

Mathematical Modeling and Computer Simulation in Blood Coagulation

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Key Words

Blood coagulation · Mathematical modeling · Computer simulation · Thrombin generation · Blood flow · Enzyme cascades · Metabolic control

Abstract

Over the last two decades, mathematical modeling has become a popular tool in study of blood coagulation. The *in silico* methods were able to yield interesting and significant results in the understanding of both individual reaction mechanisms and regulation of large sections of the coagulation cascade. The objective of this paper is to review the development of theoretical research in hemostasis and thrombosis, to summarize the main findings, and outline problems and possible prospects in the use of mathematical modeling and computer simulation approaches. This review is primarily focused on the studies dealing with: (1) the membrane-dependent reactions of coagulation; (2) regulation of the coagulation cascade, including effects of positive and negative feedback loops, diffusion of coagulation factors, and blood flow.

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Introduction

The first stage in studies of a biological (and not only biological) system is determination of the elements of this system and their interactions. As the system structure becomes clearer, other questions arise: how does it function?; how interactions of individual elements result in fulfilling the system task?; what is the role of a specific component or reaction in the functioning of the system? The questions of the first type are usually answered using experiments, while for the questions of the second type experimental research often gives indirect and ambiguous responses. This can be most probably explained by complexity of biological systems and numerous regulatory interactions between their elements. Understanding these systems and prediction of their behavior become impossible without modeling. One of the most interesting and modern methods of the research of metabolic network functioning is use of mutant or knockout animal lines, which are defective in the gene encoding some protein of the studied system. Creation of these lines takes years and is extremely expensive; therefore, running over all elements becomes a titanic task. Mathematical modeling is a valuable tool in this research, because it provides rapid and inexpensive way to simulate planned experi-

ments and select target genes. The role of mathematical and computer modeling gradually becomes more and more important in the selection of the most effective experimental approaches, and blood coagulation is not an exception in this sense.

The last two decades witnessed an explosion of mathematical modeling and computer simulation research in blood coagulation. Two possible reasons could be suggested to explain the popularity of theoretical methods. First, as indicated above, efficient theoretical analysis is possible only when a sufficient amount of empirical information is collected. They say that the work of a theoretician begins when a biochemist believes the study is finished. Although this is a bit exaggerated, there is much truth in this saying. In blood coagulation cascade, the last discovery of an unknown component or a principal reaction took place in early 1990s [1, 2]. Second, the complexity of biochemical objects makes analysis of mathematical models extremely complicated without computer simulation. Thus, the recent development of microcomputers was another stimulus for the use of modeling in blood coagulation, where even now the calculating capacity puts certain limits to the modeling approach: a 3.6-MHz processor often requires several hours to simulate a 5-minute duration experiment on two-dimensional fibrin clot formation in flowing blood.

In this review, we attempt to summarize and discuss the methods used and the results obtained in theoretical studies of coagulation. The review is primarily focused on two applications of mathematical models: (1) analysis of the mechanisms of the membrane-dependent reactions of tenases and prothrombinase and (2) studies of the regulation of the blood coagulation cascade.

Blood Coagulation

The function of coagulation is to create a fibrin clot localized at the site of vessel wall damage. This process is initiated upon contact of circulating serine protease factor VIIa (fVIIa)¹ with transmembrane protein tissue factor (TF), which becomes available to plasma upon vascular damage. The fVIIa-TF complex, or extrinsic tenase, activates fIX and fX from zymogens into active proteases. The bottom enzyme of the cascade, thrombin, con-

verts fibrinogen into fibrin, which polymerizes to form a clot, preventing a blood loss. Thrombin also activates factors V, VII, VIII, XI, protein C and platelets, initiating numerous positive and negative feedback loops. Activated platelets secrete various substances and provide binding sites for coagulation proteins to form enzymatic complexes of intrinsic tenase and prothrombinase. Thrombin also binds to thrombomodulin, an endothelial transmembrane protein, and their complex becomes a potent activator of protein C. Active factors, except for nonenzymatic fV and fVIII, are inhibited by plasma inhibitors, the principal ones being antithrombin III and tissue factor pathway inhibitor (TFPI). In addition to the TF pathway, coagulation can be activated via contact pathway upon contact with foreign materials. Although significant in many *in vitro* experiments and pathological states, this pathway is believed to be unessential for physiological hemostasis.

Recent reviews detailing the structure of the blood coagulation system are available elsewhere [1, 2]. Here, we shall focus on those aspects of hemostasis and thrombosis, which are of special interest for the subject of this review: the nonstationarity, spatial heterogeneity, and the effects of blood flow [3]. Nonstationarity means that the system does not function in a stable working state, in contrast to metabolic 'housekeeping' systems. It either rests or is activated, and in the latter case concentrations of its components continuously change until the limiting factor precursors have run out. Therefore, analysis of small deviations from a stationary condition, which formed the basis for metabolic control theory [4], is not possible for blood coagulation. Although metabolic control theory was extended to some time-dependent processes [5–7], computer simulation remains, with few exceptions [8–12], the predominant method of investigation of mathematical models of the clotting cascade.

Spatial heterogeneity is another complicating aspect of coagulation *in vivo*. While in many metabolic systems concentrations of reactants are the same in all parts of the reaction volume and, therefore, they could be described by ordinary differential equations, different reactions of blood coagulation take place in different regions. Clotting is initiated on the TF-expressing cells, from which active enzymes diffuse to the activated platelets, where the assembly of the membrane complexes and major propagation reactions take place; thrombomodulin-dependent activation of protein C is localized to endothelium. This means that a correct representation of coagulation should use partial differential equations and their solution in a two- or three-dimensional region. Again, the abilities of

¹ Abbreviations used: TF = tissue factor; TFPI = tissue factor pathway inhibitor; fVIIa = factor VIIa; fXI = factor XI; fV(a) = factor V(a); fX(a) = factor X(a); fIX(a) = factor IX(a); fVIII(a) = factor VIII(a); fXII(a) = factor XII(a); APTT = activated partial thromboplastin time; PT = prothrombin time.

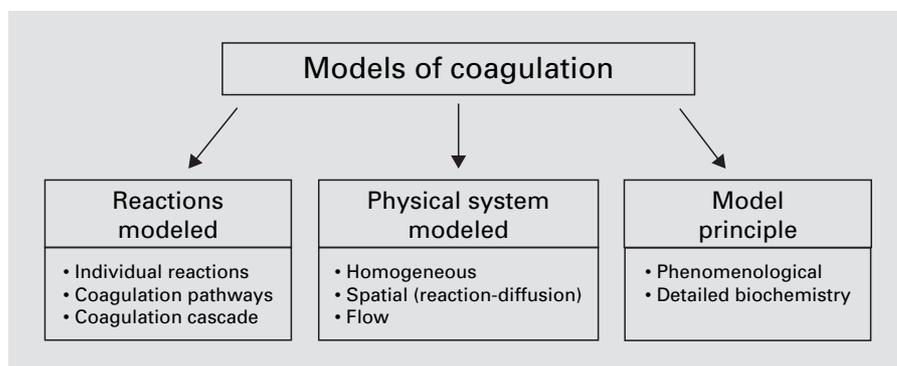


Fig. 1. Classification of the mathematical models of blood coagulation by their biochemistry, physical properties of the modeled systems, and the degree of simplification.

metabolic control theory for spatially nonuniform systems are limited [13]. Finally, formation of fibrin clots *in vivo* occurs in the presence of flow [3], and thus the Navier-Stokes-type equations, including convection, are usually utilized.

Models of Coagulation

An attempt of simple classification of coagulation models is shown in figure 1. First, the models describe different subsystems of the cascade: from isolated individual reactions to the whole cascade. Second, they are differentiated by physical properties of the described system: models of both *in vivo* and *in vitro* systems could be either homogeneous, spatial [either (pseudo)one-, two-, or three-dimensional], and could also include blood flow. Finally, the models could be approximately differentiated into two types by the degree of simplification. The first group contains ‘simple models’, which usually include few equations, either phenomenological or based on extremely simplified reaction schemes. These models usually are not verified by experiments. They are convenient because of their susceptibility to theoretical analysis or even solution. On the other hand, models of the second group are the sets of tens of differential equations, which could be solved only numerically. Computer solution of these models can be used for experimental planning and analysis, and for clinical applications.

Why a Cascade?

The function of the complex reaction network of coagulation is to provide thrombin where and when it is needed to form the fibrin. Understanding the roles of the

system components (feedback loops, inhibitors, platelets, etc.) in this regulation is important for a therapeutic control of thrombotic and bleeding disorders. Initial theoretical studies of the system structure were stimulated by the hypothesis that reactions of coagulation form a cascade [14, 15]. The study of Levine [9] analyzed the kinetics of a simple enzymatic cascade consisting of second-order reactions, with each enzyme being inhibited in a first-order reaction. The system response (kinetics of the bottom enzyme of the cascade) was a pulse with an amplitude proportional to the degree of stimulation, and the steepness of the initial kinetics increased with the increase of the number of stages in the cascade. Thus, the amplification role of the cascade was demonstrated. Later, rapidly increasing volume of experimental knowledge about the coagulation system induced modifications of this model. Thrombin inhibition by fibrin and fibrinolysis were added by Moro and Bharucha-Reid [16] and Martorana and Moro [17]. On the other hand, Khanin and Semenov [10, 18] supplemented the model of Levine with positive feedback loops of *fV* and *fVIII* activation by thrombin. The obtained differential equation sets were reduced and subjected to the analysis of singularities, which showed that an enzymatic cascade in the presence of inhibition of active factors and positive feedback loops had an activation threshold. A systematic analysis of positive feedback loops in a hierarchy of simple cascade systems imitating the coagulation cascade and its subsections was performed by Beltrami and Jesty and colleagues [8, 19]. In agreement with studies carried out by Khanin and Semenov [10, 18], in which the enzymes generated were subject to inactivation, these systems exhibited threshold properties. Their threshold criteria were obtained. A long-range feedback loop (corresponding to *fXI* activation by thrombin in the real system) was shown to affect the system’s response above the threshold, and at

high catalytic rates, to decrease the threshold value, thus activating the otherwise subthreshold system. It might be possible that the contact pathway is not a pathway of coagulation initiation, but a pathway of regulation of the system's state depending on the state of other physiological systems. Theoretical prediction of a threshold in the autocatalytic enzyme system with inhibition was confirmed in the study from the same group, and can be found in the present issue [20]. It demonstrates, both experimentally and theoretically, a threshold in the fXII autoactivation in the presence of fXIIa irreversible inhibition.

Among other observations, the study [19] found oscillatory behavior in the systems with sequential positive feedback loops; it was abolished in the presence of the long-range feedback. Interestingly, a study from our laboratory has also found damped oscillations in a simple computer simulation model of intrinsically activated blood coagulation [21]. However, this behavior was observed only in the presence of a potent negative feedback loop (the rate of protein C activation by thrombin was 70-fold higher than normal). Recently, this finding was reproduced by another group in an independently developed computer model: oscillatory behavior was observed at the protein C activation rate 200-fold higher than normal [22].

General analysis of the coagulation cascade in terms of controllability [12] has shown that coagulation system is uncontrollable, meaning that it is insensitive to the changes in the system parameters. The authors explained this feature by the sequential nature of the cascade and presence of multiple feedbacks. This insensitivity might be of physiological importance, because it means that the system functioning would not be impaired under changed conditions.

The initial analysis of platelets in the cascade [23] used a simple model, including α -granule fV secretion and stimulation of the membrane-dependent reactions by platelets (the rates of all activation reactions were assumed to be proportional to the activated platelet concentration). The singularity analysis of the reduced model has shown that the initial concentration of activated platelets affects the activation threshold. When the concentration of activated platelets exceeds some value, the system becomes unstable and has no threshold, i.e., coagulation in this case can be initiated by infinitely low concentrations of active factors. Modulation of activation thresholds in enzyme systems by the availability of the membrane-binding sites was confirmed and more systematically analyzed by Fogelson and Kuharsky [24]; a threshold response of coagulation to platelet concentration was also demonstrated in a detailed model of the

blood coagulation system [25]. Another recent model study has shown that the activation threshold is a function of the platelet activation rate [11]. Finally, it has been shown both theoretically and experimentally in studies of our group, there is also a calcium concentration threshold in coagulation [26, 27].

Conclusions and Discussion

The studies analyzing the basic structure of the clotting system demonstrated the following: (1) a simple cascade functions as a system giving nonlinear, explosion-like kinetics, (2) addition of a combination of positive feedback loops and inactivation of the enzymes generated to the cascade is necessary to obtain an activation threshold (i.e., activation signal below some level does not trigger the system), (3) the activation threshold depends on the parameters of platelet activation, on the concentration of platelets, and on calcium concentration, (4) models of simple systems similar to blood coagulation often demonstrate an oscillatory behavior, (5) the structure of the coagulation cascade seems to make it insensitive to the changes of the system parameters.

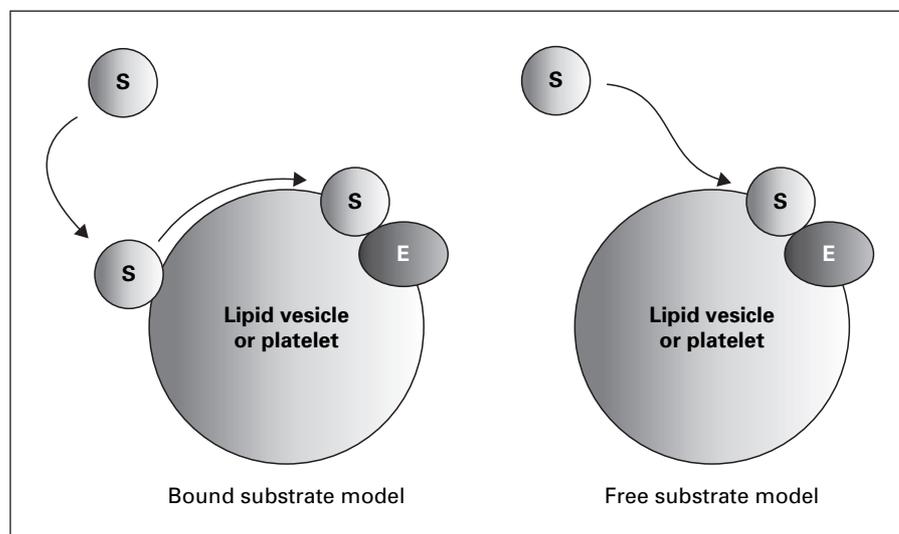
Some of these conclusions were confirmed experimentally. For example, it is well established that, for most coagulation factors, only significant deficiency (>90%) results in bleeding tendencies, demonstrating the system insensitivity [2, 28]. Other results (e.g., the oscillatory behavior) were not reported in experimental studies.

The predicted activation threshold is probably the most intriguing and important prediction. Unfortunately, there is no clear experimental demonstration of this important effect yet. On the one hand, it seems unlikely that this very excitable system functions without threshold, as this means constant activation of circulating blood. On the other hand, blood or even plasma in vitro rapidly clots without addition of activators unless the system is significantly changed (e.g., by removing calcium ions). A probable explanation is that contact activation of coagulation has no threshold [29] or that blood is activated during the collection and cannot return to the normal state due to the hysteretic properties of the contact activation pathway [30].

Mechanisms of the Membrane-Dependent Reactions

Vital reactions of blood coagulation catalyzed by extrinsic and intrinsic tenases, prothrombinase, and activated protein C are membrane dependent: the substrates,

Fig. 2. Two possible mechanisms of substrate delivery to the membrane-bound enzyme: substrate either binds the enzyme directly from the solution or it binds the membrane first and then interacts with the enzyme by means of two-dimensional diffusion.



enzymes, and cofactors of these reactions form catalytic complexes on plasma phospholipid membranes provided by platelets, lipoproteins, etc. [1, 2, 31]. In the presence of membranes, the rates of these reactions are increased by several orders of magnitude [1, 31]. Complexity of these reactions hampers a direct experimental analysis of their mechanisms, making mathematical modeling especially important in these studies. One of these particular problems is the role of the membrane in the mechanism of substrate delivery in these reactions (fig. 2): whether the substrate is delivered to the enzyme directly from the solution or first binds the membrane and is then delivered by two-dimensional diffusion?

A model of prothrombin activation by prothrombinase including fV feedback activation by thrombin was proposed by Liniger et al. [32] in 1980. Another computer model of prothrombinase, 'Clotspeed', was proposed in 1984 and was based on the bound substrate conception [33]. Prothrombin in the model could bind the membrane and interact there with the enzyme (the Xa-Va complex). Thus, the stimulating effect of the membrane was explained by the increased local substrate concentration near the membrane. This model was also able to successfully explain the linear dependence of Michaelis constant on phospholipid concentration. Another group used analytical model to distinguish the free substrate and bound substrate models of prothrombinase [34]. Their analysis predicted that both models result in a linear Michaelis constant dependence on phospholipid concentration, but at different phosphatidylserine content these lines would behave differently, forming either a set of parallel lines

(for the bound substrate model) or a set of nonparallel lines with identical values at low phospholipid concentration (for the free substrate model). Experimental test of these predictions has shown that prothrombinase is likely to follow the free substrate model.

The prothrombin-activating complex consists of three components, the enzyme, the substrate, and the cofactor, and can be assembled via different pathways. This was studied by Boskovic et al. [35], who used mathematical modeling to distinguish between possible pathways of prothrombinase assembly in the absence of phospholipids. They have developed models for various combinations of prothrombinase components, compared them with experiments, and have shown that the correct order of assembly is the formation of the binary cofactor-substrate fVa-prothrombin complex and the cofactor-enzyme fVa-fXa complexes, followed by the final ternary prothrombin-activating complex formation.

A model of intrinsic tenase [36] was designed to describe the effect of fXa and fVIIIa activity loss on the fXa generation curve. The problem of substrate delivery for intrinsic fX activation was addressed by Scandura and Walsh [37]. They analyzed fX activation by fIXa on activated platelets in the absence of the cofactor, comparing three possible models: (1) free enzyme, membrane-bound substrate; (2) free substrate, bound enzyme; (3) enzyme and substrate are both bound. Comparison of the corresponding mathematical models with experiments has shown that the system follows the bound substrate, bound enzyme scheme. The bound substrate quantitative mathematical model for the complete intrinsic tenase complex

phospholipids and on platelets was recently suggested by our group [38]. This work has proposed that the assembly of the intrinsic fX-activating complex, by analogy with prothrombinase, is likely to begin with the formation of the cofactor-substrate and -enzyme complexes.

A model for the mechanism of assembly of the extrinsic tenase complex was developed by Nemerson and Gentry [39] in 1986. Their analysis combined with experiments has shown that, in this reaction, the ordered addition mechanism functions: the enzyme-cofactor complex is formed first, followed by the substrate binding, catalysis, and product release by the enzyme-cofactor complex. The problem of the substrate delivery in this reaction was analyzed by Gentry et al. [40] in 1995. They have developed a stochastic model, compared two possibilities (the true substrate is either free or membrane-bound) under various conditions (intrinsic catalytic rate, lipid diffusion rate, etc.), and proposed possible experimental methods to distinguish the contributions of these pathways.

Extrinsic tenase is regulated by TFPI, assumed to function in a two-step fashion: first, to inhibit fXa, then to inhibit the VIIa-TF complex. Baugh et al. [41] determined kinetic constants of this pathway, performed experiments on fXa activation inhibition by TFPI, developed a mathematical model, and demonstrated that this two-step mechanism cannot describe experimental results. They suggested a possible reaction pathway to explain this difference. Later, a model of the TFPI pathway was developed by our group [42]; we have suggested a hypothetical mechanism able to explain the divergence. A recent work by Lu et al. [43] studied the regulation of factors IX and X activation by extrinsic tenase in the presence of TFPI and antithrombin III. The combination of experiments and simulations has suggested that fIXa is the primary product of the extrinsic tenase in the presence of inhibitors, although factors IXa and X are activated with similar kinetic constants.

Conclusions and Discussion

The membrane-dependent reactions are catalyzed by multiprotein complexes and have complex kinetics. In particular, use of mathematical models was productive in the studies of the three most often occurring problems: (1) the order of assembly of the enzyme-cofactor-substrate complex; (2) the mechanism of the substrate delivery to this complex; (3) the regulation of these reactions by decay of the activity of their cofactors, enzymes, and products. A number of similarities in the studied systems were found in these works.

Models of Thrombin Generation Assay and Clot Time Tests

Thrombin generation assay is one of the most popular in vitro models of blood coagulation. In this assay, coagulation in plasma (whole blood, a reconstituted system, etc.) is activated by TF (thrombin, kaolin, etc.), and the change of thrombin concentration with time is monitored using a fluorogenic or a chromogenic thrombin-sensitive substrate. Recent reviews of thrombin generation are available elsewhere [44]. In 1991, Willems et al. [45] developed the first biochemically adequate model of the coagulation cascade for thrombin generation in plasma, where the reaction scheme accurately represented existing biochemical knowledge; reactions constants were taken from experimental reports, and the simulation results (thrombin and fVa kinetics) were compared with experiments. Coagulation was activated via the extrinsic pathway, and only a section of the cascade was simulated, from fXa to thrombin formation. FXa formation was approximated with a function $[Xa](t) = A \cdot t \cdot \exp(-at)$, where $[Xa]$ is the fXa concentration, t is time, and A and a are parameters. This model was shown to have an activation threshold (the threshold value of A was 1–10 pM at $\alpha = 1 \text{ min}^{-1}$). The threshold was more sensitive to fXa and prothrombinase inactivation rates than to thrombin inactivation or protein C activation.

Another approach was used by Jones and Mann [46]. Instead of simulating coagulation in blood or plasma (very complex systems), they created a model of the reconstituted system composed of factors IX, X, V, VIII, and II at their mean plasma concentrations and phospholipids. The authors concluded that reactions of the model are sufficient to describe the experimental kinetics of the system, that the activator affects the lag time of thrombin generation more significantly than the maximal thrombin generation rate, that fVIII and the intrinsic pathway become important at low activator concentration and regulated thrombin generation at the propagation phase, that fXa and thrombin are equally efficient in fV activation, but thrombin is more important in fVIII activation than fXa (illustration of this conclusion is shown in fig. 3). Later, the same group reported a modification of the original model, including antithrombin III and TFPI [47]. This model was also successful in describing experimental data and demonstrated the presence of an activation threshold. A Monte-Carlo simulation approach for the analysis of this model has been proposed in the paper from Diamond laboratory, which can be found in this issue [48]. The original model of Jones and

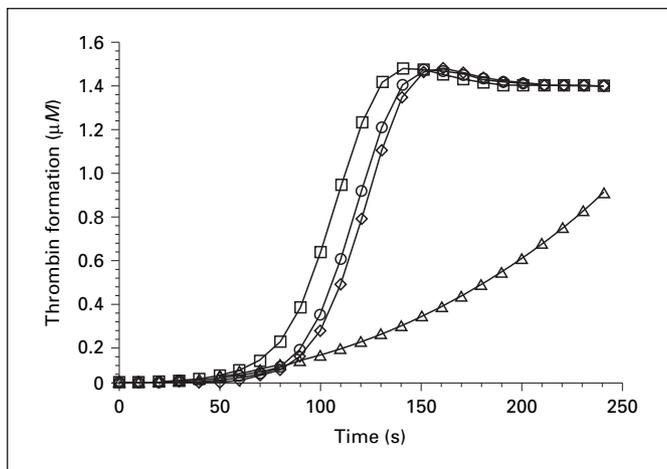


Fig. 3. Distinguishing contributions of fXa and thrombin to fV and fVIII activation. Computer simulation of thrombin formation in a reconstituted system without inhibitors activated by 5 pM TF: \square = control; \diamond = thrombin activation of fV = 0; \circ = fXa activation of fV = 0; \triangle = thrombin activation of fVIII = 0. The figure is reproduced by kind permission of the American Society of Biochemistry and Molecular Biology, copyright 1994 [46].

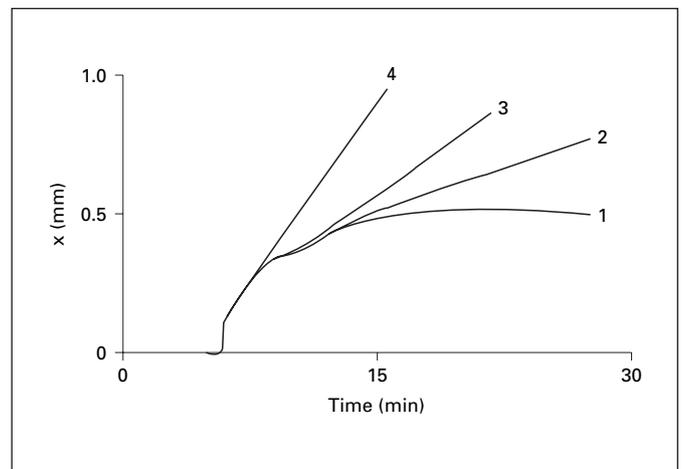


Fig. 4. Effect of fXI activation by thrombin on clot expansion in a reaction-diffusion system. Theoretical clot size versus time plot for plasma clot formation activated by glass in a reaction-diffusion system. Computer simulations were performed for the following effective rates of fXIa production by thrombin: (1) 0; (2) $10^{-5} \cdot \text{min}^{-1}$; (3) $10^{-4} \cdot \text{min}^{-1}$; (4) $7.8 \cdot 10^{-3} \cdot \text{min}^{-1}$. The figure is reproduced by kind permission of Elsevier Science, copyright 1996 [21].

Mann [46] was also used by other groups for clinical applications to analyze the mechanism of action of synthetic protease inhibitors [49, 50]. Finally, the recent study of thrombin generation in reconstituted systems simulated coagulation at different lipid concentrations [51] (previous works assumed saturating phospholipids). This model predicted existence of a threshold phospholipid concentration in the system.

Rather ancient and still widely used experimental models of coagulation are prothrombin time and activated partial thromboplastin time (APTT) tests. In these assays, plasma is mixed with activators of extrinsic or contact pathways, respectively, in the presence of excess calcium and phospholipids, and clot formation time is determined. The first model of prothrombin time was proposed by Pohl et al. [52]. This model included the complete extrinsic pathway from extrinsic tenase to fibrin formation; intrinsic tenase, fXI, and protein C were absent. Unknown rate constants were determined by χ^2 approximation. The model was designed for interpretation of clinical data. An approach to use mathematical modeling of thrombin generation for the estimation of unknown parameters in the system was also proposed [53]. Another approach for the estimation of system parameters is based on the optimality principle, which was proposed recently [54].

Another prothrombin-time-based model [55] studied sensitivity of the tests and kinetics of the reactants during the assay. Two models of activated partial thromboplastin time were developed independently in 2001 [56, 57]. One of these works focused on the general simulation of the assay and its sensitivity [56]. In particular, the study discussed the mechanism of fXII activation and suggested that the predominant mechanism is activation of fXII by trace fXIIa (and not fXII autoactivation on procoagulant surfaces or kallikrein-mediated activation). Another study used activated partial thromboplastin time to evaluate the rate of fXI autoactivation and activation by thrombin [57]. They conclude that fXI autoactivation is more important than thrombin-mediated activation in this assay.

Conclusions and Discussion

Models of coagulation in homogenous experimental systems were able to provide insight into the mechanisms of regulation of blood coagulation cascade. Ability to change all parameters in the system allows clarification of the roles of different reaction, estimation of relative reaction significance, etc. On the other hand, the existing models do not include recent experimental findings, which can significantly affect the views on system regulation: role of red blood cells in thrombin generation [58],

acceleration of coagulation reactions by lipoproteins [59], the fact that actually only a small (~5%) subpopulation of activated platelets binds coagulation factors [60], etc.

Spatial Organization of Blood Clotting

Physiological blood coagulation occurs under conditions of dynamic fluid environment: it functions in the presence of blood flow and is spatially nonuniform. The first studies analyzed these two problems separately. The first attempt to model thrombin generation under flow conditions was independently performed by two groups in 1994 [61, 62]. At the same time, our group proposed the first simple phenomenological model of clot formation in a spatially nonuniform reaction-diffusion system [63]. A more detailed model of coagulation activated via the intrinsic pathway [21, 64] simulated an *in vitro* reaction-diffusion system without flow, where clotting was activated via intrinsic pathway. The study has shown that fXI activation by thrombin can result in autocatalytic expansion of clotting in plasma (fig. 4). The effect of fXI on the spatial stage of clotting was recently confirmed experimentally [65]. Studies using models with different degree of detailing [21, 64, 66, 67] analyzed spatial dynamics of clotting upon activation via either intrinsic or extrinsic pathways. The model analysis was performed in combination with investigations on a set-up monitoring clot formation in a thin layer of nonstirred, recalcified plasma [68]. The results have shown that coagulation in a reaction-diffusion system consists of three spatially and temporally separated stages: initiation, propagation, and termination of clot expansion. In particular, it has been shown that fVIIIa-mediated feedback regulates the propagation stage of blood clotting, which is in many respects independent of initiation [65, 68]. Thus, hemophilias A and B appear to be the diseases of defective clot spatial propagation, while initiation of clotting occurred normally.

Investigation of spatial clotting dynamics demonstrated uniqueness of this system among the other nonlinear dynamic models. Therefore, the model of blood coagulation was reduced [69] to three differential equations and its behavior was a subject of several nonlinear dynamic studies [70–72]. This resulted in demonstration of a number of new unusual regimes of excitation propagation and self-organization.

Conclusions and Discussion

Spatial aspect was found to be very important for blood coagulation biochemistry. Insignificant in homogeneous systems, reactions can become important in the reaction-diffusion systems; for example, fXI activation by thrombin may be important far from the activator, because there are none others than fXIa activators of fIX. Different pathways of the metabolic network of blood clotting become important at spatially distinct regions of the forming clot. Studies of processes responsible for clot localization promise the most significant progress in the nearest future.

Effects of Blood Flow on Coagulation Processes

Baldwin and Basmadjian [61] predicted existence of three parameter regions with different thrombin production patterns and suggested an explanation for the difference between arterial and venous thrombi by the fact that these systems correspond to different regions. Activation threshold analysis in a proteolytic feedback system [73] was shown to increase with the increase of flow rate and to decrease with the increase of activation zone size. The study of Kuharsky and Fogelson [25] simulated the complete coagulation cascade assuming a thin layer near the site of damage with uniform mixing. This study also demonstrated that the concentration of thrombin produced is decreased with the increase of flow. The role of flow-mediated transport in the regulation of coagulation is the subject of another work of this group, published in this issue [74].

The simple model proposed in our group in 1994 with modifications (addition of fibrin formation as an integral of thrombin) was later used to study thrombus formation under flow conditions [75–78]. The model took into account change of flow pattern due to the clot growth. The modeling was done using a two-dimensional assumption. The authors demonstrated formation of complex thrombi structures and have shown that blood flow velocity affects not only threshold and thrombin generation rate, but also can lead to formation of multiple thrombi. Different scenarios of fluid blood transition to a solid state as a result of stability loss at different flow velocities were analyzed.

A recent work of Anand et al. [79] suggested a reaction-diffusion model of clot formation and lysis under flow conditions, using quasi-one-dimensional thrombus formation in a cylindrical vessel. Preliminary results for

this model have also been obtained and presented. In the present issue, this model is further developed and analyzed [80]. Another paper in this issue [81] analyzes the reduced model of Zarnitsina et al. [69] in two-dimensional flow. It demonstrates the role of vessel wall thrombomodulin and of rapid flows in the inhibition and localization of thrombi: in narrow channels, thrombomodulin alone could inhibit thrombin wave propagation.

Conclusions and Discussion

Simulation of coagulation under flow yielded a limited number of results, in particular, that blood flow increases the activation threshold and decreases the amount of thrombin generated; the activation threshold is decreased when the size of the activation zone is increased. On the other hand, at the present moment there are no models, which could describe formation of both platelet plug and fibrin clot in a vessel with endothelium. The most challenging aspect of understanding of blood clotting is that coagulation is able to prevent bleeding in vessels, which differ by four to five orders of magnitude in their size and flow rates. Available calculating capacity limits the reaction-diffusion-convection simulations to the use of either two-dimensional or quasi-one-dimensional assumptions.

With the increase of calculation power and appearance of new experimental methods to monitor clotting in vivo [82], which require adequate mathematical models for interpretation, it is possible to expect rapid progress in modeling clot formation in flow.

Conclusions

Mathematical models became an efficient tool in the studies of blood coagulation. They are powerful in the experimental analysis, allowing identification of the reaction mechanisms which could not be distinguished by direct experimental methods, as is the case with the substrate delivery in membrane-dependent reactions. The models are useful in the analysis of the regulation of the coagulation cascade. They can be used in clinical applications and drug design. Although specific applications require development of different models, one of the most important goals at this stage seems to be development of an integrated in vivo clotting model, incorporating plasma coagulation reactions, platelets-coagulation factors interaction, fibrin and platelet plug formation under flow conditions.

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