Physics – Uspekhi **45** (6) 619 – 636 (2002)

REVIEWS OF TOPICAL PROBLEMS PACS numbers: **05.45. – a**, **87.10 + e**, 87.19.Uv

A new class of stopping self-sustained waves: a factor determining the spatial dynamics of blood coagulation

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DOI: 10.1070/PU2002v045n06ABEH001090

Contents

Abstract. Two self-sustained wave regimes newly found in blood coagulation models are discussed: (1) oscillating-amplitude self-sustained waves, and (2) waves initially propagating as classical (constant-velocity constant-amplitude) self-sustained waves and then abruptly stopping at a fairly large distance from the point of activation. Depending on model parameters the latter waves either damp out or turn into stationary, spatially localized peaks. Analysis of blood coagulation models suggests that blood is an active medium with very unusual properties.

1. Introduction

Nonlinear reaction-diffusion systems exhibit a great variety of dynamic behavior and different forms of self-organization. The possibility of chemical reactions in a diffusion medium implies the presence of an energy source at each point of the space. Such systems are usually referred to as *active media*. Their behavior may be strikingly distinct from that of conventional physical media. The processes that take place in *active media* may be different in nature, not necessarily chemical ones. The laser is the best known example of a physical system. Its active medium is created by an electrical discharge in gases, irradiation of a gas or a crystal by light, bombardment with a beam of electrons, and other modes of energy 'pumping' from the outside, besides chemical reactions. However, consideration of processes proceeding in such systems leads to the same class of equations that are used for the description of nonlinear reaction-diffusion systems. During the last 40 years, many mathematical models have been proposed to describe various physical [1 – 6], chemical [7 – 10], biological [11 – 16], and even social [17] systems. They have allowed the elucidation of certain general laws governing active media. Indeed, these studies contributed to the creation of a new scientific discipline, nonlinear dynamics, that encompasses practically all fields of modern natural science.

A 'reaction-diffusion' type system is described by a parabolic equation

$$\mathbf{U}_t = D\mathbf{U}_{xx} + \mathbf{F}(\mathbf{U})\,. \tag{1}$$

Here, $\mathbf{U}$ is the vector, D is the diagonal matrix of diffusion coefficients, and $\mathbf{F}(\mathbf{U})$ is the function describing chemical or physical processes. In what follows, it will be frequently convenient to consider $\mathbf{F}(\mathbf{U})$ as a 'chemical' function although this does not actually imply any real chemical requirements for this function, nor does this compromise the generality of the consideration.

How the 'chemical' part of a system behaves can be deduced from the consideration of a particular case of the absence of diffusion terms. In this case, the system of equations (1) turns to a system of ordinary differential equations (2) that describes the system's behavior at 'a

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Received 15 August 2001, revised 12 November 2001
Uspekhi Fizicheskikh Nauk **172** (6) 671 – 690 (2002)
Translated by Yu V Morozov; edited by M S Aksent'eva

point', that is in such a small part of the space where diffusion averages all concentrations:

$$\dot{\mathbf{U}} = \mathbf{F}(\mathbf{U})\,. \tag{2}$$

This situation is easy to reproduce in experiment. To this effect, it is sufficient to thoroughly mix a medium; this procedure makes a 'point' as large as an ordinary 'laboratory dish'. Hence, the two different names of system (2): *system at a point* (point system) and *fully mixed system*.

1.1 A simple example

Analysis of models of various reaction-diffusion systems has demonstrated that many types of their behavior can be described by rather a simple model. We shall take advantage of this fact to describe key events in active media regardless of details and specific features of a concrete real physical or chemical medium. Such a model system is composed of two chemical components frequently referred to as the *activator* and *inhibitor* freely diffusing in one-, two- or three-dimensional space. As a rule, an activator is able to accelerate self-production; such a process is known as *autocatalytic* reaction. Autocatalysis is responsible for strong nonlinearity of the system.

Let us consider some basic properties of 'reaction-diffusion' systems taking a simplest FitzHugh – Nagumo (FHN)-type model as an example [18 – 20]. Numerous models of this type have been described. They have much in common in terms of behavior. Most data discussed in this section are well known from text-books and may be skipped by the learned reader. We included a summary of general information in this paper because it appears useful to compare the behavior of the blood coagulation system with the simplest model of an active medium.

A characteristic feature of FHN and similar models is cubic nonlinearity when a reaction function is given for the first variable and linear dependence in the second equation:

$$\begin{aligned} \frac{\partial u}{\partial t} &= D_u \frac{\partial^2 u}{\partial x^2} + f(u) - v\,, \\ \frac{\partial v}{\partial t} &= \varepsilon(u - av + b)\,, \end{aligned} \tag{3}$$

where $f(u) = -u(u-1)(u-n)$, $0 < n < 1/2$ (see Ref. [20]), and ε is the small parameter.

On the one hand, this model is an extension of the known Kolmogorov – Petrovskiĭ – Piskunov model [21]. On the other hand, it is a simplification of the Hodgkin – Huxley model describing propagation of impulses in nerve fibres [22]. It is maintained that equations of the FitzHugh – Nagumo type may be used to describe propagation of impulses in nerve and cardiac muscle fibres and also in neuristor circuits [1]. The system being considered is not fully consistent with the notion of reaction-diffusion systems because variables of the model may assume a negative value whereas there is no such thing as negative concentration. It is worth noting that negativity of 'concentrations' has no effect on the qualitative behavior of the system. By choosing different roots of function $f(u)$, it is possible to shift the range of changes of variable values to the positive region.

The phase plane of the FHN point system under consideration (3) is shown in Fig. 1. One of the isoclines is S-shaped. Systems with such an isocline or analogs resulting from a turn at 90° or mirror reflections appear to be the simplest systems, dynamics of which can not be reduced to the local behavior in the vicinity of fixed points. In such systems, isoclines may intersect each other at one, two or three fixed points. When there is a single intersection point (as shown in Fig. 1) lying on one of the descending branches of the isocline, the system has a single stable stationary state of the node or focus type. Such a case is the simplest one, but even here there are strong nonlocal effects. In a system with an S-shaped isocline showing the threshold behavior, an impulse may be generated, the amplitude and shape of which are practically independent of the initial perturbation.

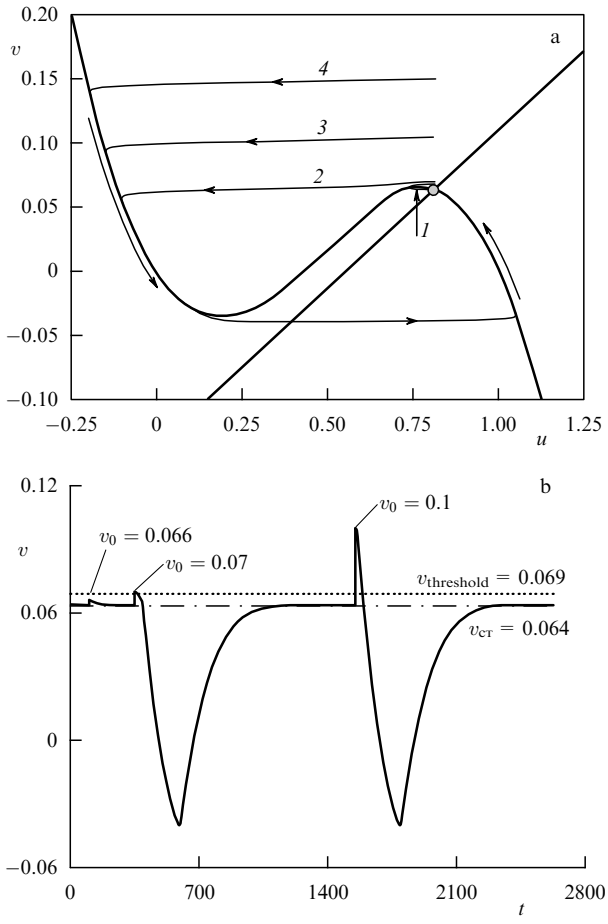

Figure 1. Phase portrait of a FHN system in the case of a single stable stationary state (a) and kinetics of the system's responses to perturbations (b). System (3) has a single stationary point, a stable node ($\varepsilon = 0.001$, $a = 4$, $b = -0.55$, $n = 0.4$). Let us consider a perturbation resulting in an up-jump of the stationary value of variable v. After this perturbation exceeds a certain threshold level (in this case 0.069), it rapidly decays. The response of the system to a rise in v up to 0.066 is described by curve *1* in (a). It is difficult to see in this figure. It is better apparent on a plot of this variable versus time (b). If the perturbation exceeds the threshold level (e.g. if it equals 0.07), the return to the stationary point is described by curve *2* (a). This trajectory shows a weak dependence on the magnitude of perturbation, being largely determined by the S-shape of the isocline. Compare curves *2*, *3*, and *4* for which the initial value of v is 0.07, 0.10, and 0.15 respectively. Figure (b) shows the time dependence of variable v for the three initial perturbations two of which are above the threshold level. They correspond to the initial perturbations described by curves *1*, *2*, and *3* respectively (a). As can be seen, the kinetics of the system changes considerably beyond the threshold while its response in terms of shape and amplitude is virtually independent of the suprathreshold perturbation when its amplitude continues to increase.

1.2 Self-sustained waves

In the spatial case [see system (3)], similar to the 'point' one, there is a single stationary state, isotropic and coincident with the stationary state of a 'point' system.

Perturbation in a small part of such a medium produces responses similar to those of a point system (see Fig. 1). The system has a threshold (magnitude of perturbation) above which it responds in a qualitatively different manner than below. The system shows traditional behavior until the threshold is reached, that is the deviation is proportional to the perturbation and rapidly dissipates after the latter is withdrawn. The behavior drastically changes above the threshold level. Specifically, a 'self-sustained' wave is generated, being one of the best known dynamic objects in active media [1, 13]. In response to a suprathreshold increase of the variable v at the left boundary of the segment, its concentration falls giving rise to an impulse closely resembling one in a 'point' system (see Figs 1 and 2). Owing to diffusion, this impulse begins to propagate in space. Figure 2 illustrates a one-dimensional case. However, a one-dimensional section has the same shape in a space of any dimension. The solution in the form of a traveling impulse is in a sense a stationary one. At given parameter values and a suprathreshold perturbation of the system, a wave is generated which propagates with a constant amplitude and speed.

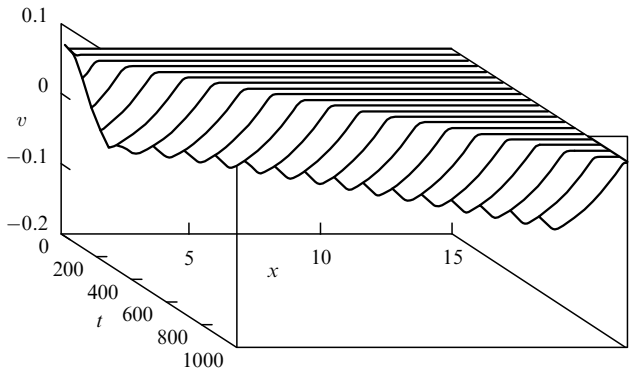

Figure 2. Propagation of a self-sustained wave. Local suprathreshold increase of second variable concentration up to 0.07 near the left boundary of the segment (see Fig. 1a, b) gives rise to a running impulse. In response to the initial rise in the concentration of variable v, its concentration first decreases sharply and then returns to the stationary state. The form of the response of both variables at each point of the space resembles the response of a homogeneous system at the same parameters. This change of concentrations disperses in the space as a wave. All wave parameters, i.e. amplitude, impulse shape, and speed, are constant throughout the entire propagation time. The parameters of the model are the same as in Fig. 1, $D_u = 0.001$. The time interval between the profiles is 50.

It differs from waves in the passive and conservative media more common in classical physics in that it travels without attenuation as far as the medium border, shows practically no dependence on the initial conditions, and behaves unusually in many other respects [13, 23–25]. As a rule, the collision of two such self-sustained waves results in annihilation, i.e. both disappear [23–25]. There is no interference (additive interaction) between self-sustained waves. Sometimes, they do not annihilate after collision but reflect off each other or show a more complicated behavior [26–32]. In a two- or three-dimensional space, self-sustained waves wind into spirals and thus create stable sources of self-sustained waves of 'infinite' duration. There are many good books and reviews devoted to self-sustained waves and active media to which the readers are referred [1, 13, 23, 24, 33].

The FHN model considered in the preceding section can be supplemented by inhibitor diffusion:

$$\begin{aligned} \frac{\partial u}{\partial t} &= D_u \frac{\partial^2 u}{\partial x^2} + f(u) - v\,, \\ \frac{\partial v}{\partial t} &= D_v \frac{\partial^2 v}{\partial x^2} + \varepsilon(u - av + b)\,, \end{aligned} \tag{4}$$

where $f(u) = -u(u-1)(u-n)$.

The solution in the form of a running impulse or self-sustained wave also exists in the case when the inhibitor diffuses with a coefficient lower than the diffusion coefficient of the activator. This is not a rigid condition; however, the solution for self-sustained waves always requires that the inhibitor propagate slower than the activator. Assuming that the inhibitor diffuses much faster than the activator, one arrives at one more nontrivial phenomenon: self-organization of spatial structures.

1.3 Dissipative structures

These structures were discovered by Turing in 1952 [11]. Today, they are referred to as *Turing's dissipative structures* or, for simplicity, *Turing structures*. It was shown that, certain conditions for the right sides of model (1) being fulfilled, the spatially uniform stationary distribution of variables determined by the steady-state of the point model loses stability after the addition of diffusion terms. In a linear approximation, this leads to a saddle-type instability and the growth of a certain kind of perturbations.

Let us modify the parameters of the model so that the single fixed point becomes a stable focus and lies on a branch of the isocline in the first equation with a positive derivative with respect to the activator ($\varepsilon = 0.01$, $b = -0.48$). Such parameters make it possible to satisfy the necessary conditions of the Turing bifurcation. The corresponding point model at given parameter values has a single fixed point, i.e. stable focus. Weak perturbation of the initial spatially uniform distribution corresponding to fixed point values results in an enhancement of perturbations by the Turing mechanism and the formation of dissipative structures (Fig. 3a). Figure 3b shows established spatial distributions of the activator and inhibitor.

In an initially isotropic system, a small change of parameter leads to spatial structures, i.e. self-organization. Why do regions with elevated concentrations of matter (see Fig. 3) stably exist in a medium with free diffusion? This is possible because the 'activator' is able to accelerate self-production and thus make up for the losses due to the outflow of matter by diffusion. The inhibitor can 'localize' a part of the space with a high activator concentration due to the larger diffusion coefficient. A much higher diffusion rate of the inhibitor is an indispensable condition for self-organization in such media. Turing's work provided a basis for a large number of studies on self-organization mechanisms in nature [1, 8, 34].

In the absence of diffusion, system (3) may undergo nondecaying oscillations (self-sustained oscillations). They occur when a single fixed point lies on the ascending branch of the cubic S-shaped isocline. In the case of diffusion, the

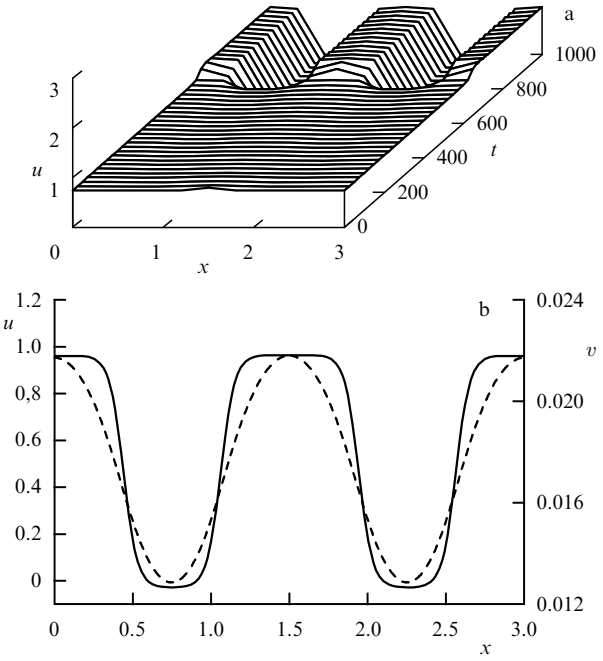

Figure 3. Dissipative structures in an FHN model [system (4)]. (a) Formation of a Turing structure for the first variable of the model; (b) spatially nonuniform distributions of concentrations of both variables for model (4) established by $t = 1000$ (solid line — activator u, dashed line — inhibitor v). Parameter values used for model (4): $\varepsilon = 0.01$, $a = 4$, $b = -0.48$, $n = 0.4$, $D_u = 0.001$, $D_v = 0.07$. The problem was considered on a segment with $L = 3$. The initial conditions were given by a small local perturbation in the center of the segment for the first variable. All the rest of the points of the space were in a stationary state corresponding to the fixed point of the system $u = 0.742$, $v = 0.065$.

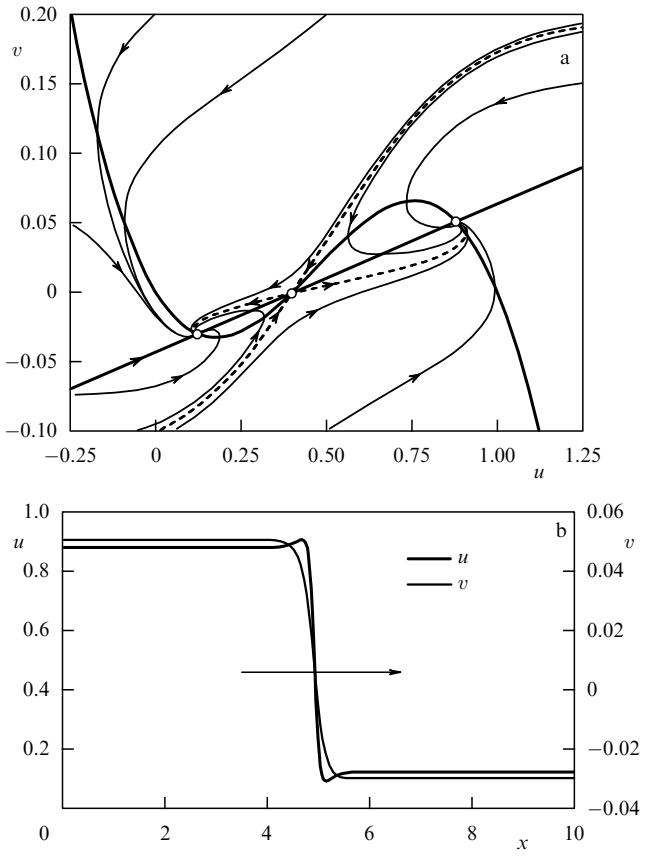

Figure 4. Bistability case: phase portrait (a), trigger wave (b). Figure (a) presents a phase portrait of system (3) without diffusion in the case of three fixed points ($\varepsilon = 0.02$, $a = 9.3995$, $b = -0.405$, $n = 0.4$); it shows isoclines of the first and second equations (3) and phase trajectories. The dashed lines represent saddle separatrices. The coordinates of fixed points are as follows: 0.88; 0.051 (upper point), 0.12; −0.0299 (lower point), 0.395; −0.001 (intermediate point). Figure (b) shows that the addition of diffusion excites a trigger wave transforming the stable down-state to the up-state ($\varepsilon = 0.02$, $a = 9.3995$, $b = -0.405$, $n = 0.4$, $D_u = 0.001$, $D_v = 0.005$).

regime of self-sustained oscillations is substituted by complex oscillations in space and various synchronization phenomena. Their consideration is however beyond the scope of the present paper.

1.4 Bistability

In the absence of diffusion, systems (3) and (4) may have up to three fixed points. A case of two fixed points corresponds to the contact of isoclines and is not a rough one. In the case of three fixed points, two of them are as a rule stable, while the third one (saddle) lies between them (Fig. 4a). Such a medium is called *bistable*; it contains two coexisting stable stationary states, each having its own region of attraction. The boundary between these regions is made up by incoming separatrices of the saddle (dashed lines in Fig. 4a). Starting from different initial conditions, that is from different phase plane regions, it is possible to reach either the up or down state (Fig. 4a).

When the diffusion of both the activator and the inhibitor 'turns on' at the appropriate parameter values and diffusion coefficient ratio, the system may have two coexisting stable spatially uniform states, each with its own characteristic threshold. In other words, there is a threshold perturbation level above which the system is unable to return to the initial state and 'switches over' to a new one. It is clear from Fig. 4a that threshold is a vague notion. The perturbation needed to prevent a system from returning to the initial state depends on its direction in the phase plane. Therefore, the threshold depends on a combination of perturbations of both variables that affects the system. The boundary of the attraction region of a given state in the phase space is an exact measure of perturbation. For all this, the notion of threshold is convenient by virtue of its simplicity and demonstrability.

In such a medium, it is possible to excite trigger waves that transform one stationary state to another. They have a good physical analogy in the form of phase transition waves. The following rule holds for bistable systems with cubic nonlinearity: a system tends to exist in a spatially uniform state having a larger threshold. In a sense, the larger threshold is equivalent to a deeper potential well. There is an explicit analogy between the two even though these systems are not conservative and the potential has no special meaning for them [35–37].

At the mutual position of isoclines shown in Fig. 4a, the threshold of the up (far-from-zero) stable state is higher than the threshold of the down (close to zero) state, and it is possible to excite a wave that triggers the lower state to the upper one (Fig. 4b). To this effect, a part of the space needs to be perturbed to above the threshold level. Then, the traveling wave will shift the whole spatial section from the lower to upper state. By shifting the coordinates of the saddle point to the right, one obtains a situation in which the down-state threshold is higher than the up-state threshold and it is possible to excite a wave switching the latter to the former.

This rule does not hold invariably. An interesting phenomenon [24] referred to by western authors as *non-equilibrium Bloch–Ising bifurcation* [37] can be observed as parameter ε decreases, i.e. at a marked slowdown of the inhibitor in bistable diffusion media. By means of special selection of initial conditions, it is possible to excite two waves, one transforming the upper state to the lower one the other transforming the lower state to the upper one, at the same parameter values below this bifurcation. A study of this bifurcation [37] and the behavior of an FHN-type system below it in a two-dimensional case was reported in Refs [38–41].

The distribution patterns of trigger waves are reminiscent of those of self-sustained waves in that their fronts move at a constant velocity. Interacting trigger fronts collide and, as a rule, annihilate. In certain situations, they can repulse one another [37] or give rise to a self-sustained wave [37, 41] or stationary peak [37].

The appearance of stationary peak is of special interest. It is formed at low velocities of colliding waves. By way of example, Fig. 5 presents a stationary peak profile resulting from the interaction of two off-waves. Such a nonisotropic distribution of matter looks surprising. In some degree, it resembles Turing structures. However, it is represented by a solitary peak whereas in Turing's case the entire space is filled with structures which gives an impression of a self-sustained wave. Certainly, it is possible to obtain a single peak in the Turing case too, by taking a sufficiently short segment. But the stationary peak shown in Fig. 5 does not depend on the length of the segment. It may rise abruptly out of the 'plain', tens or more times higher than the peak. Its parameters (amplitude and width of the distribution of variables in space) are independent of the medium dimensions if the segment length is larger than the peak size. It appears that the peak is stabilized owing to the inhibitor being distributed over a wider area in space than the activator preventing the latter's expansion. It is noteworthy that the peaks can be obtained in a monostable medium as well [1].

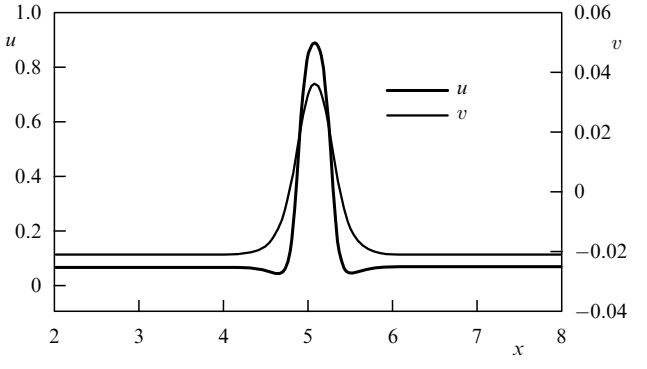

Figure 5. Activator and inhibitor profile of an established stationary peak resulting from interaction of two waves that trigger the upper state to the lower one. Parameters: $\varepsilon = 0.02$, $a = 9.3995$, $b = -0.2637$, $n = 0.4$, $D_u = 0.001$, $D_v = 0.005$.

1.5 Results of consideration of a simple example

All the three classes of dynamic behavior considered in previous sections (self-sustained waves, dissipative structures, and bistability) are impracticable in linear systems. They arise because nonlinearity produces instabilities in the phase space by the agency of which the system tends to avoid equilibrium. In the simple case considered above, this region is formed by the ascending branch of the S-shaped isocline. Such behavior is associated with self-accelerating processes, an 'enhancement' of perturbations by the system. We confine ourselves to citing the above data on classical active media and turn to a new example, the main theme of this work.

The objective of the present paper is to analyse mechanisms of the experimentally observed formation of a fibrin clot, that is the movement of a self-sustained wave that abruptly stops after initial propagation. A simplest case from the theory of active media has been considered in a preceding section. It will be shown below what blood coagulation has in common with processes taking place in the active media described earlier and what distinguishes it from the classical notions and requires the development of new concepts in the theory of nonlinear dynamical systems.

Hypothesis of the self-sustained wave nature of coagulation. Investigations into blood coagulation processes have demonstrated that they show many properties of active media [34, 35]. The production of active factors contributing to thrombus formation is possible at all points of the space. The blood coagulation cascade is integrated by positive feedback relationships responsible for strong self-activation of the process. It has all the components necessary for thrombus development governed by mechanisms that involve self-sustained waves. There is an important difference, however. A thrombus can not grow infinitely. Normally, its growth is always localized in space.

Our studies on blood coagulation have allowed us to advance a hypothesis that blood is an *active medium of a new type* [42–47]. In this medium, not only classical self-sustained waves are generated but also waves that initially propagate as classical ones but then abruptly stop at a certain distance from the point of activation, not propagating as far as the boundaries [44, 57]. Mathematical models formalizing our ideas of coagulation describe an active medium in which the known objects may coexist with new dynamic and stationary ones. In this way, the existence and propagation of self-sustained waves with varying amplitudes were discovered [44, 57] along with the formation of ring patterns and 'spots', i.e. stationary nonisotropic distributions of concentrations of matter in space [48–51]. Similar studies designed to broaden our knowledge of active media are underway using other models [52–63] and experimental [64–72] systems. It appears that only now are we beginning to understand how little we know about the possible types of dynamic behavior of systems having energy sources at each point of the space.

We believe that the blood coagulation system is not a unique one. Similar mechanisms may be responsible for unusual phenomena in biology [15, 16, 73–78], chemistry [79–86], and physics [66–70, 72]. For this reason, we suppose that the results of our dynamic blood coagulation studies may be of interest to a wide circle of students of natural sciences.

2. Molecular basis of coagulation

2.1 Clot formation — synthesis of fibrin polymers

How is a thrombus formed? Normally, a hemorrhage is stopped within 1–3 min after damage to small blood vessels. This primary hemostasis is due to the narrowing of the vessel channels and their obstruction at the site of injury

by a primary thrombus, i.e. a compact aggregate of platelets (one type of blood cell). Further development of the blood clot that completely blocks bleeding (secondary hemostasis) is due to the synthesis of the protein fibrin. Its polymerization quickly gives rise to a compact gel, a major thrombus constituent. Normal, 'liquid', blood is lacking in fibrin but contains, instead, a large amount of fibrinogen, its precursor. The splitting of small fragments from fibrinogen results in its conversion to fibrin. This process is shown in Fig. 6a. The enzyme thrombin catalyzes breakdown of four peptide bonds of fibrinogen and thus releases four small fragments, fibrinopeptides A and B. The resulting fibrin monomers undergo fast polymerization to long branched chains (Fig. 6b). In this way, a network of fibrin polymers develops at the site of injury, and the entire medium passes to a 'solid', aggregate state called gel, a commonly known state exemplified by 'la gelee' (jelly) of French cuisine. Blood cells turn out to be enclosed in the fibrin network. Collectively, these structures make up a thrombus that seals the lesioned wall of the vessel. Gel formation is almost immediately followed by biochemical reactions giving rise to the cross-links between fibrin polymer threads. They consolidate the clot into a genuine solid body.

Naturally, the enzyme converting fibrinogen to fibrin is not present in blood in an active form. Instead, its precursor, prothrombin occurs in blood plasma. Similar to fibrinogen, prothrombin is activated by means of chemical cleavage of a small fragment. There is rather a complicated system of biochemical reactions that ensures 'correct' regulation of the work of the system, that is its activation upon vessel injury, spatial-temporal dynamics of thrombus growth and its arrest.

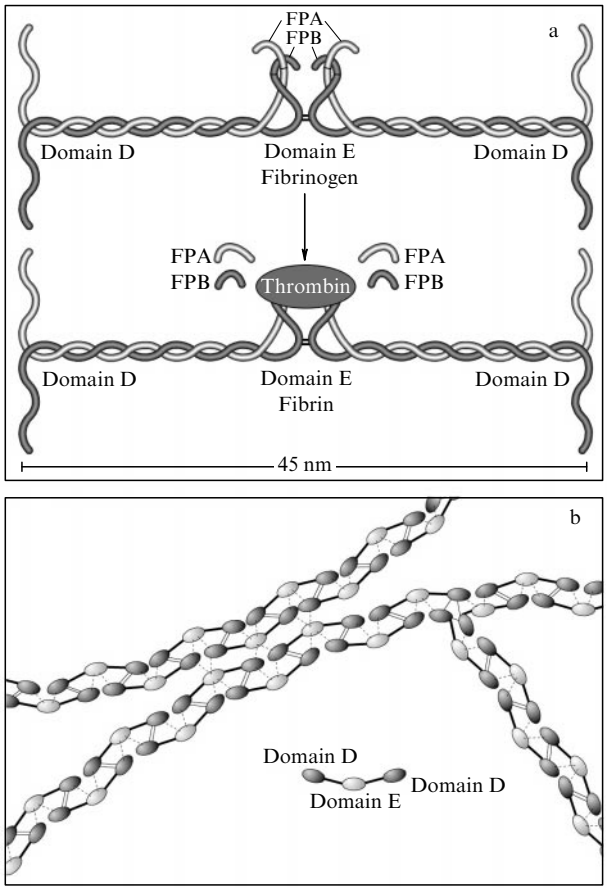

Figure 6. Synthesis of fibrin polymer. (a) Thrombin-induced cleavage of fibrinopeptides A and B (FPA, FPB) off fibrinogen; (b) subsequent polymerization of fibrin monomers into long branched chains [simplified from Mosesson M W *J. Lab. Clin. Med.*, 1990].

The main problem considered below is by what and how it is decided that blood needs 'to be or not to be' liquid in a selected portion of the circulatory system. Why is blood liquid in a normally functioning organ but undergoes clotting under the effect of biochemical reactions triggered where the vessel is damaged? By what and how is the clot size and localization determined?

2.2 Two modes of activation of coagulation. Intrinsic and extrinsic pathways of coagulation and their role

Figure 7 is a schematic representation of the blood coagulation cascade [86]. The main clotting factors are denoted by Roman numerals. All of them are proteins circulating in blood as inactive precursors. Practically all these factors are activated by the cleavage of a small fragment from the molecule, similar to fibrinogen or prothrombin. Figure 7 shows activated clotting factors denoted as *a*. According to this scheme, they activate each other in response to a vascular lesion initiating a cascade of consecutive reactions. For example, thrombin is activated by factor X_a formed at a preceding stage of the cascade. Traditionally, two pathways of activation leading to the production of factor X_a are distinguished. The intrinsic pathway is a long train of consecutive reactions starting from factor XII. The other, extrinsic, pathway proceeds from tissue factor or thromboplastin which is a protein located at the surface of practically all cells of the human body with the exception of endothelial cells making up the inner lining of normal blood vessels.

Coagulation is activated where the inner lining is damaged and blood gets in contact with cells having thromboplastin on their surface (see Fig. 7). This is the main (extrinsic) pathway of coagulation. It is known to be almost invariably involved in blood clotting whenever the vessels are damaged. It ensures fast (1 – 2 min) and efficient activation of coagulation. It follows from Fig. 7 that clotting may be also triggered by activation of factor XII, i.e. via the intrinsic pathway. Factor XII is always present in blood (hence the name of this pathway — intrinsic) and can be activated upon contact with a material alien to the body, e.g. glass. The intrinsic pathway is considered to be a 'reserve' one. The activation via this pathway is weaker than in the previous case and needs more time to be completed (within 7 – 10 min after initiation). It is however well-known that people lacking intrinsic factors of the 'reserve' pathway are vulnerable to hemorrhage because of deficient coagulation, even if the 'main pathway' remains normal.

Blood coagulation disorders are collectively called *hemophilias*. Many of them are hereditary disorders. One of the forms of hemophilia is the very common hemophilia A caused by the absence of factor VIII. Many lay people know about this disorder because it is due to an inherited defect which has been transmitted through succeeding generations in certain royal families of Europe. Tsarevich Alexis, the last heir to the Russian throne, suffered from this disease. Almost equally widespread is hemophilia B, caused by the deficit of factor IX, another element of the 'reserve' pathway. Clinical manifestations of the two disorders are very much alike.

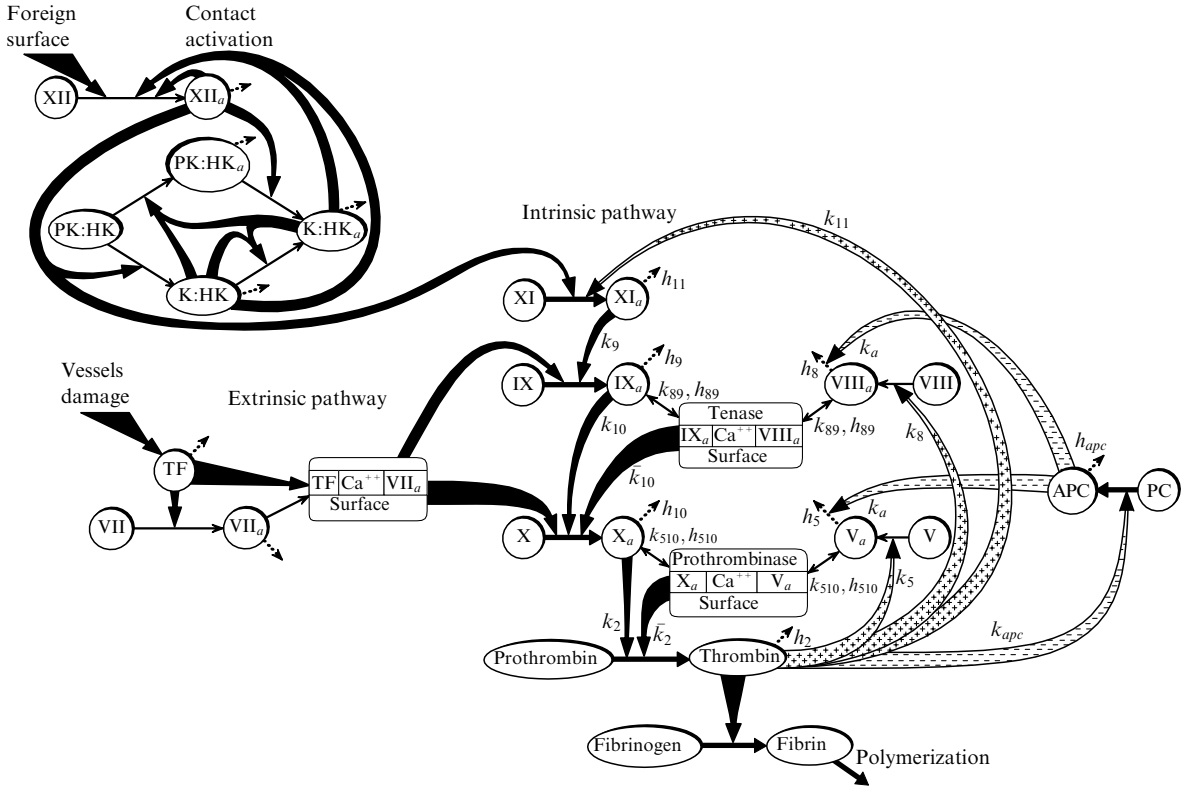

Figure 7. Schematic representation of biochemical reactions involved in blood coagulation. Roman numerals denote precursors of clotting factors and *a* — their activated forms. APC — activated protein C, TF — tissue factor. Plus and minus-arrows show positive and negative feedback relationships respectively. Dotted arrows indicate leakage of active factors in the case of inhibition. *k* and *h* are velocity constants of reactions and factor inactivation respectively.

One of the questions that has long remained unanswered in the science of hematology is why the deficiency in clotting factors of the 'reserve' pathway is so crucial for the coagulation process at all. There is the main, extrinsic pathway via which clotting is effectively initiated in case of a vessels damage. Why, then, is coagulation compromised in subjects lacking factors of the 'reserve' (intrinsic) pathway despite the normally functioning extrinsic pathway? We shall try to answer this question below.

2.3 Specific kinetic features of molecular processes of coagulation

Cascades of enzymatic reactions are widespread in biological objects. They occur in many systems, both at the cellular and other levels. Chemically, these cascades are a sequence of reactions mediated by proteolytic enzymes, such as the blood coagulation cascade, or by enzymes phosphorylating other proteins and termed *proteinkinases*.

Let us consider major features of the blood coagulation cascade. It is schematically represented in Fig. 8.

Naturally, the kinetics of active factor concentrations in such an enzymatic cascade in response to any perturbation is strongly nonlinear. It has been shown for a cascade of consecutive reactions that at an initial stage ($t \to 0$) a rise in the concentration of a final product is described by a power function in the form of t^p, where the index p denotes the number of steps in the cascade [87]. This is due to the fact that each step yields an enzyme. Each second, one enzyme molecule produces many molecules of the product which are also enzymes synthesizing large amounts of their respective product, etc. In other words, the cascade is a powerful amplifier with nonlinear properties. Amplification energy is derived from ongoing chemical reactions. Such a linear cascade has two stationary states, one zero and the other fully activated. The zero state is unstable and is readily

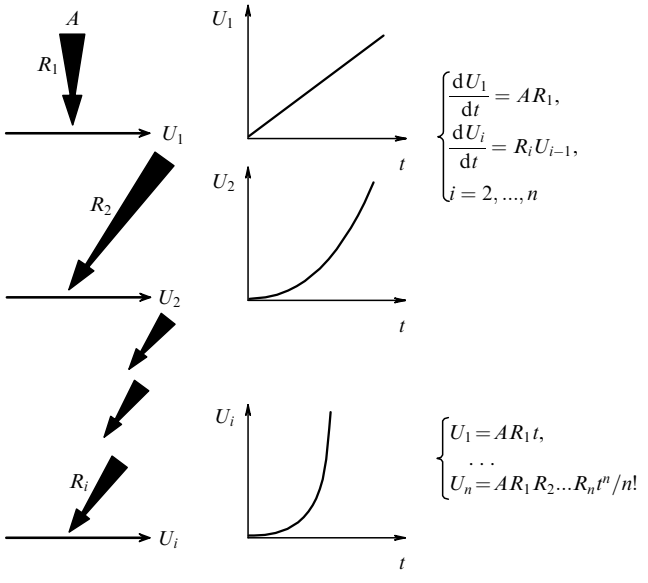

Figure 8. Kinetics of active factors (U_i) formed at different stages of the blood coagulation cascade.

transformed to the fully activated one upon a slightest fluctuation of the incoming signal. This situation corresponds to complete blood coagulation.

The above cascade properties (self-acceleration and instability) make blood coagulation akin to active media. The ability to intensify a perturbation creates prerequisites for the formation of a most important property of coagulation, the threshold response to system activation. As long as activation is below threshold blood remains liquid to provide protection of the organism against spontaneous clotting. As in the simplest example considered in the Introduction, the 'threshold behavior' of the coagulation system and bistability (stability of two coexisting blood states, liquid and solid) are induced by inhibitors and processes that arrest self-acceleration. They are sure to be present in the system and will be discussed below.

Blood coagulation reactions are integrated by strong positive feedback relationships. To induce bistability and ensure blood coagulation, nature did not confine itself to imparting nonlinearity to the enzymatic cascade. The property of nonlinearity is multiply reinforced by strong positive feedback relationships. Figure 7 shows that factors VIII and V stand apart form the others. They are not direct elements of the cascade, nor do they exhibit enzymatic activity. They are actually cofactors or effectors that significantly, by several orders of magnitude, increase the activity of factors IX and X. Cofactors V and VIII are initially inactive but their activation by thrombin leads to the formation of positive feedback loops in the system. In Fig. 7 they are shown by plus-arrows. By virtue of this mechanism, thrombin self-production is accelerated 10^5 times.

It has been shown in experiment that thrombin can activate factor XI [88, 89] at the top of the cascade thus giving rise to one more positive feedback loop in the coagulation system (see Fig. 7). This reaction makes the system autonomous. In other words, thrombin is capable of maintaining self-production even in the absence of activation via both intrinsic and extrinsic pathways. What is actually needed for the purpose is an initial primer, e.g. a small amount of thrombin itself.

Stability of the blood liquid state is maintained by inhibitors. The sequence of reactions considered in the preceding paragraphs (see Fig. 7) can stably exist in the inactive liquid state only if the lifetime of active forms of clotting factors is relatively short. Otherwise, the blood quickly coagulates. To prevent clotting, all active enzymes are rapidly inactivated by special inhibitor proteins contained in blood, e.g. antithrombin. Inactivation is effected by the binding of the inhibitors to active factors. This process is illustrated in Fig. 7 by dotted arrows originating from the corresponding factors. Such a 'flow-through' system, where active factors undergo fast and irreversible inactivation, needs to be continuously replenished by new inactive precursors. This function is performed by the liver.

Ensemble of protein C *reactions — negative feedback in blood coagulation*. Besides inhibitors, there are a number of reactions that collectively make up a negative feedback system. They are shown in the figure by minus-arrows. When activated, protein C, one more clotting factor, is capable of splitting cofactors VIII and V. This activity breaks positive feedback loops and reduces the activation rate by several orders of magnitude. Interestingly, activation of protein C, i.e. switching on the negative feedback mechanism, is effected by thrombin, that is the same key factor of blood coagulation which produces fibrin for clot formation and accelerates self-production through positive feedback. Thus, all roads lead to thrombin!

2.4 Homogeneous kinetics of blood coagulation

Blood coagulation experiments are usually designed as follows. A blood sample added to a tube is supplemented by an appropriate agent (e.g. a clotting activator obtained from under vascular endothelium), and the resulting solution is mixed for some time and thereafter left for the coagulation process to develop. In the end, a clot (i.e. gel) is formed (Fig. 9a). Under these conditions, clotting is a homogeneous process that involves the entire blood volume. A different picture is observed when a blood vessel is damaged (Fig. 9b). Blood leaks through the lesioned endothelium and comes in contact with tissues underlying it. The resultant activation of clotting factors induces coagulation at the sight of injury. The growth of the clot stops after it attains a certain size, seals the lesion, and arrests leakage. Sufficiently large vessels continue to function normally despite the lesion which in the meantime undergoes reparation. Thus, the coagulation process under natural conditions occurs as the growth of a certain spatial structure; this poses an invariably inhomogeneous problem. We shall start from a simpler homogeneous case.

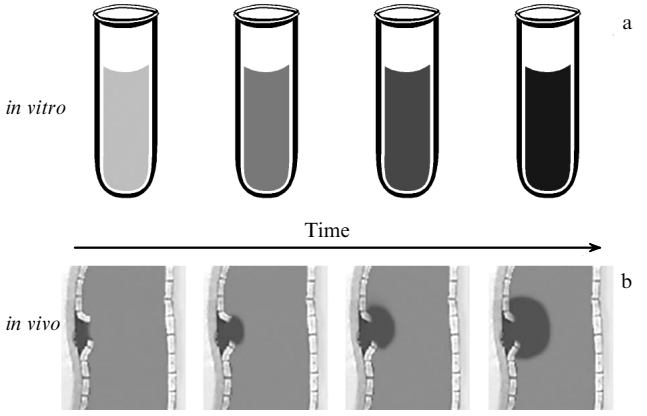

Figure 9. Thrombus formation in a tube (*in vitro*) (a) and in a blood vessel (*in vivo*) (b). In the tube, the process involves the entire blood volume (shading is proportional to thrombus solidification). In the vessel, only a portion of the blood at the site of injury undergoes solidification.

The scheme in Fig. 7 requires almost 40 differential equations to be fully described. There are a few mathematical models of the system differing in the degree of detail of the description. By means of reasonable simplifications (e.g. on the assumption of constant precursor concentrations), it is possible to reduce the number of equations to eight and use this system for the quantitative description of *in vitro* experiments [45, 46]. At the same time, all major kinetic properties of the system of interest in a homogeneous case can be qualitatively described by only two differential equations. The system that will be used, by way of example, to consider characteristic properties of the blood coagulation process is actually a reduced version of the model described elsewhere [45]. This reduced model includes two variables, thrombin (u)

and activated protein C (v):

$$\frac{du}{dt} = K_1\left(1 + K_2\frac{u}{1+K_3 v}\right)\left[K_4 u\left(1 + K_5\frac{u}{1+K_3 v}\right) + A\right] \times \left(1 - \frac{u}{u_0}\right) - K_6 u, \quad (5)$$

$$\frac{dv}{dt} = K_7 u - K_8 v,$$

$$K_1 = \frac{k_2}{h_{10}}, \quad K_2 = \frac{\overline{k}_2 k_{510} k_5}{k_2 h_{510} h_5}, \quad K_3 = \frac{k_a}{h_5} = \frac{k_a}{h_8},$$

$$K_4 = \frac{k_9 k_{10} k_{11}}{h_9 h_{11}}, \quad K_5 = \frac{\overline{k}_{10} k_{89} k_8}{k_{10} h_{89} h_8}, \quad K_6 = h_2,$$

$$K_7 = k_{apc}, \quad K_8 = h_{apc},$$

where u_0 is the concentration of prothrombin (thrombin precursor), A is the continuous influx of factor X_a that characterizes in this model the degree of system activation via the extrinsic pathway (see Fig. 7), and K_i is the combination of elementary constants shown in Fig. 7.

Table 1. Values of constants used in model (5).

K_1	K_2	K_3	K_4
2.45 min^{-1}	447.66 nM^{-1}	3.87 nM^{-1}	1.65×10^{-4} min^{-1}
K_5	K_6	K_7	K_8
4.89 nM^{-1}	2.3 min^{-1}	0.0014 min^{-1}	0.1 min^{-1}

Here, the expression within the first brackets in the equation for thrombin describes a positive feedback loop involving cofactor V and the expression in the second brackets a positive feedback loop involving cofactor VIII (see Fig. 7). The expression within the square brackets contains reactions related to the positive feedback loop via thrombin activation of factor XI (see constant K_4) as well as the influx of factor X_a. The expression within the third brackets describes the limitation on the thrombin level imposed by the concentration of its precursor in blood plasma u_0. According to the equation, protein C is activated by thrombin and inactivated in proportion to its own level. All the constants correspond to those used in the earlier description of the complete model [45, 46]. One of the main properties of blood coagulation demonstrated by the above system of equations is the threshold response of the system to activation. The threshold response of the blood coagulation system was first predicted theoretically by M A Khanin [90, 91]. Figure 10a shows characteristic kinetics of clotting factors in response to activation signals of different amplitudes. Each consecutive curve number corresponds to an increase of factor X_a influx by 0.0005 nM min^{-1}. After the activation signal reaches a certain level, a dramatic change of kinetics occurs and the concentration of the active factor begins to grow exponentially. At subthreshold activation signals, the system's response is roughly proportional to the amount of activation. A very low plasma thrombin level fails to substantially increase fibrin production, and the blood remains 'liquid'. A suprathreshold activation results in a jump of concentration to a value 4–5 orders of magnitude above the subthreshold one. Figure 10b illustrates the modulation of thrombin concentrations in response to suprathreshold signals. It can be seen that the amplitude of

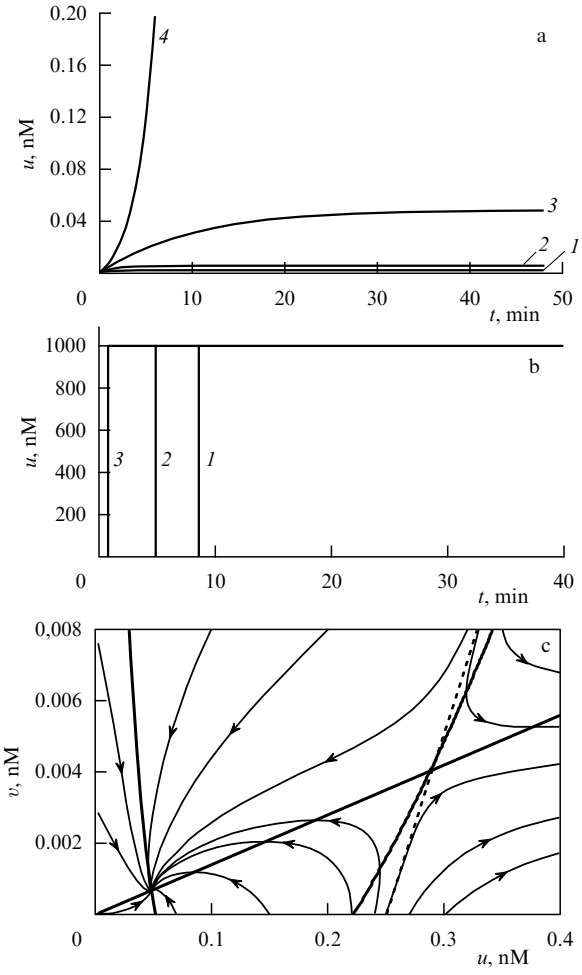

Figure 10. (a) Kinetics of thrombin synthesis at subthreshold (curves *1*, *2*, *3*) and suprathreshold (curve *4*) levels of activation (respective influx values: *1* — 0.001 nM min^{-1}; *2* — 0.0015 nM min^{-1}; *3* — 0.002 nM min^{-1}; *4* — 0.0025 nM min^{-1}); (b) explosive kinetics of thrombin synthesis in case of suprathreshold activation followed by plateau for the lack of exhaustion of precursors in the model (curve *1* — influx equals 0.0025 nM min^{-1}; *2* — 0.003 nM min^{-1}, *3* — 0.01 nM min−1); (c) phase portrait of model (5) for the case of subthreshold activation ($A = 0.002$ nM min^{-1}). Dark-colored lines are isoclines; light-colored lines are phase trajectories. The dashed line is a saddle separatrix. \

the response remains unaltered as the activation signal increases whereas the time needed to reach a maximum response changes significantly.

Comparison of the model and experimental findings reveals a qualitative similarity. In experiment, the behavior is more complicated; specifically, for suprathreshold activation signals, the thrombin concentration falls rapidly after it reached a maximum value. Such a behavior is due firstly to the exhaustion of active factor precursors and secondly to the fast inactivation of synthesized thrombin by inhibitors present in blood in large amounts. There is no explicit exhaustion of precursors in the model even though the thrombin concentration is limited by the plasma level of its precursor (u_0). The amount of thrombin necessary for solid clot formation in the real coagulation process is much smaller than the maximum achievable in the model. Therefore, the entire process depends on the initial phase of thrombin synthesis in which the behavior of the model resembles that in vivo. As in the model, the magnitude of the activation

signal shows a stronger influence on the time necessary to reach a maximum thrombin level than on its peak amplitude. Analysis of complete models taking into consideration exhaustion of precursors confirms this conclusion [46].

Figure 10c presents a phase portrait of system (5) for a case of subthreshold activation (corresponding to curve *3* in Fig. 10a). In this case, the system has three fixed points. The figure shows only two of them located near the origin of coordinates. The third one, a stable node, lies four orders of magnitude from the second point along axis u, that is outside the selected scale, and corresponds to the coagulated state of plasma. The complete phase portrait of this system showing the third fixed point is practically indistinguishable from the phase portrait in Fig. 11b where the near fixed points are absent. The large scale difference (a few orders of magnitude) makes these points practically indistinguishable. The stable fixed point close to zero corresponds to the transition of the system (at zero initial values of variables) to low (subthreshold) stationary concentrations of active clotting factors. It follows from Fig. 10c that, in this case, the thrombin concentration begins to grow quickly if its initial values were in excess of 0.25 nM as determined by the saddle separatrix separating the two stable fixed points of the system.

The shape of the isocline of the first equation in system 5 changes with increasing system activation level. Figure 11a illustrates the transition from subthreshold to suprathreshold activation. The changes are largely confined to the zone of small values of variable u. As the influx of factor X_a increases, the left branch of the isocline ascends. As soon as it touches the isocline of the second equation, the two fixed points closest to zero disappear. A complete isocline for the case of suprathreshold activation is presented in Fig. 11b showing the respective phase portrait of system (5).

Systems of type (5) are rigid from the computational standpoint. The presence of S-shaped isoclines in such systems accounts for their tendency to fluctuations. System (5) is no exception. A rise of the constant k_{apc} leads to a self-sustained oscillation regime (Fig. 12). Comparison of the model and in vitro experiment (a fully mixed system) indicates that the simplest model provides an adequate qualitative description of the majority of the observed effects. The system generates a thrombin impulse in response to small suprathreshold perturbations [92, 93]. As in the model, the amplitude of this impulse is many orders of magnitude higher than the thrombin concentrations in liquid blood. Also, it shows a weak dependence on the activation signal which largely influences the clotting time (the lower the suprathreshold activation the more time coagulation takes to complete). It has been shown above (Fig. 10b) that the simplest model behaves in a similar way.

We shall not dwell at length on the homogeneous kinetics of this system because we think it is much more interesting to consider the spatial aspect of the problem.

3. Phenomenological model

How will the system of chemical reactions represented in Fig. 7 behave in a spatial problem? In the case of activation in

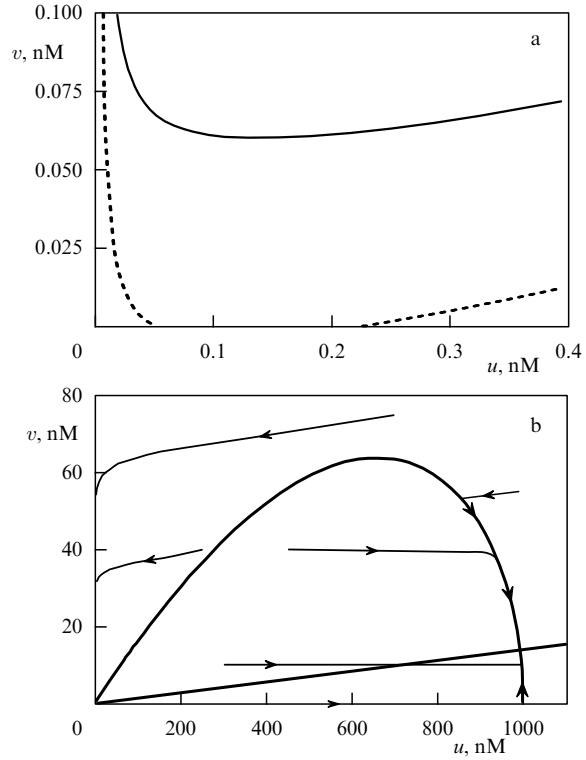

Figure 11. (a) Change of the isocline shape in the first equation of system (5) upon a rise in the level of its activation (influx of factor X_a). The solid line represents suprathreshold activation ($A = 0.0025$ nM min^{-1}); the dashed line is subthreshold activation ($A = 0.002$ nM min^{-1}). (b) Full phase portrait of system (5) in the case of suprathreshold activation represented by the solid line in (a). [Pay attention to the difference between the scales in figures (a) and (b)].

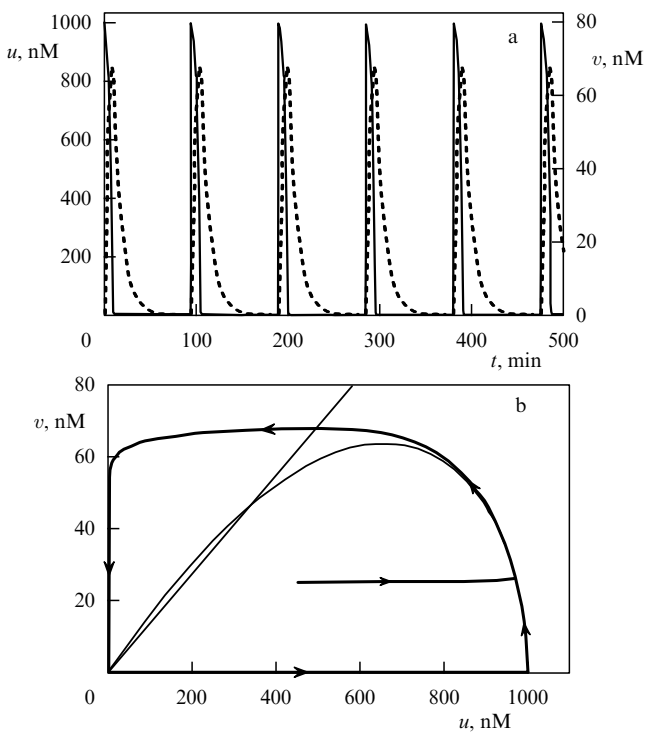

Figure 12. 10-fold increase of the constant of protein C activation by thrombin ($K_7 = k_{apc} = 0.014$ min^{-1}, $A = 0.0025$ nM min^{-1}) leads to self-sustained oscillations of active clotting factors. (a) Kinetic curves of thrombin (solid line) and activated protein C (dashed line). (b) Respective phase portrait of system (5): the light-colored lines are isoclines, the dark-colored lines are the limit cycle trajectory.

one part of the space, active clotting factors can spread either by diffusion or by drift in the blood flow. Equations (5) can be extended by the addition of diffusion terms. Then, activation of coagulation in a certain part of the space will induce propagation of a self-sustained wave of clot formation. Such behavior is typical of active media.

Although the greatly simplified model of the coagulation system obtained in our studies adequately describes the homogeneous kinetics of the system, it is evidently in conflict with the knowledge that a clot is always finite and localized.

We have considered many complete versions of the description of biochemical reactions involved in blood coagulation and could see that thrombin always spreads as a self-sustained wave or trigger wave. This is a rough effect independent of our simplifications, the key point being the ability of thrombin to activate self-production. Due to this ability, the blood is an active medium, and active clotting factors can not only migrate from the site of injury to circulation but can also be formed at each point of the space. All the inhibitors represented in Fig. 7 that so effectively block thrombin synthesis and rapidly decrease its concentration in a homogeneous case prove unable to do the same in a spatial situation. The expanding front of the inhibitors cannot overtake the thrombin front.

The first attempt to resolve this paradox was made in the works of Ataullakhanov and Guriya [43, 44]. These authors proposed a phenomenological model of coagulation [43] based on a simple idea that a self-sustained wave can catch up with another such wave. The equations of this model have the form

$$\frac{\partial u}{\partial t} = \frac{K_1 u^2}{u + K_2} - K_3 u - K_4 u v + D_u \Delta u\,,$$
$$\frac{\partial v}{\partial t} = K_5 u\left(1 - \frac{v}{K_6}\right)\left[1 + \left(\frac{v}{K_7}\right)^2\right] - K_8 v + D_v \Delta v\,, \qquad (6)$$
$$\frac{\mathrm{d}F}{\mathrm{d}t} = K_9 u\,.$$

In this model, the ability of thrombin for self-accelerated production is taken into consideration in a simple way. By analogy with the previous model (5), thrombin is denoted by the variable u. It can accelerate self-production, i.e. autocatalysis takes place. Also taken into account is thrombin inactivation effected through its binding to plasma inhibitors. The second variable, v, is a hypothetical inhibitor which is absent in an explicit form from the previously considered system of biochemical reactions (see Fig. 7). The hypothesis suggests that this inhibitor must also inactivate thrombin but differs from other inhibitors in terms of kinetics. It is induced by thrombin and thereafter accelerates self-production (the expression within the square brackets in the first term of the equation for v). According to the second equation of system (6), the production of inhibitor v is proportional to the thrombin level which means that it is synthesized only in the zone of thrombin action. This inhibitor, similar to all active clotting factors, is characterized by a certain inactivation rate. For the convenience of watching the process, the third variable F (fibrin analogy) is introduced; it marks the clot formation site. The third variable is actually an indicator (integrated thrombin value). The third equation has no effect on process dynamics.

The idea of the phenomenological model consists in that a self-sustained wave can be overtaken (and damped out) by the following one traveling at a higher velocity. The former wave (thrombin wave) creates conditions for the formation of the latter (hypothetical inhibitor). Because this inhibitor is also capable of autocatalysis, it has a degree of autonomy; that is its velocity depends on the kinetics of its own reactions. This model is qualitatively consistent with experimental findings. The thrombin wave initially propagates and then stops abruptly. This fact would be of little interest in itself (there are many ways to damp the thrombin wave out) were it not for a number of unusual clot growth regimes predicted by the model and later confirmed in experiment [42]. By changing the constant K_2 of the model that determines the thrombin production threshold, it is possible to pass from the 'normal' formation of a clot of finite size to the continuous growth regime. Incidentally, the model predicts an interesting dynamic regime, i.e. a self-sustained wave with a pulsed amplitude. This is an implicit extension of the notion of a self-sustained wave, an ulterior definition. In a model of 'normal' thrombus growth containing a slightly less active inhibitor (e.g. due to a decreased production rate), this amplitude will not reduce the amplitude of the thrombin self-sustained wave to zero; rather, this amplitude will remain somewhat higher than the threshold value but insufficient to induce inhibitor production. Thus, a thrombin self-sustained wave propagating from the site of injury may escape the influence of the inhibitor and acquire its maximum amplitude again under the effect of reactions of the blood coagulation cascade. Thereafter, the entire process is repeated starting from active formation of the inhibitor that leads to a decrease of the thrombin self-sustained wave amplitude, etc.

Figure 13a illustrates the spatial dynamics of thrombin wave distribution. Evidently, the wave amplitude undergoes well-apparent pulsation. There are points where the ampli-

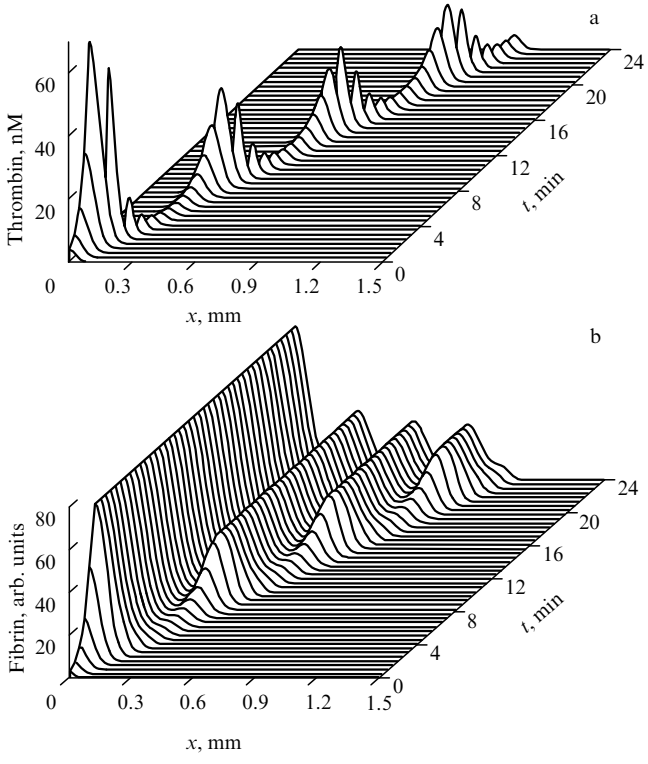

Figure 13. Propagation of a thrombin wave with a pulsed amplitude (a) and the corresponding formation of a layered fibrin clot (b). See Table 2 for parameters.

Table 2.

K_1	K_2	K_3	K_4	
2 min^{-1}	0.95 nM	0.05 min^{-1}	5 min^{-1} nM^{-1}	
K_5	K_6	K_7	K_8	K_9
0.0015 min^{-1}	5 nM	0.05 nM	0.35 min^{-1}	2.8 min^{-1}

tude is never large; fibrin formation at such points is rather low. Therefore, the running thrombin wave leaves behind a sort of matrix in the form of fibrin that gives an idea of changes of its amplitude. The resulting structure is a stratified thrombus in which liquid layers alternate with solid ones. Figure 13b shows the spatial distribution of fibrin in this situation. It can be seen that the resultant area of synthesized fibrin has a layered structure. At some points, the thrombin level was high while at others it was low; accordingly, fibrin production was high at the former points and low at the latter. The most important thing about this situation is that the fibrin amplitude can change by an order of magnitude.

The question is whether in vivo coagulation occurs at variance with the model. How does a fibrin clot grow? What are its dynamic characteristics (size and velocity)? Does it have a similar density at the point of activation, in the center and periphery of the thrombus? Do the unusual events predicted by the model ever take place in experiment? It turns out that the spatial aspect of coagulation has never interested researchers, a strange and unusual situation in modern biology that appears to encompass all conceivable problems (suffice it to say that thousands of publications concerning blood coagulation alone appear every year). It is the phenomenological model that gave incentive to experimental studies on spatial coagulation dynamics.

4. Dynamics of thrombus development *in vitro*

We examined blood in the absence of mixing. If coagulation is activated in a thin blood layer coated over a dish floor, a clot is formed around the activator. Clotting is readily induced by glass; therefore, we frequently use glass surfaces to activate coagulation. Glass beads are dropped into a Petri dish containing a thin blood layer. After the blood is decanted half an hour later, the beads covered with red clots (true thrombi) remain at the bottom of the dish (Fig. 14a). The thrombi attach the beads to the bottom. They are known to readily 'adhere' to practically any surface which is a great advantage in terms of protection of the organism. Practically speaking, blood is not a very convenient object for experimenting because it contains a large amount of light-absorbing erythrocytes. They make it difficult to continuously measure clot dimensions. It has been shown that the plasma remaining after the removal of blood cells is as subject to coagulation as the whole blood. It is transparent, and a growing fibrin clot scatters light. Therefore, it is easy to continuously observe clot development in blood plasma. Figure 14e presents a cinematogram of the clot growth on glass bead in a thin plasma layer. The snapshots were taken at 3-minute intervals. As can be seen, the clot grows for same time after which the growth is arrested. The resultant clot at the bead surface is about 0.6 mm in size. Thus, the possibility has been demonstrated to reproduce, in a relatively simple experiment, coagulation dynamics reminiscent in many respects of the real process taking place in the body.

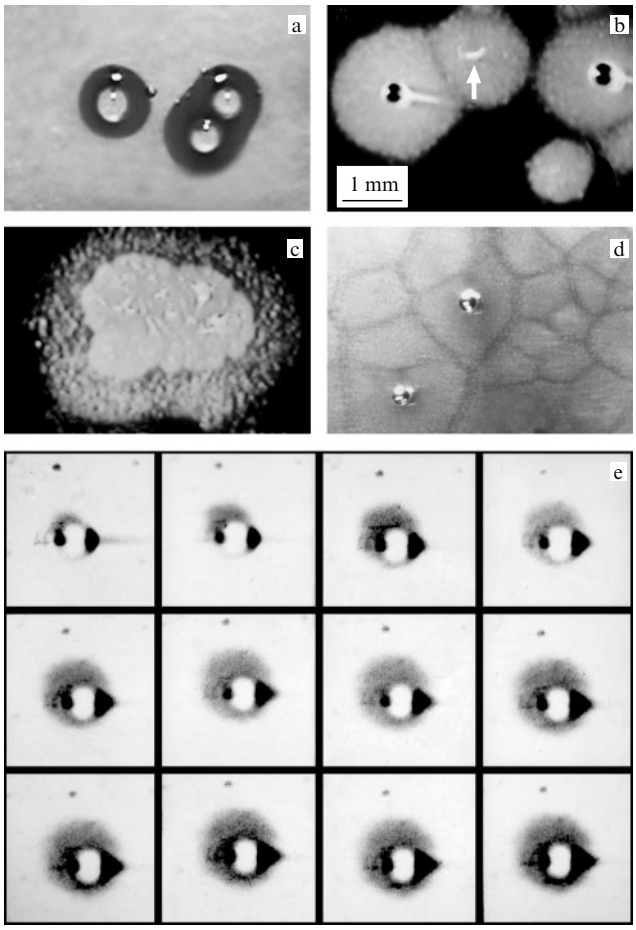

Figure 14. Thrombus formation in whole blood (a) and platelet-depleted plasma (b, d) identified from light scattering patterns. (a, b) Thrombi growing around glass beads (0.5 – 0.6 mm in diameter) and a collagen fibre (arrow) respectively; (c) clotting induced by a small parcel of dry thrombin (100 – 200 μg); (d) inhibition zones formed 120 min after activation of coagulation by glass beads; (e) snapshots taken at 3-minute intervals and showing clot formation on a glass bead (light scattering) in platelet-depleted plasma (the black triangle is a patch of light near the bead). In all experiments, the plasma layer is 0.5 mm thick.

Investigations into this process have shown that activation of coagulation under such conditions via both intrinsic (by glass) and extrinsic (by a vessel wall fragment) pathways results in blood clots of roughly the same size [42, 94]. Very different activation signals have a similar effect. The plasma may be activated by a glass bead or a thin collagen fibre (Fig. 14b) or even by dry thrombin (Fig. 14c). In the latter case, the clot is as thick as on a glass bead even though the amount of thrombin introduced into the system is large enough to coagulate all the blood present in the human body. However, no further growth of the clot can be observed. Indeed, it is arrested at a certain distance from the point of activation which does not depend on the activation signal. This finding was the first confirmation that clot development is governed by mechanisms involving self-sustained waves because only in this case do both the waves and the structures being formed show a weak dependence on the magnitude of the activation signal.

We undertook an experimental verification of other predictions of the phenomenological model. Surprisingly, they all proved to hold true. A difference between experi-

mental conditions and the human body consists in that 30–40 min after the beginning of the experiment coagulation occurs in the absence of activators. There are 'spontaneous centers' (Fig. 14b, bottom right) from which clotting spreads, the clot growth dynamics being identical with that of clots growing at the surface of strong activators. The whole space is filled with clots within 60–90 min after the initiation of the experiment. Interestingly, the final structure is divided into parts by dark stripes where blood remains in the liquid state (Fig. 14d). In accordance with the phenomenological model, areas around a completely formed clot must for some time contain elevated amounts of the hypothetical inhibitor. Indeed, coagulation in these areas can not be induced even by strong activation signals. The most interesting experimental findings are stratified structures predicted by model (6) [43]. In certain cases of simple glass-activated coagulation, the clot stops growing after a period of initial development and is surrounded by liquid blood (Fig. 15). Thereafter, another clot begins to develop at some distance from the previous one. Thus, the second layer is formed with a liquid zone around it.

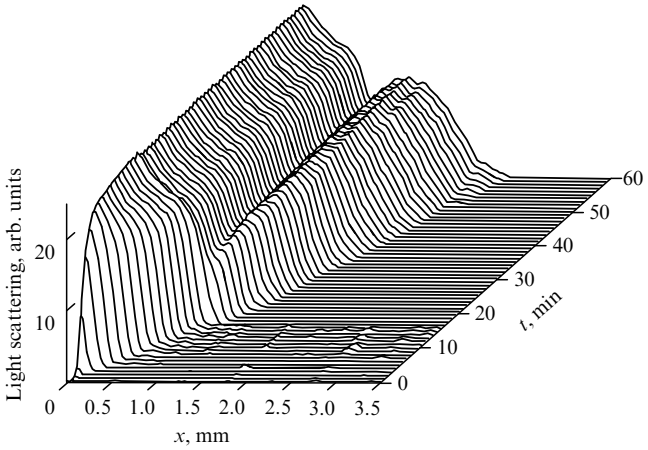

Figure 15. Formation of a layered thrombus in experiment. ATIII (2 U) was added to platelet-free recalcified blood plasma, pH = 7.4, and coagulation was activated by glass. Fibrin clot growth was followed by measuring light scattering. The interprofile interval is 1 min.

Therefore, predictions of the phenomenological model having no firm biochemical foundation for the existence of an inhibitor receive experimental confirmation! Hence, the immediate impulse to search for this inhibitor. It is however a difficult task since, theoretically, such an inhibitor must be a short-lived entity. Otherwise, its concentration in blood would be so high as to completely prevent clotting. In a word, it is clear that the molecular mechanisms of stopping of blood coagulation present the most puzzling aspect of the problem.

5. Hypothesis of thrombin activity switching. A new model

5.1 Biochemical prerequisites for the hypothesis

Let us now return to the analysis of molecular mechanisms of biochemical reactions leading to blood coagulation. Publications that appeared a few years ago made researchers switch their attention from putative autocatalysis of the inhibitor to the properties of thrombin itself. Thrombin is known to play the role of 'two-faced Janus' in the blood coagulation process. It acts both as a procoagulant (a substance promoting clot growth) and as an anticoagulant (a substance inhibiting clot growth). This dual function of thrombin is well illustrated by Fig. 7. One of the thrombin 'faces' is the activation of self-production (plus-arrows), the other is the inhibition of self-production (minus-arrows). It turned out that thrombin exists in two structurally different forms. One effectively converts fibrinogen to fibrin and activates cofactors (the plus-arrows in Fig. 7) but is a weak activator of protein C ('anticoagulation reactions' indicated by minus-arrows). The other structural form of thrombin is an efficacious activator of protein C but fails to effectively split fibrinogen.

Such an ability to switch over from one activity to the other is consistent with the notion of a second self-sustained wave, i.e. a wave of a certain substance overtaking the thrombin wave. The role of this substance is not to exclude thrombin but to transform its procoagulant state to an anticoagulant one. Unfortunately, the ability of thrombin to 'change faces', that is to switch over from one state to the other, was studied under conditions that are not encountered in the body [95, 96]. Specifically, it was induced by a synthetic chemical compound having no analogs in biological tissues [95]. At the same time, a natural protein was discovered that effectively stimulates this ability [97]. However, this protein is located at the surface of the cells that make up the inner lining of blood vessels. Therefore, it is always in contact with blood. Its ability to modulate thrombin action does not depend on thrombin concentration whereas the hypothesis claims that a substance influencing thrombin must be in turn activated (or synthesized) by thrombin.

Today, there is no substance meeting all these requirements. Nevertheless, we tried to study how the transition of thrombin from one state to the other may influence coagulation dynamics. We constructed a mathematical model based on the scheme in Fig. 7 and supplemented by reactions shown in Fig. 16 [47]. Also, we postulated that the cleavage of protein C yields (as it really does) a peptide binding to thrombin and promoting its transition from one form to the other; it thus closes the feedback loop in the system (see Fig. 16). This peptide differs from the hypothetical inhibitor of the phenomenological model. Nevertheless, the system has a feedback accounting for autocatalysis in protein C production. First, the blood coagulation cascade produces one form

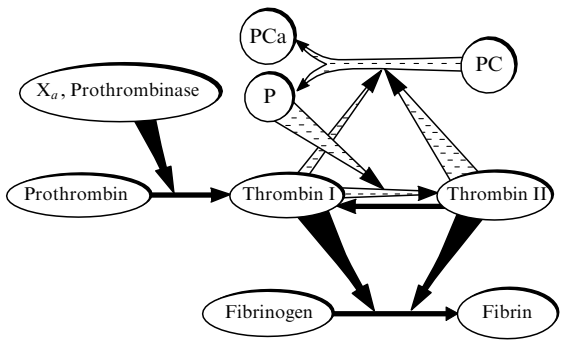

Figure 16. Schematic representation of reactions involved in the transformation of thrombin activity. Peptide P, a product of protein C (PC) activation, converts the procoagulant form of thrombin (thrombin I) to the anticoagulant form (thrombin II). Minus-arrows depict the feedback loop.

of thrombin (thrombin I) and activated protein C production rate is relatively low. Then thrombin I splits protein C to produce a peptide that transforms it to thrombin II and accelerates self-production. In this way, a self-accelerating process is triggered in the system which transforms thrombin to the form having impaired ability to maintain coagulation. In other words, thrombin action is somewhat neutralized. For the purpose of the model, a peptide resulting from protein C activation was chosen to serve as such a modulator. It is understandable that its function can be performed by any substance produced in the negative feedback loop via protein C, e.g. a product of cofactor V_a or $VIII_a$ inactivation by protein C.

The initial model included equations for all clotting factors shown in Figs 7 and 16. Subsequent comparative analysis of the reaction constants allowed this model to be simplified to a system of three equations. This model is not the phenomenological anymore. All constants and variables of the new model are consistent with the molecular nature of blood coagulation [47]. The sole hypothesis in support of this model postulates the existence of a thrombin modulator that switches it from one state to the other.

The simplified dimensionless model is represented by a system of three parabolic-type equations involving partial derivatives (Δ — the Laplace operator) and has the form [47]

$$\begin{aligned}\frac{\partial u}{\partial t} &= D\Delta u + K_1 uw(1-u)\frac{(1+K_2u)}{(1+K_3v)} - u\,,\\ \frac{\partial v}{\partial t} &= D\Delta v + K_5u^2 - K_6v\,,\\ \frac{\partial w}{\partial t} &= D\Delta w + u - K_4w\,.\end{aligned} \tag{7}$$

The model contains three variables: the activator u (the product of autocatalysis), the inhibitor v (its production rate is proportional to the square of activator), and the variable w (which maintains formation of activator; its production rate is proportional to the activator). The activator is a sum of the first and second forms of thrombin; the inhibitor is activated protein C; the variable w is clotting factor XI_a (see Fig. 7). It should be noted that this model implies no apparent autocatalysis of the inhibitor, unlike the phenomenological model (6). To differentiate between the two, we shall refer to the new model as mechanistic since it is based on the real chemical mechanisms underlying blood coagulation.

Regimes of 'normal' thrombus development, infinite growth (as far as medium borders), and the thrombin wave with a pulsed amplitude were obtained in both model (7) and the phenomenological model (6). In addition, model (7) was found to contain one more regime previously unreported as occurring in blood coagulation models and in dynamic system models at large. Therefore, we present the results of a dynamic study of this model in a separate section.

5.2 Analysis of mechanistic model of coagulation

Analysis of 'point model'. In order to analyse the observed regimes, we compared the behavior of system (7) in space and the bifurcation diagram of the corresponding point system ($D = 0$). Variables v and w were expressed through u from the corresponding equations of model (7) (at $D = 0$) and substituted into the first equation of the model. The resulting function is the 4th power polynomial the roots of which determine the fixed points of the system:

$$f(u) = u\left(\frac{K_1K_2}{K_4}\,u^3 + \left(\frac{K_1}{K_4}\,(1-K_2) + \frac{K_3K_5}{K_6}\right)u^2 - \frac{K_1}{K_4}\,u + 1\right). \tag{8}$$

It follows from formula (8) that the system always contains a zero fixed point. This point is stable at any parameter values and corresponds to the 'liquid' state of the blood. Moreover, the 4th power polynomial (8) always has one negative root because the expression within the brackets equals 1 at $u = 0$ and the value of the corresponding 3d power polynomial as $u \to -\infty$ tends to $-\infty$. Accordingly, the number of positive real roots of polynomial (8) (including the zero one) that determines the fixed points of system (7) can vary from 1 to 3 depending on the parameters of the model. The phase portrait of the system is organized so that the trajectory with the initial conditions in the positive quadrant never invades the negative value region.

Let us consider the behavior of the system in the plane of parameters (K_2; K_6). These parameters determine the rates of activator production and inhibitor inactivation respectively. Fig. 17a illustrates alteration of the type of the far-from-zero fixed point in system (8). The system has a single fixed (zero) point in the shaded region *0*. Intersection of the right boundary of region *0* gives rise to two more fixed points. The point closer to the origin of coordinates remains unstable over

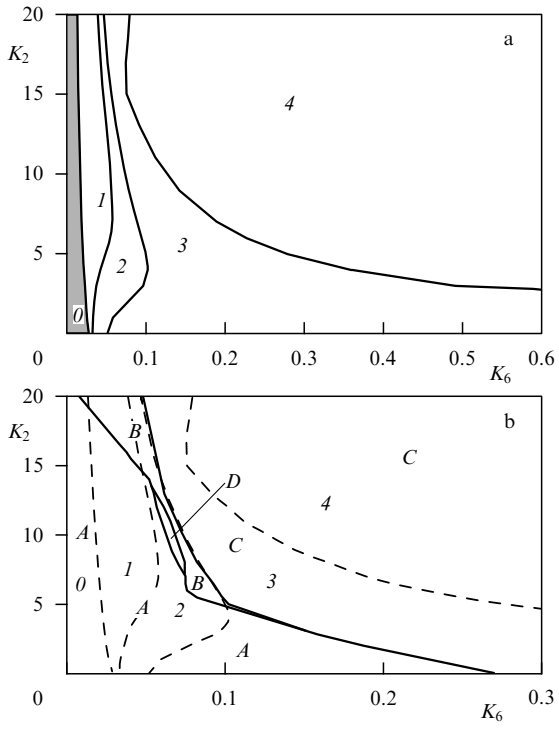

Figure 17. (a) Bifurcation diagram of model (7). The solid lines show boundaries separating different types of far-from-zero 'upper' fixed point of the system. In shaded region *0* the system has a stable single fixed (zero) point in the positive quadrant. In regions *1* and *2*, the far-from-zero point is unstable (*1* — saddle-node, *2* — unstable focus); it is stable in regions *3* and *4*. The line between regions *2* and *3* is the Poincaré – Andronov – Hopf bifurcation line. (b) Arbitrarily breaking up the plane of parameters (K_2; K_6) into four types (A, B, C, D) of model (7) responses to the initial perturbation (other parameters of the model are presented in Table 3). The dashed lines show boundaries corresponding to the solid lines of the preceding figure.

the entire field of the constants. The lines show bifurcation boundaries of the far-from-zero fixed point. It is unstable in regions *1* and *2* but stable in regions *3* and *4*. The line between regions *2* and *3* is the Poincaré – Andronov – Hopf bifurcation line. Regions *3* and *4* correspond to bistability, when the system has two stable points separated by an unstable one. A decrease of K_2 leads to the expansion of region *2*. Numerical experiments showed that this region gives place to a cascade of bifurcations of doubling period and undergoes transition to chaotic oscillations according to the Feigenbaum scenario. At the selected values of other parameters, the regions of limit cycles and chaos exist only in a very narrow range of K_6 values adjoining the Andronov-Hopf bifurcation line on the left. The constants used in the model are presented in Table 3.

Table 3.

K_1	K_2	K_3	K_4	K_5	K_6
6.85	varies	2.36	0.087	17.0	varies

Spatial dynamics of coagulation. The behavior of system (8) was examined on a segment $L = 10$ ($\Delta = \partial^2/\partial x^2$). It was supposed that the diffusion coefficient is the same for all the three variables ($D = 0.00026$). Activation of the system was simulated by a local enhancement of variable u on the left boundary of the segment being considered. Figure 17b represents an arbitrary breaking up of the previously examined region of changing parameters (K_2; K_6) into four spatial behavior regimes A, B, C, D. The solid lines show the boundaries between different types of spatial regimes, the dashed lines are bifurcation boundaries of the point system. A variety of spatial behavior regimes of system (7) may be roughly categorized into four types that occur in the respective regions of the (K_2; K_6) parametric plane.

Zone A. In this zone, the initial perturbation of the system decays. Both the velocity and the amplitude of an impulse running from the activation zone decrease so that it vanishes. Zone *A* (at $K_2 < 14$) includes regions *0* and *1* and most of region *2* of bifurcation boundaries of the point system. In these regions, the 'homogeneous' system has a single stable point (zero). The impulse spreads further when initial parameters move upward and to the right across zone *A*. Moreover, the impulse moves with practically constant velocity and amplitude, and then stops abruptly and vanishes (Fig. 18). Such a regime agrees with the experimentally observed [42] 'normal' growth of a fibrin clot. At $K_2 < 5$, zone *A* also includes a part of region *3* containing one more stable fixed point (the bistability zone in the point system). The zero stable point in region *3* corresponds to the 'liquid' state of the blood and the nonzero one to the 'solid' state, i.e. a clot. It is worthwhile to note that the choice of other initial conditions (other activations) leads to the attraction region of the second nonzero fixed point, that is to obtain a trigger wave and transform blood to a coagulated state.

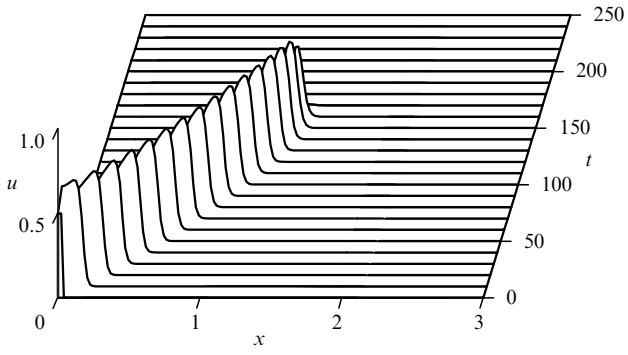

Figure 18. Propagation of a self-sustained wave followed by an abrupt stop in zone *A* ($K_2 = 15.0$, $K_6 = 0.044$, other parameters are given in Table 3).

Zone B. In the upper part of zone *B*, a 'classical' (constant-velocity, constant-amplitude) self-sustained impulse propagates. Here, the thrombin wave travels an infinitely long distance without attenuation simulating a continuously growing clot. Such a situation is not infrequent in *in vitro* experiments when a fibrin clot fills the entire space. Zone *B* includes upper parts of regions *0* and *1* and almost the entire region *2* adjoining its right boundary at $K_2 > 5$. At $K_2 < 7$, the solution depends in a complex manner on the initial conditions; simultaneously, the transition to turbulent regimes occurs. Also, propagation of a pulsed-amplitude impulse is observed in zone B, along with the formation of the 'stratified' thrombi mentioned above and confirmed in experiment [42]. There are several scenarios for the formation of such thrombi (Fig. 19).

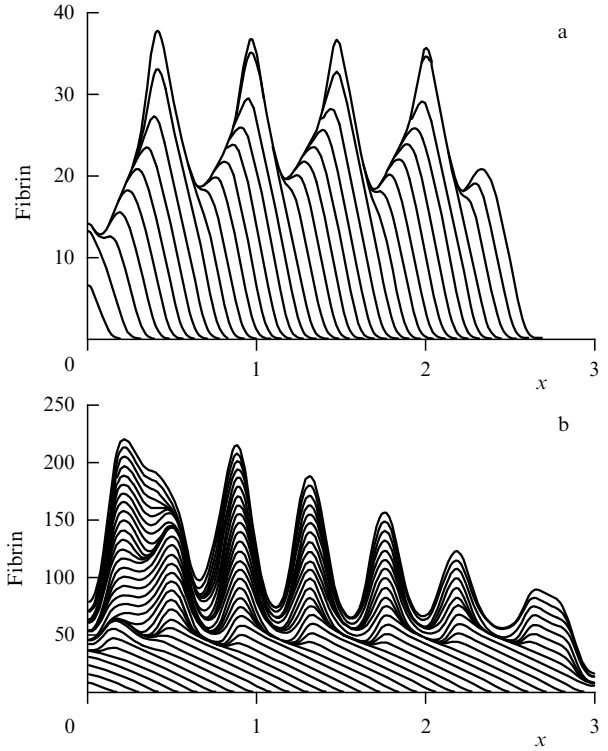

Figure 19. Two different scenarios of the formation of the 'stratified' thrombi predicted by model (7). Fibrin is represented as an integrated thrombin value, in analogy to the phenomenological model (6) ($\mathrm{d}F/\mathrm{d}t = u$). (a) Computation at constant values $K_2 = 7$, $K_6 = 0.075$; other constants are given in Table 3; consecutive ring formation. A thrombin self-sustained wave travels to the right of the activation boundary and splits; the backward impulse contributes to the formation of the next ring before it decays. (b) Computation at constant values $K_2 = 8.2$, $K_6 = 0.077$; other constants are given in Table 3. The traveling wave leaves behind a pulsed space of active factor concentrations, and ring formation occurs simultaneously over the entire length of the developing fibrin clot. Higher fibrin concentrations are achieved than in (a) during the same time; this results in a denser thrombus. All the remaining parameters (segment length equalling 3, interprofile time intervals equalling 20, and initial activation on the left border) are similar in both cases.

Generally speaking, the lower part of zone *B* should have been divided by more boundaries not shown in Fig. 17. This is a zone of chaotic behavior on the whole. Strictly speaking, the pulsed impulse propagation regime should not be regarded as chaotic since it is a boundary regime. Also, wave-splitting regimes are feasible in zone B, with a running impulse breaking into two; the pair of impulses thus formed breaks in turn into two more pairs, etc.; as a result, the entire space turns out to be chaotically filled with traveling, standing, and oscillating structures. The dynamic patterns of such a system in this zone are poorly known.

Zone C trigger wave propagates. The left boundary of this zone almost exactly coincides with the Andronov – Hopf bifurcation line. It should be recalled that zone *C* in a point system is a bistable zone characterized by the presence of two stable fixed points, zero (corresponding to 'liquid' blood) and nonzero (corresponding to 'solid' fibrin clot). Above a certain activation threshold, the entire space of the system is filled with thrombin.

Zone D is a small area between zone *A* where the initial perturbation decays and zone *B* where self-sustained impulses propagate. In zone *D*, an impulse traveling from the activation region does not dissipate after a stop but undergoes stabilization and exists for an infinitely long time. Figure 20a illustrates the transition of the system to such a spatially nonuniform stationary solution. It also presents a thrombin spatial distribution profile (Fig. 20b). Of special interest is the structure with all three variables of the model. Figure 20b, where the distribution of corresponding variables is normalized to their maximum values shows that thrombin (u) has the narrowest profile (spatial distribution). The inhibitor shows the broadest distribution; it bounds the structure and prevents the spread of thrombin. It is worthwhile to note that this structure forms in the case of equality of the diffusion coefficients of all three variables of the model. Such a mode of formation of solitary structures (peaks) in 'reaction-diffusion'-type systems has not been previously described.

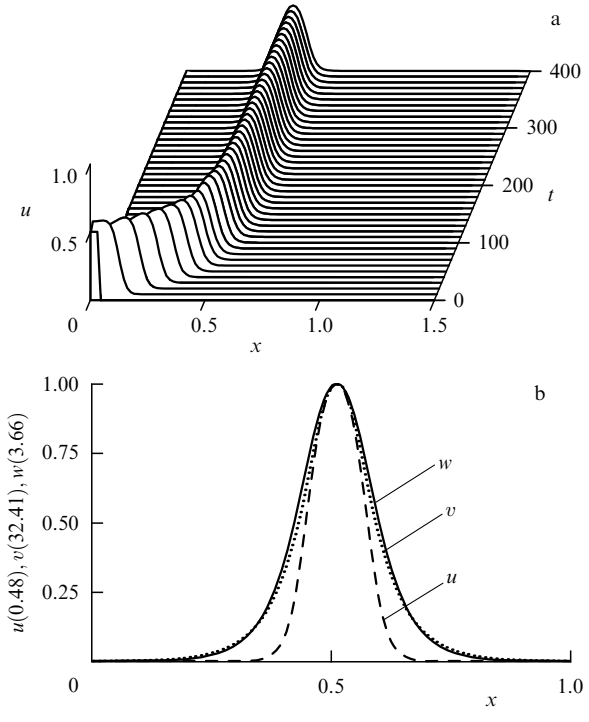

Figure 20. Formation of a standing impulse of active clotting factors at a distance from the activation boundary in zone *D* ($K_2 = 11.0$, $K_6 = 0.062$, other parameters are presented in Table 3); (a) impulse formation; (b) distributions of all three variables of the model corresponding to the stationary impulse and normalized to their maximum values (the maximum of each variable is given in brackets).

6. Conclusion

Based on theoretical and experimental studies of spatial dynamics of blood coagulation, we arrived at the conclusion that blood may be regarded as an active medium. It is however an unusual active medium where the excitation propagates over a finite distance and retains many features of traditional self-sustained waves. In the context of the science of coagulation, it means that the blood clotting process consists of three distinct phases; initiation, elongation (growth), and termination of clot development. Each phase is dominated by a different set of biochemical reactions. Moreover, the phases are space and time-specific occurring in different parts of the clot and changing each other.

Initiation of coagulation. The process may start by means of the intrinsic or extrinsic pathway. All initiation reactions occur at the injured or foreign surface or in close proximity to it. As a result, thrombin in the procoagulant state accumulates in a high concentration near the surface.

Clot elongation. High thrombin concentration at the surface activates intrinsic pathway factors regardless of the way of initiation of coagulation. In fact, the intrinsic pathway is not so much the mode of activation as the mode of generation of a thrombin self-sustained wave to support the growth phase independently of activation signals. This pathway determines the clot growth rate.

Stopping and termination of clot development. This third phase depends on reactions responsible for the stop of a thrombin self-sustained wave. We believe that the key role is played by a so far unknown product of some reaction in the negative feedback loop via protein C. This product transforms one form of thrombin to the other and thus catalyzes self-acceleration of thrombin inactivation and the arrest of coagulation. The size of the clot is a function of the kinetic parameters of this process; it is practically unrelated to the nature and strength of the activation signal. This phase is very poorly understood and remains to be clarified. It is described on the assumption of unknown factors or reactions.

The above hypothesis was used to explain a number of puzzling phenomena.

To begin with, the function of intrinsic pathway reactions in the coagulation process consists of clot elongation. This offers an immediate solution to the puzzle of hemophilias. In accordance with the canonical viewpoint, blood coagulation should not suffer in subjects deficient in clotting factors VIII or IX because its activation induced by a vessels damage occurs via the 'extrinsic' pathway; it triggers the cascade starting from factor X. Such patients have an intact extrinsic pathway and may be expected to form a normal clot. By contrast, we think that hemophilia is a thrombus growth defect rather than an abnormal activation. While the latter remains normal, clotting is compromised and the thrombus does not grow for the lack of the self-supporting process for which 'intrinsic' pathway factors are indispensable. This accounts for the lack of adequate clot formation that does not proceed farther than a thin film at the wound surface

which fails to prevent bleeding completely. An experimental study of the spatial dynamics of coagulation in patients with hemophilia is currently underway in our laboratory. The preliminary results indicate that the clot elongation phase is indeed compromised in these patients. The clot practically stops growing after the process is normally activated.

Secondly, the hypothesis provides a physiological explanation of the existence of two different thrombin states.

Thirdly, examination of coagulation models suggests regimes in which the normal development of the thrombus is disturbed. Two such regimes appear to be of special interest. One of them has been described in the phenomenological model (6) [44] as a continuous clot growth characterized by marked variations of the thrombin wave amplitude accounting for the 'layered' structure of the resultant clot. This regime appears to develop in serious pathological cases leading to the so-called disseminated intravascular coagulation syndrome manifested as numerous small thrombi developing in patients with quite different and unrelated diseases. It is a very dangerous clinical condition in which normal regulatory mechanisms of blood circulation are overwhelmed rapidly leading to a fatal outcome. This syndrome is known to occur in patients with cancer, renal insufficiency, infection, any form of shock or other diseases.

Studies of the spatial dynamics of blood coagulation have provided important new knowledge of this interesting but very specific process; furthermore, they have resulted in the discovery of a previously unknown type of general dynamic behavior. It appears that the spatial dynamics of three-component reaction-diffusion systems may bring to light as many unexpected facts as the dynamics of three ordinary differential equations revealed in its time. In this context, the following is worthy of note. Having to do with an active medium in which the behavior of the system at a point is described by two differential equations, it is possible to predict spatial events from the phase portrait (homogeneous behavior) of the system. If an impulse is observed in the homogeneous case, in the spatial case this impulse will be most likely running at a constant velocity over an infinite distance or, alternatively, it will decay if the diffusion coefficient it too large. In the case of two stationary states, i.e. bistability, a trigger wave will be traveling. Such a situation is impossible in the general case of three differential equations. In certain regions of a parametric space, the spatial dynamics of such system can be predicted from its homogeneous behavior. However, predictions become impracticable under more complex regimes. Let us consider a stationary impulse regime. In a homogeneous system an impulse is generated; in a parametric space, a large region corresponds to this regime. Is it possible to predict, from the 'point' system behavior, the fate of the initial perturbation in this or that region of the parametric space? Indeed, it may either decay after covering a certain distance, stop and give rise to a stationary structure, or travel infinitely far. We can not answer this question, nor can we even say whether there is a solution in the form of stationary structures in this system. The system's behavior at a point is practically identical under each of the three regimes.

It has long been known that the transition from two ordinary differential equations to three implies an important qualitative leap. Specifically, the dynamic behavior of a fully determined system of three ordinary differential equations may be chaotic. The discovery of dynamic chaos in the second half of the 20th century revolutionized the theory of dynamic systems and substantially modified our basic views of the nature of things by confusing random and deterministic phenomena. The existence of totally new regular regimes of distribution and self-organization in three-component systems proved a complete surprise. This discovery leads far beyond the blood coagulation problem. We believe that further studies on three-component system dynamics will bring many new and unexpected facts to different fields of natural science.

The authors are deeply grateful to E E Shnol', G T Guriya, and A I Lobanov for helpful discussions. Thanks are also due to O L Morozova for her assistance in computations, and M V Ovanesov and N G Korotina who provided experimental materials. This work was partly supported by the Russian Fund for Basic Research (No 00-04-48855).

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