Section A-Research paper
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ANALYSIS OF THE EFFECT OF ULTRASOUND FIELD FORCES ON ERYTHROCYTES - AS A MODEL OF THEIR BEHAVIOR IN THE VASCULAR SYSTEM

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Abstract

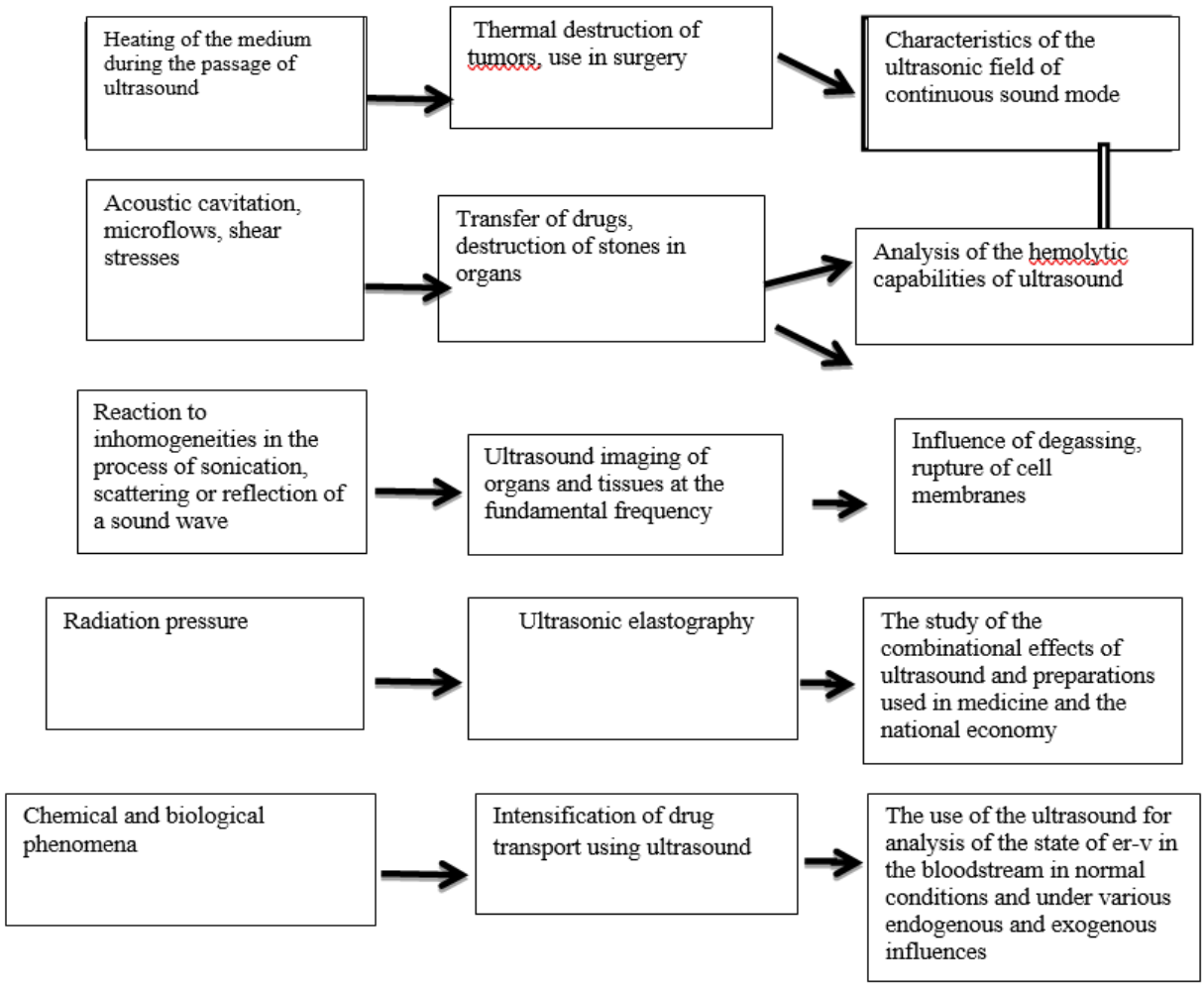
The results of our own research on the function of erythrocyte deformation in the microcirculation process were analysed along with literary sources. It has been established that both the characteristics of the plasma membrane and the resistance to flow in the vascular system contribute to the effective deformability of erythrocytes. The impact of US field forces on blood cells served as an in vitro model for the mechanical impact on erythrocytes. An analogy was drawn between how erythrocytes behave in the bloodstream and how therapeutic ultrasonography affects blood cells, and a model of the forces at play was put out.

Keywords: Ultrasonography, Vascular, Hemolysis, Erythrocytes, Cardiovascular.

I. INTRODUCTION

At the moment, ultrasonography has solidified its place in the medical services industry. Around 60 million patients worldwide have ultrasound exams each year, which are currently among the most safe and informative procedures available, according to WHO estimates. The application of ultrasound in biology and medicine allows for direct control over living tissues to gather data. Cells and tissues are altered as a result of these interactions, which are dependent not only on the characteristics of the tissue under investigation but also on those of the ultrasonic field. The biological impact of physical impact during ultrasonic sounding should be assessed by minimising changes in the environment's and the object's properties, such as temperature, acoustic pressure, deformability, etc. The osmolarity of red blood cells' surroundings (blood plasma) changes depending on the severity of the disorder. Erythrocyte membrane permeability and absorption capacity are altered during endogenous intoxication. The

cytoskeleton's structure and the shape of erythrocytes both change concurrently, and this allows for the discovery of variations in the resistance of cells with distinct rheologies [1]. Erythrocyte osmotic and chemical resistance is assessed using the widely used Terskov and Gitelson method [2]. At the same time, the characteristics of the erythrocyte stroma alter as a clinical indicator in several pathologies (malignant tumours of varied location, diabetes mellitus, cardiovascular pathologies, burns, etc.) [29,30]. The similarities and variations in the mechanisms behind different forms of erythrocyte lysis were revealed through investigation. It is possible to think about acid hemolysis as a multi-stage process. At the initial stage of hemolysis, conformational changes in integral membrane and membrane proteins are seen upon contact with an acidic extracellular media, as seen in fig. . Haemoglobin denaturation takes place at the second stage, which is known as a phase of profound erythrocyte membrane integrity loss and additional disruption of protein-lipid and lipid-lipid membrane connections. Phenomena in Us Field Use of Ultrasound in Medicine Work Tasks.



Scheme 1. Effects created by the ultrasonic field when it is used in medicine.

Osmotic hemolysis can be conceptualised as a single-stage process that results in cell deformability and the release of haemoglobin into the cell's environment, according to analysis of the process' mechanism. Both times, the process of cell apoptosis is preceded by the transport of the surrounding solution into the cell in line with the gradient of concentration.

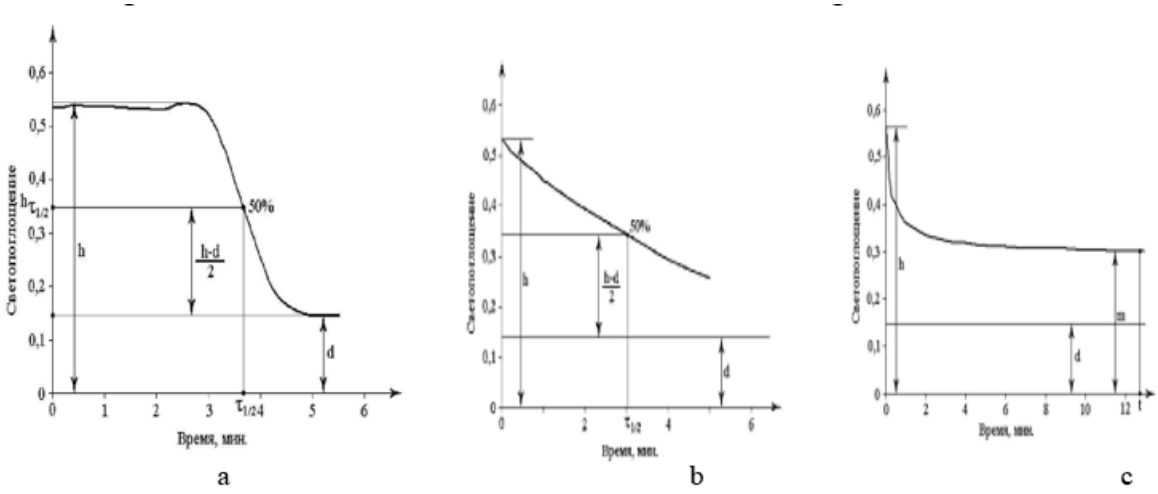


Fig. 2. Dynamics of acidic (a), ultrasonic (c) and osmotic (c) hemolysis, where h is the initial light absorption, d is the residual light absorption, t_{50} is the time of 50% heme

At the same time, there are similar mechanisms that are used to alter the characteristics of cell membranes in response to a variety of stimuli, including stress. We have investigated the hemolytic resistance of erythrocytes in the presence of US, membrane-active substances, ageing, and stress.

II. MATERIALS AND METHODS

Determination of the stability of erythrocytes by osmotic and acid hemolysis.

Study of osmotic hemolysis of erythrocytes. 0.4 ml of a 5% erythrocyte suspension was mixed with 2 ml of a hypoosmotic sodium chloride solution to calculate the amount of hemolysis. Erythrocyte hemolysis was assessed for each sample in solutions of 0.3%, 0.4%, and 0.5% NaCl. The sample was then centrifuged for 5 minutes at 3000 rpm, and the optical density of the supernatant was assessed using a T92+ spectrophotometer at a wavelength of 535 nm for haemoglobin absorption. Based on the data acquired, the proportion of hemolyzed erythrocytes was estimated from the hemolysis curves. Using an FEC photoelectrocalorimeter on a red light filter, it was also possible to monitor changes in the optical density of the cell suspension (cell volume). The output signal might be recorded thanks to the KSP-4 recorder. Using the formula D=lg(100/T), the optical density of the suspension was determined from the transmission value (%). Kinetic method of ultrasonic hemolysis, proposed to characterize the mechanical properties of erythrocyte membranes**.**

The method we developed for automatic photoelectric registration of the hemolysis of red blood cells under the influence of continuous ultrasound at a frequency of 0.88 mHz within the intensity range of 0.1 - 1.0 W/cm2 at a constant temperature was used to study the resistance of erythrocytes. The increase in light transmission in a cuvette containing a suspension of erythrocytes during their ultrasonic hemolysis was measured and recorded using a setup made up of a T-5 ultrasonic therapeutic device, an FEC-M photoelectric colorimeter, a potentiometer, and a KSP-4 recorder in order to study the kinetics of hemolysis.

III. RESULTS AND DISCUSSION

The Gompertz law is followed by the kinetic curves for the release of haemoglobin, giving them an S-shape. Similar shapes can be seen in the % hemolysis curves for osmotic, thermal, acidic, and photohemolysis. In the range of 10 to 90%, several writers have discovered a linear relationship between the quantity of hemolyzed erythrocytes and the time of hemolysis during immune hemolysis caused by complement. Our studies with continuous ultrasound at J=0.2-0.4 W/cm2 and f=0.88 MHz also supported the existence of a rectilinear section of the curve in the range of 10-70%, which is ostensibly connected to the specificity of the action of ultrasound at different intensities. . Control investigations revealed that the rectilinear part of the hemolysis curve dramatically rises (5-95%) with an increase in ultrasonic intensity to 1 W/cm2.

A number of authors [3,4,5,6] have demonstrated that under some circumstances, ultrasonic exposure can result in changes to the cells and tissues that ultrasonic waves pass through. There is a distinct threshold intensity of ultrasonic exposure, claims [6]. Cell lysis starts when this limit is reached. Hemolysis in the US field is likely brought on by either cavitation or severe hydrodynamic forces. However, in the case of collapsing cavitation and in regions of turbulent flows, hemolysis can also take place at therapeutic intensities [11, 12]. It has been demonstrated that erythrocytes' stromal mechanical characteristics alter when external influences operate on them. It was discovered that these parameters depended on the erythrocytes' own characteristics (age, shape, and rheology), on the sounding environment, the impact factor, and the existence of pathology in the body [3,13]. Erythrocytes' (RBCs') primary job is to carry oxygen from the lungs to the tissues. These cells are biconcave, without a nucleus, and contain haemoglobin in their mature condition. Erythrocytes have the capacity to alter their form in response to both endogenous and external influences. As a result of the mechanical effects of the vascular bed, they can travel through capillaries during blood circulation and experience sufficiently substantial deformations. The integration of membrane proteins into erythrocytes' shape and deformability ensures their structural and functional integrity. Two different types of proteins contribute to the construction of the erythrocyte membrane: proteins that make up the premembrane cytoskeleton and integral membrane proteins that are integrated into the lipid bilayer [14]. The performance of erythrocytes' primary

function, the transport of gases (O2 and CO2) through the circulatory system, is significantly influenced by their deformability. Blood viscosity and microcirculatory resistance both significantly rise when the deformability of blood cells slightly declines [34,35]. As a result, decreased deformability of erythrocytes is a sign of several microvascular illnesses, including diabetes and cancer [15,29,30].

In the human body, oxygen is intensively transferred from the lungs to all organs, tissues, and cells throughout the blood flow process, which lasts for 23–25 seconds. Only about 0.3% of the blood's volume is made up of dissolved oxygen, which makes up the remaining 18–20% of the oxygen content. The erythrocytes, which can concentrate up to 20–22 mM O2 in their cytoplasm, are responsible for the blood's high oxygen capacity. Regarding the processes of this process, it should be emphasised that during the second phase, oxygen is delivered to erythrocytes in the lungs' capillaries and released into the tissue capillaries. The erythrocyte membrane is easily permeable to oxygen under normal circumstances, which is based on the high diffusion permeability of the erythrocyte membrane's lipid bilayer for O2 (basal permeability) [16]. A high permeability of the erythrocyte membrane for oxygen is provided by protein pores (channels) of the cell's plasma membrane, which is suggested to be the result of the "loosening" action of cholesterol molecules by the second theory. Basal diffusion through the lipid bilayer occurs at a low rate, and this is supported by the low rate of basal diffusion through the lipid bilayer [17]. It has been demonstrated that the permeability of BLM derived from erythrocyte shadows for O2 is quite high (1.13 ± 0.32), independent of the lateral pressure in the layer. even at the highest surface pressure (47 mN/m). 10^{-2} m/s. This indicates that the high oxygen permeability of the erythrocyte membrane occurs without the involvement of specific protein channels or pores in O_2 transport and enables erythrocytes to quickly and easily carry out their primary function, which is to provide the body with the required amount of oxygen. It should be mentioned that different areas of the vascular bed have different blood's rheological properties. The macro- and microrheological parameters of whole blood viscosity, viscoelasticity, deformability, and erythrocyte aggregation must be highlighted while analysing blood flow in both major vessels ($d \geq 200\mu m$) and the microvascular bed ($\leq 200\ \mu m$). Erythrocyte suspension in plasma and the blood of mammals and humans both exhibit non-Newtonian fluid behaviour [9, 18], whose flow through vessels is modelled by a power law. The whole blood's plasma viscosity, which ranges from 1.8 to 2.2 mPa/s, is 1.5 times more than that of an isotonic solution, which is 1.10 mPa/s. The deformability and stability of the membranes guarantee that blood cells can sustain severe and passive loads while undergoing continuous circulation in blood arteries [19,21]. In this regard, it is important to note that the resistance to blood flow in the vascular system appears to be correlated with a reduction in the viscosity of the erythrocyte suspension and, consequently, with an increase in shear rate, indicating a significant role for flow deformation of erythrocytes in producing viscous resistance to blood flow in the vascular system without taking the action of such rheological factors into account. as aggregation, hematocrit, and suspension

medium viscosity. This establishes that blood behaves differently from Newtonian fluids, as evidenced by the fact that viscosity decreases with increasing speed or shear stress. This results from two processes [20]: flow deformation of erythrocytes coupled with their orientation along the flow line at medium and particularly high shear rates above 50 s^{-1}, and the formation of erythrocyte aggregates at low shear rates that totally disintegrate at shear rates of roughly 80–120 s^{-1}. *Deformability of erythrocytes in the bloodstream.* Erythrocytes transport haemoglobin and exchange oxygen with organs and tissues. Over the course of their three to four-month lifespan, each of the 5,000,000 cells in 1 ml of blood circulates more than 1000 times daily. Erythrocytes with a diameter of less than ~8 m in the bloodstream can be subjected to severe shear loads at the same time because d-microvessels are much smaller than the size of the cell itself. Erythrocyte deformability is influenced by their lifespan (age) and depends on their rheological parameters, internal viscosity, and membrane mechanical properties [18].

Shear forces, which depend on the extracellular fluid's viscosity and shear rate, and hematocrit also have an impact on deformation. High hematocrit levels enhance the suspension's viscosity, which mostly causes an increase in erythrocyte deformation. A weakening of the deformation is shown at a particular hematocrit value [26]. It rises as a result of shear liquefaction brought on by the deformation and orientation of flow cells. Taking into account the aforementioned, we proposed conditional modelling of the shear forces acting on blood cells in the vascular bed along with the impact of US field forces acting on the erythrocyte suspension, and we presented the correlation of the shear forces acting on cells both *in vivo* and *in vitro.*

Mechanical factors such as vascular resistance, which is influenced by vascular tone, blood viscosity, shear stresses on the vessel wall, pressure changes, and the synergistic action of vascular reactions and rheological factors all play a role in the movement of blood in the bloodstream. Due to their flexibility, erythrocytes can adopt the most varied structure in the circulatory bed, shifting in response to the flow of plasma or to the form of the vessel. The erythrocyte's capacity for deformation and for aggregation are both significant characteristics. Only normocytes may deform normally. Laminar flow changes to turbulent flow when the rheological characteristics of erythrocytes are altered, which also disturbs their order in the bloodstream. Erythrocytes clumping together because of the size change can severely impair capillary blood flow in microvessels. Erythrocytes are also the blood cells that are least susceptible to the harmful effects of ultrasonography, in contrast to other blood cells. It is thought that either cavitation or high hydrodynamic stresses are the causes of US hemolysis. In the case of collapsing cavitation and in regions of turbulent flows, hemolysis can also happen at therapeutic intensities [8,22]. The primary harmful elements, acoustic microcurrents and shear pressures, have not been proven to alter the chemical composition of the sonication medium in the tested sonication mode [11]. It was shown that external stimuli can alter the mechanical characteristics of the erythrocyte stroma. These factors depend

on the characteristics of the erythrocytes (age, shape, and rheology), as well as on the sounding circumstances, the impact factor, and the presence of pathology in the body [23]. The physiotherapeutic mode (J=0.1-0.3W/cm2, f=0.88 MHz) with continuous exposure, used in medical facilities, is the best mode of exposure to ultrasound on erythrocytes. Under these circumstances, the adverse effects of ultrasound on cells are negligible and practically undetectable [25]. It appears that blood flow occurs at a fast rate in the aorta and arteries (cavitation regime), declines in the veins, and eventually reaches a minimum in the capillaries (pre-cavitation regime, mode). Erythrocytes are also more at danger of being destroyed in capillaries than they are in the aorta and arteries. We attempted to make a comparison between both processes by taking into account the aforementioned information and our own findings about the research of erythrocyte resistance in the bloodstream in vivo and in the field of action of ultrasonic waves in vitro. The circulatory system is made up of vessels with various rheological and physico-chemical properties, which in turn affect blood flow and red blood cell composition. In this instance, the blood is flowing through the circulatory system at varying pressures and speeds. This is because erythrocytes are sensitive to a variety of factors during blood flow, including mechanical, hydrodynamic, and metabolic impacts. In order to evaluate the permeability of biological membranes in the physiotherapeutic mode of sounding, we have devised a spectrophotometric approach [24,26,36]. Different writers' analyses of how big blood arteries work revealed that they can exhibit static, quasi-static (low frequencies), and oscillatory modes (high-frequency vibrations). Critical blood flow rates are seen simultaneously in veins under normal circumstances and in arteries when there is functional or diagnostic compression, or pathological alterations [27]. Large arteries typically have blood flow rates of 1.5–2.9 m/s, although in severe disorders, these rates can be significantly greater.

At sound frequencies between 60 -180 Hz, it is seen that various vascular disorders cause the critical blood velocity to fall to 0.5 m/s connected to a reduction in Young's modulus. Analysis of the blood flow in the veins revealed an oscillation frequency of about ~235 Hz, and other vessels have a significant frequency spread of about ~25–500 Hz with vessel radii R0 ~10–3–1.2x10–2 m, an inverse relationship between critical blood velocities and relative vessel wall thickness q (q=0.02-0.04), and so on. The minimal blood flow velocity in tortuous vessels is U_0=0.12 m/s, which is approximately two times less. Due to the vessel's constant flow, g=US=const, mechanical compression of the vessel and a reduction in its lumen S cause an increase in blood velocity U. The reported speed of pulse wave propagation in large arterial arteries is 4 times faster (4-14 m/s) than in large venous channels (1-2 m/s) at the same time. lead to additional hydrodynamics violations and other pathological processes. This implies that the vessel's tortuosity and bending may cause more hydrodynamic violations as well as other pathological processes. It should be emphasised that cardiopulmonary bypass (CPB) is currently one of the most frequently employed techniques in cardiac surgery.

In the meantime, intravascular hemolysis and damage to the CPB apparatus' produced components are brought on by extended blood circulation [25,28]. The blood flow in the aorta and arteries is high-speed and resembles the behaviour of cells in the cavitation mode. It then gradually decreases in the veins and reaches a minimum in the capillaries, approximating the pre-cavitation mode [34]. Pressure, blood flow rate, and vessel diameter all decline in this sequence. At the same time, it is evident that erythrocytes are more at risk of being destroyed in capillaries than in the aorta and arteries when comparing the lengths of the aorta and the total length of all capillaries in the body. At the same time, turbulent blood flow and extreme shear deformation, jet stream action, hydrodynamic forces, surface tension, the impact of high hydrostatic pressure in CPB devices, and negative pressure in the coronary region all contribute to intravascular destruction of erythrocytes during CPB [10]. Erythrocytes' mechanical resistance and deformability control how they behave in blood flow. Red blood cells are obviously destroyed during CPB because erythrocytes lack the capacity to alter their shape at blood flow rates near to physiological, which are mostly realised *in vivo* in the vessels of the microvasculature and *in vitro* in the oxygenator and filters. In an ultrasonic field, a model of mechanical cell annihilation is put forth. Erythrocytes are reportedly affected by mechanical, hydrodynamic, and biochemical forces when in the bloodstream.

Table 2. Correspondence of the parameters of the ultrasound field with the mechanical parameters of the cardiovascular system

ULTRASOUND		Cardiovascular system
Pre-cavitation regime	Cavitation regime	Heart
0,01-0,2 Vt/sm^2	0,3 and more	aorta, arteries
0.88MHs	> 1 MHs	veins, capillaries

Figure 3 presents a scheme of turbulent blood flow in the aorta and arteries.

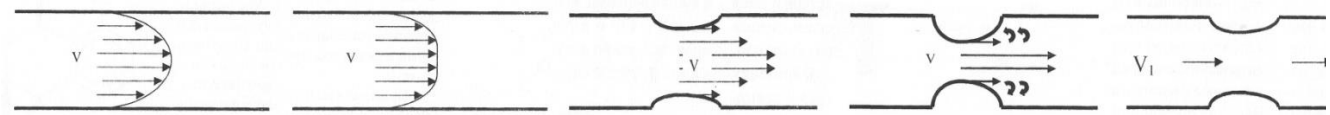


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Figure 3. Scheme of turbulent flow of blood in the aorta and arteries.

As depicted in Figure, a turbulent blood flow is seen in the aorta, arteries, and stenoses (as well as in the vessels), which corresponds to the cavitation mode. The blood flow speed then decreases (a, v, d) in the veins and reaches a minimum in the capillaries, which corresponds to the pre-cavitation mode. Pressure, blood flow speed, and blood vessel diameter all change in this order. At the same time, it makes sense that erythrocytes are more vulnerable to being destroyed in capillaries than in the aorta and arteries when comparing the size of the aorta and the length of all capillaries in the body. It is possible to extrapolate and compare the impact of eddy currents, shear stresses, and microflows arising in the ultrasound field with friction, collisions, vortex movements, and pulsation during the movement of blood vessel cells, i.e. when mechanical factors act on vascular bed cells. At the same time, data on the behaviour of erythrocytes under various sounding settings correspond with the rheological properties of cells in various regions of the blood stream. According to the analysis described above, there is a connection between a cell's ability to withstand various ultrasound exposure modes and its behaviour inside the bloodstream, which can be used as a model for erythrocyte behaviour within the bloodstream. It appears that the methods by which ultrasound causes morphological and functional damage to biological cells are based on mechanical elements, such as shock waves, microflows, and acoustic currents, which are crucial to understand for successful use of ultrasound in medicine and the microbiological industry.

Thus, it is demonstrated that the influence of outside influences on erythrocytes results in modifications to their stromal mechanical properties. It has been discovered that these parameters depend on the erythrocytes' own characteristics (age, shape, and rheology), as well as on the sounding environment, the impact factor, and the existence of pathology in the body [13,25]. As an analogy to how cells are destroyed in the circulation, we have suggested a model of mechanical cell annihilation in an ultrasonic environment. The impact of mechanical variables on cells occurs in both the ultrasonic field (eddy currents, shear stresses, and microflows) and when moving in blood arteries (friction, collisions, eddy motions, and pulsation). Consequently, it can be assumed that the mechanisms of morphological and functional damage to biological cells caused by ultrasound are based on mechanical factors, such as shock waves, microflows, and acoustic currents, which are crucial to understand for its effective use in medicine and the microbiological industry.

IV. CONCLUSION

It has been demonstrated that ultrasonic waves can be used in physiotherapeutic procedures to diagnose cell membrane damage and assess the health of cells. The study examined and analysed the regularities of changes in the physical and chemical properties of erythrocytes in various modes of sounding, as well as in the action of some physical factors of the external environment. Priority components of ultrasonic waves in the mechanism of impact on blood cells were identified and a physical

model was developed; investigate the influence of physical factors on the functional state of erythrocyte membranes in experimental leukaemias and the correlation of erythrocyte behaviour in the field of actigraphy; identify the contribution of erythrocytes of different ages to the hemolytic picture of red blood (erythron) in pathologies and the relationship between them; reveal general regularities of influence of physico-chemical characteristics on blood cell membranes. It is demonstrated how the mechanical components of the cardiovascular system and the ultrasonic field in the physiotherapeutic mode of sounding have an impact on erythrocytes generally. It is feasible to determine the impact that each of these groups has on the hemolytic pattern of human and animal blood thanks to the division of red blood cells into age fractions that takes place in the bloodstream of humans and animals in vivo.It was feasible to propose it as an analogue of the model of cell behaviour in the human bloodstream thanks to the results of the investigation of several modalities of ultrasonic sonication of blood cells. In light of the erythrocyte age composition, tumour location, drug concentration, chemical structure, and impact of environmental factors on biological objects, the proposed technique enables evaluation of changes in the functional state of cell membranes. This offers numerous options for applying the ultrasonic kinetic approach as a screening technique in various biomedical research fields.

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