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MASTER'S THESIS

DETERMINATION OF THE KINETICS OF THROMBIN FORMATION DURING WHOLE BLOOD COAGULATION

within the Basic Educational Programme of Master's Degree «Physics Methods and Information Technologies in Biomedicine»
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ABSTRACT

Keywords: hemostasis, blood clotting system, blood clotting factors, physical and mathematical model of hemocoagulation, thromboelastography, resonant acoustic method, thrombin kinetics.

The purpose of this work is to develop a method for evaluating thrombin generation based on the study of the dynamics of the viscoelastic properties of native blood during hemocoagulation, using the resonant acoustic method and NPTEG technology.

«Hemostasis is a complex hierarchically subordinate system that regulates the maintenance of the optimal functional state of the hemostatic potential (HP) – an integrative component of the full cycle of hemocoagulation, providing the necessary blood flow and restoring the integrity of the vascular wall when it is damaged» [8]. «Disorders of the hemostatic system that alter the viscoelastic characteristics of circulating blood are extremely dangerous and can lead to fatal complications: bleeding or thrombosis, which gives special priority to fundamental and applied blood clotting research» [14].

Among the methods of studying the blood coagulation system, of particular interest are "global" methods that allow integratively and *ex tempore* to assess the state of HP.

Fibrin, formed as a result of the interaction of thrombin and fibrinogen, plays a decisive role in changing the viscoelastic properties of blood. Consequently, the production of thrombin, as a result of competing enzymatic reactions, sets the kinetics of fibrin formation, providing a change in the aggregate state of the blood. For this reason, when analyzing the state of HP, special attention is paid to assessing the kinetics of thrombin formation and inactivation as a trigger of hemocoagulation.

With this in mind, this paper proposes a method for evaluating the generation of thrombin based on the results of a study of the kinetics of the final product of blood coagulation – fibrin, the operating time of which determines the dynamics of viscoelastic characteristics during coagulation. «The most suitable for

such an assessment is the domestic technology of low-frequency piezothromboelastography (LPTEG), which is based on a resonant acoustic method for determining the viscoelastic characteristics of blood during coagulation under the action of periodic shear deformations obtained using ultrasonic transducers» [8].

Main publications on the topic of the master's thesis:

1. Demkin V.P., Mel'nichuk S.V., Udut V.V., Demkin O.V., Tyutrin I.I. Physical principles of the method of low-frequency piezothromboelastography for studying rheological properties of whole blood // Russian physics journal, 2019. – Vol. 62. – №6. – pp. 972–983. Doi: 10.1007/s11182-019-01803-y.

2. Demkin V.P., Melnichuk S.V., Gavar A.V., Demkin O.V., Rudenko T.V., Udut E.V., Tyutrin I.I., Udut V.V. Spectral Regularities of Viscoelastic Parameters of Whole Blood Exposed to Periodic Shear Stress // Bulletin of Experimental Biology and Medicine, 2020. – V. 169. – № 2. pp. 293 – 296. Doi: 10.1007/s10517-020-04871-0.

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4. Demkin O.V. Modeling of the kinetics of thrombin formation based on the viscoelastic properties of whole blood // Aerophysics. Photonics and quantum optical technologies. Plasma physics. Solid state physics. Thermophysics. Physical methods in natural sciences and materials science. Elementary particle physics, astrophysics and cosmology. Instrumental methods and techniques of experimental physics : Materials of the 60th International Scientific Student Conference on April 10-20, 2022 / Novosibirsk State University. – Novosibirsk : CPI NSU, 2022. – pp. 148–149. (In Russian)

5. Tsybulina A.O., Demkin O.V. Rheological model of blood and assessment of hemostatic potential based on viscoelastic properties of whole blood // Aerophysics. Photonics and quantum optical technologies. Plasma physics. Solid state physics. Thermophysics. Physical methods in natural sciences and materials science. Elementary particle physics, astrophysics and cosmology. Instrumental methods and techniques of experimental physics : Materials of the 60th International Scientific Student Conference on April 10-20, 2022 / Novosibirsk State University. – Novosibirsk : CPI NSU, 2022. – pp. 165–166. (In Russian)

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CONTENTS

LIST OF ABBREVIATIONS

APTT	–	activated partial thromboplastin time
AH	–	arterial hypertension
AP	–	arterial pressure
HP	–	hemostatic potential
ICC	–	intensity of contact coagulation
IPC	–	intensity of polymerization of the clot
IRLC	–	intensity of retraction and lysis of the clot
ICD	–	intensity of coagulation drive
ITC	–	the intensity of total coagulation
CTAA	–	coefficient of total anticoagulation activity
CTA	–	constant of thrombin activity
MAC	–	maximum amplitude of the clot
LPTEG	–	low-frequency piezothromboelastography
TGT	–	thrombin generation test
TEG	–	thromboelastography
PT	–	personal computer
PT	–	prothrombin time
RASB	–	regulation of the aggregate state of blood
GP	–	gelling point
TT	–	thrombin time
η	–	dynamic viscosity
τ	–	tangential stress
$\dot{\gamma}$	–	shear strain rate
$\vec{u}$	–	mechanical displacement
ε	–	Cauchy – Green strain tensor
σ	–	normal stress
$\vec{f}$	–	deformation force

ρ – medium density

ν – frequency

Dx – displacement of the end of the resonator needle along the axis x

ω – the circular frequency set by a piezoelectric generator of mechanical vibrations

ω_0 – natural oscillation frequency of the resonator needle in the liquid

β – attenuation coefficient of needle-resonator oscillations in a viscous liquid

INTRODUCTION

«Currently, there is a sufficient amount of data indicating that thrombohemorrhagic complications (especially in diseases of the cardiovascular system) are the leading cause of disability and mortality of the able-bodied population worldwide» [1-3]. «Despite the huge volume of fundamental and applied research conducted in the world on this topic and the results achieved, the effectiveness and safety of antithrombotic prevention and therapy is far from perfect and is not always accompanied by the desired clinical efficacy» [4-7]. «The achievement of high efficiency is largely determined by the level of modern ideas about the pathogenesis of thrombophilia and thrombosis, the possibilities of their timely diagnosis, as well as knowledge of the pharmacodynamics of antithrombotic drugs. In this regard, the adequacy of methods for assessing hemostatic potential acquires a key role, and the success of prevention and treatment of thrombohemorrhagic complications ultimately depends on the efficiency, informativeness, accessibility and reliability of these methods. In solving this problem, it is of particular importance to attract knowledge about the physical properties of blood, since its aggregate state changes during the coagulation process with significant changes in its viscoelastic properties. The detailed understanding of the process of regulation of the aggregate state of blood (RASB) and the acquisition of new fundamental knowledge requires the addition of the existing laboratory research paradigm by methods of mathematical modeling of hemocoagulation processes and the determination of the physical mechanisms of changes in the rheological properties of blood during clotting» [8, 9]. «Development of such methods for the diagnosis of hemocoagulation and verification of the corresponding model of physico-chemical processes with the possibility of prompt obtaining objective information about the state of the hemostatic potential (HP) of the subject and the response to antithrombotic therapy, will allow pathogenetically justified to determine and change the regimens (dose, discreteness) of prevention and treatment» [10].

To date, the presence of a large number of methods available in the arsenal of hemostasis laboratories creates the illusion of comprehensive and all-encompassing monitoring of hemostatic potential [11, 12].

«However, the existing local and global methods of laboratory diagnostics of the hemostasis system have a number of significant drawbacks: low sensitivity and lack of standardization, duration of sample preparation, conducting studies on a model of citrate plasma or stabilized blood» [9,13]. The unstructured, inaccurate and fragmented analysis results obtained by local and global methods practically exclude a holistic view of the state of the hemostasis system. «One of the widely used global tests is the method of low-frequency piezothromboelastography (LPTEG), which allows an integrative assessment of the state of hemostatic potential (HP) based on measuring the dynamics of viscoelastic characteristics of whole blood at all stages of its coagulation process: from "damage" to the vascular wall of a vein during blood sampling to the formation of a fibrin-platelet clot» [10].

In papers [8,10, 13, 14, 15,], «the complex mathematical model and physical foundations of the method of low-frequency piezothromboelastography under periodic shear stresses for studying the viscoelastic properties of whole (native) blood and its application for the diagnosis of hemostatic potential are described. A method is proposed for determining the viscoelastic properties of blood by measuring the amplitude-frequency and phase characteristics of oscillations of the piezoelectric needle-resonator (Mednord piezothromboelastograph apparatus), taking into account rheological changes in non-Newtonian fluid under conditions of periodic shear stresses» [8]. «In the course of the conducted studies, a strong correlation was revealed between the amplitude of the signal of the direct piezoelectric effect of the piezoelectric sensor of the thrombopiezoelastograph and the value of the dynamics viscosity coefficient» [10, 14, 16]. «Comparison of the calculated values of the viscosity coefficient with its reference values for water and glycerin determined by direct rheometric measurements showed their good agreement, which indicates the

sensitivity of the method to changes in the viscoelastic characteristics of the liquid. On the one hand, due to the change in the aggregate state of blood from liquid to solid-elastic, the change in its viscoelastic characteristics can occur within a wide range. On the other hand, the piezoelectric sensor is a complex mechanical system, the sensitivity of which is determined not only by the impedance of the unloaded piezoelectric, but also by the configuration of the resonator needle, which leads to a significant change in its resonance frequency spectrum, and, consequently, the sensitivity of the method itself»[8, 10, 14].

In this study, a new approach is proposed to determine the HP of whole (native) blood, «based on a complex physico-mathematical model of the hemocoagulation process as a sequential change in its rheological properties and analysis of data on the kinetics of the final product of blood coagulation – fibrin, the development of which determines the dynamics of viscoelastic characteristics of blood during coagulation» [17]. «It is known that fibrin, formed as a result of the interaction of thrombin and fibrinogen, plays a main role in changing the viscoelastic properties of blood. For this reason, when analyzing the state of HP, special attention is paid to the kinetics of thrombin formation as a trigger of hemocoagulation. Consequently, the production of thrombin, as a result of competing enzymatic reactions, sets the fibrin formation, providing a change in the aggregate state of the blood. With this in mind, we propose a method for estimating thrombin generation based on the dynamics of the blood viscosity coefficient during hemocoagulation» [9, 17, 18].

In this regard, the purpose of this dissertation research is to develop a method for evaluating thrombin generation based on studying the dynamics of viscoelastic properties of whole (native) blood in the process of hemocoagulation, using the resonant acoustic method and LPTEG technology.

To achieve the research goal, the following tasks have been solved [10,13, 14, 15].

1. Study of the technology of low-frequency piezothromboelastography to determine the rheological properties of whole blood;

2. Development and application of the resonant acoustic method for calculating the blood viscosity coefficient.

3. Conducting of experimental studies of changes in the viscoelastic properties of whole blood during hemocoagulation.

4. Development of a method for generating thrombin in the process of coagulation of whole blood.

5. Conducting of laboratory and clinical studies of the kinetics of thrombin formation in the process of hemocoagulation of native blood by the LPTEG method.

The research is designed to update and supplement the fundamental data on the mechanisms of the hemocoagulation process and on the understanding of kinetics of thrombin formation as the basis of a new approach to the assessment of hemostatic potential. The use of this approach to elucidate the mechanisms of changes in the viscoelastic characteristics of whole blood, reflecting the state of HP, makes it possible to control HP and manage its normalization.

Unlike conventional coagulation tests, the thrombin formation test can be used for a general evaluation of hemostasis, the results of which can then be used to assess local characteristics of hemostasis, such as prothrombin time, activated partial thromboplastin time and fibrinogen and thromboplastin levels. other clotting factors. The introduction of this method will contribute to a better understanding and evaluation of general hemostatic processes.

1 Hemostasis system and methods of its diagnosis

The hemostasis system is a very complicated biological system in the body for maintaining the physiological balance of multidirectional processes that ensure the integral property of blood to maintain a liquid state (normal) and to coagulate, in order to prevent extravasation of blood in certain, extreme situations for the body [12,19]. «The control of the conjugacy of the participation of the functions of the hemostasis system, which are opposite in their orientation, is provided by the system of regulation of the aggregate state of the blood (RASB), the regulation of which, in turn, is provided by the interaction of the central and peripheral nervous system and the endocrine system» [13].

«A derivative of the functioning of the hemostasis system is the hemostatic potential, which is a reflection of the integrative activity of the vascular-platelet, coagulation, anticoagulation and fibrinolytic links of the hemostasis system that determine the rheological properties of blood» [16]. According to the predominance of activity of individual state of the hemostasis system, HP can be positive (predominance of coagulation orientation), negative (predominance of anticoagulation mechanisms) or neutral (functional balance of the combination of hemocoagulation and anticoagulation mechanisms), while the level of individual factors of the hemostasis system can be extremely variable. The displacement of the HP from the optimal level automatically activates the regulation factors that ensure the activation of compensation mechanisms, the result of which is the return of the HP to the "specified" optimal level. «Thus, with a deviation of HP towards a positive value, the resulting correction occurs by increasing the concentration level of anticoagulation factors and/or a decrease in the level of coagulation factors, and conversely, with a deviation of HP in the negative direction, that is an increasing of the coagulation factors concentration and/or a decreasing of the concentration of anticoagulation factors is observed in the physiological state. Being a functional system, the hemostasis system determines the optimal level of HP in a physiological and pathological state and coordinates the functioning of structures

and mechanisms aimed at achieving the optimal level of HP in various conditions (external and internal), analyzes the proportionality of the result obtained and, if necessary, additionally activates new components, stimulates the necessary activation/inhibition mechanisms in order to achieve sufficient adaptive result» [13].

«Almost any nosology is accompanied by a variation of the HP state , especially in urgent conditions in which critical disorders of the hemostasis system can lead to death» [20]. «The study of the functional state of HP plays an important role in the diagnosis of hypercoagulation and thrombophilia, the processes of disseminated intravascular coagulation and bleeding, as well as the dynamic control of antithrombotic therapy during conservative and surgical treatment. The state of the vascular-platelet and coagulation parts of hemostasis and related changes in the rheological properties of blood play an very important role in the pathogenesis and development of many diseases» [4, 5].

«To date, researchers are "armed" with a fairly weighty set of "local" and "global" methods to evaluate the hemostasis system. Unfortunately, even with the correct observance of the preanalytical stage, the local tests used to diagnose the state of the HP in reality provide extremely limited fragmentary information about its functioning about the process and, unfortunately, do not provide a complex approach for evaluation of the state of the RASB system.

That is why the renaissance of "global" tests and methods analyzing the process of hemocoagulation *ex tempore* is not accidental» [16]. «Such methods provide for two extremely important points:

− working with whole (native) blood;

− registration of the coagulation process under changing the viscous and viscoelastic properties of blood changes.

In this case, development the of domestic technology is of particular importance which was named as the technology of low-frequency piezothromboelastography (LPTEG)» [14,16]. «This technology makes it possible to evaluate all phases of blood clotting in real time and quantify the intensity of

pro- and anticoagulant potential, can be a tool for evaluation the pharmacodynamics of antithrombotic drugs – the key to the prevention and treatment of thrombohemorrhagic complications» [8, 16].

1.1 Blood clotting system

«The blood clotting system is one of the most important physiological systems of the body. Its functioning ensures, normally, the maintenance of blood in a liquid flowing state, as well as local blood clotting - thrombosis - at the site of damage to the vessel» [8]. Blood clotting (Hemocoagulation) is the most important stage of the hemostasis system, responsible for stopping bleeding when the vascular system of the body is damaged. The combination of various blood clotting factors interacting with each other in a very complex way forms a blood clotting system [21].

There are three main mechanisms for stopping bleeding in case of vascular damage, which, depending on the conditions, can function simultaneously, with the predominance of one of the mechanisms [9]:

− vascular-platelet hemostasis caused by vascular spasm and their mechanical blockage by platelet aggregates. On the collagen molecules exposed as a result of damage to the vessel walls, adhesion (adhesion), activation and aggregation (gluing together) of platelets occur. In this case, a so-called "white thrombus" is formed, that is, a thrombus with a predominance of platelets;

− coagulation hemostasis is triggered by a tissue factor from the tissues surrounding the damaged vessel, and is regulated by blood clotting factors. It provides a blockage of the damaged part of the vessel with a fibrin clot [8,16];

− fibrinolysis is the dissolution of a blood clot after the restoration of a damaged vessel wall [16].

The blood clotting system consists of thirteen proteins, which are called blood clotting factors (Table 1); they are usually denoted by Roman numerals (for

example, FVII – factor VII), the activated form is indicated by adding the index "a" (FVIIa – activated factor VII). «Of these, seven are activated to serine proteases (factors XII, XI, IX, X, II, VII and precallikrein), three are cofactors of these reactions (factors V, VIII and high–molecular kininogen HMK), one is a cofactor/receptor (tissue factor, factor III), another is trasglutaminase (factor XIII) and, finally, fibrinogen (factor I) is a substrate for the formation of fibrin» [9, 17], the end product of blood clotting reactions (Table 1.1).

Table 1.1 – The main factors of blood clotting [9]

Factor	Name of the factor	Average blood plasma content, mg/l	Half-life time
FI	Fibrinogen	3000	3 days
FII	Prothrombin	100	4-5 days
FIII	Tissue factor	traces of the substance	
FY	Proaccelerin	10	25 hours
FYII	Proconvertin	0,5	5 hours
FYIII	Antihemophilic factor A	0,1	10 hours
FIX	Antihemophilic factor B	5	20 hours
FX	Stewart-Prauer factor	10	3 days
FXI		5	3 days
FXII	Hageman factor	30	3 days
FXIII	Fibrinstabilizing factor	10	12 days
	Precallikrein	50	
	High molecular weight kininogen	70	

Blood clotting involves an effectively regulated series of transformations of inactive zymogens into active enzymes, which eventually leads to the formation of thrombin and the conversion of fibrinogen into fibrin. Note that the "internal" blood clotting pathway is a slow process, since it involves a large number of clotting factors.

«The final result of the work of the blood coagulation system is the conversion of fibrinogen into fibrin under the action of thrombin. The production of thrombin as a result of a cascade of biochemical reactions sets the dynamics of fibrin production processes and changes in the viscoelastic properties of blood. For this reason, when analyzing the state of the HP, special attention is paid to evaluate the kinetics of thrombin formation and inactivation as a trigger of hemocoagulation» [16, 17].

1.2 Hemocoagulation and its phases

To date, the prevailing model of hemocoagulation is a model based on the cellular theory of blood clotting [22]. According to this theory, hemocoagulation consists of three overlapping phases – initiation, amplification and propagation [23] (Figure 1.1).

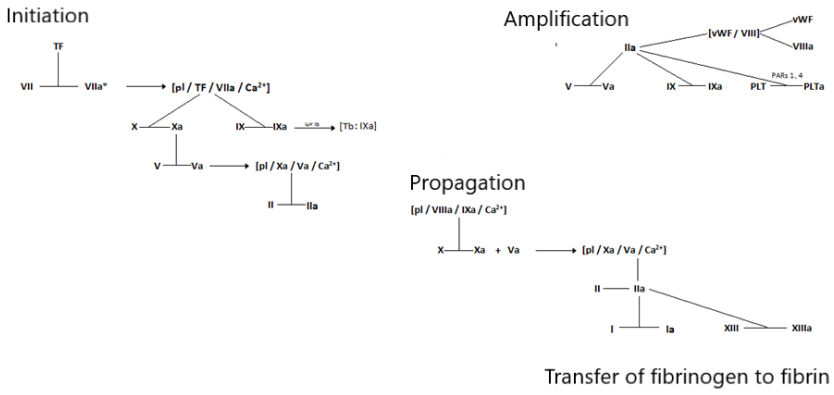


Figure 1.1 – Cascade of biochemical reactions of plasma hemostasis [23]

The essence of the initiation phase, as the name implies, is the activation of the blood clotting process. Two main ways of hemocoagulation initiation are known – internal (Hageman pathway) and external (tissue factor pathway) [9, 17]. The internal pathway, the Hageman pathway begins with the contact activation of (mainly) the XII factor of blood clotting, which initiates the XI factor, and

subsequently the IX factor of blood clotting. It is the activated IX factor, entering into a complex with the activated VIII coagulation factor, that forms a complex – internal tenase [VIIIa-IXa], which activates the X factor of coagulation and forms prothrombinase. However, to date, it has been proven that the internal pathway, unlike the pathway of the tissue factor, contributes to the spatial spread of the clot, but is not the leading pathway in the initiation of hemocoagulation. Thus, it is the external pathway, the path of the tissue factor, that is the main pathway for the beginning of blood clotting. When the vessel is damaged, the blood plasma contacts the tissue factor, resulting in the activation of factor VII and the formation of an external tenase complex [TF-VIIa]. This complex, in the presence of calcium ions, initiates a hemocoagulation cascade, activating factors X and IX on the surface of the subendothelium [24].

«It is the activation of the X factor by the [TF-VIIa-Ca2+] complex that leads to the formation of a small amount of thrombin, insufficient for the transformation of fibrinogen into fibrin, but sufficient to trigger a positive feedback loop in which thrombin enhances its own production. When the subendothelium is exposed, in addition to the activation of the plasma cascade, vascular-platelet hemostasis is activated, characterized by adhesion, activation and aggregation of platelets. Platelet activation also occurs under the action of a small amount of thrombin activated in the initiation phase. In addition, thrombin activates factors V, VIII and XI. The active forms of these factors and the factor IXa moving from the subendothelium, formed in the initiation phase, bind to the surface of the adhered platelets. This process involves the amplification phase, in which the formation of active cofactors (factors Va and VIIIa) occurs, and the reaction surface shifts from the subendothelium to the adhered platelets. Activation of factor VIII, occurring in the amplification phase, allows the formation of a tenase complex [IXa-VIIIa-Ca2+] on the platelet surface. This complex leads to the activation of factor X, which, in turn, leads to the formation of a prothrombinase complex [Xa-Va-Ca2+], which increases the generation of thrombin in the damaged area. After that, a positive feedback is triggered, which consists in the fact that under the influence of

the accumulated thrombin, the activation of XIa accelerates, which affects factor IX. At the same time, there is an increase in activated cofactors (factors Va and VIIIa). At the same time, on the platelet surface, there is an increase in the amount of tenase [IXa-VIIIa-Ca2+]. Also, under the influence of thrombin, there is a similar increase in prothrombinase complexes [Xa-Va-Ca2+]. Together, all these processes lead to an acceleration of hemocoagulation by several thousand times» [9, 25].

Thus, the third stage, the propagation stage, is characterized by the formation of thrombin, the amount of which is sufficient for the transformation of fibrinogen into fibrin.

1.3 Local and global methods of hemostasis evaluation

«To date, researchers have been provided with a wide range of methods of amidolytic and enzyme immunoassay to evaluate the hemostasis system and HP. However, a limited range of these methods is used in routine clinical practice (activated partial thromboplastin time, prothrombin time, thrombin time, fibrinogen, soluble fibrin monomer complexes, D-dimers). The amidolytic technique carries information about the activity of the enzyme, enzyme immunoassays carry information about the amount of the enzyme, and the coagulation technique characterizes the rate of interaction between some enzymes under conditions of relatively standardized activation. It is clear that the cumulative results scattered over the measurement give only an approximate characteristic of the hemostatic potential. Expanding the arsenal of techniques (evaluation of endothelial producers, determination of the level of a number of blood clotting factors), increasing diagnostic value, does not exclude fragmentation of ideas about the process and does not give a holistic view of the hemostasis system» [13, 16].

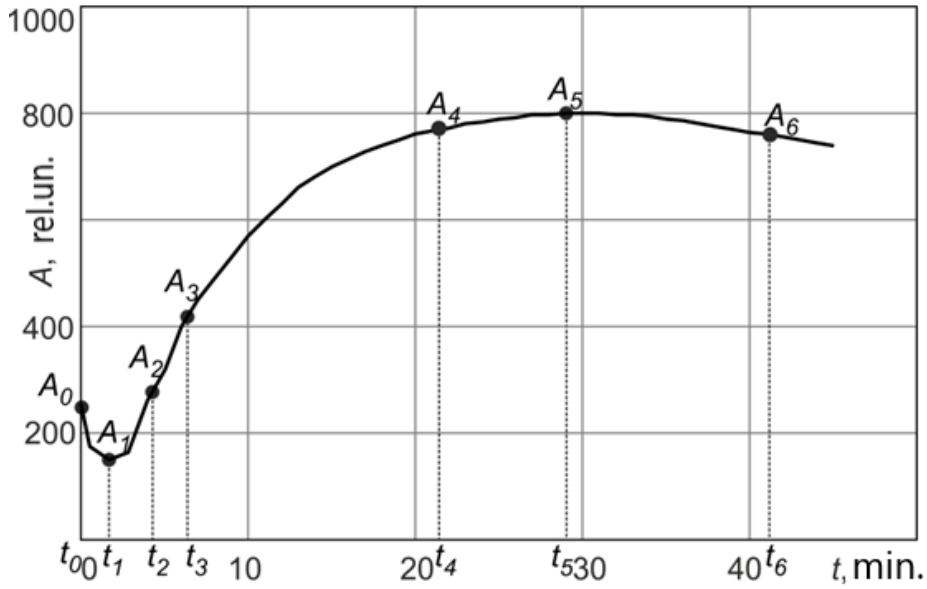
«The group of global methods for measuring hemostatic potential based on physical detection methods, along with the LPTEG method, includes: the

thromboelastography (TEG) method» [26] «and the thrombin generation test (TGT)» [27, 28], «designed for integrative assessment of plasma and cellular components of native blood involved in all stages of fibrinogenesis, from initiation before the formation of p/s fibrin and its possible lysis, otherwise for the assessment of the hemostasis system as a whole» [29].

«The LPTEG method measures the resistance of whole (native) blood to forced oscillations of the resonator needle, which reflects the change in the aggregate state of blood over time» [16].

«As an example, Figure 1.2 shows a graph of changes in the aggregate state of the blood of a healthy volunteer by the LPTEG method, on which the signal amplitude of the piezoelectric sensor A of the process under study is estimated in relative units along the ordinate axis, and the time of the study *t* in minutes along the abscissa axis» [30]. «The dynamics of the process under study – the transition of blood during coagulation from a liquid state to a solid-elastic one – is determined by changes in the aggregate state of blood and is recorded in the form of an integrated LPTEG curve (Figure 1.2), where each point of which (*Ai*) is determined by the state of the system at a specific time of the study (t_i)» [8, 16].

«When measuring the signal of a piezoelectric sensor, the following parameters are recorded and determined: *A0* – the initial value of the signal amplitude at time *t0*, in relative units; *t1* – the reaction period (the time from the beginning of the study to the maximum decrease in the amplitude *A1*,); *t2* – the time to reach the amplitude *A2; A2* – an increase in the signal amplitude by 100 rel. units; *t3* – blood clotting time (gelling point), determined automatically when measuring the *tg* angle of the curve by 50%; *A3* – the magnitude of the signal amplitude at the gelling point; *A4* is the amplitude value 10 minutes after reaching the gelling point; *t5* is the time to reach the maximum amplitude *(A5)* (the time of formation of the fibrin-platelet structure of the clot); *A6* is the signal amplitude value 10 minutes after reaching the maximum amplitude» [8,14].



(*A0–A5*) – NPTEG amplitude at the stages of fibrin formation;

A6 – amplitude at the 10th minute of clot lysis; *(t1–t5)* – the time intervals of the stages of fibrinogenesis; *(t3)* – the gelling point (blood clotting time).

Figure 1.2 – Whole blood LPTEG indicators of a healthy volunteer

«According to the formulas indicated in Table 2, the following indicators are calculated in relative units: the initial stage of coagulation – the intensity of contact coagulation (ICC); the constant of thrombin activity (CTA); the intensity of coagulation drive (ICD); the intensity of polymerization of the clot (IPC); the coefficient of total anticoagulation activity (KTAA); the intensity of retraction and lysis of the clot (IRLC); the maximum amplitude of the clot (MA).

Table 2 – Calculated indicators of LPTEG

Indicator	Decoding the value of the indicator
ICC	$ИСС = \frac{A1-A0}{t1}$ ИСС, rel.un. – intensity of contact coagulation; A_1, rel.un. – maximum decrease in the amplitude of the curve during the reaction period «*t1*»; A_0, rel.un. – the initial value of the amplitude of the curve at time *t0*; t_1, min. – время от начала исследования до достижение минимальной амплитуды кривой НПТЭГ – А1. This indicator mainly reflects the aggregation activity of the shaped elements of the blood, the I and II phases of coagulation, or its suspension stability (SS).
ICD	$ИСD = \frac{A3-A1}{t3}$ ИСD, rel.un. – intensity of coagulation drive; A_3, rel.un. – the magnitude of the amplitude of the curve at the "gelling point"; A_1, rel.un. – maximum decrease in the amplitude of the curve during the reaction period «t_1»; t_3, min. – время свертывания крови – «точка желирования», фиксируемая автоматически при изменении tg угла кривой на ~ 60 %. This indicator characterizes mainly the proteolytic stage of the III phase of hemocoagulation. The *A* – part of the NPTEG curve near the gelling point (a change in the tg angle of the curve by ~ 60%) reflects the beginning of the polymerization process, which at the gelling point (GP) leads to the formation of a fibrin gel – the main structural framework of a hemostatic clot.

Table 2 continuation – Calculated indicators of LPTEG

CTA	$CTA = \frac{A2}{t2-t1}$ CTA, rel.un. – constant of thrombin activity; *A2*, rel.un. – increase in the amplitude of the curve by 100 rel.un.; *t2*, min. – time to reach the amplitude of *A2* curve; *t1*, min. – the time from the beginning of the study to the achievement of the minimum amplitude of the LPTEG – *A1* curve. The use of this indicator in the analysis of LPTEG is due to the need to determine a universal criterion for assessing the intensity of the proteolytic stage of fibrin formation.
IPC	$IPC = \frac{A4-A3}{10\ min}$ IPC, rel.un. –the intensity of polymerization of the clot; *A4,* rel.un. – the value of the amplitude after 10 minutes from the "gelling point"; *A3,* rel.un. – the value of the amplitude at the "gelling point". Displaying the intensity of the polymerization stage. The use of a time interval equal to 10 minutes is due to the need to unify the method, since the formation of transverse covalent bonds is a fairly long stage of post-gel formation.

Thus, LPTEG piezothromboelastograms allow analyzing all stages of fibrinogenesis and assessing the state of hemostatic potential» [8, 16].

«TGT test is based on the main properties of the blood aggregation control system to generate thrombin, the dynamics of changes in the concentration of which determines the total effect of the interaction of all factors of the coagulation system» [27, 28, 31]. «The physical principle of the thrombin generation test is to determine the amount of thrombin (in nmol), which is formed during the recalcification of citrate blood plasma in the presence of a fixed concentration of tissue factor and fluorogenic substrate» [28, 32].

«The TEG method is based on the analysis of the dependence of blood viscosity on time during the formation of a clot. A citrate blood sample taken from a vein is required for the analysis. The principle of the method is the incubation of

whole blood at 37 ° C in a heated cylindrical cuvette, which oscillates for 10 seconds at an angle $4^{\circ}45'$ in a bowl with a pin freely suspended and connected to a wire» [32, 26].

«The output measured characteristic for TGT is the concentration of thrombin, for TEG – the amplitude of the rotation angle of the sensor rod immersed in citrate recalcified blood, for LPTEG (measurement in native blood) – the amplitude of the electrical oscillations of the piezoelectric sensor.

The clotting time can reach tens of minutes and falls on the point of formation of the viscoelastic gel (*A4*) on the LPTEG curve. The TEG method is not without drawbacks, the main of which are the problem of standardization, insufficient sensitivity of the method in assessing the main links of the hemostasis system, especially in the case of functional hypoxia, hypocalcemia and hypothermia» [32].

2 Rheological properties of blood

Rheology (from the Greek ρεος, "flow, flow" and -logy) is a branch of physics that studies deformations and fluidity of matter [33].

The term "Rheology" was first introduced by Eugene Bingham, an American chemist. He showed that for many real liquids, the critical stress level τ_0 must be reached in order for the liquid to start flowing. Below this critical voltage, the liquid behaves like a solid. The rheological properties of blood depend on many factors.

«The rheological properties of blood depend on a number of factors: the concentration of blood cells and their aggregation parameters, the composition of the plasma and its spatial distribution, the kinetic characteristics of blood flow, the rate of elastic shear deformations, and external factors; moreover, various factors can have a mutual influence on their value» [14].

«The presence of these factors generally ensures the classification of blood as a non—Newtonian fluid with two main rheological properties - viscosity and plasticity. The main difference between non-Newtonian fluids is the dependence of viscosity on the shear strain rate» [9]. Currently, there are a large number of rheological models describing this dependence for various shear rates. Consideration of this dependence is necessary when modeling vascular hemodynamics, because the rate of shift in one period of the cardiac cycle in the arteries ranges from 0 to 1000 s-1.

It is known that the viscosity and elasticity of blood change significantly during its coagulation. «To date, significant progress has been made in the development of theoretical methods and experimental tools for studying hemorheology and a deep understanding of the interaction of blood components that determine its viscoelastic properties» [34-38]. «At the same time, the problem of determining changes in the viscoelastic properties of whole blood during clotting remains open due to the lack of diagnostic tools. In existing devices for determining the rheology of whole blood, studies are carried out on its stabilized

samples (capillary and rotary rheometers), and when assessing elastic properties, thromboelastographs are used to evaluate changes in this characteristic after the formation of a clot» [6]. The measurement of the viscoelastic properties of whole blood is very limited in time due to the natural process of coagulation. In these cases, stabilizing drugs (heparin, EDTA, etc.) are used during measurements to prevent coagulation [34, 35], which can lead to changes in hemorheological properties and to certain errors in the measurement of the viscosity coefficient.

2.1 Blood viscosity, rheological models of blood

«Viscosity is an important property of liquids that describes the resistance of a liquid to spreading; it is related to internal friction in a liquid. The most common type of fluidity is shear flow, in which liquid layers move relative to each other under the action of shear stress, which is defined as the force acting on a unit area of the liquid, and allows you to obtain a velocity gradient over the thickness of the sample, called the shear rate. The shear viscosity or dynamic viscosity associated with this process is determined by the ratio of shear stress to shear rate.

Simple unstructured liquids are classified as Newtonian, which means that their viscosity does not depend on the magnitude of the applied shear. Examples can be water and simple hydrocarbons. As the complexity of the fluid increases, the fluids may exhibit more complex behavior and exhibit a non-Newtonian response, in which the viscosity depends on the magnitude of the applied shear. These types of liquids are commonly referred to as structured or complex liquids. This non-Newtonian behavior is characteristic of many liquids, including blood, which are usually liquids that liquefy during shear, where the viscosity decreases with increasing shear rate» [13].

«Blood is a concentrated suspension of several basic cellular elements: erythrocytes, leukocytes and platelets in an aqueous polymer and ionic medium - plasma consisting of 93% water and 3% particles: electrolytes, organic molecules, numerous proteins (albumin, globulins and fibrinogen) and waste products» [8, 38]. «Erythrocytes are biconcave discs with an average diameter of 6 to 8 microns

and a maximum thickness of 1.9 microns. The average volume of an erythrocyte is 90 μ^3. Their number per cubic millimeter of blood is approximately 5 to 6 x 10^6, and they represent approximately 40 to 45% by volume of normal human blood and more than 99% of all blood cells. The proportion of red blood cells is called hematocrit. The main function of red blood cells is the transport of oxygen and carbon dioxide.

White blood cells are roughly spherical and much larger than red blood cells, but they exist in smaller numbers in the blood: their diameter ranges from 6 to 17 microns, and they are approximately 7 to 11 x 10^3 per cubic millimeter in a normal adult. The main function of red blood cells is the transport of oxygen and carbon dioxide. White blood cells are roughly spherical and much larger than red blood cells, but they exist in smaller numbers in the blood: their diameter ranges from 6 to 17 microns, and they are approximately 7 to 11 x 10^3 per cubic millimeter in a normal adult. White blood cells play a vital role in fighting infection and are thus able to migrate from blood vessels and into tissues.

Platelets are small disk non-nuclear cell fragments, much smaller than erythrocytes and leukocytes (approximately 2 to 3 μ^3). Platelets are a vital component of the blood clotting mechanism. The total volume concentration of leukocytes and platelets is only about 1%. The presence of cellular elements of blood and their interaction leads to significant changes in the rheological properties of blood.

Blood plasma, which consists mainly of water, is a Newtonian fluid. However, whole blood has complex mechanical properties that become especially significant when the particle size is much larger or at least comparable to the size of the lumen. In this case, what happens at the level of microcirculation (in small arterioles and capillaries), blood cannot be represented as a homogeneous liquid, but as a suspension of blood cells (especially erythrocytes) in plasma. The presence of blood cell elements and their interaction leads to significant changes in the rheological properties of blood, and reliable measurements must be carried out to obtain appropriate microstructural models» [37]. «Numerous researchers have

found that blood viscosity gradually decreases as the velocity gradient increases. This dependence manifests itself at relatively low velocity gradients up to 60-70 s^{-1}. At velocity gradients of 60-70 s^{-1} and higher, the decrease in viscosity practically ceases, and it becomes "constant" or, as it is often called, asymptotic» [36]. «The viscosity curve characteristic of blood is concave towards the deformation velocity axis. Therefore, judging by the flow curve, pseudoplasticity is inherent in blood. Given that blood has a yield point, it (using the terminology accepted in rheology) can be attributed to nonlinear-visco-plastic media.

The main contribution to the elastic properties of blood is made by the aggregation ability of erythrocytes forming a spatial structure. At low shear deformation rates, the spatial structure of erythrocytes does not collapse and causes the threshold behavior of shear deformation, at which the plastic properties of blood manifest themselves. At high velocities $\gamma > 200$ s^{-1}, the spatial structure of erythrocytes is destroyed, which causes the effect of shear thinning of blood, leading to a decrease in viscosity. For example, in systole, with an increase in blood flow, red blood cells (erythrocytes) dissociate and deform more efficiently, because the faster they move, the less viscous the blood becomes. The slower the cells move (as in diastole), the more viscous the blood becomes.

Figure 2.1 shows the dependence of the dynamic coefficient of blood viscosity on the shear rate» [37].

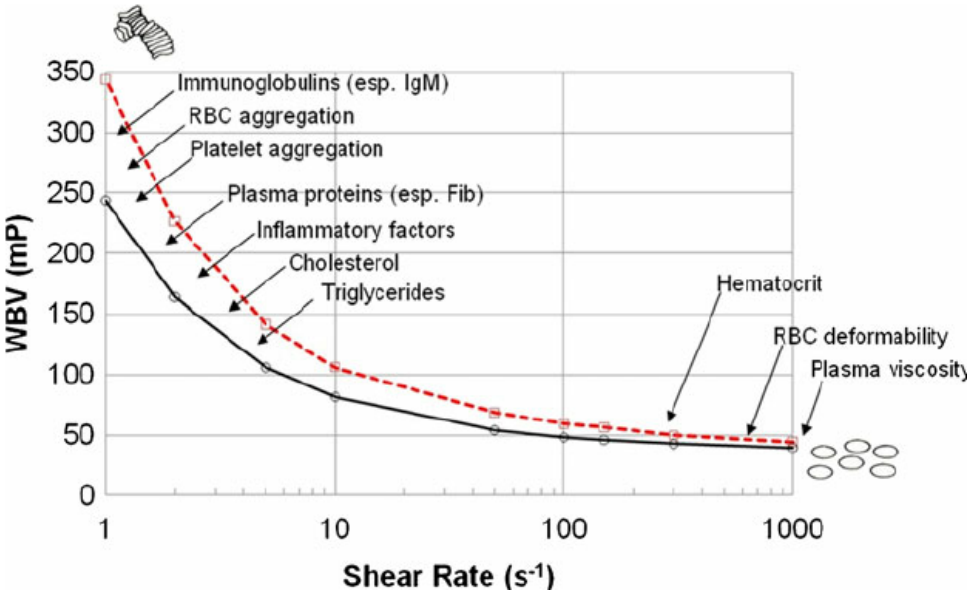


Figure 2.1 – The dependence of the viscosity of whole blood on the shear rate

As can be seen from Figure 2.1, at different shear rates, blood viscosity is affected by various rheological factors. «In areas of circulation with a high shear rate, blood viscosity is mainly determined by hematocrit, erythrocyte deformability and blood plasma viscosity. In areas with a low shear rate, where blood slows down, plasma protein molecules and cells interact in such a way that aggregates or rolls are formed, and platelets and other intermolecular compounds aggregate.

Whole blood has two main rheological properties – viscosity and plasticity and, therefore, belongs to the class of non-Newtonian fluids» [39]. «The most well-known non-Newtonian characteristic of blood is its dilution during shear deformation: at low shear rates, blood seems to have a high apparent viscosity (due to aggregation of red blood cells) at high shear rates, there is a decrease in blood viscosity (due to deformability of red blood cells). The viscoelastic behavior of blood is less important at higher shear rates. Understanding the relationship between blood composition and its physical properties is important for developing a suitable model for describing blood behavior» [36].

«Whole blood is well described in the rheological model as a liquid with viscous and elastic properties» [40]. The main rheological equation for such liquids

is $\tau = \eta\dot{\gamma},$ where $\tau -$ is the tangential stress tensor; $\dot{\gamma} -$ is the shear rate; $\eta -$ is the viscosity.

«To obtain a rheological equation, consider the dependence of the tangential stress developing in neighboring layers of a moving fluid τ on their velocity v in accordance with Newton's formula.

$$\tau = \eta \frac{dv}{dy} \quad , \tag{2.1}$$

where η- coefficient of dynamic viscosity of the liquid ($\eta = const,$ for a Newtonian fluid). Let′s give this formula a different look.

$$\tau = \eta \frac{d}{dy}\left(\frac{dx}{dt}\right) = \eta \frac{d}{dt}\left(\frac{dx}{dy}\right) = \eta \frac{d\gamma}{dt} = \eta\dot{\gamma} \ , \tag{2.2}$$

Most biological fluids, including whole blood, belong to the class of non-Newtonian fluids. For them, $\eta(\dot{\gamma})$ is a nonlinear function of $\dot{\gamma}$ and depends on a number of factors: the concentration and composition of the liquid, kinetic characteristics, the rate of elastic shear deformations, external factors; moreover, various factors can have a mutual influence on their magnitude.

For a non-Newtonian fluid, $\eta = \eta(\dot{\gamma})$. Thus, equation (2.3)

$$\tau = \eta(\dot{\gamma})\dot{\gamma} \tag{2.3}$$

is the basic rheological equation.

The spatiotemporal distribution of blood viscosity is characterized by the dynamic value (apparent viscosity) of the coefficient of internal friction $\eta(r,t).$ The significant dependence of viscosity on internal and external factors reflects fundamental differences in the types of blood flow and its kinetics and, accordingly, generates a variety of rheological models of blood. Phenomenological models are reduced to the corresponding rheological equations that determine the functional relationship between the dynamic viscosity η, stress τ and the shear

strain rate $\dot{\gamma}$. Most of the existing rheological models can be derived from the phenomenological equation» [13, 39]:

$$\tau^n = \tau_0^m + \eta^m \dot{\gamma}^n, \tag{2.4}$$

where τ_0^m– limited shear stress, *m* и *n* they are selected from comparison with the experiment.

Another kind of equation of the semiempirical rheological model has the form:

$$\begin{aligned} &\eta = \eta_\infty + (\eta_0 - \eta_\infty) \cdot f(\dot{\gamma}) \\ &f(\dot{\gamma}) = 1 \ (if \ \dot{\gamma} \to 0) \\ &f(\dot{\gamma}) = 0 \ (if \ \dot{\gamma} \to \infty) \end{aligned}, \tag{2.5}$$

here η_0 и η_∞ correspond to the viscosity of blood at $\dot{\gamma} \to 0$ и $\dot{\gamma} \to \infty$.

Currently, there are many rheological models reflecting the fundamental differences in the types of blood flow depending on internal and external factors. As an example, the effect of hematocrit and plasma chemical composition on the value of dynamic viscosity is taken into account in the rheological models of Quemada [41] and Walburn-Schneck [42]. As a comparison of these models, we calculated the blood viscosity coefficients in our studies to assess the effect of these factors on the change in the viscosity coefficient, the Quemada model [41] was used, in which hematocrit was taken into account

$$\eta = \eta_p \left(1 - \frac{1}{2} \frac{k_0 + k_\infty \sqrt{\dot{\gamma}/\gamma_c}}{1 + \sqrt{\dot{\gamma}/\gamma_c}} H_t \right)^{-2}, \tag{2.6}$$

Where values η_p, γ_c, k_0, k_∞ have been took from [39],
and the Walburn-Schneck model with two parameters: hematocrit and the concentration of globulin proteins in plasma

$$\eta = C_1 e^{C_2 H_t} e^{C_4 \frac{TPMA}{H_t^2}} \dot{\gamma}^{-C_3 H_t}, \tag{2.7}$$

where C_1= 0.000797 Pa ·s , C_2 = 0.0608 , C_3 = 0.00499 ,C_4 = 14.585 l/g ,

TPMA – is protein concentration without albumin, Ht – hematocrit.

«The calculation of the dynamic viscosity coefficient according to formulas (2.6) and (2.7) in our case in the range of shear rates of 75 s^{-1} shows a good correspondence of the calculation results to experimental data» [31, 37].

«Blood coagulation is an extremely complex biochemical process that starts when the vascular wall is damaged and leads to the polymerization of fibrin with the formation of a clot that stops bleeding. The phase transformation of blood from a liquid state to a solid-elastic one during the polymerization of fibrin and the formation of transverse intermolecular bonds, its retraction and subsequent lysis is determined by changes in the aggregate state of blood, and, accordingly, changes in its viscoelastic properties. Thus, by changing the viscosity coefficient, it is possible to judge the dynamics of phase transformations of blood in the process of coagulation» [16].

2.2 Resonant acoustic method for determining the viscoelastic properties of blood

«The technology of low-frequency piezothromboelastography for determining viscoelastic characteristics in the process of blood clotting is based on methods of low-frequency elastography. Elastography is used to differentiate tissues and fluids by their viscoelastic properties through mechanical action and analysis of deformations obtained using ultrasound diagnostic scanners or MRI scanners.

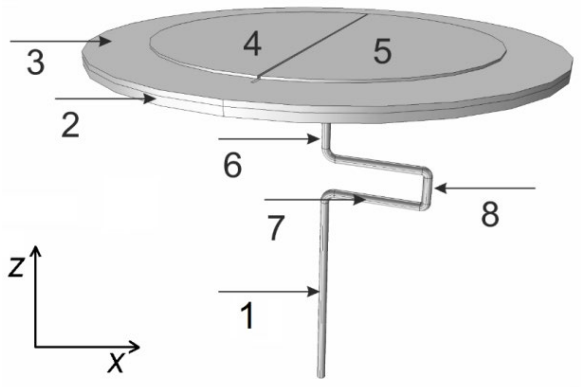
Ultrasound elastography has been used in medical practice quite recently. Although the diagnostic equipment has modern tools and technologies, the potential of ultrasound elastography is far from being exhausted. One of the most important areas of such research is ultrasound elastography of the whole blood coagulation process. Hemocoagulation is an extremely complex biochemical process triggered when the vascular wall or blood cells are damaged, and leads to

the polymerization of fibrin, accompanied by the formation of a clot that stops bleeding» [16, 43].

«In the LPTEG technology, the change in the aggregate state of whole blood is determined and recorded as a dependence of the oscillation amplitude of the piezoelectric sensor of the piezothromboelastograph.

The principle of operation of this device is based on the registration of changes in the resistance of the examined blood to resonant vibrations of the piezoelectric sensor resonator needle (Figure 2.2), fixed on a piezoelectric element, which is a brass base on which a layer of piezoceramics is fixed, divided into two circular segments, and lowered by the second end into a cuvette with the patient's blood. The resonator needle in its middle part is made with a bend in the form of a loop» [8].

«A voltage varying according to the harmonic law is applied to one of the piezoelectric segments. Under the influence of this voltage, the piezoelectric makes mechanical vibrations, which are transmitted to the needle. When the end of the resonator needle is immersed in the liquid, the amplitude-phase characteristics of the voltage on the recording piezoelectric element will change. This is due to the influence of the viscoelastic properties of the liquid on the amplitude-phase characteristics of the mechanical vibrations of the resonator needle, on the change in its own and resonant oscillation frequency» [14].



1 – resonator needle; 2 – brass ring; 3 – brass disc; 4, 5 – piezoelectric semicircular plates;
6, 7, 8, 9 – cylindrical sections of the rod with a rectangular loop
Figure 2.2 – Piezoelectric sensor

Consequently, by changing the amplitude-phase characteristics of the voltage on the recording piezoelectric element, it is possible to obtain information about the magnitude of the viscoelastic characteristics of the liquid.

«The physical principles of the resonant acoustic method for determining the viscoelastic characteristics of a liquid are as follows.

The oscillations of the resonator needle of a piezoelectric sensor can be considered as forced oscillations of a physical body (a physical pendulum) in a viscoelastic medium under the influence of a force varying according to a harmonic law, and the influence of the medium will be reflected in the change in the amplitude-phase characteristics and the natural and resonant frequency of oscillations of this pendulum. Thus, from the measured amplitude-phase characteristics of the piezoelectric sensor, it is possible to calculate its resonant and natural frequencies, and, consequently, the viscoelastic characteristics of the blood under study and their dynamics during coagulation» [13, 14, 44].

Consequently, by changing the amplitude-phase characteristics of the voltage on the recording piezoelectric element, it is possible to obtain information about the magnitude of the viscoelastic characteristics of the liquid.

«In classical viscometry, the determination of the viscoelastic properties of blood is based on measuring the dependence of the shear stress $\tau(\dot{\gamma})$, as well as the threshold values of τ and $\dot{\gamma}$. To account for the elastic properties of blood, the method of complex representation of the shear modulus $G = G' + iG''$, is used, where G', G'' are the modulus of elasticity (storage modulus) and the modulus of viscosity (loss modulus), respectively. Similarly, for the viscosity coefficient, taking into account the elastic properties of blood, we can write $\eta^* = \eta' + i\eta''$, where $\eta', \eta'' -$ are the viscosity coefficient and the elasticity coefficient, respectively» [34, 35].

«To determine the real and imaginary parts of the complex viscosity value, the method of oscillatory viscometry or the method of dynamic mechanical analysis (DMA) is used, when the dynamics of a viscoelastic medium is considered under the action of a force varying in time according to the harmonic

law: $\tau = \tau_0 sin\omega t$,where ω is the frequency of the driving force.In this case, the time and frequency dependences of $\tau^*, \dot{\gamma}$ и η^*. are investigated» [8, 44].

«In this work, an approach based on a mathematical model of forced cylinder oscillations in a viscoelastic fluid is applied to solve this problem. To account for the viscous and elastic properties of blood, we used the method of complex representation of the viscosity coefficient $\eta^* = \eta' + i\eta''$, where $\eta', \eta'' -$ is the viscosity coefficient and the elasticity coefficient associated with the shear modulus G': $\eta'' = \frac{G'}{\omega}$, where ω is the frequency of shear stresses.When the lower part of the pendulum is immersed in a viscous liquid, the amplitude-frequency and phase characteristics of its oscillations shift towards lower frequencies, depending on the viscoelastic parameters of the liquid and the frequency of forced oscillations.

To determine the complex viscosity index of a liquid from the amplitude-phase characteristics of vibrations of a resonator needle, we have developed a mathematical model according to which the oscillation of the end of a resonator needle immersed in a liquid can be represented as vibrations of a cylinder of height *h* and radius *R,* performed in a direction perpendicular to the axis of the cylinder, in a viscoelastic medium η* under the action of a periodic forcing force strength» [10, 13].

«We denote the forcing periodic force acting on the resonator needle from the piezoelectric element, for $f = f_0 e^{i\omega t}$, where ω – the frequency set by a piezoelectric element-a generator of mechanical vibrations. Under the influence of this force, the lower end of the resonator needle performs harmonic oscillations in an elastic medium $x = x_0 e^{i\omega t}$. When the lower end of the resonator needle is immersed in a viscoelastic liquid, the amplitude-frequency characteristics of its oscillations can be determined from the equation» [8, 14]

$$\ddot{x} + 2\beta\dot{x} + (\omega_0')^2 x = \varepsilon_0 e^{i\omega t}, \qquad (2.8)$$

where ω_0' - the natural oscillation frequency of the resonator needle in the liquid, which is determined through the shear modulus *G;* β – attenuation coefficient,

depending on η'; $\varepsilon_0 = \frac{f_0}{m}$, $m -$ the mass of the lower end of the resonator needle immersed in liquid.

The solution of equation (2.8) is $x = \tilde{x}_0 e^{i(\omega t - \varphi)}$,

where

$$\tilde{x}_0 = \frac{\varepsilon_0}{\sqrt{[(\omega_0')^2 - \omega^2]^2 + 4(\beta\omega)^2}} \quad (2.9)$$

$$tg\varphi = \frac{2\beta\omega}{(\omega_0')^2 - \omega^2} \quad (2.10)$$

To calculate the coefficient β, we take into account that for ultrasonic vibrations, the values of the Reynolds number are equal to: for water, *Re* ~220; for glycerin, *Re* ~0.2; blood *Re* ~60, which allows us to conclude about the laminar flow regime of the lower part of the moving rod in these liquids. Consequently, the calculations can use a model of the viscous friction force proportional to the velocity of the lower section of the rod relative to the liquid medium.

Figure 2.3 shows a cross section of a cylinder of radius *R* and height *h* moving in a viscous liquid at a velocity of υ [8]. The viscous friction force equal to $dF_v = \eta \frac{dv_\tau}{dy} dS$ acts on the dS element, where $v_\tau = v\, sin\alpha -$ is tangential component of the cylinder velocity. Then $dF_v = 2\eta \frac{dv}{dy} hRsin\alpha d\alpha$. Integrating this expression, we get $F_v = 4\eta \frac{dv}{dy} hR$. «To calculate the velocity gradient, we use the expression for the velocity of propagation of shear waves in a viscous medium $= \sqrt{\frac{2\eta\omega}{\rho}}$, where ρ – liquid density. Then we can put $\frac{dv}{dy} = \frac{v}{l^*}$, where $l^* = \frac{2\pi c}{\omega}$ – is the thickness of the fluid layer involved in the movement. As a result , the formula for the coefficient β is obtained» [8]:

$$2\beta = 4\eta/(\pi l^* \rho_0 R), \quad (2.11)$$

where ρ_0 – density of the resonator needle material, $l^* = \frac{2\pi c}{\omega}$ – the thickness of the fluid layer involved in the movement, $c = \sqrt{\frac{2\eta\omega}{\rho}}$ – the speed of shear waves,

ρ – liquid density. From formulas (2.10) and (2.11) it is possible to determine the real part of the viscosity coefficient $\eta' = \eta$.

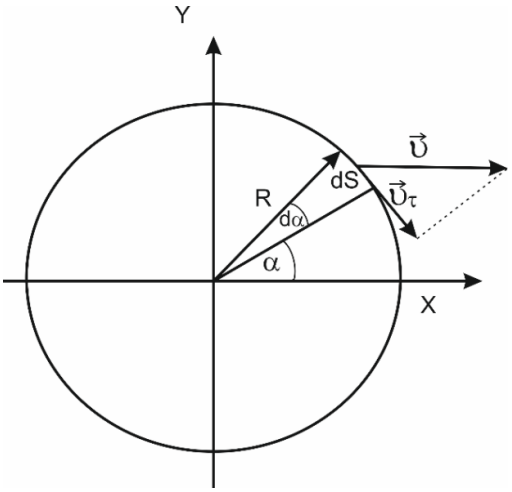


Figure 2.3 – Section of a cylinder oscillating in a viscous liquid

To determine the real part of the complex shear modulus $G^* = G' + iG''$, which characterizes the elastic properties of the fluid, we use the formula for the tangential stress $\tau = G' \frac{x}{l^*}$, where x is the magnitude of the shear strain along the X axis. As a result, for the natural frequency of oscillation of the resonator needle in the liquid ω_0' we obtain the formula:

$$\omega_0' = \sqrt{\frac{4G'}{\pi l^* \rho_0 R}} \tag{2.12}$$

The formula (2.12) can be used to calculate the shear modulus $G' = G$ according to the value determined from the experiment ω_0'.

As a result, the modulus of the complex viscosity coefficient η^* taking into account the ratios $\eta' = \frac{G''}{\omega}$; $\eta'' = \frac{G'}{\omega}$, can be calculated by the formula

$$\eta^* = \sqrt{\eta^2 + \left(\frac{G'}{\omega}\right)^2}. \tag{2.13}$$

The piezoelectric sensor of the piezothromboelastograph "Mednord" is a complex mechanical system. Piezoceramic elements in the form of two semicircular segments are "loaded" onto a brass disk to which a resonator needle is attached, immersed in a viscous liquid. Accordingly, the natural frequency of such a system differs significantly from the natural oscillation frequency of a free piezoelectric element.

«The conductivity of a piezoelectric element in an alternating current circuit increases with increasing frequency and linearly depends on the latter. However, at some frequencies, this dependence of conductivity is violated and is characterized by a sharp increase in conductivity, followed by its sharp drop. These changes in conductivity have a resonant character, and resonances occur on a number of multiple harmonics, and for each harmonic two resonances (sequential and parallel) are observed, corresponding to the voltage resonance and the current resonance in the equivalent electrical circuit of the piezoelectric element» [45]. «The resonant frequency f_r is shifted to the low frequency region from the antiresonance frequency f_a and depends on the electromechanical coupling coefficient» [46].

The thickness of the piezoelectric elements included in the piezoelectric sensor of the piezotromboelastograph device is 0.02 cm. Then the upper limit of the frequency band of the piezo sensor reception, calculated by the formula $f_{max} = 0.44\frac{c_l}{l}$, where c_l is the speed of sound in ceramics, $l = 0.02$ cm is the thickness of the piezo plate, will be equal to 10.7 MHz. Therefore, this type of piezoelectric converter is broadband.

Thus, the presence of several resonances in a broadband piezoelectric sensor makes it possible to select a resonant frequency corresponding to the shear strain rate, the most suitable for studying the influence of rheological factors of blood and evaluation the hemostatic potential [10, 47].

«To study the spectral patterns of the behavior of the viscoelastic characteristics of whole blood, an analysis of the amplitude-frequency characteristics of the oscillations of the needle resonator of the piezothromboelastograph Mednord in air and water in a wide frequency range of 0-80 kHz was carried out» [10, 13].

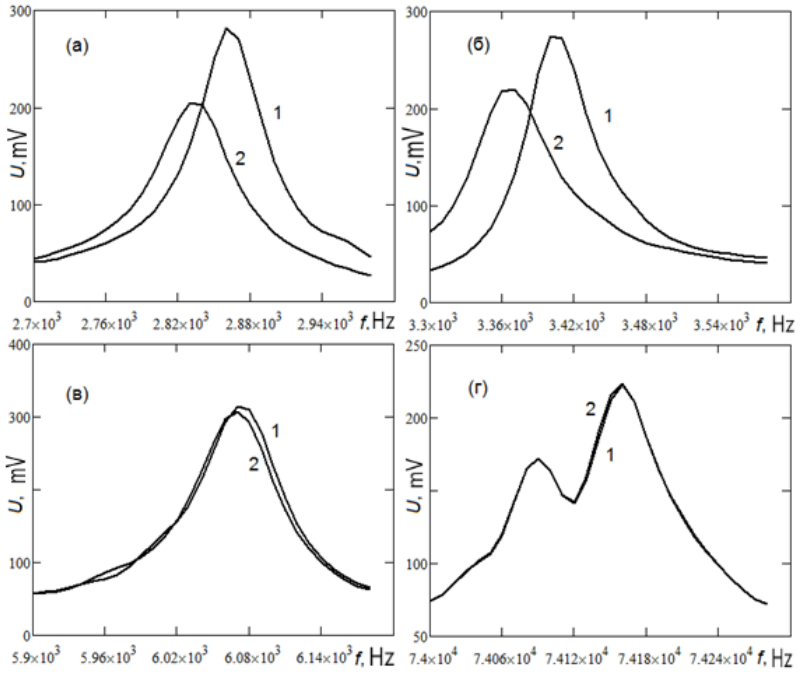
«Measurements of the received signal were carried out using a digital oscilloscope. The amplitude of the signal applied to the piezoelectric element was 100 mV. It was found that resonances of the oscillation amplitude of the resonator needle for this type of piezothromboelastograph Mednord are observed in the air for frequencies 2864, 3391, 6070 Hz and 74 kH.

Figure 2.4 shows the amplitude-frequency response of the piezoelectric sensor for different oscillation frequencies of the resonator needle» [10].

«Figure 2.4 (a) shows that the amplitude-frequency response in water has the form of resonant curves shifted in frequency relative to vibrations in air. With increasing frequency, this difference disappears, and at f = 74 kHz, the curves practically do not differ. The maximum oscillation amplitude of the resonator needle in the air is three times greater than the amplitude of the signal supplied to the piezoelectric element, which indicates the resonance state of the piezoelectric sensor. A comparison of curves 1 and 2 shows that the resonant frequency of the piezoelectric sensor and the resonant oscillation amplitude of the resonator needle decrease in water due to viscosity.

Figure 2.4 (b) shows that the amplitude-frequency characteristics of the received signal in air and water for the antiresonance frequency $f_a = 3391$ and 3360Hz have the same patterns as for the resonant frequencies f_r= 2864 and 2840 Hz. During the transition from air to water, the change in the amplitude of the electric signal at the antiresonance frequency is comparable in magnitude to the changes in the amplitude of the electric signal at the resonant frequency, however, the change in the antiresonance frequency $\Delta\omega_a$ = 31 Hz is 1.3 times greater than the change in the resonant frequency $\Delta\omega_r$ = 24 Hz.This indicates that the use of an antiresonance frequency is preferable for studying the

viscoelastic properties of the medium, because at this frequency the resonant acoustic method turns out to be more accurate» [10].



(a) – resonance: 1 – air (2864 Hz); 2 – water (2840 Hz).

(b) – antiresonance: 1 – air (3391 Hz); 2-water (3360 Hz).

(c) – resonance: 1 – air (6070 Hz); 2-water (6070 Hz).

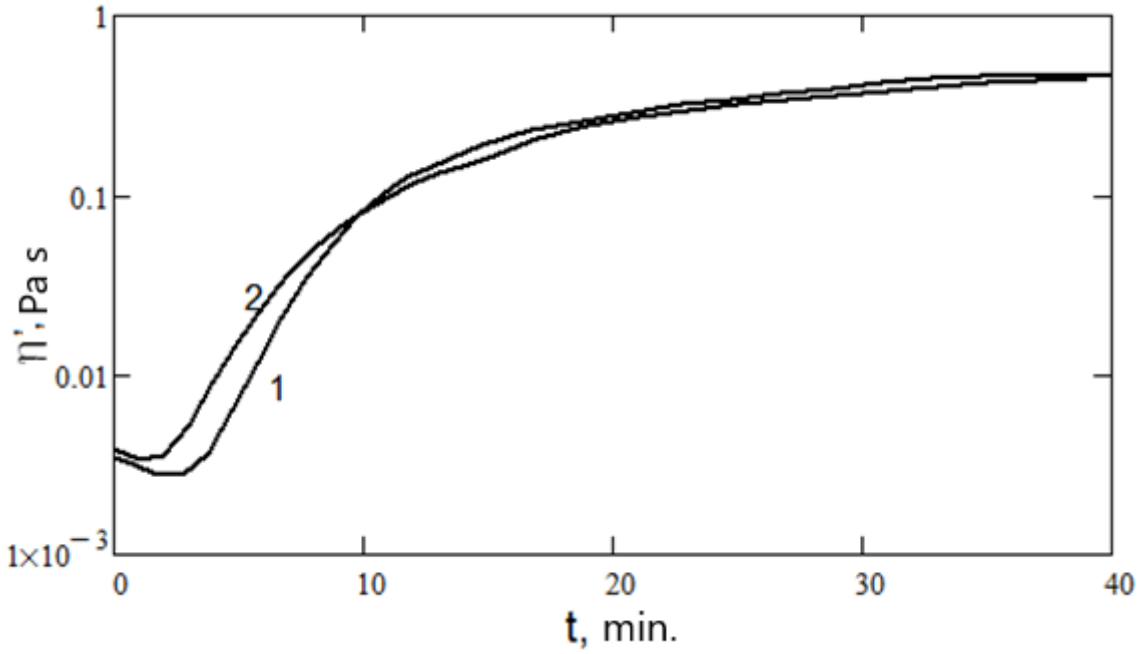
(d) – resonance (74 kHz): 1 – air; 2-water.

Figure 2.4 – Dependence of the oscillation amplitude of the resonator needle on the frequency

«With a further increase in frequency, the influence of the viscosity of the medium does not affect the amplitude-frequency characteristics of the oscillation of the resonator needle. As an example, Figures 2.4(c) and 2.4 (d) show the amplitude-frequency characteristics of a piezoelectric sensor for a frequency of 6070 Hz and a frequency of 74 kHz. As can be seen from these figures, the amplitude-frequency response of the piezoelectric sensor does not change during the transition from air to water. This is explained by the fact that the thickness of the liquid layer adjacent to the needle is $l^* = \frac{2\pi c}{\omega}$, where c is the velocity of shear

waves, sharply decreases and the resistance of the medium relative to shear deformations can be neglected» [10].

«Figure 2.5 shows the calculated dependence of the viscosity coefficient of whole blood of a healthy volunteer η' on time for the frequency f_r and f_a of oscillation of the resonator needle» [13].

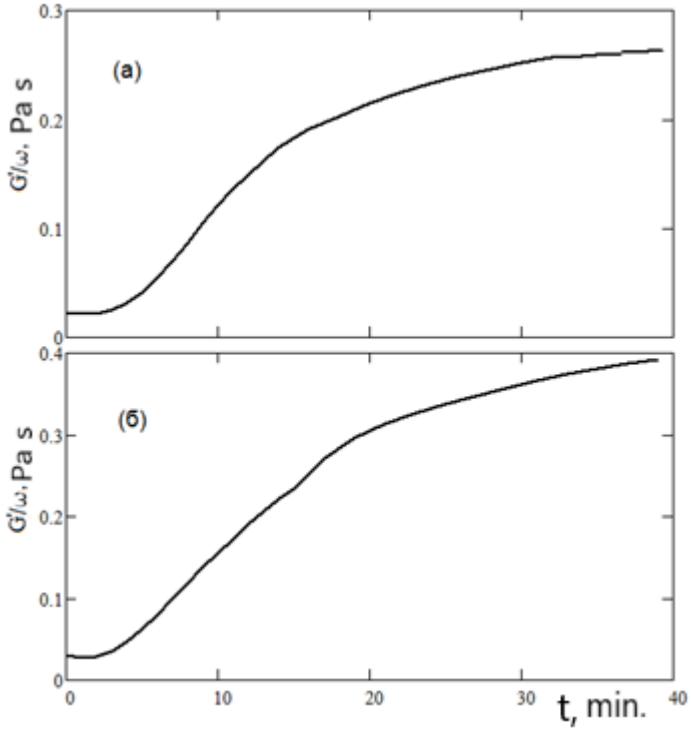


1 – f_r=2864 Hz; 2 – f_a=3391 Hz.

Figure 2.5 – Dependence of the blood viscosity coefficient η' on time for the frequency f_r and f_a of oscillation of the resonator needle

It can be seen from the figure that up to the gelling point, the viscosity coefficient is sensitive to the frequency of shear vibrations, and then the differences in the coefficients η',, for f_r=2864 Hz; f_a=3391 Hz disappear.

Figure 2.6 shows the dependence of the shear strain modulus G'/ω of whole blood on time for f_r and f_a oscillations of the resonator needle.

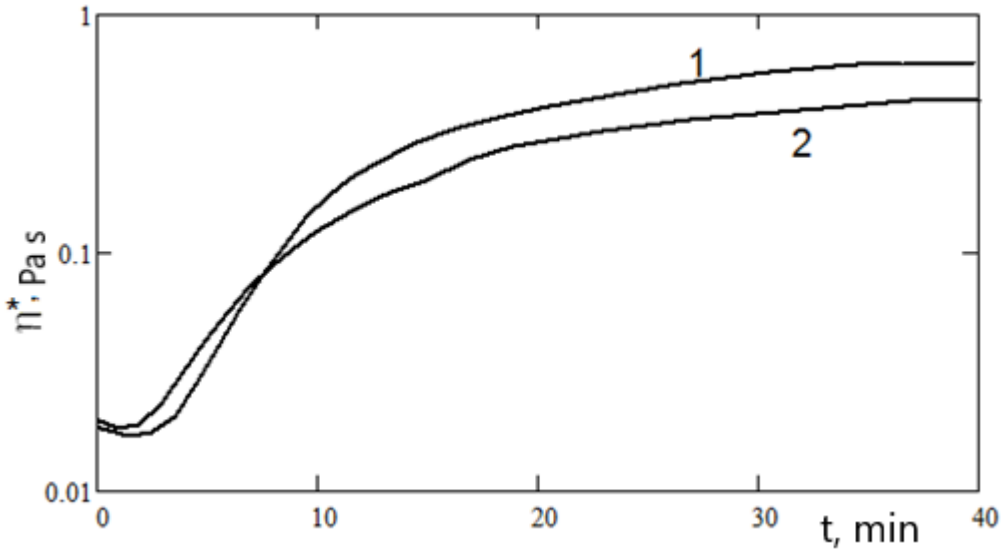


(а) – $f_r = 2864$ Hz; (б) – $f_a = 3391$ Hz

Figure 2.6 – Dependence of the shear strain modulus G'/ω of whole blood on time for f_r and f_a oscillations of the resonator needle

«The modulus of elasticity G' in the process of blood coagulation increases sharply, reaching a maximum value during the polymerization of fibrin and the formation of transverse intermolecular bonds, its retraction and further lysis. It should be noted that with an increase in the frequency of shear vibrations by 20%, the elastic modulus increased by 50%. This behavior of blood relative to shear deformations confirms the presence of a low-frequency viscoelastic relaxation process in it, due to the formation of a volumetric fibrin-platelet structure of the clot.

Figure 2.7 shows the dependence of the complex coefficient of blood viscosity η^* on time for f_r and f_a oscillations of the resonator needle» [13].



1 – $f_r = 2864$ Hz; 2 – $f_a = 3391$ Hz

Figure 2.7 – Dependence of the complex coefficient of blood viscosity η^* on time for f_r and f_a oscillations of the resonator needle

«It follows from the calculations carried out that the shear modulus G', reflecting the elastic properties of blood, in the frequency range under consideration makes a significant contribution to the complex viscosity index. During coagulation, the proportion of the elastic component in relation to blood viscosity increases 10-fold: from 5% at the beginning of the coagulation process to 50% when a fibrin-platelet clot is formed» [10].

The obtained amplitude-frequency patterns of the behavior of the viscoelastic characteristics of whole blood make it possible to use a resonant acoustic method to determine the viscoelastic properties of whole blood and their dynamics during coagulation in a wide range of shear vibration frequencies.

CONCLUSION

As a result of the work done, all the planned tasks were solved and the following results were obtained:

1. A theoretical and experimental study of the viscoelastic properties of native blood under oscillating shear stresses has been carried out. The dynamics of the process under study – the transition of blood from a liquid state to a solid-elastic one is determined by changes in the aggregate state of blood and is recorded as a dependence of the amplitude and phase of oscillations of the resonator needle in the blood on the time during the period hemocoagulation.

2. A mathematical model and a resonant acoustic method for calculating the complex viscosity coefficient of whole blood based on ultrasound elastography of its characteristics during coagulation have been developed. The high sensitivity of the method to changes in the viscoelastic properties of reference liquids is confirmed.

3. The calibration of the readings of the improved hardware and software complex of the Mednord piezothromboelastograph on an aqueous solution of glycerin and the dependence of the blood viscosity coefficient on the coagulation time calculated on this basis gave good agreement with the results of numerical calculations of blood viscosity using mathematical modeling.

4. Calibration of the readings of the improved hardware and software complex of the Mednord piezothromboelastograph on an aqueous solution of glycerin was carried out. The dependence of the blood viscosity coefficient on the coagulation time calculated on this basis gave a good agreement with the results of numerical calculations of blood viscosity using mathematical modeling.

5. Calculations of the real and imaginary parts of the complex viscosity index by resonant acoustic method were carried out. A comparison of the calculations with the available rheometric measurement data shows their good agreement. The obtained results and comparison with the experiment confirm the possibility of using the resonant acoustic method to determine the viscoelastic

properties of whole blood and analyze their dynamics during coagulation in a mode as close as possible to in vivo.

6. «It is shown that in the process of blood coagulation, the real and imaginary parts of the coefficient η^* increase by orders of magnitude, reaching the maximum value during the formation of the fibrin-platelet structure of the clot. It follows from the calculations carried out that the shear modulus reflecting the elastic properties of blood in the frequency range under consideration can make a significant contribution to the complex viscosity index. During coagulation, the proportion of the elastic component in relation to blood viscosity increases 10-fold: from 5% at the beginning of the coagulation process to 50% when a fibrin-platelet clot is formed» [10].

7. Amplitude-frequency patterns of the behavior of viscoelastic characteristics of whole blood make it possible to use a resonant acoustic method to determine the effect of plasma and cellular factors of blood clotting on the hemostatic potential in a wide range of shear vibration frequencies and shear strain rates.

8. The method for evaluation the concentration of thrombin in the process of fibrinogenesis based on piezothromboelastography using a resonant acoustic method to determine the viscoelastic properties of whole blood has been developed. The results of calculating the concentration of thrombin (TGT) by this method are compared with the results of the thrombin generation test and the thromboelastography test (TEG).

9. Clinical, laboratory and numerical experiments were carried out to calculate the kinetics of thrombin and hemostatic potential with different enzyme composition of blood in conditions of physiological norm and pathology (hyper- and hypocoagulation). Method determination of the kinetics of thrombin formation during whole blood coagulation has sufficient sensitivity and provides information about the concentration of thrombin at all phases of fibrinogenesis in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application.

The proposed method for estimating the concentration of thrombin is based on the postulate of a directly proportional dependence of the change in the concentration of thrombin and the rate of change in the complex coefficient of blood viscosity, determined by the results of measurements of the dynamics of viscoelastic characteristics based on low-frequency piezothromboelastography during coagulationThis method has sufficient sensitivity and provides information about the concentration of thrombin at all phases of fibrinogenesis in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application.

The results of the work have been published in 4 articles cited in the international databases Scopus and Web of Science and presented at 4 international and Russian conferences.

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Служебная записка

Выпускная квалификационная работа «Determination of the kinetics of thrombin formation during whole blood coagulation» / «Исследование кинетики образования тромбина в процессе коагуляции цельной крови» студента группы №052163 физического факультета Демкина О.В., выполненная на базе НИИФиРМ им. Е.Д.Гольдберга Томского НИМЦ, содержит в себе сведения, представляющие интеллектуальную собственность, принадлежащую данной организации. Полученные результаты до настоящего времени не опубликованы. Прошу разрешить размещение текста ВКР Демкина О.В. в электронной библиотеке ТГУ (репозитории) с изъятием раздела «Результаты».

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Ministry of Science and Higher Education of the Russian Federation NATIONAL RESEARCH TOMSK STATE UNIVERSITY (NR TSU) Faculty of Physics ADMIT TO THE DEFENCE AT THE SEB Director of the BEP Doctor of Physics and Mathematics, Professor _____V.P. Demkin "____"_____20__ MASTER'S THESIS DETERMINATION OF THE KINETICS OF THROMBIN FORMATION DURING WHOLE BLOOD COAGULATION within the Basic Educational Programme of Master's Degree "Physics Methods and Information Technologies in Biomedicine" subject area 03.04.02 - Physics Demkin Oleg Vladimirovich Research supervisors Doctor of Medicine, Professor, Corresponding Member of the Russian Academy of Sciences _____V.V. Udut signature "____"_____2023 Doctor of Physics and Mathematics, Professor _____V.P. Demkin signature "____"_____2023 Author student of group No 052163 _____ O.V. Demkin signature Tomsk - 2023 ABSTRACT Keywords: hemostasis, blood clotting system, blood clotting factors, physical and

mathematical model of hemocoagulation, thromboelastography, resonant acoustic method, thrombin kinetics. The purpose of this work is to develop a method for evaluating thrombin generation based on the study of the dynamics of the viscoelastic properties of native blood during hemocoagulation, using the resonant acoustic method and NPTEG technology. "Hemostasis is a complex hierarchically subordinate system that regulates the maintenance of the optimal functional state of the hemostatic potential (HP) - an integrative component of the full cycle of hemocoagulation, providing the necessary blood flow and restoring the integrity of the vascular wall when it is damaged" [8]. "Disorders of the hemostatic system that alter the viscoelastic characteristics of circulating blood are extremely dangerous and can lead to fatal complications: bleeding or thrombosis, which gives special priority to fundamental and applied blood clotting research" [14]. Among the methods of studying the blood coagulation system, of particular interest are "global" methods that allow integratively and ex tempore to assess the state of HP. Fibrin, formed as a result of the interaction of thrombin and fibrinogen, plays a decisive role in changing the viscoelastic properties of blood. Consequently, the production of thrombin, as a result of competing enzymatic reactions, sets the kinetics of fibrin formation, providing a change in the aggregate state of the blood. For this reason, when analyzing the state of HP, special attention is paid to assessing the kinetics of thrombin formation and inactivation as a trigger of hemocoagulation. With this in mind, this paper proposes a method for evaluating the generation of thrombin based on the results of a study of the kinetics of the final product of blood coagulation - fibrin, the operating time of which determines the dynamics of viscoelastic characteristics during coagulation. "The most suitable for such an assessment is the domestic technology of low-frequency piezothromboelastography (LPTEG), which is based on a resonant acoustic method for determining the viscoelastic characteristics of blood during coagulation under the action of periodic shear deformations obtained using ultrasonic transducers" [8]. Main publications on the topic of the master's thesis: 1. Demkin V.P., Mel'nichuk S.V., Udut V.V., Demkin O.V., Tyutrin I.I. Physical principles of the method of low-frequency piezothromboelastography for studying rheological properties of whole blood // Russian physics journal, 2019. - Vol. 62. - №6. - pp. 972-983. Doi: 10.1007/s11182-019-01803-y. 2. Demkin V.P., Melnichuk S.V., Gavar A.V., Demkin O.V., Rudenko T.V., Udut E.V., Tyutrin I.I., Udut V.V. Spectral Regularities of Viscoelastic Parameters of Whole Blood Exposed to Periodic Shear Stress // Bulletin of Experimental Biology and Medicine, 2020. - V. 169. - № 2. pp. 293 - 296. Doi: 10.1007/s10517-020-04871-0. 3. Demkin V.P., Zhukovskaya A.A., Rudenko T.V., Melnichuk S.V., Demkin O.V., Udut V.V. Dynamics of rheological properties of blood in resonant acoustic piezoelastography // Rational pharmacotherapy collection of scientific materials of the XVI International Scientific Congress, St. Petersburg, October 14- 16, 2021. St. Petersburg, 2021. - P. 55-58. (In Russian) 4. Demkin O.V. Modeling of the kinetics of thrombin formation based on the viscoelastic properties of whole blood // Aerophysics. Photonics and quantum optical technologies. Plasma physics. Solid state physics. Thermophysics. Physical methods in natural sciences and materials science. Elementary particle physics, astrophysics and cosmology. Instrumental methods and techniques of experimental physics : Materials of the 60th International Scientific Student Conference on April 10-20, 2022 / Novosibirsk State University. - Novosibirsk : CPI NSU, 2022. - pp. 148-149. (In Russian) 5. Tsybulina A.O., Demkin O.V. Rheological model of blood and assessment of hemostatic potential based on viscoelastic properties of whole blood // Aerophysics. Photonics and quantum optical technologies. Plasma physics. Solid state physics. Thermophysics. Physical methods in natural sciences and materials science. Elementary particle physics, astrophysics and cosmology. Instrumental methods and techniques of experimental physics : Materials of the 60th International Scientific Student Conference on April 10-20, 2022 / Novosibirsk State University. - Novosibirsk : CPI NSU, 2022. - pp. 165-166. (In Russian) 6. Tsybulina A.O., Demkin O.V., Kotlovskaya L.Yu. Assessment of hemostatic potential using mathematical apparatus of fuzzy logic // III JOINT SCIENTIFIC FORUM OF PHYSIOLOGISTS, BIOCHEMISTS AND MOLECULAR BIOLOGISTS (Sochi, 3-8 October 2022). SCIENTIFIC WORKS. Volume 3. - Moscow: Publishing House "Pero", 2022. - pp.121-122. (In Russian) 7. Demkin V.P., Melnichuk S.V., Demkin O.V., Kotlovskaya L.Y., Rudenko T.V., Tsibulina A. O., Zhukovskaya A. A., Tyutrin I.I., Udut V.V. Method for Evaluating Thrombin Concentration Based on the Data of Monitoring the Viscoelastic Properties of Native Blood in the Process of Hemocoagulation // Russian Physics Journal , 2022. - Vol. 65. - P. 1149-1154. Doi: 10.1007/s11182022-02744-9. 8. Demkin V.P., Melnichuk S.V., Zavadovsky K.V., Suyundukova A.T., Rudenko V.V., Demkin O.V. Method of local hemodynamics for assessing the hemodynamic significance of tandem stenoses in bifurcations of coronary vessels// Izvestiya vuzov. Physics - 2023. -Vol.66. −No.3. −p.44-50. (In Russian) CONTENTS LIST OF ABBREVIATIONS 6 INTRODUCTION 8 1 Hemostasis system and methods of its diagnosis 12 1.1 Blood clotting system 14 1.2 Hemocoagulation and its phases 16 1.3 Local and global methods of hemostasis assessment 18 2 Rheological properties of blood 24 2.1 Blood viscosity, rheological models of blood 25 2.2 Resonant acoustic method for determining the viscoelastic 31 properties of blood ..... 3 Kinetics of thrombin formation during hemocoagulation 43 3.1 Theoretical foundations of the thrombin generation method 44 3.2 Kinetics of thrombin formation based on the processing of data 47 obtained by the LPTEG method ..... 3.3 Comparison of the results of numerical calculations of the 51 thrombin concentration with the experiment ..... CONCLUSION 56 REFERENCES 59 LIST OF ABBREVIATIONS APTT - activated partial thromboplastin time AH - arterial hypertension AP - arterial pressure HP - hemostatic potential ICC - intensity of contact coagulation IPC - intensity of polymerization of the clot IRLC - intensity of retraction and lysis of the clot ICD - intensity of coagulation drive ITC - the intensity of total coagulation CTAA - coefficient of total anticoagulation activity CTA - constant of thrombin activity MAC - maximum amplitude of the clot LPTEG - low-frequency piezothromboelastography TGT - thrombin generation test TEG - thromboelastography PT - personal computer PT - prothrombin time RASB - regulation of the aggregate state of blood GP - gelling point TT - thrombin time η - dynamic viscosity τ - tangential stress □□ - shear strain rate □□u - mechanical displacement ε - Cauchy - Green strain tensor σ - normal stress □□f - deformation force ρ - medium density ν - frequency Dx - displacement of the end of the resonator needle along the axis x ω - the circular frequency set by a piezoelectric generator of mechanical vibrations □□0 - natural oscillation frequency of the resonator needle in the liquid β - attenuation coefficient of needle-resonator oscillations in a viscous liquid INTRODUCTION "Currently, there is a sufficient amount of data indicating that thrombohemorrhagic complications (especially in diseases of the cardiovascular system) are the leading cause of disability and mortality of the able-bodied population worldwide" [1-3]. "Despite the huge volume of fundamental and applied research conducted in the world on this topic and the results achieved, the effectiveness and safety of antithrombotic prevention and therapy is far from perfect and is not always accompanied by the desired clinical efficacy" [4-7]. "The achievement of high efficiency is

largely determined by the level of modern ideas about the pathogenesis of thrombophilia and thrombosis, the possibilities of their timely diagnosis, as well as knowledge of the pharmacodynamics of antithrombotic drugs. In this regard, the adequacy of methods for assessing hemostatic potential acquires a key role, and the success of prevention and treatment of thrombohemorrhagic complications ultimately depends on the efficiency, informativeness, accessibility and reliability of these methods. In solving this problem, it is of particular importance to attract knowledge about the physical properties of blood, since its aggregate state changes during the coagulation process with significant changes in its viscoelastic properties. The detailed understanding of the process of regulation of the aggregate state of blood (RASB) and the acquisition of new fundamental knowledge requires the addition of the existing laboratory research paradigm by methods of mathematical modeling of hemocoagulation processes and the determination of the physical mechanisms of changes in the rheological properties of blood during clotting" [8, 9]. "Development of such methods for the diagnosis of hemocoagulation and verification of the corresponding model of physico-chemical processes with the possibility of prompt obtaining objective information about the state of the hemostatic potential (HP) of the subject and the response to antithrombotic therapy, will allow pathogenetically justified to determine and change the regimens (dose, discreteness) of prevention and treatment" [10]. To date, the presence of a large number of methods available in the arsenal of hemostasis laboratories creates the illusion of comprehensive and allencompassing monitoring of hemostatic potential [11, 12]. "However, the existing local and global methods of laboratory diagnostics of the hemostasis system have a number of significant drawbacks: low sensitivity and lack of standardization, duration of sample preparation, conducting studies on a model of citrate plasma or stabilized blood" [9,13]. The unstructured, inaccurate and fragmented analysis results obtained by local and global methods practically exclude a holistic view of the state of the hemostasis system. "One of the widely used global tests is the method of low-frequency piezothromboelastography (LPTEG), which allows an integrative assessment of the state of hemostatic potential (HP) based on measuring the dynamics of viscoelastic characteristics of whole blood at all stages of its coagulation process: from "damage" to the vascular wall of a vein during blood sampling to the formation of a fibrin-platelet clot" [10]. In papers [8,10, 13, 14, 15,], "the complex mathematical model and physical foundations of the method of low-frequency piezothromboelastography under periodic shear stresses for studying the viscoelastic properties of whole (native) blood and its application for the diagnosis of hemostatic potential are described. A method is proposed for determining the viscoelastic properties of blood by measuring the amplitude-frequency and phase characteristics of oscillations of the piezoelectric needle-resonator (Mednord piezothromboelastograph apparatus), taking into account rheological changes in non-Newtonian fluid under conditions of periodic shear stresses" [8]. "In the course of the conducted studies, a strong correlation was revealed between the amplitude of the signal of the direct piezoelectric effect of the piezoelectric sensor of the thrombopiezoelastograph and the value of the dynamics viscosity coefficient" [10, 14, 16]. "Comparison of the calculated values of the viscosity coefficient with its reference values for water and glycerin determined by direct rheometric measurements showed their good agreement, which indicates the sensitivity of the method to changes in the viscoelastic characteristics of the liquid. On the one hand, due to the change in the aggregate state of blood from liquid to solid-elastic, the change in its viscoelastic characteristics can occur within a wide range. On the other hand, the piezoelectric sensor is a complex mechanical system, the sensitivity of which is determined not only by the impedance of the unloaded piezoelectric, but also by the configuration of the resonator needle, which leads to a significant change in its resonance frequency spectrum, and, consequently, the sensitivity of the method itself"[8, 10, 14]. In this study, a new approach is proposed to determine the HP of whole (native) blood, "based on a complex physico-mathematical model of the hemocoagulation process as a sequential change in its rheological properties and analysis of data on the kinetics of the final product of blood coagulation - fibrin, the development of which determines the dynamics of viscoelastic characteristics of blood during coagulation" [17]. "It is known that fibrin, formed as a result of the interaction of thrombin and fibrinogen, plays a main role in changing the viscoelastic properties of blood. For this reason, when analyzing the state of HP, special attention is paid to the kinetics of thrombin formation as a trigger of hemocoagulation. Consequently, the production of thrombin, as a result of competing enzymatic reactions, sets the fibrin formation, providing a change in the aggregate state of the blood. With this in mind, we propose a method for estimating thrombin generation based on the dynamics of the blood viscosity coefficient during hemocoagulation" [9, 17, 18]. In this regard, the purpose of this dissertation research is to develop a method for evaluating thrombin generation based on studying the dynamics of viscoelastic properties of whole (native) blood in the process of hemocoagulation, using the resonant acoustic method and LPTEG technology. To achieve the research goal, the following tasks have been solved [10,13, 14, 15]. 1. Study of the technology of low-frequency piezothromboelastography to determine the rheological properties of whole blood; 2. Development and application of the resonant acoustic method for calculating the blood viscosity coefficient. 3. Conducting of experimental studies of changes in the viscoelastic properties of whole blood during hemocoagulation. 4. Development of a method for generating thrombin in the process of coagulation of whole blood. 5. Conducting of laboratory and clinical studies of the kinetics of thrombin formation in the process of hemocoagulation of native blood by the LPTEG method. The research is designed to update and supplement the fundamental data on the mechanisms of the hemocoagulation process and on the understanding of kinetics of thrombin formation as the basis of a new approach to the assessment of hemostatic potential. The use of this approach to elucidate the mechanisms of changes in the viscoelastic characteristics of whole blood, reflecting the state of HP, makes it possible to control HP and manage its normalization. Unlike conventional coagulation tests, the thrombin formation test can be used for a general evaluation of hemostasis, the results of which can then be used to assess local characteristics of hemostasis, such as prothrombin time, activated partial thromboplastin time and fibrinogen and thromboplastin levels. other clotting factors. The introduction of this method will contribute to a better understanding and evaluation of general hemostatic processes. 1 Hemostasis system and methods of its diagnosis The hemostasis system is a very complicated biological system in the body for maintaining the physiological balance of multidirectional processes that ensure the integral property of blood to maintain a liquid state (normal) and to coagulate, in order to prevent extravasation of blood in certain, extreme situations for the body [12,19]. "The control of the conjugacy of the participation of the functions of the hemostasis system, which are opposite in their orientation, is provided by the system of regulation of the aggregate state of the blood (RASB), the regulation of which, in turn, is provided by the interaction of the central and peripheral nervous system and the endocrine system" [13]. "A derivative of the

functioning of the hemostasis system is the hemostatic potential, which is a reflection of the integrative activity of the vascular-platelet, coagulation, anticoagulation and fibrinolytic links of the hemostasis system that determine the rheological properties of blood" [16]. According to the predominance of activity of individual state of the hemostasis system, HP can be positive (predominance of coagulation orientation), negative (predominance of anticoagulation mechanisms) or neutral (functional balance of the combination of hemocoagulation and anticoagulation mechanisms), while the level of individual factors of the hemostasis system can be extremely variable. The displacement of the HP from the optimal level automatically activates the regulation factors that ensure the activation of compensation mechanisms, the result of which is the return of the HP to the "specified" optimal level. "Thus, with a deviation of HP towards a positive value, the resulting correction occurs by increasing the concentration level of anticoagulation factors and/or a decrease in the level of coagulation factors, and conversely, with a deviation of HP in the negative direction, that is an increasing of the coagulation factors concentration and/or a decreasing of the concentration of anticoagulation factors is observed in the physiological state. Being a functional system, the hemostasis system determines the optimal level of HP in a physiological and pathological state and coordinates the functioning of structures and mechanisms aimed at achieving the optimal level of HP in various conditions (external and internal), analyzes the proportionality of the result obtained and, if necessary, additionally activates new components, stimulates the necessary activation/inhibition mechanisms in order to achieve sufficient adaptive result" [13]. "Almost any nosology is accompanied by a variation of the HP state , especially in urgent conditions in which critical disorders of the hemostasis system can lead to death" [20]. "The study of the functional state of HP plays an important role in the diagnosis of hypercoagulation and thrombophilia, the processes of disseminated intravascular coagulation and bleeding, as well as the dynamic control of antithrombotic therapy during conservative and surgical treatment. The state of the vascular-platelet and coagulation parts of hemostasis and related changes in the rheological properties of blood play an very important role in the pathogenesis and development of many diseases" [4, 5]. "To date, researchers are "armed" with a fairly weighty set of "local" and "global" methods to evaluate the hemostasis system. Unfortunately, even with the correct observance of the preanalytical stage, the local tests used to diagnose the state of the HP in reality provide extremely limited fragmentary information about its functioning about the process and, unfortunately, do not provide a complex approach for evaluation of the state of the RASB system. That is why the renaissance of "global" tests and methods analyzing the process of hemocoagulation ex tempore is not accidental" [16]. "Such methods provide for two extremely important points: − working with whole (native) blood; − registration of the coagulation process under changing the viscous and viscoelastic properties of blood changes. In this case, development the of domestic technology is of particular importance which was named as the technology of low-frequency piezothromboelastography (LPTEG)" [14,16]. "This technology makes it possible to evaluate all phases of blood clotting in real time and quantify the intensity of pro- and anticoagulant potential, can be a tool for evaluation the pharmacodynamics of antithrombotic drugs - the key to the prevention and treatment of thrombohemorrhagic complications" [8, 16]. 1.1 Blood clotting system "The blood clotting system is one of the most important physiological systems of the body. Its functioning ensures, normally, the maintenance of blood in a liquid flowing state, as well as local blood clotting - thrombosis - at the site of damage to the vessel" [8]. Blood clotting (Hemocoagulation) is the most important stage of the hemostasis system, responsible for stopping bleeding when the vascular system of the body is damaged. The combination of various blood clotting factors interacting with each other in a very complex way forms a blood clotting system [21]. There are three main mechanisms for stopping bleeding in case of vascular damage, which, depending on the conditions, can function simultaneously, with the predominance of one of the mechanisms [9]: − vascular-platelet hemostasis caused by vascular spasm and their mechanical blockage by platelet aggregates. On the collagen molecules exposed as a result of damage to the vessel walls, adhesion (adhesion), activation and aggregation (gluing together) of platelets occur. In this case, a so-called "white thrombus" is formed, that is, a thrombus with a predominance of platelets; − coagulation hemostasis is triggered by a tissue factor from the tissues surrounding the damaged vessel, and is regulated by blood clotting factors. It provides a blockage of the damaged part of the vessel with a fibrin clot [8,16]; − fibrinolysis is the dissolution of a blood clot after the restoration of a damaged vessel wall [16]. The blood clotting system consists of thirteen proteins, which are called blood clotting factors (Table 1); they are usually denoted by Roman numerals (for example, FVII - factor VII), the activated form is indicated by adding the index "a" (FVIIa - activated factor VII). "Of these, seven are activated to serine proteases (factors XII, XI, IX, X, II, VII and precallikrein), three are cofactors of these reactions (factors V, VIII and high-molecular kininogen HMK), one is a cofactor/receptor (tissue factor, factor III), another is trasglutaminase (factor XIII) and, finally, fibrinogen (factor I) is a substrate for the formation of fibrin" [9, 17], the end product of blood clotting reactions (Table 1.1). Table 1.1 - The main factors of blood clotting [9] Blood clotting involves an effectively regulated series of transformations of inactive zymogens into active enzymes, which eventually leads to the formation of thrombin and the conversion of fibrinogen into fibrin. Note that the "internal" blood clotting pathway is a slow process, since it involves a large number of clotting factors. "The final result of the work of the blood coagulation system is the conversion of fibrinogen into fibrin under the action of thrombin. The production of thrombin as a result of a cascade of biochemical reactions sets the dynamics of fibrin production processes and changes in the viscoelastic properties of blood. For this reason, when analyzing the state of the HP, special attention is paid to evaluate the kinetics of thrombin formation and inactivation as a trigger of hemocoagulation" [16, 17]. 1.2 Hemocoagulation and its phases To date, the prevailing model of hemocoagulation is a model based on the cellular theory of blood clotting [22]. According to this theory, hemocoagulation consists of three overlapping phases - initiation, amplification and propagation [23] (Figure 1.1). Figure 1.1 - Cascade of biochemical reactions of plasma hemostasis [23] The essence of the initiation phase, as the name implies, is the activation of the blood clotting process. Two main ways of hemocoagulation initiation are known - internal (Hageman pathway) and external (tissue factor pathway) [9, 17]. The internal pathway, the Hageman pathway begins with the contact activation of (mainly) the XII factor of blood clotting, which initiates the XI factor, and subsequently the IX factor of blood clotting. It is the activated IX factor, entering into a complex with the activated VIII coagulation factor, that forms a complex - internal tenase [VIIIa-IXa], which activates the X factor of coagulation and forms prothrombinase. However, to date, it has been proven that the internal pathway, unlike the pathway of the tissue factor, contributes to the spatial spread of the clot, but is not the leading pathway in the initiation of hemocoagulation. Thus, it is the external pathway, the path of the tissue factor, that is

the main pathway for the beginning of blood clotting. When the vessel is damaged, the blood plasma contacts the tissue factor, resulting in the activation of factor VII and the formation of an external tenase complex [TF-VIIa]. This complex, in the presence of calcium ions, initiates a hemocoagulation cascade, activating factors X and IX on the surface of the subendothelium [24]. "It is the activation of the X factor by the [TF-VIIa-Ca2+] complex that leads to the formation of a small amount of thrombin, insufficient for the transformation of fibrinogen into fibrin, but sufficient to trigger a positive feedback loop in which thrombin enhances its own production. When the subendothelium is exposed, in addition to the activation of the plasma cascade, vascular-platelet hemostasis is activated, characterized by adhesion, activation and aggregation of platelets. Platelet activation also occurs under the action of a small amount of thrombin activated in the initiation phase. In addition, thrombin activates factors V, VIII and XI. The active forms of these factors and the factor IXa moving from the subendothelium, formed in the initiation phase, bind to the surface of the adhered platelets. This process involves the amplification phase, in which the formation of active cofactors (factors Va and VIIIa) occurs, and the reaction surface shifts from the subendothelium to the adhered platelets. Activation of factor VIII, occurring in the amplification phase, allows the formation of a tenase complex [IXa-VIIIaCa2+] on the platelet surface. This complex leads to the activation of factor X, which, in turn, leads to the formation of a prothrombinase complex [Xa-Va-Ca2+], which increases the generation of thrombin in the damaged area. After that, a positive feedback is triggered, which consists in the fact that under the influence of the accumulated thrombin, the activation of XIa accelerates, which affects factor IX. At the same time, there is an increase in activated cofactors (factors Va and VIIIa). At the same time, on the platelet surface, there is an increase in the amount of tenase [IXa-VIIIa-Ca2+]. Also, under the influence of thrombin, there is a similar increase in prothrombinase complexes [Xa-Va-Ca2+]. Together, all these processes lead to an acceleration of hemocoagulation by several thousand times" [9, 25]. Thus, the third stage, the propagation stage, is characterized by the formation of thrombin, the amount of which is sufficient for the transformation of fibrinogen into fibrin. 1.3 Local and global methods of hemostasis evaluation "To date, researchers have been provided with a wide range of methods of amidolytic and enzyme immunoassay to evaluate the hemostasis system and HP. However, a limited range of these methods is used in routine clinical practice (activated partial thromboplastin time, prothrombin time, thrombin time, fibrinogen, soluble fibrin monomer complexes, D-dimers). The amidolytic technique carries information about the activity of the enzyme, enzyme immunoassays carry information about the amount of the enzyme, and the coagulation technique characterizes the rate of interaction between some enzymes under conditions of relatively standardized activation. It is clear that the cumulative results scattered over the measurement give only an approximate characteristic of the hemostatic potential. Expanding the arsenal of techniques (evaluation of endothelial producers, determination of the level of a number of blood clotting factors), increasing diagnostic value, does not exclude fragmentation of ideas about the process and does not give a holistic view of the hemostasis system" [13, 16]. "The group of global methods for measuring hemostatic potential based on physical detection methods, along with the LPTEG method, includes: the thromboelastography (TEG) method" [26] "and the thrombin generation test (TGT)" [27, 28], "designed for integrative assessment of plasma and cellular components of native blood involved in all stages of fibrinogenesis, from initiation before the formation of p/s fibrin and its possible lysis, otherwise for the assessment of the hemostasis system as a whole" [29]. "The LPTEG method measures the resistance of whole (native) blood to forced oscillations of the resonator needle, which reflects the change in the aggregate state of blood over time" [16]. "As an example, Figure 1.2 shows a graph of changes in the aggregate state of the blood of a healthy volunteer by the LPTEG method, on which the signal amplitude of the piezoelectric sensor A of the process under study is estimated in relative units along the ordinate axis, and the time of the study t in minutes along the abscissa axis" [30]. "The dynamics of the process under study - the transition of blood during coagulation from a liquid state to a solid-elastic one - is determined by changes in the aggregate state of blood and is recorded in the form of an integrated LPTEG curve (Figure 1.2), where each point of which (Ai) is determined by the state of the system at a specific time of the study (ti)" [8, 16]. "When measuring the signal of a piezoelectric sensor, the following parameters are recorded and determined: A0 - the initial value of the signal amplitude at time t0, in relative units; t1 - the reaction period (the time from the beginning of the study to the maximum decrease in the amplitude A1,); t2 - the time to reach the amplitude A2; A2 - an increase in the signal amplitude by 100 rel. units; t3 - blood clotting time (gelling point), determined automatically when measuring the tg angle of the curve by 50%; A3 - the magnitude of the signal amplitude at the gelling point; A4 is the amplitude value 10 minutes after reaching the gelling point; t5 is the time to reach the maximum amplitude (A5) (the time of formation of the fibrin-platelet structure of the clot); A6 is the signal amplitude value 10 minutes after reaching the maximum amplitude" [8,14]. (A0-A5) - NPTEG amplitude at the stages of fibrin formation; A6 - amplitude at the 10th minute of clot lysis; (t1-t5) - the time intervals of the stages of fibrinogenesis; (t3) - the gelling point (blood clotting time). Figure 1.2 - Whole blood LPTEG indicators of a healthy volunteer "According to the formulas indicated in Table 2, the following indicators are calculated in relative units: the initial stage of coagulation - the intensity of contact coagulation (ICC); the constant of thrombin activity (CTA); the intensity of coagulation drive (ICD); the intensity of polymerization of the clot (IPC); the coefficient of total anticoagulation activity (KTAA); the intensity of retraction and lysis of the clot (IRLC); the maximum amplitude of the clot (MA). Table 2 - Calculated indicators of LPTEG Indicator Decoding the value of the indicator ICC A1−A0 ИСС = t1 ИСС, rel.un. - intensity of contact coagulation; A1, rel.un. - maximum decrease in the amplitude of the curve during the reaction period "t1"; A0, rel.un. - the initial value of the amplitude of the curve at time t0; t1, min. - время от начала исследования до достижение минимальной амплитуды кривой НПТЭГ - A1. This indicator mainly reflects the aggregation activity of the shaped elements of the blood, the I and II phases of coagulation, or its suspension stability (SS). ICD A3−A1 ИСD = □□3 ИСD, rel.un. - intensity of coagulation drive; A3, rel.un. - the magnitude of the amplitude of the curve at the "gelling point"; A1, rel.un. - maximum decrease in the amplitude of the curve during the reaction period "t1"; t3, min. - время свертывания крови - "точка желирования", фиксируемая автоматически при изменении tg угла кривой на ~ 60 %. This indicator characterizes mainly the proteolytic stage of the III phase of hemocoagulation. The A - part of the NPTEG curve near the gelling point (a change in the tg angle of the curve by ~ 60%) reflects the beginning of the polymerization process, which at the gelling point (GP) leads to the formation of a fibrin gel - the main structural framework of a hemostatic clot. Table 2 continuation - Calculated indicators of LPTEG CTA A2 CTA = □□2−□□1 CTA, rel.un. - constant of thrombin activity; A2, rel.un. - increase in the amplitude of the curve by 100 rel.un.; t2, min. - time to reach the amplitude of

A2 curve; t1, min. - the time from the beginning of the study to the achievement of the minimum amplitude of the LPTEG - A1 curve. The use of this indicator in the analysis of LPTEG is due to the need to determine a universal criterion for assessing the intensity of the proteolytic stage of fibrin formation. IPC A4−A3 IPC = 10 min IPC, rel.un. -the intensity of polymerization of the clot; A4, rel.un. - the value of the amplitude after 10 minutes from the "gelling point"; A3, rel.un. - the value of the amplitude at the "gelling point". Displaying the intensity of the polymerization stage. The use of a time interval equal to 10 minutes is due to the need to unify the method, since the formation of transverse covalent bonds is a fairly long stage of post-gel formation. Thus, LPTEG piezothromboelastograms allow analyzing all stages of fibrinogenesis and assessing the state of hemostatic potential" [8, 16]. "TGT test is based on the main properties of the blood aggregation control system to generate thrombin, the dynamics of changes in the concentration of which determines the total effect of the interaction of all factors of the coagulation system" [27, 28, 31]. "The physical principle of the thrombin generation test is to determine the amount of thrombin (in nmol), which is formed during the recalcification of citrate blood plasma in the presence of a fixed concentration of tissue factor and fluorogenic substrate" [28, 32]. "The TEG method is based on the analysis of the dependence of blood viscosity on time during the formation of a clot. A citrate blood sample taken from a vein is required for the analysis. The principle of the method is the incubation of whole blood at 37 ° C in a heated cylindrical cuvette, which oscillates for 10 seconds at an angle 4°45′ in a bowl with a pin freely suspended and connected to a wire" [32, 26]. "The output measured characteristic for TGT is the concentration of thrombin, for TEG - the amplitude of the rotation angle of the sensor rod immersed in citrate recalcified blood, for LPTEG (measurement in native blood) - the amplitude of the electrical oscillations of the piezoelectric sensor. The clotting time can reach tens of minutes and falls on the point of formation of the viscoelastic gel (A4) on the LPTEG curve. The TEG method is not without drawbacks, the main of which are the problem of standardization, insufficient sensitivity of the method in assessing the main links of the hemostasis system, especially in the case of functional hypoxia, hypocalcemia and hypothermia" [32]. 2 Rheological properties of blood Rheology (from the Greek □□□□□□□□, "flow, flow" and -logy) is a branch of physics that studies deformations and fluidity of matter [33]. The term "Rheology" was first introduced by Eugene Bingham, an American chemist. He showed that for many real liquids, the critical stress level τ0 must be reached in order for the liquid to start flowing. Below this critical voltage, the liquid behaves like a solid. The rheological properties of blood depend on many factors. "The rheological properties of blood depend on a number of factors: the concentration of blood cells and their aggregation parameters, the composition of the plasma and its spatial distribution, the kinetic characteristics of blood flow, the rate of elastic shear deformations, and external factors; moreover, various factors can have a mutual influence on their value" [14]. "The presence of these factors generally ensures the classification of blood as a non-Newtonian fluid with two main rheological properties - viscosity and plasticity. The main difference between non-Newtonian fluids is the dependence of viscosity on the shear strain rate" [9]. Currently, there are a large number of rheological models describing this dependence for various shear rates. Consideration of this dependence is necessary when modeling vascular hemodynamics, because the rate of shift in one period of the cardiac cycle in the arteries ranges from 0 to 1000 s-1. It is known that the viscosity and elasticity of blood change significantly during its coagulation. "To date, significant progress has been made in the development of theoretical methods and experimental tools for studying hemorheology and a deep understanding of the interaction of blood components that determine its viscoelastic properties" [34-38]. "At the same time, the problem of determining changes in the viscoelastic properties of whole blood during clotting remains open due to the lack of diagnostic tools. In existing devices for determining the rheology of whole blood, studies are carried out on its stabilized samples (capillary and rotary rheometers), and when assessing elastic properties, thromboelastographs are used to evaluate changes in this characteristic after the formation of a clot" [6]. The measurement of the viscoelastic properties of whole blood is very limited in time due to the natural process of coagulation. In these cases, stabilizing drugs (heparin, EDTA, etc.) are used during measurements to prevent coagulation [34, 35], which can lead to changes in hemorheological properties and to certain errors in the measurement of the viscosity coefficient. 2.1 Blood viscosity, rheological models of blood "Viscosity is an important property of liquids that describes the resistance of a liquid to spreading; it is related to internal friction in a liquid. The most common type of fluidity is shear flow, in which liquid layers move relative to each other under the action of shear stress, which is defined as the force acting on a unit area of the liquid, and allows you to obtain a velocity gradient over the thickness of the sample, called the shear rate. The shear viscosity or dynamic viscosity associated with this process is determined by the ratio of shear stress to shear rate. Simple unstructured liquids are classified as Newtonian, which means that their viscosity does not depend on the magnitude of the applied shear. Examples can be water and simple hydrocarbons. As the complexity of the fluid increases, the fluids may exhibit more complex behavior and exhibit a non-Newtonian response, in which the viscosity depends on the magnitude of the applied shear. These types of liquids are commonly referred to as structured or complex liquids. This non-Newtonian behavior is characteristic of many liquids, including blood, which are usually liquids that liquefy during shear, where the viscosity decreases with increasing shear rate" [13]. "Blood is a concentrated suspension of several basic cellular elements: erythrocytes, leukocytes and platelets in an aqueous polymer and ionic medium - plasma consisting of 93% water and 3% particles: electrolytes, organic molecules, numerous proteins (albumin, globulins and fibrinogen) and waste products" [8, 38]. "Erythrocytes are biconcave discs with an average diameter of 6 to 8 microns and a maximum thickness of 1.9 microns. The average volume of an erythrocyte is 90 μ3. Their number per cubic millimeter of blood is approximately 5 to 6 x 106, and they represent approximately 40 to 45% by volume of normal human blood and more than 99% of all blood cells. The proportion of red blood cells is called hematocrit. The main function of red blood cells is the transport of oxygen and carbon dioxide. White blood cells are roughly spherical and much larger than red blood cells, but they exist in smaller numbers in the blood: their diameter ranges from 6 to 17 microns, and they are approximately 7 to 11 x 103 per cubic millimeter in a normal adult. The main function of red blood cells is the transport of oxygen and carbon dioxide. White blood cells are roughly spherical and much larger than red blood cells, but they exist in smaller numbers in the blood: their diameter ranges from 6 to 17 microns, and they are approximately 7 to 11 x 103 per cubic millimeter in a normal adult. White blood cells play a vital role in fighting infection and are thus able to migrate from blood vessels and into tissues. Platelets are small disk non-nuclear cell fragments, much smaller than erythrocytes and leukocytes (approximately 2 to 3 μ3). Platelets are a vital component of the blood clotting mechanism. The total volume concentration of leukocytes and platelets is only about 1%. The presence of cellular

elements of blood and their interaction leads to significant changes in the rheological properties of blood. Blood plasma, which consists mainly of water, is a Newtonian fluid. However, whole blood has complex mechanical properties that become especially significant when the particle size is much larger or at least comparable to the size of the lumen. In this case, what happens at the level of microcirculation (in small arterioles and capillaries), blood cannot be represented as a homogeneous liquid, but as a suspension of blood cells (especially erythrocytes) in plasma. The presence of blood cell elements and their interaction leads to significant changes in the rheological properties of blood, and reliable measurements must be carried out to obtain appropriate microstructural models" [37]. "Numerous researchers have found that blood viscosity gradually decreases as the velocity gradient increases. This dependence manifests itself at relatively low velocity gradients up to 60-70 s- 1 -1 . At velocity gradients of 60-70 s and higher, the decrease in viscosity practically ceases, and it becomes "constant" or, as it is often called, asymptotic" [36]. "The viscosity curve characteristic of blood is concave towards the deformation velocity axis. Therefore, judging by the flow curve, pseudoplasticity is inherent in blood. Given that blood has a yield point, it (using the terminology accepted in rheology) can be attributed to nonlinear-visco-plastic media. The main contribution to the elastic properties of blood is made by the aggregation ability of erythrocytes forming a spatial structure. At low shear deformation rates, the spatial structure of erythrocytes does not collapse and causes the threshold behavior of shear deformation, at which the plastic properties of blood manifest themselves. At high velocities γ > 200 s-1, the spatial structure of erythrocytes is destroyed, which causes the effect of shear thinning of blood, leading to a decrease in viscosity. For example, in systole, with an increase in blood flow, red blood cells (erythrocytes) dissociate and deform more efficiently, because the faster they move, the less viscous the blood becomes. The slower the cells move (as in diastole), the more viscous the blood becomes. Figure 2.1 shows the dependence of the dynamic coefficient of blood viscosity on the shear rate" [37]. Figure 2.1 - The dependence of the viscosity of whole blood on the shear rate As can be seen from Figure 2.1, at different shear rates, blood viscosity is affected by various rheological factors. "In areas of circulation with a high shear rate, blood viscosity is mainly determined by hematocrit, erythrocyte deformability and blood plasma viscosity. In areas with a low shear rate, where blood slows down, plasma protein molecules and cells interact in such a way that aggregates or rolls are formed, and platelets and other intermolecular compounds aggregate. Whole blood has two main rheological properties - viscosity and plasticity and, therefore, belongs to the class of non-Newtonian fluids" [39]. "The most well-known non-Newtonian characteristic of blood is its dilution during shear deformation: at low shear rates, blood seems to have a high apparent viscosity (due to aggregation of red blood cells) at high shear rates, there is a decrease in blood viscosity (due to deformability of red blood cells). The viscoelastic behavior of blood is less important at higher shear rates. Understanding the relationship between blood composition and its physical properties is important for developing a suitable model for describing blood behavior" [36]. "Whole blood is well described in the rheological model as a liquid with viscous and elastic properties" [40]. The main rheological equation for such liquids is □□ = □□□□̇, where □□ − is the tangential stress tensor; □□̇ − is the shear rate; □□ − is the viscosity. "To obtain a rheological equation, consider the dependence of the tangential stress developing in neighboring layers of a moving fluid τ on their velocity v in accordance with Newton's formula. □□□□ □□ = □□ , (2.1) □□□□ where □□-coefficient of dynamic viscosity of the liquid (□□ = □□□□□□□□□□, for a Newtonian fluid). Let's give this formula a different look. □□ □□□□ □□ □□□□ □□□□ □□ = □□ = □□ = □□ = □□□□̇ , (2.2) □□□□ □□□□ □□□□ □□□□ □□□□ Most biological fluids, including whole blood, belong to the class of nonNewtonian fluids. For them, □□(□□̇) is a nonlinear function of □□̇ and depends on a number of factors: the concentration and composition of the liquid, kinetic characteristics, the rate of elastic shear deformations, external factors; moreover, various factors can have a mutual influence on their magnitude. For a non-Newtonian fluid, □□ = □□(□□̇). Thus, equation (2.3) □□ = □□(□□̇)□□̇ (2.3) is the basic rheological equation. The spatiotemporal distribution of blood viscosity is characterized by the dynamic value (apparent viscosity) of the coefficient of internal friction η(r,t). The significant dependence of viscosity on internal and external factors reflects fundamental differences in the types of blood flow and its kinetics and, accordingly, generates a variety of rheological models of blood. Phenomenological models are reduced to the corresponding rheological equations that determine the functional relationship between the dynamic viscosity η, stress □□ and the shear strain rate □□̇. Most of the existing rheological models can be derived from the phenomenological equation" [13, 39]: τn =τ0m +ηmγ̇n, (2.4) where □□0□□- limited shear stress, m и n they are selected from comparison with the experiment. Another kind of equation of the semiempirical rheological model has the form: η=η∞ +(η0 −η∞)· f (γ̇) f (γ̇)=1 (if γ̇□□→ 0) f (γ̇)= 0 (if γ̇□□→ ∞) , (2.5) here □□0 и □□∞ correspond to the viscosity of blood at □□̇ → 0 и □□̇ → ∞. Currently, there are many rheological models reflecting the fundamental differences in the types of blood flow depending on internal and external factors. As an example, the effect of hematocrit and plasma chemical composition on the value of dynamic viscosity is taken into account in the rheological models of Quemada [41] and Walburn-Schneck [42]. As a comparison of these models, we calculated the blood viscosity coefficients in our studies to assess the effect of these factors on the change in the viscosity coefficient, the Quemada model [41] was used, in which hematocrit was taken into account −2 η=ηp □□□□1− 12k01++k∞γ̇γγ̇□□cγc Ht □□□□ , (2.6) Where values ηp, γc, k0, k∞ have been took from [39], and the Walburn-Schneck model with two parameters: hematocrit and the concentration of globulin proteins in plasma □□□□□□□□ □□ = □□1□□□□2□□□□□□□□4 □□2□□ □□−□□3□□□□, (2.7) where C1= 0.000797 Pa ·s , C2 = 0.0608 , C3 = 0.00499 ,C4 = 14.585 l/g , TPMA - is protein concentration without albumin, Ht - hematocrit. "The calculation of the dynamic viscosity coefficient according to formulas (2.6) and (2.7) in our case in the range of shear rates of 75 s-1 shows a good correspondence of the calculation results to experimental data" [31, 37]. "Blood coagulation is an extremely complex biochemical process that starts when the vascular wall is damaged and leads to the polymerization of fibrin with the formation of a clot that stops bleeding. The phase transformation of blood from a liquid state to a solid-elastic one during the polymerization of fibrin and the formation of transverse intermolecular bonds, its retraction and subsequent lysis is determined by changes in the aggregate state of blood, and, accordingly, changes in its viscoelastic properties. Thus, by changing the viscosity coefficient, it is possible to judge the dynamics of phase transformations of blood in the process of coagulation" [16]. 2.2 Resonant acoustic method for determining the viscoelastic properties of blood "The technology of low-frequency piezothromboelastography for determining viscoelastic characteristics in the process of blood clotting is based on methods of low-frequency elastography. Elastography is used to differentiate tissues and fluids by their viscoelastic properties through mechanical action and analysis of deformations obtained using ultrasound diagnostic scanners or MRI scanners. Ultrasound elastography has been

used in medical practice quite recently. Although the diagnostic equipment has modern tools and technologies, the potential of ultrasound elastography is far from being exhausted. One of the most important areas of such research is ultrasound elastography of the whole blood coagulation process. Hemocoagulation is an extremely complex biochemical process triggered when the vascular wall or blood cells are damaged, and leads to the polymerization of fibrin, accompanied by the formation of a clot that stops bleeding" [16, 43]. "In the LPTEG technology, the change in the aggregate state of whole blood is determined and recorded as a dependence of the oscillation amplitude of the piezoelectric sensor of the piezothromboelastograph. The principle of operation of this device is based on the registration of changes in the resistance of the examined blood to resonant vibrations of the piezoelectric sensor resonator needle (Figure 2.2), fixed on a piezoelectric element, which is a brass base on which a layer of piezoceramics is fixed, divided into two circular segments, and lowered by the second end into a cuvette with the patient's blood. The resonator needle in its middle part is made with a bend in the form of a loop" [8]. "A voltage varying according to the harmonic law is applied to one of the piezoelectric segments. Under the influence of this voltage, the piezoelectric makes mechanical vibrations, which are transmitted to the needle. When the end of the resonator needle is immersed in the liquid, the amplitude-phase characteristics of the voltage on the recording piezoelectric element will change. This is due to the influence of the viscoelastic properties of the liquid on the amplitude-phase characteristics of the mechanical vibrations of the resonator needle, on the change in its own and resonant oscillation frequency" [14]. 1 - resonator needle; 2 - brass ring; 3 - brass disc; 4, 5 - piezoelectric semicircular plates; 6, 7, 8, 9 - cylindrical sections of the rod with a rectangular loop Figure 2.2 - Piezoelectric sensor Consequently, by changing the amplitude-phase characteristics of the voltage on the recording piezoelectric element, it is possible to obtain information about the magnitude of the viscoelastic characteristics of the liquid. "The physical principles of the resonant acoustic method for determining the viscoelastic characteristics of a liquid are as follows. The oscillations of the resonator needle of a piezoelectric sensor can be considered as forced oscillations of a physical body (a physical pendulum) in a viscoelastic medium under the influence of a force varying according to a harmonic law, and the influence of the medium will be reflected in the change in the amplitude-phase characteristics and the natural and resonant frequency of oscillations of this pendulum. Thus, from the measured amplitude-phase characteristics of the piezoelectric sensor, it is possible to calculate its resonant and natural frequencies, and, consequently, the viscoelastic characteristics of the blood under study and their dynamics during coagulation" [13, 14, 44]. Consequently, by changing the amplitude-phase characteristics of the voltage on the recording piezoelectric element, it is possible to obtain information about the magnitude of the viscoelastic characteristics of the liquid. "In classical viscometry, the determination of the viscoelastic properties of blood is based on measuring the dependence of the shear stress □□(□□), as well as the threshold values of □□ and □□ . To account for the elastic properties of blood, the method of complex representation of the shear modulus □□ = □□′ + □□□□′′ , is used, where □□′, □□′′ are the modulus of elasticity (storage modulus) and the modulus of viscosity (loss modulus), respectively. Similarly, for the viscosity coefficient, taking into account the elastic properties of blood, we can write □□∗ = □□′ + □□□□′′, where □□′, □□′′ − are the viscosity coefficient and the elasticity coefficient, respectively" [34, 35]. "To determine the real and imaginary parts of the complex viscosity value, the method of oscillatory viscometry or the method of dynamic mechanical analysis (DMA) is used, when the dynamics of a viscoelastic medium is considered under the action of a force varying in time according to the harmonic law: □□ = □□0□□□□□□□□□□ ,where ω is the frequency of the driving force.In this case, the time and frequency dependences of □□∗, □□′ и □□∗. are investigated" [8, 44]. "In this work, an approach based on a mathematical model of forced cylinder oscillations in a viscoelastic fluid is applied to solve this problem. To account for the viscous and elastic properties of blood, we used the method of complex representation of the viscosity coefficient □□∗ = □□′ + □□□□′′, where □□′, □□′′ − is the viscosity coefficient and the elasticity coefficient associated with the shear ′: □□′′ = □□′ , where ω is the frequency of shear stresses.When the lower modulus □□ □□ part of the pendulum is immersed in a viscous liquid, the amplitude-frequency and phase characteristics of its oscillations shift towards lower frequencies, depending on the viscoelastic parameters of the liquid and the frequency of forced oscillations. To determine the complex viscosity index of a liquid from the amplitudephase characteristics of vibrations of a resonator needle, we have developed a mathematical model according to which the oscillation of the end of a resonator needle immersed in a liquid can be represented as vibrations of a cylinder of height h and radius R, performed in a direction perpendicular to the axis of the cylinder, in a viscoelastic medium η* under the action of a periodic forcing force strength" [10, 13]. "We denote the forcing periodic force acting on the resonator needle from the piezoelectric element, for □□ = □□0□□□□□□□□, where □□ - the frequency set by a piezoelectric element-a generator of mechanical vibrations. Under the influence of this force, the lower end of the resonator needle performs harmonic oscillations in an elastic medium □□ = □□0□□□□□□□□. When the lower end of the resonator needle is immersed in a viscoelastic liquid, the amplitude-frequency characteristics of its oscillations can be determined from the equation" [8, 14]¨ □□ + 2□□□□ + (□□0′)2□□ = □□0□□□□□□□□, (2.8) where □□0′ - the natural oscillation frequency of the resonator needle in the liquid, which is determined through the shear modulus G; □□ - attenuation coefficient, depending on □□′; □□0 = □□□□0 , □□ − the mass of the lower end of the resonator needle immersed in liquid. The solution of equation (2.8) is □□ = □□0□□□□(□□□□−□□), where □□0 □□0 = (2.9) [□□0′2−□□2]2+4(□□□□)2 2□□□□ □□□□□□ = (□□′)2−□□2 (2.10) 0 To calculate the coefficient β, we take into account that for ultrasonic vibrations, the values of the Reynolds number are equal to: for water, Re ~220; for glycerin, Re ~0.2; blood Re ~60, which allows us to conclude about the laminar flow regime of the lower part of the moving rod in these liquids. Consequently, the calculations can use a model of the viscous friction force proportional to the velocity of the lower section of the rod relative to the liquid medium. Figure 2.3 shows a cross section of a cylinder of radius R and height h moving in a viscous liquid at a velocity of □□ [8]. The viscous friction force equal to □□□□□□ □□□□□□ = □□ □□□□ □□□□ acts on the dS element, where □□□□ = □□ □□□□□□□□ − is tangential □□□□ component of the cylinder velocity. Then □□□□□□ = 2□□ □□□□ *h*□□□□□□□□□□□□□□□. Integrating this □□□□ expression, we get □□□□ = 4□□ □□□□ *h*□□. "To calculate the velocity gradient, we use the expression for the velocity of propagation of shear waves in a viscous medium 2□□□□ □□□□ □□ ∗ = 2□□□□ - is = , where ρ - liquid density. Then we can put , where □□ □□ □□□□ □□ □□ the thickness of the fluid layer involved in the movement. As a result , the formula for the coefficient β is obtained" [8]: 2□?? =4η/(πl* ρ0R), (2.11) where ρ0 - density of the resonator needle material, □□ = - the thickness □□ 2□□□□ of the fluid layer involved in the movement, □□ = - the speed of shear waves, □□ ρ - liquid density. From formulas (2.10) and (2.11) it is possible to determine the real part of the viscosity coefficient □□′ = □□. Figure 2.3 - Section of a cylinder

oscillating in a viscous liquid To determine the real part of the complex shear modulus □□∗ = □□′ + □□□□′′, which characterizes the elastic properties of the fluid, we use the formula for the tangential stress □□ = □□′ □□□□∗, where x is the magnitude of the shear strain along the X axis. As a result, for the natural frequency of oscillation of the resonator needle in the liquid □□0′ we obtain the formula: □□0′ = □□□□∗□□0□□ (2.12) The formula (2.12) can be used to calculate the shear modulus □□ ′ = □□ according to the value determined from the experiment □□0′ . As a result, the modulus of the complex viscosity coefficient □□∗ taking into account the ratios □□′ = □□′′; □□′′ = □□′, can be calculated by the formula □□ □□ □□∗ = □□2 + □□′2. (2.13) □□ The piezoelectric sensor of the piezothromboelastograph "Mednord" is a complex mechanical system. Piezoceramic elements in the form of two semicircular segments are "loaded" onto a brass disk to which a resonator needle is attached, immersed in a viscous liquid. Accordingly, the natural frequency of such a system differs significantly from the natural oscillation frequency of a free piezoelectric element. "The conductivity of a piezoelectric element in an alternating current circuit increases with increasing frequency and linearly depends on the latter. However, at some frequencies, this dependence of conductivity is violated and is characterized by a sharp increase in conductivity, followed by its sharp drop. These changes in conductivity have a resonant character, and resonances occur on a number of multiple harmonics, and for each harmonic two resonances (sequential and parallel) are observed, corresponding to the voltage resonance and the current resonance in the equivalent electrical circuit of the piezoelectric element" [45]. "The resonant frequency □□□□ is shifted to the low frequency region from the antiresonance frequency □□□□ and depends on the electromechanical coupling coefficient" [46]. The thickness of the piezoelectric elements included in the piezoelectric sensor of the piezotromboelastograph device is 0.02 cm. Then the upper limit of the frequency band of the piezo sensor reception, calculated by the formula □□□□ □□□□□□□□ = 0.44 □□ , where c□□ is the speed of sound in ceramics, □□ = 0.02 cm is the thickness of the piezo plate, will be equal to 10.7 MHz. Therefore, this type of piezoelectric converter is broadband. Thus, the presence of several resonances in a broadband piezoelectric sensor makes it possible to select a resonant frequency corresponding to the shear strain rate, the most suitable for studying the influence of rheological factors of blood and evaluation the hemostatic potential [10, 47]. "To study the spectral patterns of the behavior of the viscoelastic characteristics of whole blood, an analysis of the amplitude-frequency characteristics of the oscillations of the needle resonator of the piezothromboelastograph Mednord in air and water in a wide frequency range of 080 kHz was carried out" [10, 13]. "Measurements of the received signal were carried out using a digital oscilloscope. The amplitude of the signal applied to the piezoelectric element was 100 mV. It was found that resonances of the oscillation amplitude of the resonator needle for this type of piezothromboelastograph Mednord are observed in the air for frequencies 2864, 3391, 6070 Hz and 74 kH. Figure 2.4 shows the amplitude-frequency response of the piezoelectric sensor for different oscillation frequencies of the resonator needle" [10]. "Figure 2.4 (a) shows that the amplitude-frequency response in water has the form of resonant curves shifted in frequency relative to vibrations in air. With increasing frequency, this difference disappears, and at f = 74 kHz, the curves practically do not differ. The maximum oscillation amplitude of the resonator needle in the air is three times greater than the amplitude of the signal supplied to the piezoelectric element, which indicates the resonance state of the piezoelectric sensor. A comparison of curves 1 and 2 shows that the resonant frequency of the piezoelectric sensor and the resonant oscillation amplitude of the resonator needle decrease in water due to viscosity. Figure 2.4 (b) shows that the amplitude-frequency characteristics of the received signal in air and water for the antiresonance frequency □□□□ = 3391 and 3360Hz have the same patterns as for the resonant frequencies □□□□= 2864 and 2840 Hz. During the transition from air to water, the change in the amplitude of the electric signal at the antiresonance frequency is comparable in magnitude to the changes in the amplitude of the electric signal at the resonant frequency, however, the change in the antiresonance frequency Δ□□□□ = 31 Hz is 1.3 times greater than the change in the resonant frequency Δ□□□□ = 24 Hz.This indicates that the use of an antiresonance frequency is preferable for studying the viscoelastic properties of the medium, because at this frequency the resonant acoustic method turns out to be more accurate" [10]. (a) - resonance: 1 - air (2864 Hz); 2 - water (2840 Hz). (b) - antiresonance: 1 - air (3391 Hz); 2-water (3360 Hz). (c) - resonance: 1 - air (6070 Hz); 2-water (6070 Hz). (d) - resonance (74 kHz): 1 - air; 2-water. Figure 2.4 - Dependence of the oscillation amplitude of the resonator needle on the frequency "With a further increase in frequency, the influence of the viscosity of the medium does not affect the amplitude-frequency characteristics of the oscillation of the resonator needle. As an example, Figures 2.4(c) and 2.4 (d) show the amplitude-frequency characteristics of a piezoelectric sensor for a frequency of 6070 Hz and a frequency of 74 kHz. As can be seen from these figures, the amplitude-frequency response of the piezoelectric sensor does not change during the transition from air to water. This is explained by the fact that the thickness of the liquid layer adjacent to the needle is □□ , where c is the velocity of shear □□ waves, sharply decreases and the resistance of the medium relative to shear deformations can be neglected" [10]. "Figure 2.5 shows the calculated dependence of the viscosity coefficient of whole blood of a healthy volunteer □□′ on time for the frequency □□□□ and □□□□ of oscillation of the resonator needle" [13]. 1 - □□□□=2864 Hz; 2 - □□□□=3391 Hz. Figure 2.5 - Dependence of the blood viscosity coefficient η′ on time for the frequency □□□□ and □□□□ of oscillation of the resonator needle It can be seen from the figure that up to the gelling point, the viscosity coefficient is sensitive to the frequency of shear vibrations, and then the differences in the coefficients □□′,, for □□□□=2864 Hz; □□□□=3391 Hz disappear. Figure 2.6 shows the dependence of the shear strain modulus □□′/□□ of whole blood on time for □□□□ and □□□□oscillations of the resonator needle. (a) - □□□□ = 2864 Hz; (б) - □□□□ = 3391 Hz Figure 2.6 - Dependence of the shear strain modulus □□ ′/□□ of whole blood on time for □□□□ and □□□□ oscillations of the resonator needle "The modulus of elasticity □□ ′ in the process of blood coagulation increases sharply, reaching a maximum value during the polymerization of fibrin and the formation of transverse intermolecular bonds, its retraction and further lysis. It should be noted that with an increase in the frequency of shear vibrations by 20%, the elastic modulus increased by 50%. This behavior of blood relative to shear deformations confirms the presence of a low-frequency viscoelastic relaxation process in it, due to the formation of a volumetric fibrin-platelet structure of the clot. Figure 2.7 shows the dependence of the complex coefficient of blood viscosity η* on time for □□□□ and □□□□ oscillations of the resonator needle" [13]. 1 - □□□□ = 2864 Hz; 2 - □□□□ = 3391 Hz Figure 2.7 - Dependence of the complex coefficient of blood viscosity η* on time for □□□□ □□□□□□ □□□□ oscillations of the resonator needle "It follows from the calculations carried out that the shear modulus □□′, reflecting the elastic properties of blood, in the frequency range under consideration makes a significant contribution to the complex viscosity index. During coagulation, the proportion of the elastic component in relation to blood viscosity increases 10-fold: from 5% at the beginning of

the coagulation process to 50% when a fibrin-platelet clot is formed" [10]. The obtained amplitude-frequency patterns of the behavior of the viscoelastic characteristics of whole blood make it possible to use a resonant acoustic method to determine the viscoelastic properties of whole blood and their dynamics during coagulation in a wide range of shear vibration frequencies. 3 Kinetics of thrombin formation during hemocoagulation "Hemostasis is a complex hierarchically subordinate system that regulates the maintenance of the optimal functional state of the hemostatic potential - an integrative component of the full cycle of hemocoagulation, providing the necessary blood flow and preventing its extravasation in case of damage to the vascular wall. Disorders of the hemostatic system that alter the viscoelastic characteristics of circulating blood are extremely dangerous and can lead to fatal complications: bleeding or thrombosis, which gives special importance to fundamental and applied studies of blood clotting" [9, 12, 16,19]. Among the methods of studying the blood coagulation system, "global" methods that allow integratively and ex tempore to evaluate HP are of particular interest [16]. Such an assessment of the state of the hemostasis system provides for two extremely important points: − work with native blood (the beginning of the analysis immediately after sampling), -a sample containing endothelial producers, clotting factors, inhibitors/activators of hemostasis and fibrinolysis, drugs, etc., affecting the coagulation process; − registration of the coagulation process under the conditions of standardized contact activation and graphical reflection of its characteristics by changing the viscoelastic properties of blood when its aggregate state changes. "Fibrin, formed as a result of the interaction of thrombin and fibrinogen, plays a decisive role in changing the viscoelastic properties of blood" [17, 21, 22]. Consequently, the production of thrombin, as a result of competing enzymatic reactions, sets the dynamics of the formation of fibrin, providing a change in the aggregate state of the blood. For this reason, when analyzing the state of HP, special attention is paid to assessing the kinetics of thrombin formation and inactivation as a trigger of hemocoagulation. At the same time, the method of performing the thrombin generation test is quite complex, requires special equipment and expensive consumables [26,32]. "Taking into account the above, it is assumed that it is possible to evaluate the generation of thrombin based on the results of a study of the kinetics of the final product of blood coagulation - fibrin, the development of which determines the dynamics of its viscoelastic characteristics during coagulation" [17]. "The most effective for such an evaluation is the domestic technology of low-frequency piezothromboelastography (LPTEG), which is based on a resonant acoustic method for determining the viscoelastic characteristics of blood during coagulation under the action of periodic shear deformations obtained using ultrasonic transducers" [9,10,17]. 3.1 Theoretical foundations of the thrombin generation method The nonstationary equation of substance transfer in the reaction-diffusion approximation or without diffusion is used as the main equation governing the blood clotting process, including a set of biochemical reactions involving factors, enzymes, zymogens - catalysts and coagulation inhibitors [48]. "These equations are based on the continuity equation □□□□□□ □□ , (3.1) □□□□ = □□□□□□□□□□ + □□□□□□ □□ where □□□□ − concentration of the i-th factor; □□□□ - diffusion flow density: □□□□ = −□□□□ □□□□□□□□ □□□□ ; (3.2) □□□□□□□□ - the rate of chemical reaction of the interaction of factors □□ и □□: □□□□□□□□ = □□□□□□□□□□□□□□□ (3.3) To date, the prevailing model of hemocoagulation is a model based on the cellular theory of blood clotting" [49]. "According to this theory, hemocoagulation consists of three overlapping phases - initiation, amplification and propagation. Figure 3.1 shows the sequence of the cascade of the main enzymatic biochemical reactions {1-5} leading to the formation of fibrin" [9,17]. Figure 3.1 - Cascade of the main biochemical reactions of blood clotting When the vessel is damaged, the blood plasma contacts the tissue factor, resulting in the activation of factor VII and the formation of an external tenase complex [TF-VIIa]. This complex, in the presence of calcium ions, initiates a hemocoagulation cascade, activating factors X and IX on the surface of the subendothelium. It is the activation of the X factor by the [TF-VIIa-Ca2+] complex that leads to the formation of a small amount of thrombin for the direct transformation of fibrinogen into fibrin (reaction {5}, Figure 3.1) and the launch of a positive feedback loop in which thrombin enhances its own operating time (reaction {2-4}, Figure 3.1), leading to nonlinear growth of thrombin concentration (thrombin generation) and, as a consequence, as a result of the reaction {5} (Figure 3.1), the production of fibrin. "Thus, thrombin is one of the main regulators of hemocoagulation in vivo, the determination of the activity of thrombin generation is able to integratively display the state of hemostatic potential. As is known, the main factor in changing 45 the viscoelastic properties of blood during coagulation is an increase in the concentration of fibrin. In accordance with the kinetic equation of the direct transformation of fibrinogen into fibrin (reaction {5}, Figure 3.1), the change in the concentration of fibrin will be determined by equation (3.4)" [17, 50] □□□□(□□□□) □□□□ = □□(□□□□□□) × □□(□□) × □□□□,□□□□ = □□ , (3.4) □□□□ □□□□ where □□(□□□□□□); □□(□□) и □□(□□□□)□□□□,□□□□ − concentrations of activated thrombin, fibrinogen and fibrin, □□□□,□□□□ - the rate of the corresponding biochemical reaction; □□ - the proportionality coefficient between the LPTEG signal curve and the rate of □□□□ change in blood viscosity . From the formula (3.4) follows: □□□□ □□ □□□□ □□(□□□□□□) = □□(□□)×□□□□,□□□□ □□□□ (3.5) "Thus, knowing the initial concentration of fibrinogen, the rate constant of the biochemical reaction {1} and calculating the rate of change of the viscosity coefficient from the integrative LPTEG curve, it is possible to determine the dynamics of thrombin production during blood coagulation" [9, 17]. At the same time, it should be noted that the production of fibrin in small quantities begins from the moment the resonator needle is immersed in a cuvette with blood. This follows from the formula that determines the dependence of the fibrin concentration on time: □□(□□□□)(□□) = ∫0□□ □□(□□□□□□)(□□′) □□(□□) □□□□,□□□□□□□′ (3.6) Formula (3.5) is the basis of the method for evaluating thrombin generation at all stages of whole blood fibrinogenesis [9,17]. 3.2 Kinetics of thrombin formation based on the processing of data obtained by the LPTEG method Thrombin is one of the main regulators of hemocoagulation in vivo. In the physiological state, thrombin circulates in the form of an inactive prothrombin enzyme, whose activation in the aspect of hemocoagulation occurs under the influence of factor Xa. Thrombin performs many functions in blood clotting processes - activates not only procoagulant factors and platelets, but also triggers a cascade of its own inactivation - the protein C system [9]. To solve the problem of determining the kinetics of thrombin generation, a method has been developed for calculating changes in thrombin concentrations based on the results of measuring the viscoelastic properties of native blood during hemocoagulation [17]. "The equipment for determining the viscoelastic characteristics of blood is shown in Figure 3.2. The generator (2) sets the frequency of forced oscillations of the piezoelectric resonator needle (1). The amplitude-frequency and phase characteristics of the piezoelectric sensor electrical signal resulting from the piezoelectric effect are recorded by an oscilloscope (3), signal processing and calculations of the viscoelastic characteristics of the liquid are performed on a computer (4). The result of measuring the dynamics of the blood clotting

process is the curve of the integrative state of the complete hemocoagulation cycle - the time dependence of the oscillation amplitude of the resonator needle recorded by a piezo sensor and the amplitude-phase characteristics of the oscillation of the resonator needle recorded by an oscilloscope" [9]. 1 - piezothromboelastograph Mednord; 2 - harmonic oscillator; 3 - oscilloscope; 4 - computer. Figure 3.2 - Equipment for measuring the blood viscosity coefficient [9] The measurement of the viscoelastic characteristics of blood is carried out as follows: blood is taken from the cubital vein with a three-component siliconized syringe with a volume of 1 ml without applying a tourniquet. After that, for 10 seconds, the contents of the syringe are placed in a 0.45 ml cuvette located in the thermostat of the Mednord piezothromboelastograph device and the study of the blood clotting process begins. After that, venous blood is taken from the cubital vein of the second arm according to the standard procedure in order to determine the concentration of fibrinogen and prothrombin. After receiving the initial NPTEG curve, the curve is adjusted taking into account the hardware function of the device. The experimental NPTEG curve contains a contribution to the change in the amplitude of the signal A(t) due to the shift of the resonant oscillation frequency of the needle-resonator of the piezoelectric sensor □□□□(□□) in a viscous liquid with respect to the frequency of the oscillator □□0 [17], □□(□□) 2 □□□□(□□) = □□01 − 2 □□0 , (3.7) □□(□□) □□ где □□0 = 2 □□ , □□ - the coefficient of dynamic viscosity, and G is the shear □□0 modulus. "The ratio of the amplitude of the signal of the piezoelectric sensor A(t) to the amplitude at the initial moment of time before the needle-resonator is immersed in a cuvette with blood in accordance with the theory of forced oscillations is determined by the formula: □□(□□□□(□□)) = □□□□(□□(□□(□□))) 1 (3.8) □□0 □□0 and it is called the hardware function of the device. The hardware function is determined by conducting an experiment to measure A(ω(t)) on a liquid with a known viscosity coefficient (glycerin). Figure (3.3) shows the experimental curve of the LPTEG dependence A(t) (blue -without adjustment for the shift of the resonant frequency) and □□корр.(□□) - (red with adjustment for the shift of the resonant frequency), calculated by the formula" [17] □□корр.(□□) = □□(□□) − □□(□□0)□□□□□□(□□) (3.9) "As can be seen from the figure 3.3, the effect of shifting the resonant oscillation frequency of the piezoelectric sensor manifests itself at the initial moments of coagulation time t < 3 min., when the increase in fibrin concentration is insignificant. Obviously, the time point t1, marked in Figure 1.2, corresponds to a situation when two competing processes: the natural frequency shift and the operating time of fibrin become equal in magnitude. At t>t1, an increase in the fibrin concentration has a predominant effect on an increase in the viscosity coefficient □□. Figure 3.3 shows that the red and blue curves at t >10 min. practically coincide" [17]. blue curve - measured values of the piezoelectric sensor electrical signal; red curve - taking into account the correction adjusted for a decrease in the electrical signal of the piezoelectric sensor, taking into account the shift of the natural frequency; the green curve is the first derivative of the red curve Figure 3.3 - The change in the viscosity of whole blood depending on the time in the initiation phase "The green curve in Figure 3.3 indicates the change in the concentration of thrombin in relative units, determined from the formula (3.5). The figure shows that at the first moments of time, the increase in the concentration of thrombin is insignificant and linearly depends on time, determined by the initial concentrations of thrombin and fibrinogen, but after 3 minutes, a nonlinear growth (generation) of thrombin is observed, which corresponds to the inclusion of positive feedback in the cascade of enzymatic chemical reactions (Figure 3.1). The maximum concentration of thrombin is reached at time t2 in Figure 1.2, at which the second time derivative of the integrative curve is zero □□□□□□□□□□ □□ □□2□□ □□□□ = □□□□×□□□□,□□□□ □□□□2 (3.10) Thus, the point t2 in Figure 1.2 is the inflection point at which the exponential growth of fibrin concentration begins to slow down, due to a decrease in the concentration of thrombin [51]. At point t2, the concentration of thrombin reaches the value of the initial concentration of prothrombin □□□□□□□□□□□□□□□(□□□□) ≡ □□□□□□ , this condition allows us to determine the coefficient α in the formula (3.5). Taking into account the formula (3.5)", [9, 17] □□□□ □□ = □□□□□□ × □□□□ × □□□□,□□□??/ □□□□□□□□□□ , (3.11) □□□□ □□2□□ where □□□□□□□□□□ the derivative defined at the point t2, где □□□□2 = 0. In this case, the formula (3.5) can be rewritten as □□□□ □□□□□□□□ = □□□□□□ □□□□□□□□ (3.12) □□□□ □□□□□□ "Thus, formula (3.12) makes it possible to determine the dependence of concentration of thrombin on time in absolute units from the known concentration of prothrombin and the measured dependence of the oscillation amplitude of the resonator needle of the piezoelectric sensor of the piezotromboelastograph" [17]. 3.3 Comparison of the results of numerical calculations of the thrombin concentration with the experiment "Figure 3.4 shows a comparison of our method for assessing the concentration of thrombin using the NPTEG technology with the method of hromboelastography (TEG) and the thrombin generation test (TGT)" [9, 26-29,32]. (1) -TGT; (2) - TEG; (3) - LPTEG. Figure 3.4 - Graphical representation of the results of global tests of the hemostasis system "Comparison of graphs of the dependence of output characteristics on time shows that the methods of TGT and TEG are characterized by the presence of a delay time between the start of the test and measurements of the output signal. For TEG, this is the clotting time, which indicates how quickly the formation of the first fibrin strands begins after the addition of a clotting activator to the blood. The time delay in the TGT method is the time measured from the introduction of a mixture of a fluorogenic substrate with ionized calcium into a container with a blood plasma sample and an activator until a significant signal deviation. The time delay in the TGT method is the time measured from the introduction of a mixture of a fluorogenic substrate with ionized calcium into a container with a blood plasma sample and an activator until a significant signal deviation. As can be seen from Figure 3.4, the time of the maximum concentration of thrombin falls on the gelling point (A2) on the LPTEG curve, and the time delay is 2.75 minutes. As a result of the processes of thrombin inactivation, its concentration decreases, so this test does not describe the final stage of clot formation - the production of fibrin and its polymerization. TGT also has a number of disadvantages related to the standardization of analysis. In particular, it is very sensitive to the pre-analytical stage of sample preparation, including the method of collection, the nature of the test tube material, the anticoagulant used and analytical parameters (tissue factor level, lipid concentration, presence of chylosis)" [9, 17, 32]. "In addition, as a result of the processes of inactivation of thrombin, its concentration decreases, so this test of TGT does not also describe the final stage of clot formation - the production of fibrin and its polymerization. Thus, due to the physical principles inherent in them, the methods of TGT and TEG cannot describe the entire cycle of blood coagulation at all stages of fibrinogenesis. On the contrary, the LPTEG test has sufficient sensitivity and provides information at all levels of hemocoagulation in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application" [16, 52, 53]. Figure 3.5 shows LPTEG curves showing the state of hemostatic potential in patients with normocoagulation (Case 1), hypercoagulation (Case 2) and hypocoagulation (Case 3) [9]: A is the original curve, without adjusting for the frequency shift; B

is a curve adjusted for frequency shift; C is a curve showing the kinetics of thrombin generation. Figure 3.5 - Thrombin kinetics determined by the LPTEG technology. The experiment was carried out on a piezothromboelastography of a copper cord with a resonant frequency of a generator of 3000 Hz [9] 53 In the figure, a scale is plotted along the ordinate axis on the left, displaying the magnitude of the piezoelectric sensor signal in relative units for curves A and B. The green curve shows the change in the concentration of thrombin during coagulation of whole blood, measured in mg/dl, plotted on the right ordinate axis.Видны заметные отличия в клинических случаях 1-3. In comparison with normocoagulation (case 1), hypercoagulation (case 2) is characterized by a shorter time of thrombin tk and a narrower half-width curve B, which means a shorter time for the formation of a blood clot. Accordingly, in case 2, the blood has a high degree of thrombosis. For hypocoagulation (case 3), the value of tk and the half-width of the curve B is greater than in case 1. Accordingly, such blood has a high degree of thrombohemorrhagic outcome. As can be seen from the comparison of curves A, B and C, our method is more accurate for assessing the hemostatic potential and diagnosing diseases of the hemostatic system. Figure 3.6 shows the results of a comparative study of the hemostasis system of a healthy volunteer using LPTEG technology with the measurement of viscoelastic characteristics of blood at a frequency of 3000 Hz and 360 Hz. Considering that the concentration of thrombin in accordance with formula 3.5 is proportional to □□(□□), figure 3.6 (B) shows the dependence of the concentration of thrombin on time during coagulation. As can be seen from figure 3.6, the dynamics of the blood coagulation process with increased viscosity is more intense. This is especially noticeable when the frequency of the generator is 360 Hz. The proposed method for estimating thrombin generation is based on knowledge about the physical properties of blood, since its aggregate state changes during clotting with significant changes in its viscoelastic characteristics. (A) (B) (A) - the dependence of the viscosity of whole blood n(t) in the process of coagulation; (B) − dependence of the derivative on the viscosity of whole blood n (1) in the process of coagulation; 1 - generator frequency 360Hz normal blood viscosity; 2 - generator frequency 360Hz increased blood viscosity; 3 - generator frequency 3kHz normal blood viscosity; 4 - generator frequency 3kHz increased blood viscosity. Figure 3.6 - Dependence of viscosity (A) and its derivative (B) of whole blood during hemocoagulation for a healthy volunteer "This method is used to study whole blood by changing the viscosity-elastic characteristics due to mechanical action on it and to analyze the resulting shear deformations obtained using ultrasound diagnostic scanners. The concentration of accumulated thrombin during coagulation is directly proportional to the rate of change of the complex viscosity coefficient determined from the dynamics of the integrative curve of the LPTEG. The maximum concentration of thrombin is reached at a time when the second time derivative of the integrative curve is zero and when the exponential growth of fibrin concentration begins to slow down, due to the processes of thrombin inactivation. This method has sufficient sensitivity and provides information about the concentration of thrombin at all phases of fibrinogenesis in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application" [17]. CONCLUSION As a result of the work done, all the planned tasks were solved and the following results were obtained: 1. A theoretical and experimental study of the viscoelastic properties of native blood under oscillating shear stresses has been carried out. The dynamics of the process under study - the transition of blood from a liquid state to a solidelastic one is determined by changes in the aggregate state of blood and is recorded as a dependence of the amplitude and phase of oscillations of the resonator needle in the blood on the time during the period hemocoagulation. 2. A mathematical model and a resonant acoustic method for calculating the complex viscosity coefficient of whole blood based on ultrasound elastography of its characteristics during coagulation have been developed. The high sensitivity of the method to changes in the viscoelastic properties of reference liquids is confirmed. 3. The calibration of the readings of the improved hardware and software complex of the Mednord piezothromboelastograph on an aqueous solution of glycerin and the dependence of the blood viscosity coefficient on the coagulation time calculated on this basis gave good agreement with the results of numerical calculations of blood viscosity using mathematical modeling. 4. Calibration of the readings of the improved hardware and software complex of the Mednord piezothromboelastograph on an aqueous solution of glycerin was carried out. The dependence of the blood viscosity coefficient on the coagulation time calculated on this basis gave a good agreement with the results of numerical calculations of blood viscosity using mathematical modeling. 5. Calculations of the real and imaginary parts of the complex viscosity index by resonant acoustic method were carried out. A comparison of the calculations with the available rheometric measurement data shows their good agreement. The obtained results and comparison with the experiment confirm the possibility of using the resonant acoustic method to determine the viscoelastic properties of whole blood and analyze their dynamics during coagulation in a mode as close as possible to in vivo. 6. "It is shown that in the process of blood coagulation, the real and imaginary parts of the coefficient η∗ increase by orders of magnitude, reaching the maximum value during the formation of the fibrin-platelet structure of the clot. It follows from the calculations carried out that the shear modulus reflecting the elastic properties of blood in the frequency range under consideration can make a significant contribution to the complex viscosity index. During coagulation, the proportion of the elastic component in relation to blood viscosity increases 10-fold: from 5% at the beginning of the coagulation process to 50% when a fibrin-platelet clot is formed" [10]. 7. Amplitude-frequency patterns of the behavior of viscoelastic characteristics of whole blood make it possible to use a resonant acoustic method to determine the effect of plasma and cellular factors of blood clotting on the hemostatic potential in a wide range of shear vibration frequencies and shear strain rates. 8. The method for evaluation the concentration of thrombin in the process of fibrinogenesis based on piezothromboelastography using a resonant acoustic method to determine the viscoelastic properties of whole blood has been developed. The results of calculating the concentration of thrombin (TGT) by this method are compared with the results of the thrombin generation test and the thromboelastography test (TEG). 9. Clinical, laboratory and numerical experiments were carried out to calculate the kinetics of thrombin and hemostatic potential with different enzyme composition of blood in conditions of physiological norm and pathology (hyper- and hypocoagulation). Method determination of the kinetics of thrombin formation during whole blood coagulation has sufficient sensitivity and provides information about the concentration of thrombin at all phases of fibrinogenesis in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application. The proposed method for estimating the concentration of thrombin is based on the postulate of a directly proportional dependence of the change in the concentration of thrombin and the rate of change in the complex coefficient of blood viscosity, determined by the results of measurements of the dynamics of viscoelastic characteristics based on low-frequency piezothromboelastography during coagulationThis

method has sufficient sensitivity and provides information about the concentration of thrombin at all phases of fibrinogenesis in whole blood from initiation to the formation of a fibrin clot, which ensures its wide application. The results of the work have been published in 4 articles cited in the international databases Scopus and Web of Science and presented at 4 international and Russian conferences. REFERENCES 1. WHO: Newsletters. 10 leading causes of death in the world, 2020. URL: https://www.who.int/ru/news-room/fact-sheets/detail/the-top-10-causes-ofdeath . (in Russian) 2. Virani S.S., Alonso A., Benjamin E.J., et al.// Circulation. -2020. -V. 141. -Iss. 9. -P. e333-470F. 3. Demkin V.P., Melnichuk S.V., Zavadovsky K.V., etc. The effect of dynamic blood viscosity on the coronary blood flow of the stenosed portion of the artery. // Izvestiya vuzov.Physics.− 2021. − Vol. 64.−no.12.−pp.172-178. (in Russian) 4. 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