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Rheological Properties of the Erythrocytes in Weakened Static Magnetic Field of the Earth *In vitro* Study

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background: The magnetobiological effect of the weakened magnetic field of the Earth is of interest due to the consequences of the long stay of astronauts in space.
Objective: The rheological properties of erythrocytes in a weakened magnetic field of the Earth are investigated.
Methods: Osmotic gradient ektacytometry, aggregometry.
Results: A study of the rheological properties of erythrocytes of blood rats in vitro, exposed at a temperature of 0°C in a natural and weakened magnetic field of the Earth, was carried out. It is established that a weakened magnetic field leads to a decrease in the rate of hemolysis, the average body volume, transformation, and decrease in the specific surface of the erythrocyte, potentiates the weakening of the deformation and aggregation properties.
Conclusions: The results of the work should be taken into account not only to predict the rheological behavior of the blood system when the natural magnetic field is weakened but also to optimize the conditions for the long-term storage of donor blood.

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1. INTRODUCTION

The interest in the work on the effect of static magnetic fields on a living organism does not dry up. Being one of the main forces of nature, magnetism is an indispensable ingredient in the original substance from which the Universe and our planet emerged. The earth's magnetic field is a factor of billions of years of biological evolution. The natural magnetic field of the Earth is only 50 μT, but it decisively affects the daily life of mankind and nature. At the University of Giessen, scientists have successfully proved that even magnetic fields with intensities in the pT-range have an undeniable effect on human well-being, like natural meteorological phenomena that exemplify the effects of even small low-intensity magnetic fields.

The biological effects of magnetic vacuum at the organism level are manifested in plants, microorganisms, insects, worms, tritons, fish, and mammals. Conformational changes of chromatin were found in human cells in a zero magnetic field [1]. The maximum magnetobiological effect on the viscosity of a suspension of E. coli cells was observed in a field close to zero [2]. It is been shown that the hypomagnetic field negatively affects the early development and functioning of the central nervous system [3,4]. Extensive research undertaken in anticipation of the Apollo-mission to the moon showed that a 10-day stay in conditions of less than 50 nT caused a decrease in the critical speed of the sliding flicker [5]. Hypomagnetic effects are fundamental importance, but their physical origin remains unclear. Unfortunately, the work on the influence of the deprivation of the magnetic field on the tissues of the body is very few, they are mainly carried out on the whole organism.

2. METHODS

Among possible approaches to studying the effects of hypomagnetic biological effects, we chose magnetic shielding instead of compensating for the Earth's magnetic field, as in our earlier study [6]. Experiments in field compensated conditions, in our opinion, should be considered as incorrectly conducted. Indeed, despite the absence of instrument registration of the field, the cells are under the simultaneous influence of two mutually exclusive vectors, and the field is absent only theoretically. The weakened field is proposed to be viewed from the angle of view of a magnetic vector potential having an independent value since the vector potential of the "zero fields" (when the magnetic and electric fields are zero) changes the course of cellular biochemical processes [7]. In our work, the reactions of red blood cells to the weakened magnetic field of the Earth were detected in vitro in the absence of an acute neuroendocrine response, i.e. the effects of the hypothalamic-pituitary-adrenal system.

The results of three series of experiments on Wistar rats of both sexes from one litter aged 3, 4 and 5 months are presented. In each series used the blood of 2 animals. All animal experiments were conducted as approved by Institutional Animal Care and Use Committee and adhered to established guidelines. After decapitation on the guillotine, as the most humane way of killing animals, samples of mixed heparinized (100 U/ml) blood in 500-μl Eppendorf-tubes filled to the top we placed on melting ice in foam insulating containers. The content of blood samples at 0 °C is the most acceptable condition for maintaining the native properties of blood during laboratory testing. Experimental samples we placed in a shielding chamber, which is a hollow cylinder, covered with layers of alloys of amorphous magnetic material AMAG-172 [8]. The magnitude of the magnetic field induction, measured by a three-component magnetometer HB0302.1A (0.1–100 μT, NPO ENT, Russia), was 0.192 μT. The control samples were located in a polyethylene container from under drinking water. Studies were performed immediately after blood collection, after 6 hours, one day, 2, 3, 4, 5, 7, 10, two, 3 and 4 weeks of storage in the chambers. The following general clinical blood parameters were recorded: the number of erythrocytes (RBC), hematocrit (EPV), erythrocyte volume (MCV), the degree of hemoconcentration 10(1-EPV)/RBC, which we suggested earlier as an indicator of stress [9]. The physical meaning of this formula is the plasma volume per 1 erythrocyte of peripheral blood. The potassium (K_{pl}) and sodium ion concentrations in the blood plasma (Na_{pl}) we measured using flame photometry. Evaluation of the deformation properties of erythrocytes we performed by osmotic gradient ektacytometry [10], the aggregation properties of erythrocytes were measured by the piezodynamic method in the blood microvolume [11]. We investigated the following deformation parameters of erythrocytes: integral deformability in isotonic osmolality (I_{def}), the water permeability of membranes (I_{min}) [12], shape index (S/V), or average cell fragility (O_{min}), the degree of hemoglobin hydration (O_{hyper}), the osmotic range of deformability (O_{hyper}-O_{min}). Aggregation indicators: minimum (T_{min}) and maximum (T_{max}) aggregate strength, aggregation half-period (τ), spontaneous aggregation rate (1/τ), and aggregation index ($K_{aggr}=T_{max}/τ$). All rheological tests were performed at $(25±0.2)^oC$. Experimental results are shown as graphs of change in mean arithmetic values with standard deviations and corresponding to a sample of trend lines. All results were expressed as the means±SD. Student's paired t-test was used to compare the data.

3. RESULTS

Fig. 1 shows changes in general clinical blood parameters. The most significant changes in the blood are presented with a follow-up of 4 days since from the fifth day of exposure there is a progressive breakdown of red blood cells.

As can be seen in Fig. 1, during the exposure process, the erythrocytes are progressively disintegrated in control samples that were stored under the ordinary magnetic field of the Earth (EMF), and in the blood that was exposed to a weakened magnetic field (HMF), it does not change statistically. This is evidenced by the accumulation of K_{pl}. RBC in HMF samples is still reduced, but less intensively compared to EMF. At the same time, the growth of EPV is noted in HMF more intensively. MCV change volume shows that the cells in the HMF swell more weakly. These interrelated changes result in the fact that in HMF the volume of plasma per erythrocyte 10(1-EPV)/RBC does not change, and in EMF it significantly exceeds the initial level.

Fig. 2 shows the profiles of the osmotic deformability, i.e. osmoscans [10] of red blood cells.

From the first 6 hours of blood storage on ice in the area of 170 mosmol/kg H_2O a clear break (the bend) appears on the osmoscans, and in the zone (200-210) mosmol/kg H_2O - barely noticeable. Osmoscanes of native blood recorded immediately after blood taking characterized by a smooth curve. Subsequently, the location of bend is shifted to the zone (200-210) mosmol/ kg H_2O. The deformation index in this zone, initially elevated in the EMF, under HMF conditions dramatically exceeds this level with a high degree of confidence by 3-4 days (Table 1).

In the process of exposure, all osmokans shift entirely in the direction of hypertonic osmolality. As can be seen from the figs., the osmocans in HMF are slightly higher in the first 6 hours compared to the EMF, and subsequently much lower. Reducing I_{def} of red blood cells during storage of blood on ice for 4 days is not observed. At a later date, the observation of osmocans is significantly modified compared to the native. A significant decrease in red blood cell deformability occurs only from the fifth day of exposure. For a more detailed analysis of the studied determinants of erythrocyte deformability, refer to Fig. 3.

Erythrocyte deformability I_{def} in HMF is lower than in EMF samples. This occurs as a result of the spherulation, or swelling (O_{min} increase) of red blood cells in HMF. However, the degree of O_{hyper} displacement to the hypertonic region in HMF and the osmotic range in which the cells still exhibit deformation properties (O_{hyper}-O_{min}) are higher than in the EMF. The osmolality shift of the manifestation of the maximum deformation properties towards the hypertonic concentrations of the suspension medium confirms the observed osmogram shift to the right (Fig. 2). Significant differences are noted in water permeability or membrane integrity (I_{min}): the permeability of erythrocyte membranes in HMF is lower than in EMF throughout the experiment.

As can be seen from the graphs in Fig. 4, the aggregation properties of erythrocytes in the blood decrease sharply from the first hours and after the daily exposure is subject to already small changes.

At the same time, the minimum (T_{min}) and maximum strength of the aggregates (T_{max}), the rate of spontaneous aggregation (1/τ), the aggregation index of the erythrocytes (K_{aggr}) maintained under HMF conditions, are significantly reduced compared to the EMF (Fig. 5).

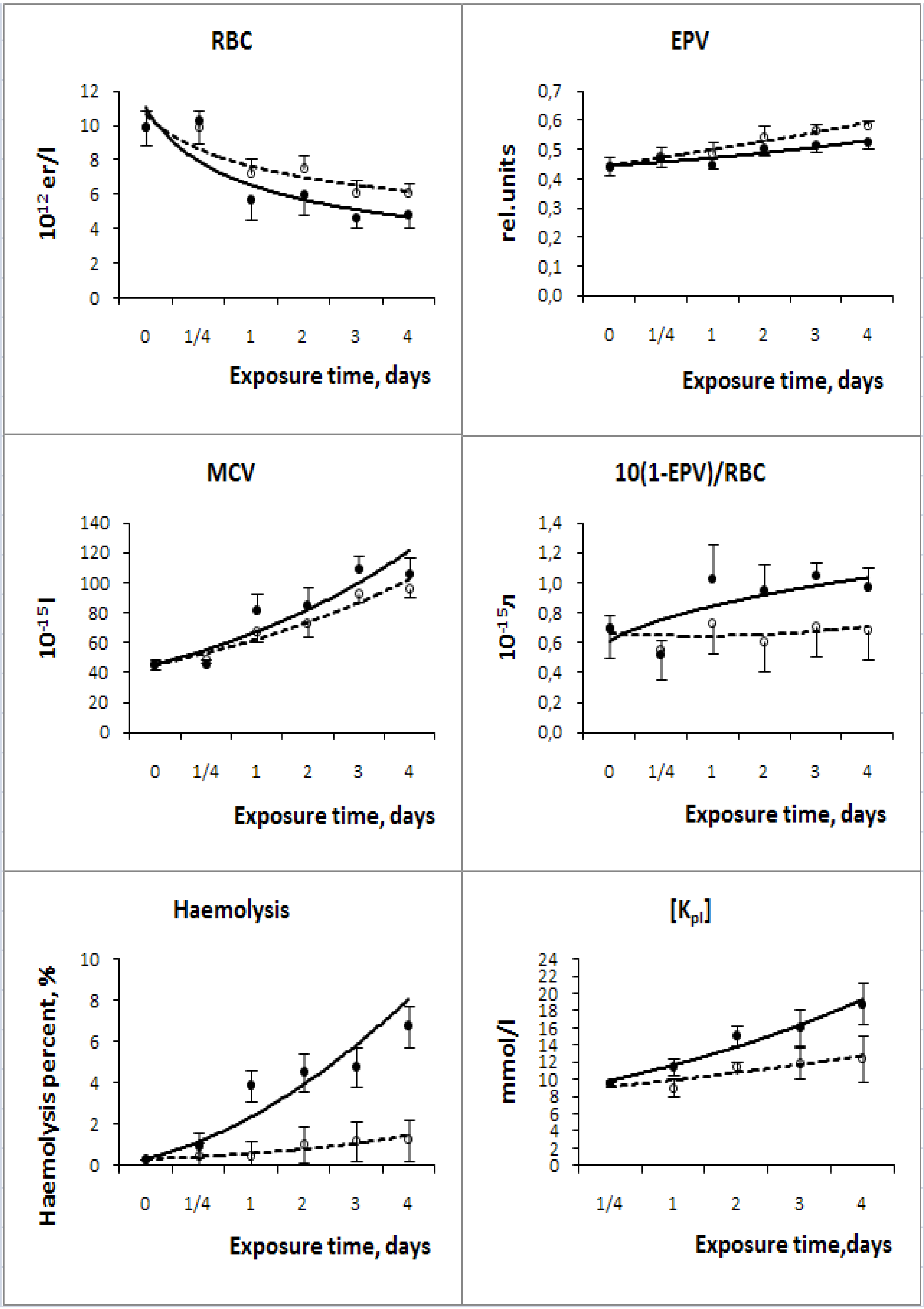

Fig. 1. Clinical indicators of erythrocytes

• - Samples in the EMF; ° - Samples in the HMF

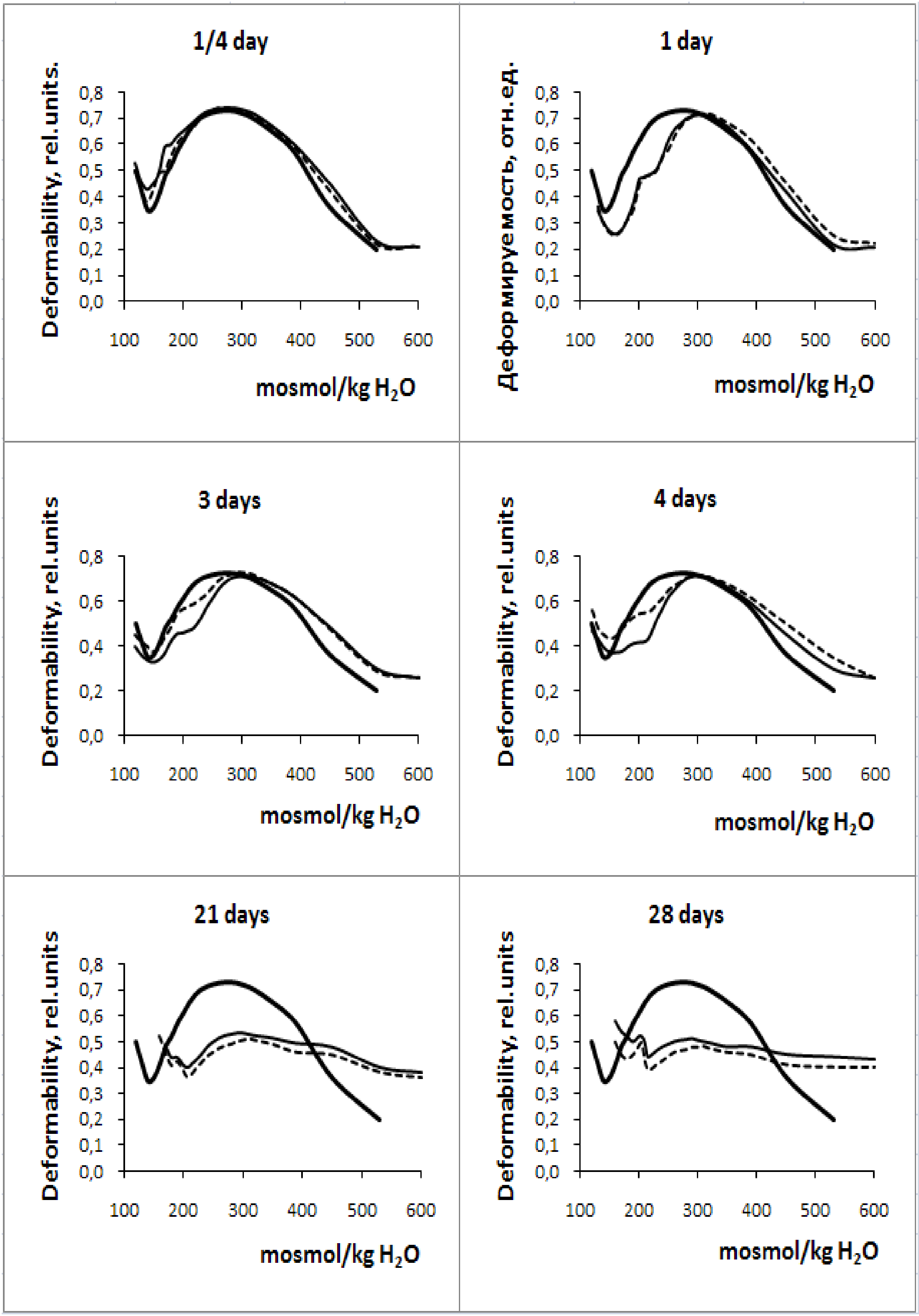

Fig. 2. Osmoscans for erythrocytes

– In native blood; – In EMF; --- In HMF

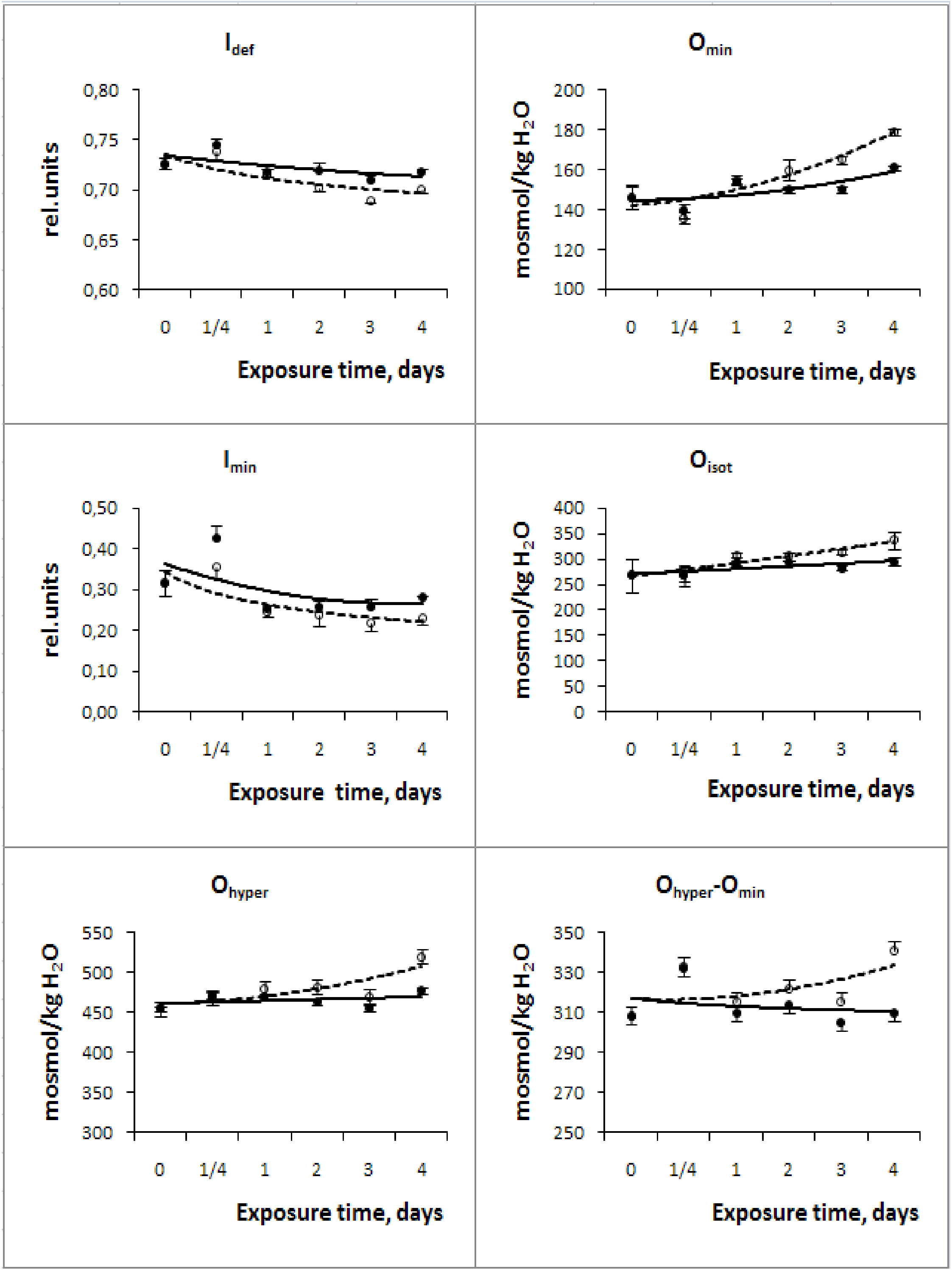

Fig. 3. Characteristic indicators of osmoscans in investigated samples
– In EMF; --- In HMF

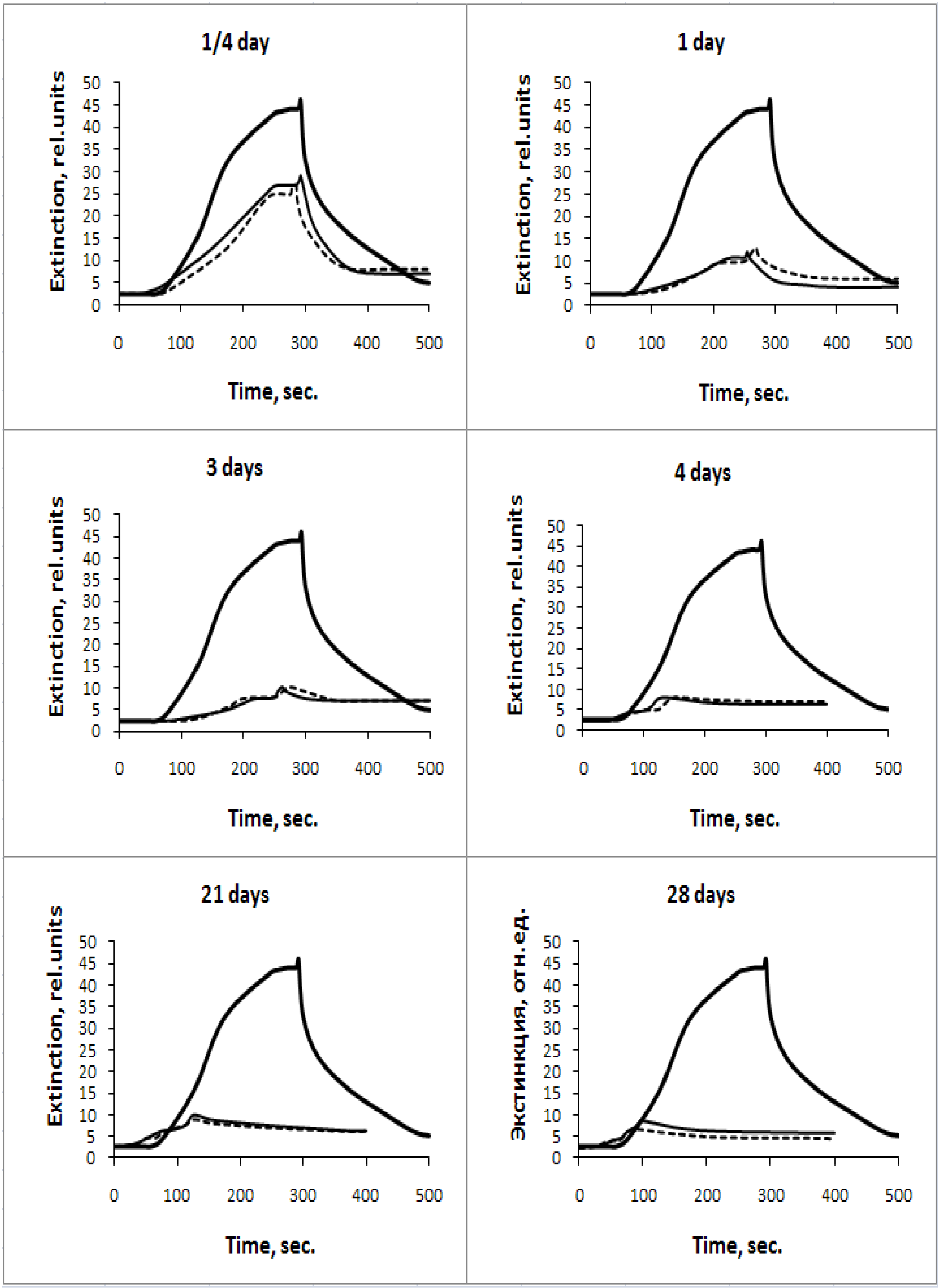

Fig. 4. Erythrocyte aggregatograms
– In native blood; – In EMF; --- In HMF

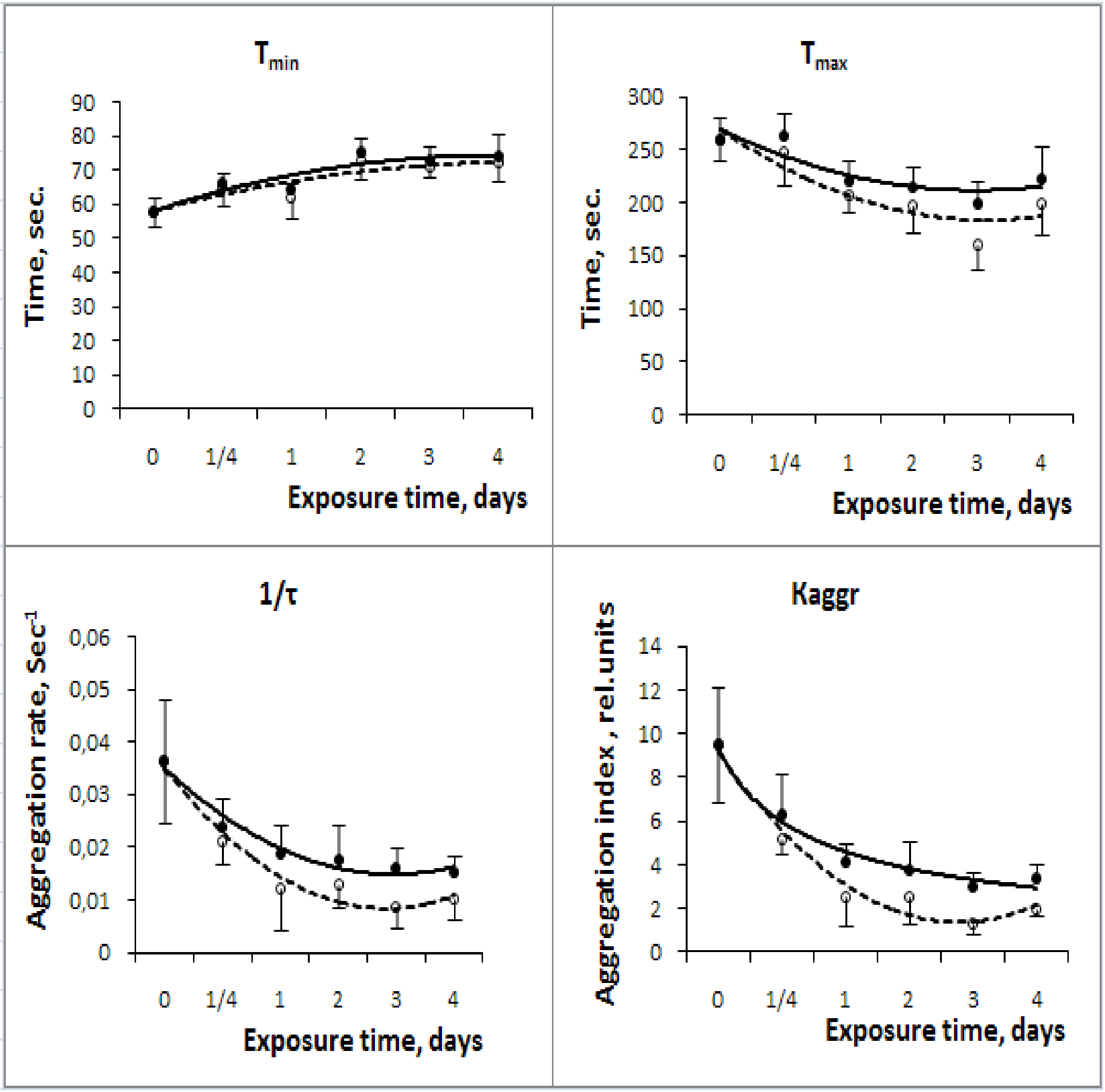

Fig. 5. Characteristic indicators of aggregatograms in investigated samples
• - In EMP; ° - In HMP

Table 1. The index of deformability in the bend zone (200-210) mosmol/kg H_2O

Exposure time, days	EMF	HMF
	Relative units	
1/4	0,590±0,012	0,500±0,010*
1	0,475±0,006	0,465±0,007
3	0,460±0,006	0,575±0,008*
4	0,415±0,005	0,545±0,006*

*Note: * - significant changes, p <0,001*

4. DISCUSSION

Experienced functional dependences of the cell response in the absence of a magnetic field are necessary for a theoretical explanation and prediction of magnetic deprivation of living organisms. Despite the abundance of empirical and widely discussed data, the mechanisms of field influence and, in particular, the underlying magnetoreception have not yet been determined. It is unclear how the change in magnetic energy, which is much smaller than the scale of thermal fluctuations, affects the rate of chemical reactions. The mechanisms of functioning of spin-correlated pairs of radicals, and the universal physical mechanism of the precession of magnetic moments, and the rotation of large fragments of macromolecules or amino acid residues, i.e. molecular rotations, rotation of the target itself and stable magnetic nanoparticles [13]. A possible target of the action of the vector potential can be both the protons of water and the indispensable component of life itself water, which is the medium for biochemical and biophysical processes in living organisms. The effects of a 40-fold weakened geomagnetic field on the redox properties of water are studied. As shown, water with a smaller number of hydrogen bonds is a stronger reactive medium, and a weakened field violates the structure of water in biological tissues [14-16]. For this reason, significant weakening of magnetic field should considered a functional breakdown of the course of physicochemical processes in a living organism, i.e. like stress. One of the common constituents of all cells is ions. Ions as magnetosensors conduct electromagnetic pulses and allow the cell to function. The tissue of the human body, the most sensitive to magnetic effects, is blood, which has the property of a diamagnetic material during oxidation and is paramagnetic during deoxygenation.

A feature of this study is the comparison of changes in the indices in some blood samples over a long period of exposure, because were evaluated purely paired changes. Statistical evaluation, made in probability, corresponding to the Student's t-test, reveals, in the overwhelming majority, significant differences in the performance of blood samples in HMF compared to the EMF in some cases ($p < 0.001$). Despite the selection of animals from the same litter, the initial values and the reaction to the action experience some deviations as result of possible gender differences, which expanded the range of variation of the trait during statistical calculations.

As our study shows, red blood cells in HMF are more resistant to volume increase, spontaneous hemolysis, and water loss, compared to natural conditions. A significantly lower I_{min} indicates inhibition of erythrocyte membrane perforation during storage. The ongoing transformation causes a decrease in the specific surface of the erythrocyte S/V, which is clearly manifested in the increase in O_{min}. This indicates a decrease in osmotic resistance and deformability of erythrocytes under HMF. However, colleagues from Novosibirsk in similar conditions found a linear increase in the osmotic stability of red blood cells, correlating with the degree of weakening of the geomagnetic field [17]. The authors performed an osmotic test for the degree of hemolysis in 0.45% NaCl in the blood, stabilized with a solution of sodium citrate, with repeated centrifugation and subsequent dilution with saline. In our opinion, the inadequacy of the applied methods when working with such delicate objects as red blood cells causes a transformation and change in the corpuscular volume, which leads to erroneous estimates of their osmotic properties.

A characteristic feature of the change in the deformation properties of erythrocytes in the experiencing blood is the appearance of the bend on osmoscans in the area (200-210) mosmol/kg H_2O of the suspension medium. Researchers have previously observed this phenomenon, for example, with xerocytosis and sickle cell anemia [18, 19], with stomacytic elliptocytosis and ovalocytosis [20, 21], red blood cell treated with increasing concentrations of lysolecithin and diamide [22], hydroxyurea [23], dinitrobenzene [24], acitretin[25]. The appearance of two hypotonic minima on osmuscans was either not given attention, either due to the presence of cell populations by different S/V ratios, or the presence of completely non-deformable cells. But the fact of the appearance of the bending on osmokans always in one zone of osmolality is suggestive of a generalized cause associated with the structural feature of the original form of the erythrocyte. The spectrin network can be locally compressed or stretched as a result of the relative displacement of anchor proteins while the total surface area of the membrane remains constant. The network stretched at rest with a biconcave shape, exerts a compressive force on the lipid bilayer when the erythrocyte swells and returns to its native form when excess space is "squeezed" in the central region. Such a "collapse" occurs by jump and in a very narrow range of osmotic concentrations of the order of 5–7 mosmol/kg H_2O. Therefore, it is recorded on an osmogram with a very scrupulous, iterative measurement. The shear effect at the network during the transformation of the erythrocyte in this zone depends on the modulus of elasticity and elasticity of the membrane. As result of increased free radical oxidation and cytoskeleton tightening in the case of pathology, such incorporation occurs with a more radical jump in the deformability index. Partial reduction of deformability, when osmolality is approaching to isotonic, explains the appearance of a break in the osmogram. The deformability in this zone in erythrocytes in HMF is significantly higher than that in erythrocytes maintained under EMF conditions. Our observations are confirmed by the recently published report that a 1.5-hour exposure of mice peritoneal neutrophils under magnetic shielding hypomagnetic conditions (a residual constant magnetic field of 20 nT) causes a decrease in intracellular production of reactive oxygen species [26]. A distinctive feature of the behavior of erythrocytes was prolonged, during 5 days of storage, the maintenance of the deformation abilities of erythrocytes, comparable to the initial values.

Sodium concentration in plasma during 4 days of observation did not change: in EMF samples it was (139±6) mmol/l and in HMF - (137±5) mmol/l. The erythrocyte hemolysis is accompanied, firstly, by the release of the content with a low sodium concentration into the plasma (a decrease Na_{pl}), secondly, by swelling of the remaining cells, i.e osmotic filling with plasma water (an increase Na_{pl}), and thirdly, activation of the sodium pump. As a result, the concentration of Na_{pl} remains constant. Only from the 5th day begins its noticeable decline in accordance with the massive destruction of red blood cells. By the end of the 28-day exposure, the sodium concentration in the EMF was (114±5) mmol/l, and in HMF (110±8) mmol/l compared to the initial concentration (141±3) mmol/l (p <0, 05). The potassium concentration was (65±3) mmol/l and (61±2) mmol/l, respectively, compared to the initial concentration (7.8± 0.5) mmol/l (p <0.0001).

We found that the blood content under HMF conditions leads to a decrease in spontaneous aggregation of erythrocytes, which is consistent with previously made in vitro observations [27]. The manifestation of this phenomenon is sharply reduced in blood samples within 6 hours of exposure at 0 °C: the number of aggregates involved in the process and all the characteristic parameters decrease. After daily exposure outside the body, these changes continue exponentially, with the gravitational sedimentation of erythrocytes dominating in the cell.

5. CONCLUSIONS

Exposure of blood in vitro under conditions of a weakened magnetic field of the Earth as compared to natural storage at 0 °C causes:

1. Reducing the rate of hemolysis of erythrocytes;
2. Acceleration of swelling and transformation with a decrease in the specific surface of the erythrocyte, leading to a weakening of the deformation properties of erythrocytes.
3. Reducing the degree of perforation and water permeability of the membrane.
4. A less noticeable increase in the stiffness of the central regions of the erythrocyte.
5. Reducing the ability of red blood cells to spontaneous aggregation.
6. The results of the work should be taken into account not only to predict the rheological behavior of the blood system when the natural magnetic field is weakened but also to optimize the conditions for the long-term storage of donor blood.

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ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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