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The Review of Contemporary Ideas about the Influence of Flow Rate on Blood Clotting[1]

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Abstract—Normal blood clotting is vitally important for mammals. The diffusion-convection transfer of clotting factors plays a key role in blood clot formation. Since the shear rates of blood flow are very high (up to 7000 sec–1), clot formation critically depends on the flow rate. The methods of study of the flow effect on clotting are indirect and the processes are rather complex; therefore, mathematical models of this process are significant for interpretation of results and understanding of the mechanisms. The review expounds the main experimental data on the effect of flow on the blood clotting cascade, some hypotheses and mathematical models explaining different regimes of the functioning of this system. The review is focused on specific problems encountered by researchers in this field. Some of the experimental works have shown that flow decreases the size of the formed fibrin clot and that the dependence of clot formation period on the flow shear rate is a threshold function. However, there are also experimental evidence that the flow can increase production of clotting factors (factor Xa), which must be expressed in the procoagulant action of the flow. Mathematical models of different aspects of clotting give no unified predictions either. Nevertheless, the combined analysis of results of detailed modeling and experiments, in our opinion, may result in understanding of the mechanisms, which determine the behavior of clotting in a flow.

Key words: blood clotting, mathematical modeling, clotting in a flow, factor Xa, fibrin.

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The hemostatic system of humans and mammals consists of the two components: vascular-platelet and plasma [1, 2]. When the vascular wall is damaged, the substances released from the cells of destroyed endothelium into blood induce, in the first place, vasoconstriction and formation of a platelet aggregate [3]. After activation, platelets become capable of adhesion to the place of lesion and mutual aggregation. As a result, a comparatively loose platelet plug is formed in the place of lesion to prevent the loss of blood cells but not plasma. The typical time of actuation of these systems is several minutes. Plasma clotting is activated at the contact of blood with the cells that underlie endothelium and develops against the background of the above events. Its actuation time is on the order of tens of minutes [4, 5] and it results in formation of a dense fibrin clot completely preventing the loss of blood.

Since clotting is activated only by the damaged part of vascular wall, i.e. spatially localized, it is accepted to reduce the effect of blood flow to the following aspects: the flow brings inactive clotting factors to the activator and carries active forms downstream; the flow provides the influx of platelets to the activator; the flow can influence the kinetics of membrane-dependent reactions by increasing the rate of substrate influx and product removal.

Blood is a viscous fluid and the flow rate profile crosswise the vessel is not a constant value: the rate is close to null near the vascular wall and maximal in the center. The flow in the near-wall area is usually described by the shear rate of the flow [6]. This parameter shows how quickly the flow rate varies from the wall to the center. In humans, this value varies from 20 s^{-1} in cava and pulmonary trunk to 7000 s^{-1} in arterioles [7].

The present review is concerned with the studies of the flow effect on plasma clotting; the analysis of the flow effect on platelet clotting is beyond the scope of survey.

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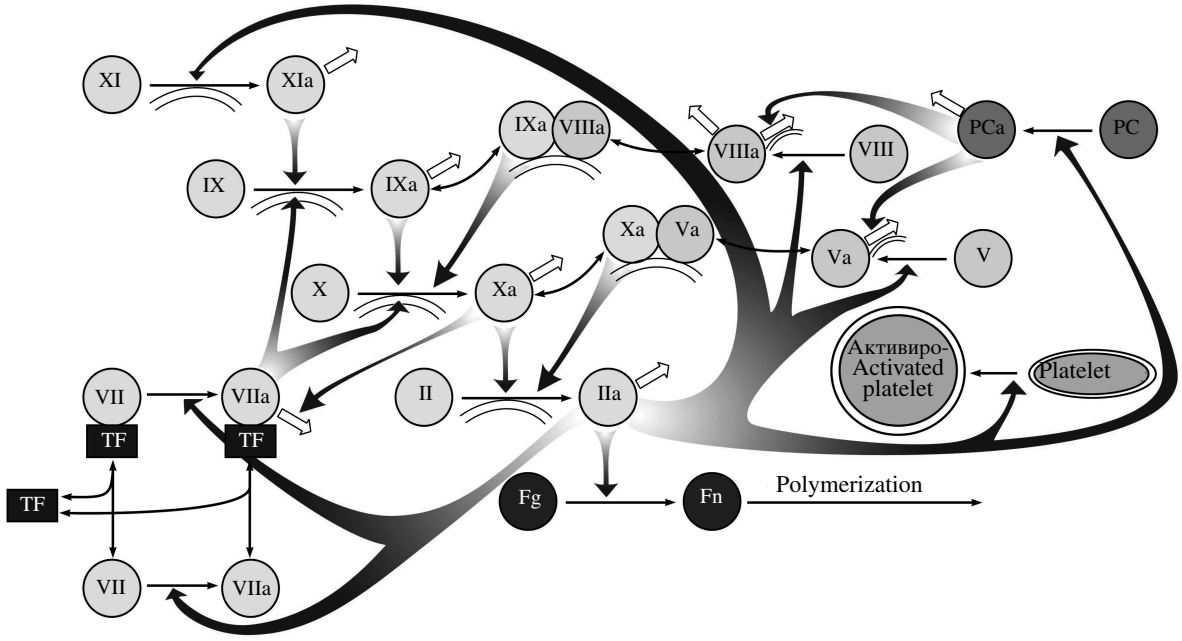

Fig. 1. Basic reactions of the plasma system of blood clotting. Enzymatic catalysis and transformations induced by it are shown by gray and black arrows, respectively. Inhibition is shown by short hollow arrows. Symbols: TF, tissue factor; Roman numerals designate the corresponding clotting factors; PC, protein C; Fg, fibrinogen; Fn, fibrin. Two neighboring designations of clotting factors are a complex formed by these factors (e.g., XaVa). Double arc under the reaction arrow (designation of a complex) shows that this reaction (complex assembly) occurs only on phospholipids surface.

DESCRIPTION OF PLASMA COMPONENT

Clotting is a complex system of enzymatic reactions in blood plasma, which is triggered at blood vessel damage [2, 5]. Figure 1 shows the most significant reactions of this system. The cells of almost all tissues of organism, except for endothelium and blood cells, express a transmembrane glycoprotein, tissue factor (TF). Normally, TF does not contact blood, but any lesion of endothelium results in the contact of plasma with TF-bearing cells. Plasma permanently contains factor VIIa, serine protease, which in itself has almost no enzymatic activity. However, factor VIIa is bound to TF with the formation of an extrinsic tenase complex (VIIa-TF) possessing a high enzymatic activity and being able to activate factors IX and X. These very events start the process of clotting.

The cascade of clotting reactions is catalyzed by serine proteases [2, 5, 8]. Serine proteases are present in blood plasma as inactive precursors termed as clotting factors and are marked by Roman numerals. These proteins can activate each other through specific cleavage in certain sites. The activated form of clotting factor is designated by the same Roman numeral with index "a". The last and major enzyme in the cascade is factor IIa, which is also named as thrombin. Thrombin is formed from prothrombin (factor II). The main regulation of the system is closed on this factor [9, 10]. Thrombin transforms the fibrinogen protein into fibrin, which is polymerized with the formation of fibrin network and conversion of blood from fluid to the gel-like state. Fibrin fibers reinforce the primary platelet thrombus and fix it in the site of vascular lesion, preventing its washing out by blood flow. The reactions of factor X activation by factor IXa and prothrombin activation by factor Xa are very slow. However, low amounts of thrombin formed in these reactions trigger the loops of positive feedbacks resulting in explosive formation of thrombin [9]. In the first place, thrombin increases the amount of extrinsic tenase by way of activation of factor VII into factor VIIa [10]. Second, thrombin activates factors VIII and V [11, 12]. Their active forms are co-factors for factors IXa and Xa, respectively. Thrombin also activates platelets, which results in the formation of complexes IXa–VIIIa (intrinsic tenase) and Xa–Va (prothrombinase) on their membranes. These complexes assembled in the presence of potassium can work quicker by 3–5 orders of magnitude than the enzymes in the absence of co-factors and provide further distribution of a clot in the zones distant from the activator.

All active enzymes of the systems are inhibited by protease inhibitors present in plasma, the major of them being antithrombin III (AT-III) and tissue factor pathway inhibitor (TFPI). AT-III is the principal inhibitor of thrombin, whereas TFPI inhibits the extrinsic tenase by a complex Xa-dependent mechanism [13, 14].

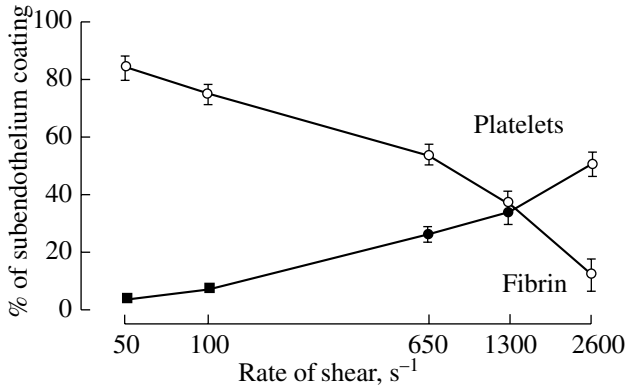

Fig. 2. Deposit of fibrin and platelets on subendothelium after 5 min of venous blood pumping at different rates of shear. Reproduced from [15]. Perfusion chamber was a ring-shaped unit: in the center of a cylinder chamber there was an everted segment of rabbit aorta with removed endothelium. The blood taken from elbow vein was pumped into the space between the walls of the cylinder chamber and aorta. The flow rate was controlled by a peristaltic pump.

THE EFFECT OF FLOW ON BLOOD CLOTTING

Experimental studies. The flow carries inactive factors to the place of activation and carries away the activated factors. Does it improve the clotting or, on the contrary, suppress the latter? One of the main results concerning the flow effect on clotting was obtained already in 1986 [15]. This work was carried out using an annular perfusion chamber, where human blood was pumped with the shear rates of 50–2600 s^{-1} through averted rabbit aorta segments, with endothelium removed by a balloon catheter. At the same time, precipitation of platelets and fibrin on subendothelium was measured. Blood was pumped directly from elbow vein to perfusion chamber without recycling. The flow rate was preset by a peristaltic pump. Perfusion time was 5 min. The presence of fibrin on subendothelium was assessed systematically at 10-μm intervals (about 1000 sections). The fact that it was just the fibrin was confirmed by electron microscopy showing the presence of 23-nm linear structures, corresponding to half of the linear size of fibrin molecule. As a result, it was established that the shear rate increase was accompanied by the decrease of percentage of surface coating with fibrin in the end of the experiment (Fig. 2), i.e. the flow impaired plasma clotting. The quicker was the flow, the more it impaired plasma clotting.

One of the widespread types of study of blood clotting in flow is based on a closed circulation system: the same blood volume is pumped many times through a chamber with the clotting activator [16]. We believe that the experiments of this type, in spite of their simplicity, give no reliable information about the clotting process. It is due to the fact that repeated recirculation of the same blood volume results in hyperactivation, because, first, activated clotting factors are not removed from blood flow (as in the organism) and, second, the effect of contact activation of the clotting system from the flow chamber walls increases with time. Clotting is a spatially inhomogeneous process, with some reactions occurring on the activator and other reactions occurring at a distance from it; hence, clotting begins on the activator and is spread deep into the area, the flow carries active factors downstream and they, after having passed through the whole cycle, appear again near the activator and intensify the clotting. Thus, it is practically impossible to separate the contributions to clot formation of the direct effect of clotting activator on flowing blood and the effect of active clotting factors brought by the flow.

The kinetics of clot formation at different shear rates of the flow has been studied in [17]. The authors pumped whole blood or platelet-rich plasma through glass capillaries with the modified inner surface and registered the time of clotting (the moment of clot appearance) depending on different values: the shear rate of flow, the inner diameter of capillary, and the size of activating site. Modification of the inner surface of capillaries was as follows. First capillaries were coated inside with butyltrichlorosilane; then 1,2-dilauroyl-*sn*-glycero-3-phosphocholine was pumped through them to form a monolayer of neutral phospholipids. Deep UV photolithography was used to remove some part of lipids and silanated surface. Then, TF-containing phosphatidylcholine vesicles were pumped to form a bilayer on the cleared surface. As a result, a shaped site with TF exposed in the flow, simulating the place of vascular wall damage, was formed on the inner surface of the capillary. The clot was registered by optical microscopy. The threshold behavior was shown: the clot was formed during approximately the same time in a certain range of flow rates but, as soon as the rate exceeded the threshold, the clot was not formed at all (during the experiment) (Fig. 3a). At the same time, the threshold was determined by the activator size: the larger was the activator, the higher shear rates were needed for threshold appearance, and vice versa (Fig. 3b).

After it has been shown that the flow impairs clotting, it would be logical to find out the clotting factors whose transfer leads to such result. It should be noted that still there is no valid study of transfer by the flow of all clotting factors, both active and inactive. There are only works on activation of factor Xa at different shear rates of the flow. Since factor Xa is a component of prothrombinase, the main activator of thrombin, the kinetics of factor Xa activation may provide important data for the understanding of the flow effect on blood clotting.

The work of Katoh et al. [18] has shown that, when factor Xa is formed as a result of activation by the inner pathway of clotting with cephaloplastin, the rate of its production decreases together with the increase of shear rate of the flow. In the case when this factor is activated directly by reptilase (from the poison of tic-

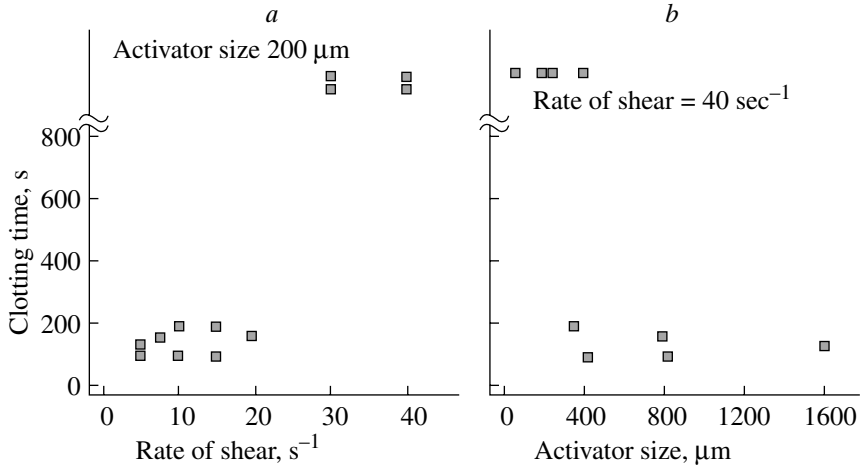

Fig. 3. Dependence of the time of clot appearance on the rate of shear at a constant size of activator (*a*) and on the size of activator at a constant rate of shear (*b*). Reproduced from [17]. Whole blood or platelet-rich plasma was pumped through a glass capillary with the inner walls coated with phospholipids. Some part of the inner surface, i.e. annular segment, was coated with phospholipids containing the tissue factor. This region was an activator of clotting. The time of clotting was determined by the moment of thrombin emission and initial formation of a fibrin clot. Fluorogenic substrate was used for the recording: being split by thrombin, it increased fluorescence in the blue channel.

polonga), the rate of factor Xa activation does not depend on shear rate of the flow.

However, according to [19], the rate of factor X activation by the extrinsic tenase increases together with the increase of shear rate of the flow. The authors used a flow reactor, which was a microcapillary coated inside with a single bilayer of phospholipids containing the known amount of tissue factor. The solutions of factor VIIa in a concentration sufficient for saturation and factor X in different concentrations were pumped through this reactor. The rate of factor X flux to the surface could be changed by varying the flow rate.

Besides the delivery of factor X to the activation zone, the effect of removal of active factor Xa from the reaction zone has been investigated. It has been shown [20, 21] that it may result in the higher activity of the extrinsic tenase complex. It is due to the fact that removal of the product from the reaction zone makes the active site free for attachment of the next substrate molecule.

Since activation of factor Xa by the extrinsic tenase is important for clotting actuation and the functioning of the intrinsic tenase and prothrombinase complexes is important for clot growth, results of the work [18] may imply that the impairment of factor Xa production by the intrinsic tenase at flow gain decreases the rate of clot growth.

Results reported in [19] demonstrate that the rate of factor Xa activation is higher in an intensive flow. However, in this study the whole factor VII was in the active state, whereas in the organism it is activated in the course of clotting. Simultaneously with the activation of factor VII and formation of the extrinsic tenase (VIIa–TF), the latter is inhibited. Thus, the described situation with too much of available active extrinsic tenase does not occur in an organism in practice. Hence, the obtained results are not quite fit for the understanding of blood clotting processes in the flow.

The works [20, 21] lead to the conclusion that the flow improves factor Xa activation and thus accelerates actuation of clotting. It does not conform with the behavior of the system described above [15, 17]. Really, clot formation is not limited by factor Xa activation; it is a much more complex process. A clot is a polymer network of fibrin activated by thrombin; before polymerization, it can be carried away by the flow. Complexity of the clotting system did not make it possible to investigate the causes of severe contradictions between different experimental variants. These results cannot be understood without application of mathematical modeling.

Mathematical modeling of blood clotting in the flow. The numerical experiment reproducing an in vitro system can be carried out with more complete models, which take into account most of the known clotting reactions. Unfortunately, the most part of works considering such complete models (e.g., [22, 23]) come to the construction of model itself and demonstration of the fact that it describes at least something. There is no experimental verification of the models, nor the search of special regimes of clotting function, nor detailed study of its different stages. We cannot but hope that it is a matter of close future. However, one should not think that the clotting has never been analyzed on full-scale clotting models. A complete clotting model has been constructed [24], and it has been shown [25] that thrombin generation under flow conditions depends on the competition between activation of factors X and IX by the extrinsic tenase complex and their removal by the flow. At the same time, clotting inhibitors ATIII and

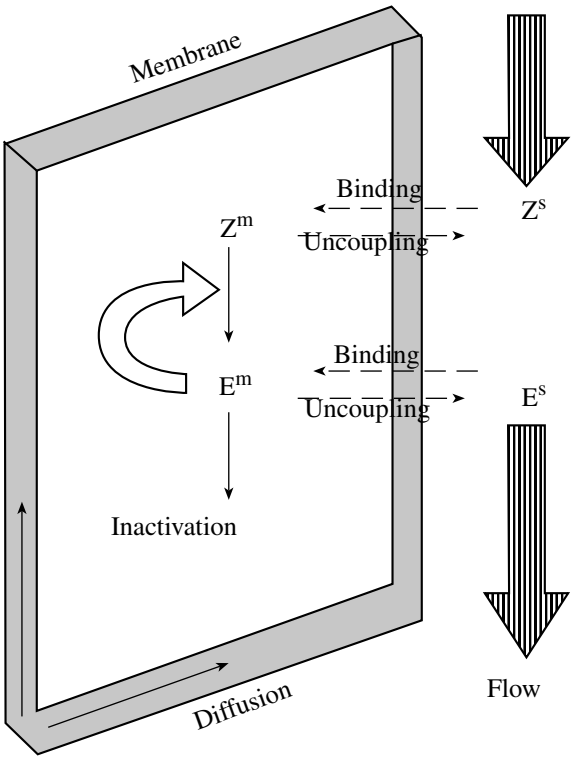

Fig. 4. Schematic representation of the system of reactions used in [26]. Z, zymogen; E, enzyme. Reproduced from [26]. Upper indices m and s mean that the substance is on the membrane and in solution, respectively. The enzyme catalyzes its formation from the zymogen on membrane surface. The enzyme and the zymogen can diffuse in the plane of the membrane, get into the membrane from solution, and get out into solution. The densities of E and Z binding sites are equal over the whole membrane surface. Diffusion rate in the medium is rather high, so that the flow of reagents in the medium does not depend on the distance from the membrane.

TFPI have no significant effect on thrombin generation. This interesting although not quite clear prediction has prompted to propose an alternative mechanism "shutdown" of TF on the wall by adhering platelets, which merely block the access to it. Such prediction is probably untrue, because registration of TFPI functioning in this work [24] has not been quite correct.

The study of blood clotting in the flow using complete models undoubtedly can withdraw the contradictions that appear during the analysis of experimental data obtained in different variants. The works considered in the previous section [20, 21] demonstrate that the flow improves the activation of factor Xa, which must lead to the improvement of clotting. It has also been shown [15, 17] that the flow impairs clot formation. Computer modeling would make it possible to define the space-time distribution of factor Xa depending on shear rate of the flow and how it affects clot formation. Besides, it would be possible to find out the factors, whose removal by the flow is critical for clotting, and to propose new experiments that could elucidate the work of mechanisms responsible for clotting triggering, clot growth, and cessation of growth under conditions of the flow. As far as we know, such investigations have not been carried out as yet. It may be due to some difficulties emerging at application of complete clotting models.

Unfortunately, all complete models are extremely difficult for external analysis: it is necessary to check 50–60 equations, boundary and initial conditions, the range of admissible assumptions used at calculations. Besides, it may be difficult for developers of a model to draw correct conclusions not only about a certain regime of the clotting system behavior, but also about general validity of the model itself. Therefore, the frequently used models are reduced, i.e. describe only some part of the clotting system or the whole system but with a number of assumptions, e.g. of existence of quick equilibrium relations between some of the variables. Such reduced models have their own serious drawbacks, but sometimes they help better understand what goes on in the clotting system.

Let us demonstrate the effectiveness of such approach by the example of work [26]. The model used in this work was constructed with the following assumptions. The system consisted of one zymogen and one enzyme catalyzing its formation from the zymogen. The zymogen and the enzyme could diffuse in the plane of the membrane (activator), could come out into the liquid phase and come in from the latter. Besides, it was supposed that the boundary layer between the membrane and the flowing medium was infinitesimal and could be neglected. Diffusion in the medium was rather quick, so that the flow of reagents did not depend on the distance to the membrane. Schematic representation of this system is given in Fig. 4. The work [26] showed that the system activation threshold was regulated by the kinetics of substrate activation and inhibition and by the concentration of binding sites on the membrane, as well as by flow rate and activator size. So, all other factors being equal, the weaker flow or larger activator can result in crossing the threshold of activation and triggering the enzyme production, whereas the stronger flow or smaller activator could prevent the start of the system. This result, fully confirmed in [17], is an evidence that even such a simple model as in work [26], seemingly incommensurable with the clotting system, can correctly predict the regimes of the clotting system behavior.

Another work [27] is based on a reduced model [28]. This model contains eight differential equations describing the space-time dynamics of activation of clotting factors. In this model, clotting activation begins with the production of factor XIa, while formation of the extrinsic tenase is not taken into account. Formation of the intrinsic tenase and prothrombinase complexes is a function of potassium concentration. It has been shown [27] that clotting (thrombin distribution) can be stopped by the inhibiting wall in narrow channels and

by rapid flow in broad channels. Since in the organism undamaged blood vessels are coated with thrombomodulin and heparan sulfate, i.e. have anticoagulant surfaces, the first result of this work may imply that cessation of clot growth in the organism is associated with undamaged wall beyond the place of lesion. At the same time, the arrest of clotting distribution by rapid flow may imply that in the organism the flow induces clot localization in a zone close to the activator.

Blood is not a perfect liquid, so here emerges the question as to how to describe its movement at modeling. However, the difference of blood from perfect liquid manifests itself only in rather small vessels (with the sizes comparable to the sizes of blood cells). The size of red blood cell is 6–8 μm, being less almost by 1.5–2 orders of magnitude than the size of the experimental channel. Hence, blood can be considered as a perfect liquid with good approximation. Therefore, in most of the works, the flow rate is assessed by the value of shear rate of blood on the channel boundary calculated for perfect liquid on the basis of volume velocity. This very approximation was used in all works considered in our review, with the exception of work [23], where a viscoelastic model was used with the relaxation time depending on deformation. This model is described in detail in [29].

Numerical modeling of the process of fibrin polymerization in the presence of flow is an independent and important problem. Its peculiarities make it necessary, with a certain degree of minuteness, to model the process of formation of a polymer fibrin network, generally consisting of the infinite number of reactions of reversible incorporation of fibrin molecules into this network. One of the works on this subject was a mathematical study [30] considering the conditions of fibrin clot formation as transition of the medium from liquid to gel-like state. The analysis was based on a one-dimensional model with the linear profile of the flow rate gradient providing convection of reagents, which could also diffuse. Thrombin is formed from prothrombin only in the activator (on the boundary of modeling zone); is consumed at a rate proportional to its concentration value; diffuses from the activator, and is carried away by the flow. It transforms fibrinogen into fibrin, which under certain conditions can be polymerized and form a clot. It has been shown [30] that the height of the formed clot depends on the rate of thrombin inhibition, permeability of the clot for the flow, and shear rate of the flow. At low rates of the flow, the clot size is limited by the availability of thrombin, while at high rates the flow carries away fibrin monomers before they are polymerized. Transition from one type of system behavior to the other is provided by permeability of the clot for the flow. This work shows that one of the pathways of clotting inhibition by the flow may be removal of nonpolymerized fibrin, which causes retardation of clot growth until full stop.

CONCLUSIONS

Let us sum up the currently known data on blood clotting in the flow: it is quite reliable that the flow impairs blood clotting and a sufficiently intensive flow can completely prevent clot formation. A number of hypotheses explain such behavior of the clotting system. It may be washing of fibrin out of the forming clot, anticoagulant properties of vascular surface, and diminution of factor Xa activation by the intrinsic tenase. Most of such hypotheses (and some experimentally confirmed data) are based on the results of modeling with simple reduced models. At the same time, complex models claiming the completeness of clotting system representation are still only being developed, and yet there are no clear and sensible suggestions obtained on their basis (all the more those verified experimentally). However, we hope that their time will come, and very soon. Then, using complete clotting models, we will be able to obtain theoretical prediction and possible ways of their experimental verification, which will provide for the thorough study of all aspects of blood clotting in the flow.

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