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SPATIAL ASPECTS OF THE DYNAMICS OF BLOOD CLOTTING — I. HYPOTHESIS*

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A hypothesis on the autowave mechanism of blood clotting is proposed and analysed. It is assumed that the growth of the thrombus is ensured by the propagation in the blood of a concentration thrombin autowave. In the zone behind the moving front of this wave conditions are created for the generation of a further autowave — wave of inhibition of clotting. The latter, moving quicker than the first at a certain distance from the site of damage, overtakes the thrombin wave, stopping further clotting. The hypothesis agrees with the existing data on the biochemistry of the coagulation system. The possible ways of its experimental verification are analysed.

Blood is a fluid. Therefore, the main task of the clotting system is to protect the blood-carrying system of the body from haemorrhaging. Any damage to the blood-carrying system must be rapidly and effectively removed. For this a clot forms about the site of damage. A small volume of blood around the damaged site rapidly and compactly passes to the solid state. The conditions for this process set a number of requirements of a general nature on the space–time dynamics of the coagulation system.

1. The clot must be solid.
2. Passage from the liquid to the solid state must occur sufficiently rapidly.
3. The clot formed must be localized about the region of damage and have a clearly contoured separation boundary from the blood.

The first two requirements are best met by bistable (trigger) dynamic systems (in the form of systems with phase transition). Such systems have two stable states. Passage from one state to the other occurs in a threshold manner, that is, with change in the parameter the system will remain in the initial state until a threshold value is reached. Then the system very rapidly passes to the new state.

The third requirement implies that factors of activation, on the one hand, must rapidly spread over the whole region of space which enters into the clot and, on the other, their concentration outside this region must be minimal. Since no special mechanisms or systems are known for the transfer of active factors in space the simple assumption of passive

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diffusion propagation of these substances suggests itself. However, this assumption runs into serious difficulties:

1. Increase through diffusion in the concentration of the active factors close to the damaged site is impeded both by their dilution on diffusion into the blood and the presence of blood flow. The most serious restriction for rise in the concentration of active factors through simple diffusion is their washing out by the blood flow. In fact, the characteristic diffusion rates of proteins are of the order 0.05 mm/min [1] and the blood flow speeds in the capillaries 1–10 mm/min [2].

2. The distribution profile in space in the concentration of the diffusing substance has no clearly defined boundaries. Because of dilution the concentration monotonically drops on moving away from the source (Fig. 1*a*). This complicates the clear localization of the boundary of the thrombus.

The question arises as to how to increase the rate of rise in the concentration of a factor at a certain distance from the site of damage. Within an essentially diffusional mechanism, this might be achieved by increasing the spatial concentration gradient. Locally, a large gradient may be set up through the rapid production in the zone of damage of a large amount of activator. However, passive diffusion in the surrounding space has the result that, with increase in the distance from the source, the gradient will rapidly drop (Fig. 1*a*). The speed of propagation of the activator will drop just as quickly.

Propagation of the activator would be quite different if a rise in its concentration at each point above a certain threshold value were accompanied by its explosive autocatalytic production at this site. In this case the system considered might be included in the category of so-called excitable (active) media [3]. In the latter, as is known, large concentration gradients may propagate in space at a constant speed.

In such media, propagation of concentration self-sustained non-linear waves — autowaves — is possible. In particular, it is well known that autowaves also exist in bistable systems [3, 4]. In this case the autowave appears as propagation in space of a wave of abrupt transition from one state to another (trigger wave). Figure 1*b* presents a typical concentration profile of the trigger autowave.

The autowave distribution of the activator of the coagulation system would have an obvious advantage over purely diffusional distribution. In fact, the speed of movement of the front of the autowave is determined by the steepness of this front and may exceed by orders of magnitude the speed of diffusion.

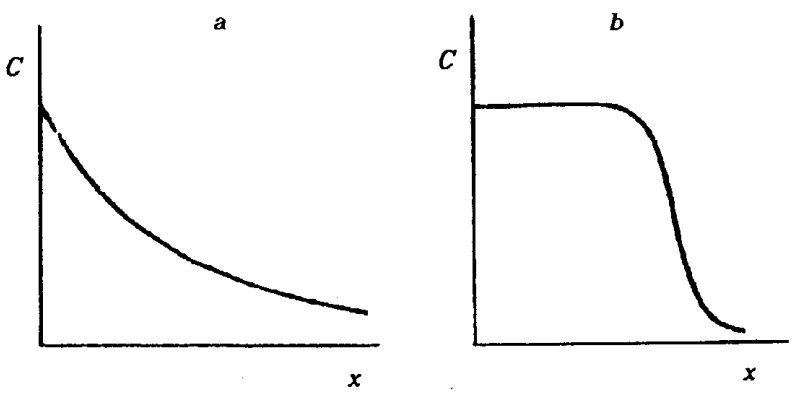

Fig. 1. Distribution of the concentration of the substance (C) along the spatial coordinate (x) on diffusional (a) and autowave (b) propagation.

The amplitude of the autowave does not change in the course of propagation. The absence of attenuation of the autowaves in active media is due to the fact that in them diffusional blurring of the wave front is compensated by production of a spreading substance at each point of the medium. A key role is usually played here by autocatalytic reactions. Diffusion of the substance from the neighbouring region of space leads to activation of its autocatalytic production at this point. In such a system the stable initial state may exist only in the presence of an excitation threshold. The kinetics of the autowave system in the absence of diffusion (total mixing) closely depends on the level of activation and has the form depicted in Fig. 2 [5]. Subthreshold excitation dissipates (curve *1*) and above threshold exponentially grows (curve *2*).

This makes it clear that simultaneously all the *a priori* demands on the space–time organization of the blood-clotting process are met by active media. Below we show that the current biochemical data on the kinetic arrangement of the clotting system allow the blood to be regarded as a kind of active medium.

Activation of blood clotting, as is known [6], leads to the release of a cascade of enzymatic reactions at the site of damage. Figure 3 shows the kinetics of formation of thrombin, a factor directly catalysing the appearance of fibrin on activation of the inner (Fig. 3*a*) [7] and outer (Fig. 3*b*) [8] pathways of the clotting system. In both cases typical autocatalytic kinetics is present.

Another important condition is the presence of the excitation threshold. In [9, 10], it was shown theoretically that the clotting system may behave in a threshold manner. This assumption was confirmed by us experimentally in [7].

From all this it may be assumed that the clotting system (and together with it also the blood) is an active medium in which, in principle, a sustained thrombin autowave might spread.

The hypothesis on the autowave nature of the propagation of thrombin runs up against an obvious difficulty. In fact, such a wave when triggered would stop only on reaching the natural boundaries of the medium. This means that local excess of the threshold of activation of the clotting system would automatically lead to blood coagulation in the whole blood-carrying system. However, growth of the real thrombus in the blood is a self-limiting process. To describe this property the mechanism of arrest of the thrombin wave at a finite distance from the zone of damage is necessary.

One may imagine several ways of arresting the coagulation wave. For example, in a medium behind the front of the thrombin autowave may be triggered a second autowave —

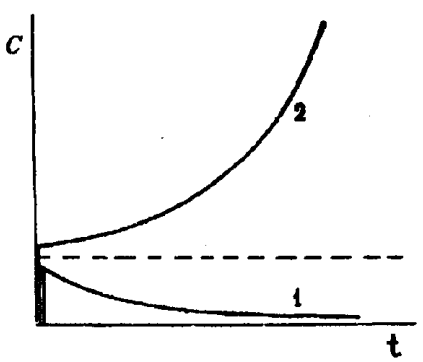

Fig. 2. Kinetics of rise in the concentration of thrombin on subthreshold (*1*) and above-threshold (*2*) activation of the clotting system. Broken line shows the threshold value of activation [5].

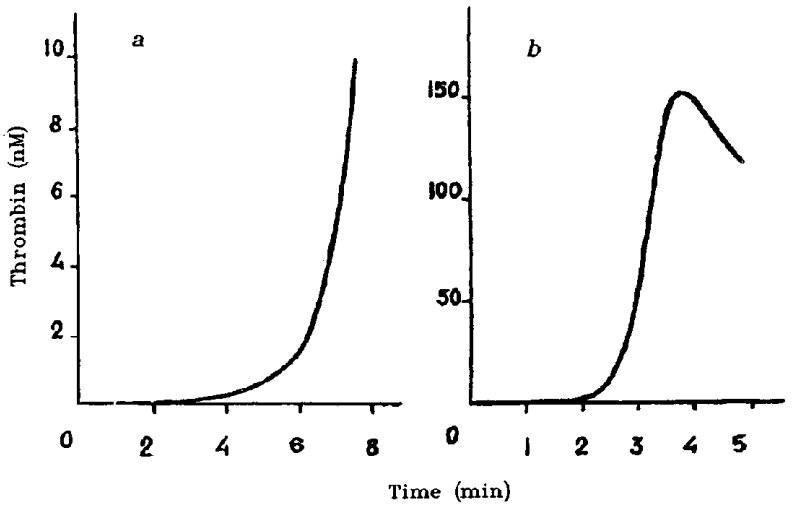

Fig. 3. Kinetics of rise in the concentration of thrombin on activation of the inner (*a*) [7] and outer (*b*) [8] pathways of the clotting system.

the wave of the substance arising after the appearance of thrombin and stopping its production. If the second wave spreads at a speed greater than the first then, later generated in the activation zone, it may catch up and arrest the first wave.

Analysis of the current biochemical data shows that, among the enzymes involved in the clotting process, the main candidate for the role of substance spreading in the second wave is protein C [11]. The process of its activation is triggered by thrombin and it itself effectively stops the formation of thrombin [12].

In principle, other variants of arrest of the thrombin wave are also worth discussing. However, in this paper we shall confine ourselves solely to analysis of the above-outlined mechanism.

To sum up let us formulate a hypothesis to be verified: blood is an active medium of a new type (medium active twice). In this medium, local activation of clotting leads to the generation and spread in space of the concentration thrombin autowave. This wave spreads at speeds far greater than diffusional. In the zone where the wave has passed, conditions appear for the generation and propagation of a second autowave — wave of protein C. The speed of the second wave is greater and, therefore, it catches up with the first wave and stops it at a certain distance from the site of initiation of clotting.

Let us see whether the proposed hypothesis contradicts the known data on the blood-clotting system. Figure 4 shows by continuous arrows the main (basic) scheme of the reactions leading to the formation of thrombin. The homogeneous kinetics (disregarding spatial effects) of activation of the clotting factors using mathematical models was analysed in [9, 13, 14]. And although in these studies only individual portions of the basic scheme were modelled, the possibility of autocatalytic growth in the concentration of thrombin and the presence of threshold behaviour were demonstrated.

Our examination of the models, taking into account the basic scheme as a whole, showed that the biochemical cause of autocatalysis in this system is the presence of positive feedback loops. The latter are ensured by the reactions ($k5$) and ($k8$) of activation of the cofactors y_5 and y_8. These models show that the threshold behaviour is governed by the presence of

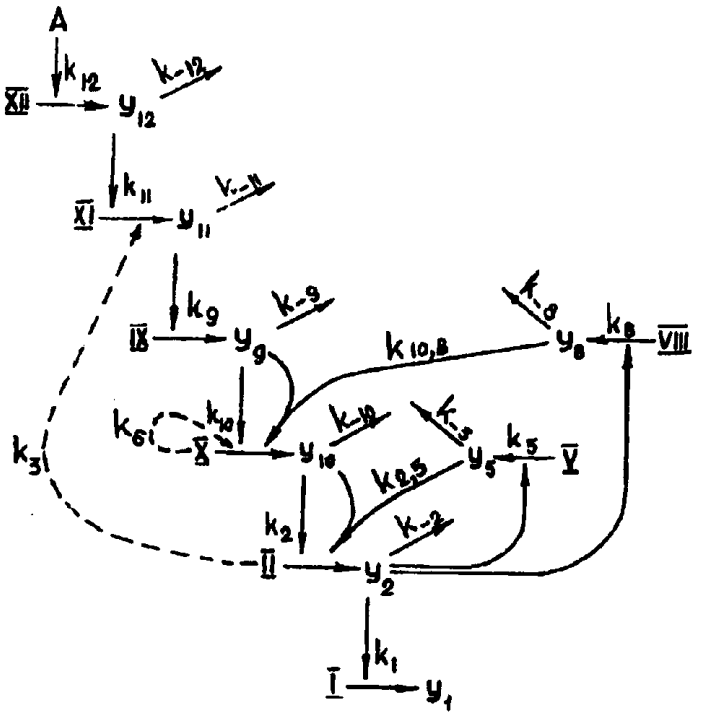

Fig. 4. Scheme of the reactions of the clotting system (explanations in text). Roman numerals denote the inactive forms (precursors) of the corresponding factors of the clotting system: y_i, active forms of the same factors. A, value of contact activation.

reactions involving inhibitors rapidly inactivating the active forms of the factors. In the scheme these are the reactions k-i where $i=2, 9, 10, 11, 12$.

Analysis of the spatial dynamics of the models based on the main scheme in Fig. 4 shows that these models have no solutions of the autowave type. However, if one takes into consideration the presence in the real system of positive feedback loops leading to activation of the main factors, then the autowave regimes of propagation of thrombin prove to be actually possible. The reactions ensuring positive feedbacks of such a type in this system are known and are shown by a broken line in Fig. 4. Reference [15] describes activation of factor XI by thrombin and [16] describes auto-activation of factor X (the reactions $k3$ and $k6$ in Fig. 4). Allowance for any of these reactions makes possible the existence of a genuine thrombin autowave.

The mechanisms of the reactions blocking synthesis of the thrombin wave have received far less attention. It is known that there are inhibitors of the active forms of the clotting factors which are constantly present in the blood [15]. The point was discussed above that they ensure the formation of the excitation threshold. However, such inhibitors are not capable of stopping the active wave because of the uniformity of the distribution in space. If such an inhibitor can quench the wave it will not allow it to be born. However, the blood has a protein which apparently plays an important role in the arrest of the thrombin wave. This is protein C [11, 12]. It effectively destroys the active forms of the cofactors V and VIII thereby stopping the formation of thrombin [11]. It is known that the main activator of protein C is thrombin itself [12]. It is also known that several proteins take part in the activational process [12].

The existence of the thrombin inhibitor activated by thrombin itself and also the complex kinetics of its activation indirectly support the autowave nature of the clotting process. In fact, if the propagation in space of thrombin were stopped simply thanks to rapid drop in the concentration of the active factors on moving away from the zone of damage, there would be no need for the complex biochemistry of the production of the inhibitor. As functionally pointless the block of the reactions of the production of protein C would have been lost in the course of evolution.

For the autowave propagation of the active protein C, it must be assumed that the kinetics of its activation, as such, is of an autocatalytic nature and threshold behaviour is characteristic of it. The existing data do not contradict this but at present there is not enough information of this chain of reactions for a final solution of the problem.

Information on the true mechanism of growth of the thrombus may be obtained in different ways. In particular, much may be gleaned by further study of the kinetics of activation of the clotting system and the mechanisms of its arrest in a homogeneous system *in vitro*. Of special interest is the kinetics of the processes of activation of protein C: do autocatalysis and threshold behaviour operate here? However, the most informative in our view is direct study of the process of clotting *in vitro* without mixing for low or even zero flow speeds.

The process of growth of the thrombus is easy to record optically since the process of polymerization of fibrin is accompanied by rise in light scatter. The presence of chromogenic and fluorescent substrates specifically split by different factors of the clotting system makes one hopeful that the propagation in space of individual factors, primarily thrombin, may also be recorded.

It would be easiest of all to investigate the one-dimensional growth of the thrombus in a small-diameter tube. The complexity of such an experimental design is linked with the small dimensions: according to our estimations growth of the thrombus in tubes of 0.1 mm and less may be considered one-dimensional.

It is far easier to observe the process of propagation of the thrombus boundary in a thin layer. If a solid object, for example, a glass bead or collagen fibre, is introduced into it thoroughly stimulating contact activation, the thrombin wave must begin to spread from it. Covering a certain distance such a wave must stop.

Behind the thrombin wave will move the wave of polymerization of fibrin. The whole volume of space traversed by the wave is filled with the fibrin polymer. The profile of this wave and the dependence of the speed of movement of the thrombin wave front appear most informative for solving the problem of the mechanism of the process.

The greatest interest and indeterminacy are associated with the mechanism of arrest of growth of the thrombus. Therefore, it is interesting to measure the profile and the dependence of the growth rate of the thrombus as a function of the distance from the zone of activation right up to arrest if such will be observed.

From the notions of stability of the process of the arrest of the thrombus boundary it appears that the zone encompassed by the second wave must be larger than the zone of polymerization ringing the region of the thrombus. This means that the second wave catching up with the first still covers a certain distance before it wanes. In this region, clotting cannot be induced. The presence of such zones around the thrombi arrested in growth (or stopped) would appear to be most convincing evidence of the existence of the second wave. Such effects might be recorded by studying the interaction of the clotting waves growing from the nearby centres. If there is no inhibition wave they will always behave as shown in Fig. 5*a*.

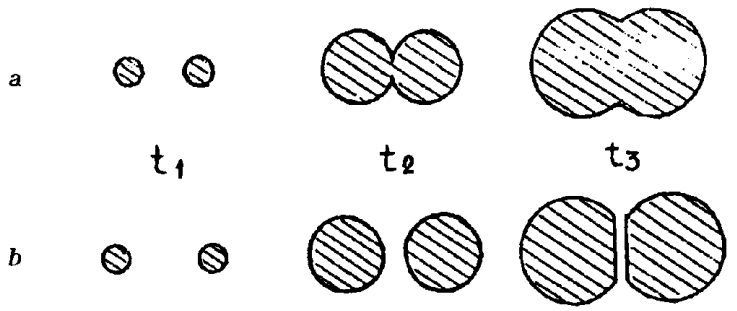

Fig. 5. Interaction of two growing thrombi at different distances between them.

If an inhibition wave exists then in the phase when it catches up with the wave of activation there may appear zones in which clotting is inhibited (Fig. 5*b*). In the case of massive overgrowth these zones must separate from each other the regions of clotting merging at the early stages. The width of these zones must be roughly identical over the whole length.

Detailed investigation of the dynamic effects and spatial structures appearing in the active medium in which the two autowaves — activation and inhibition — may exist was carried out in the second part of this work using a simple phenomenological model.

The results of the experimental study of the processes of thrombus growth *in vitro* in thin layers are outlined in the third part of this communication.

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