGE ELECTROMAGNETIC WAVES

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In this work the influence of millimeter range electromagnetic waves (MM EMW) on some important physical-chemical parameters of erythrocyte (ER) of rat blood has been studied. The change of these parameters is directly connected with aggregation of ER. It was shown that the irradiation of MM EMW by 51.8 *GHz* frequency results in decreasing of the absolute value of the surface charge density of ER, moreover, the acidic hemolysis of ER fastens. It was also shown that under the influence of MM EMW the decrease of ER density in suspension occurs along with their concentration enhancement. It was concluded that MM EMW is a factor, which contributes to the aggregation of ER.

Keywords: erythrocyte suspension, millimeter range electromagnetic waves, irradiation, physical-chemical parameters of erythrocyte, aggregation.

Introduction. Nowadays millimeter range electromagnetic waves (MM EMW) are considered to be irreplaceable anthropogenic factors of environment and the studies of their effect on biological systems are an actual topic of contemporary science [1–5]. The fact is interesting that MM EMW impact the biological systems being on any level of organization [5–10]. Though, the effect depends on the field strength, frequency and irradiation duration [5]. There exist several all-accepted hypotheses concerning to the mechanisms of MM EMW effect. According to one of them, the effect of MM EMW is mediated by water, i.e. MM EMW energy transfers to the biological system through water by resonant mechanism. This hypothesis is based on the fact that the respective effect in the biological system is invoked by frequencies resonant for water [11]. According to the second hypothesis, MM EMW have a direct impact on biological system via biological membranes, which is due to formation of electro-acoustic waves [5, 11]. It was shown that apart from water resonant frequencies there are also such frequencies, the irradiation by which leads to significant effects in biological systems [8, 9]. Nevertheless, the experimental data show that MM EMW can affect the biological systems by above-mentioned mechanisms simultaneously [12, 13].

Among other biological systems the blood takes a special place, since circulating the whole organism apart from oxygen and food it carries information as well.

In this paper the effect of MM EMW on the surface charge density and aggregation of erythrocyte (ER) has been studied.

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Materials and Methods. White outbred laboratory rats (*Rattus norvegicus*, "vistar") with 80–100 *g* weight, physiological solution, Na-citrate, human blood serum albumin ("Sigma", USA) were used in experiments. In each groups – control and experimental, 3 rats were used. Experiments were repeated three-times and the obtained results were averaged. Animals were kept in similar conditions and fed by combined food. Blood of animals was gathered in glassy dish preliminarily added by 1–2 *mL* 5% solution of Na-citrate with further addition of physiological solution; 3–3.5 *mL* blood was obtained from each animal. The blood was centrifuged during 15 *min* with 1500 *g* acceleration using Electronic centrifuge capacity. Supernatant was removed and physiological solution was added to the sediment to obtain a suspension of ER. Then the optic density of the suspension was reached to 0.7 at λ=670 *nm* wavelength. Measurement of the optic density was carried out using photoelectrocolorimeter PEK-2. Non-irradiated suspension of the Ers was used as a control sample. Irradiation was carried out by 51.8 *GHz* frequency during 20, 40, 60, 80 and 100 *min*.

Irradiation of MM EMW was carried out by G4-141 generator ("Istok", Fryazino, Russia) with 37.5–53.5 *GHz* working interval of frequencies and 64 $\mu W/cm^2$ power flux density (electromagnetic field was homogeneous), the distance between sample and waveguide was equal to 180 *mm*. After irradiation the surface charge density of the ER membranes was measured, which was judged by their mobility in the constant electric field. The surface charge density σ (K/m^2) was calculated by the following formula [14]:

$$\sigma = \frac{\bar{\omega}\eta}{k+r},$$

where $\bar{\omega}$ is electrophoretic mobility of a particle, i.e. relation of linear rate of ER in the electric field to potential gradient of the electric field, η is viscosity of disperse medium, *k* is width of double electric layer (*Å*), *r* is radius of counterion. Taking into account that the buffer ionic strength is equal to 0.15 *M*/*L*, *k* is approximately equal to 7.74 *Å*, the counterion radius can be neglected.

Part of non-irradiated and irradiated suspension was used for studying the resistance of ER. Hemolysis was carried out by hemolytic 0.004 N HCl. It was judged about hemolysis by the optic density change of the suspension at λ=670 *nm* and room temperature. Percentage of ER exposed to hemolysis in any moment of time was calculated by the following formula:

$$E = \frac{\Delta D_i}{D_0 - D_n} \cdot 100\%,$$

where *E* is quantity of ER exposed to hemolysis, %; $\Delta D_i = D_i - D_{i+1}$ is difference between two alternative values of the optic densities; $D_0 - D_n$ is difference between initial and final values of the optic density in studying suspension. Based on the calculated values of *E*, the erythrograms were constructed by which one can judge about distribution of ER by stability in time.

Densities of ER in non-irradiated and irradiated suspensions were measured as well. Measurements of densities of the samples were carried out using densitometer Anton Paar DMA 4500 vibrating tube densitometer. Measurements were carried out at 30°*C*. The error in experiments does not exceed 5–15%.

Results and Discussion. Values of the surface charge densities of membranes of ER in non-irradiated and irradiated suspensions with different durations are presented in Fig. 1.

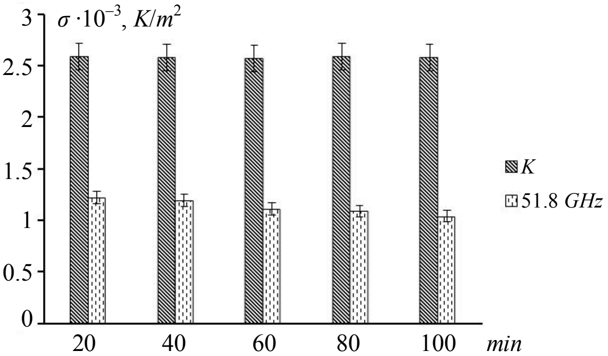

Fig. 1. Absolute values of the surface charge density of ER membranes in control (*K*, non-irradiated) and irradiated by 51.8 *GHz* frequency samples at different durations of irradiation.

As it is obvious from Fig. 1, at the irradiation the absolute values of the surface charge density decreases compared with the control. It is also seen that the enhancement of the irradiation duration in its turn leads to decreasing of the absolute value of the surface charge density. At the irradiation with 20 *min* duration the absolute value of σ decreases by 53% as compared with control, at the irradiation with 100 *min* duration the decrease occurs by 60%. The charge of membranes of ER plays a crucial role in rheological properties of blood [15, 16].

Phenomena taking place on the surface of ER and the cell charge value are accepted to be judged by their mobility rate in the electric field – electrophoretic mobility [17]. Electrophoretic mobility of ER is an integral criterion of physical-chemical properties of the blood as well as a criterion of the organism general state. The decrease of electrophoretic mobility can be conditioned by aggregation of ER, worsening of microcirculation, which finally may result in development of hypoxia of tissues [18]. In normal conditions in animal organism the stability of the surface charge of ER is preserved and their functional activity is controlled. The surface charge of ER membranes creates equilibrium between lipid-protein components of a cell membrane and those of surrounding medium [19]. Negative surface charge, created by polarized heads of phospholipids as well as sialic glycoproteins, provide repulsion of cells from each other thereby excluding the interaction between them, particularly the adhesion of ER, which has a huge biological value [20]. Taking into account that sialic glycoproteins play an important role in modulation of ER-ER interaction as well as in interaction with endothelium [21], it can be considered that the surface charge of ER has a significant role in aggregation and disaggregation of ER [20]. The decrease of negative surface charge density of ER may lead to instability of the blood cells [22] and as a consequence disorders the blood function.

According to data presented by Cortez Maghelly and Bisch [23], the stability of ER membranes to different physical-chemical factors decreases with increasing of the charge in the inner side of the membrane. With enhancement of difference of transmembrane potentials, the membrane becomes thinner, which leads to increasing the capacity of the membrane charges [24]. The width of double electric layer is changes and the absolute value of the electrokinetic potential is decreases. Based on decreasing of the surface charge density one can confirm the decrease of the absolute value of the electrokinetic potential. It, in turn, results in enhancement of the membrane permeability and reduction of ER stability toward hemolytic factor and acceleration of hemolysis.

Aggregation of ER is conditioned by decreasing the absolute value of σ, which in turn leads to increasing of erythrocyte sedimentation rate (ESR) [20]. It was shown that ESR increases at the irradiation by MM EMW, though the irradiation by 60 *GHz* frequency and 30 *min* duration leads to the enhancement of ESR twice, which is connected with the aggregation degree of ER [10, 25].

To reveal the stability changes of ER at the irradiation, acidic hemolysis was realized under the influence of MM EMW with 51.8 *GHz* frequency at different durations. Majority of physical-chemical properties of ER depend on effects of different factors (physical and chemical). Particularly, one of criteria determining the stability of Ers is duration of the hemolysis under the influence of a hemolytic agent. The hemolysis duration depends not only on membrane properties, but also on concentration of hemolytic (chemical factor) or intensity (physical factor). The process of ER hemolysis represents a function on the time $E = f(t)$, i.e. degree of hemolysis of ER. Analysis of the acidic erythrograms of ER was carried out by the following criteria: duration of hemolysis, location of the erythrogram peak and maximal quantity of ER exposed to the hemolysis. Erythrograms of the acidic hemolysis of ER at MM EMW irradiation with various durations are presented in Fig. 2.

As it is obvious from the presented Figure, in the control sample (*K*) the hemolysis duration of the ER was equal to 7 *min*. In the case of MM EMW irradiation with 20 *min* duration, a little rise of the left shoulder of the acidic erythrogram and a reduction of the hemolysis duration (3 *min*) are observed as compared with the control, which indicates the decrease of the resistance of ER membranes. However, the enhancement of maximal number of ER exposed to the hemolysis (35.7%) is observed compared with the control (25%). Most apparently, it is connected with increasing of the permeability of ER membranes for hemolytic due to the irradiation and that during short time of irradiation the restoration processes do not make progress. At MM EMW irradiation with 40 *min* duration a little increase of the right shoulder of the acidic erythrogram as compared with the control and a formation of two peaks are observed (1 *min*, 1.5 *min*). It is conditioned by the fact that during 40 *min* in membranes the restoration processes begin making progress and the physical-chemical changes taking place lead to decreasing of the membrane permeability for hemolytic. At MM EMW irradiation with 60 *min* duration a stabilization of hemolysis with characteristic duration (7 *min*) is observed. At the irradiation of ER suspension with 80 *min*, the hemolysis intensity of the ER enhances and the maximal number of ER exposed to hemolysis attains to 42.2%. Though, in this case a decrease of hemolysis duration (3.5 *min*) is observed. We assume that the

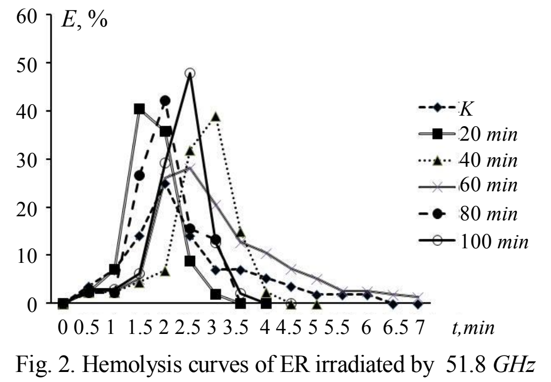

Fig. 2. Hemolysis curves of ER irradiated by 51.8 *GHz* frequency.

long-term irradiation results in destructive consequences and the restoration mechanisms turn to be insufficient for the membrane stability increase. Most apparently, conformational changes of membrane proteins of ER are induced, as a result of which the membrane permeability for hemolytic increases, that is why the hemolysis of ER becomes more intensive. At the irradiation of suspension of ER with 100 *min* duration the hemolysis intensity of ER increases more and the maximal quantity of them exposed to irradiation attains to 47.9%. It should be mentioned that in this case a decrease of hemolysis duration is also observed (3 *min*) compared with control. The above mentioned data probably are conditioned by the fact that the changes induced by the irradiation are not restored and the resistance of ER membranes decreases. Thus, the results of the studies show that MM EMW impact the resistance of the ER membranes moreover; the observed effect sharply depends on the irradiation time, since at the irradiation with 20 *min* the restoration processes are not still initiated in the membranes. At the irradiation with 40 *min* duration an increase of resistance is already observed, which is pronounced at the irradiation with 60 *min*. At 80 and 100 *min* durations the irradiation of MM EMW already acts as a stimulator of hemolysis.

The influence of MM EMW on density of ER in suspension at the irradiation with 60 *min* duration is also studied. Density dependence of ER on their number concentration in suspension is presented in Fig. 3, which in the case of the control sample enhances along with increasing of the number concentration of the ER. In the irradiated sample, vice versa, the density decreases with the increasing of the number concentration. It is known that the irradiation results in water structure change, which transfers to more structured state. It occurs due to the transition of water bound molecules with the membrane to free state with further clusterization, in the consequence of which the above mentioned changes are observed. As a result of this after the irradiation, one part of ER is subjected to the hemolysis and the aggregates are formed and the total density of suspension decreases [10, 25]. Besides, under the influence of irradiation, such changes take place that lead to increasing of the charge on the inner side of the membrane as it is stated by Cortez Maghelly and Bisch [23]. These changes are possible due to the transition of the irradiation energy throughout the electro-acoustic waves of MM EMW, which is especially more intensive at the irradiation with 80 and 100 *min* durations [11].

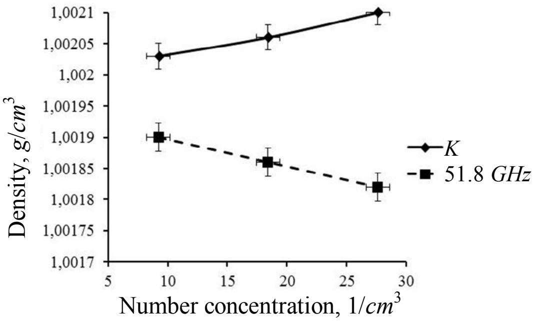

Fig. 3. Dependence of ER density in suspension on their number concentration at 25°*C*.

Conclusion. Therefore, based on the obtained data one can conclude that MM EMW irradiation is a factor for accelerating the aggregation of ER. It is indicated by the decrease of absolute value of the surface charge density of ER; the accelerated hemolysis of ER as well as by the decrease of the density of ER in the suspension, which takes place along with increasing of the concentration of ER.

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