THE CHAIN REACTION OF THE BLOOD CLOTTING MECHANISM IN RELATION TO THE THEORY OF HEMOSTASIS AND THROMBOSIS

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By J. H. Milstone, M.D.

THE PHYSIOLOGIC REQUIREMENT

If occasion arose to invent a blood clotting mechanism, it might be arranged for the blood to remain fluid in the vessels, yet promptly congeal when mixed with the juice of freshly cut tissue. This would probably work well for small punctures. However, as this mechanism was tested with larger cuts, an unexpected difficulty might appear. As the blood flowed through the break, a coagulated film would be deposited on the cut surface. This layer would seal over the wounded tissue and hinder the admixture of tissue juice with that portion of blood not yet clotted. Therefore, hemorrhage would continue through a passageway lined by freshly clotted blood.

To overcome this difficulty a chain reaction might be introduced. Then, as one layer of blood was clotting, it would incite the neighboring layer to clot. Thereby the coagulation process could be propagated from one layer to the next, and tissue juice would be needed only to exert its effect on the initial layer. The mechanism could then achieve a hemostatic plug which would grow by accretion, and which, in this respect, would resemble the natural plug depicted by Tocantins.1

Thus, by teleologic conjecture, we have arrived at some function a chain reaction might serve. Other functions can be imagined. Ordinary chemical reactions, be they stoichiometric combinations or enzymatic, begin rapidly and thereafter slow down. But, when a chain reaction is involved, one of the products accelerates the reaction, with the result that as more product is formed the reaction goes increasingly faster.* Hence, chain reactions may start with a lag period, but later are apt to proceed explosively. This offers opportunity for control during the lag period, but ensures rapid action when the lag has been passed or overcome.

THE BIOCHEMICAL MECHANISM

Whatever its prime function, a chain reaction does occur during the coagulation of blood. An experiment in which the clotting process was transmitted through a series of plasma samples was described by Gratia in 1911.2

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* The concept of chain reaction was originally introduced in connection with the photochemical combination of hydrogen and chlorine. It has been broadly applied to various series of consecutive reactions which repeat themselves over and over again. When, in addition, the reaction velocity increases as described by Glasstone (Glasstone, S.: Textbook of Physical Chemistry, ed. 2. New York, D. Van Nostrand Co., Inc., 1946. P. 1083), then the chain is said to be nonstationary. It is this type which is implied throughout the present discussion. Here, the terms 'chain reaction' and 'autocatalytic effect' are used in order to include other possible mechanisms besides a simple autocatalytic reaction; and autocatalytic reactions are regarded as a special group of nonstationary chain reactions.
been initiated in the first tube, each tube of fluid plasma was caused to clot by seeding it with a few drops of serum from the preceding tube. In 1935, Fischer, apparently unaware of Gratia’s previous discussion, reported similar serial passage experiments with minor differences in technic and results. Gratia was impressed by the formal resemblance between the propagation of bacteriophage and the propagation of the clotting process with repeated new formation of thrombin. Fischer wrote of blood coagulation as an endlessly transmissible chain reaction.

Although these demonstrations were spectacular, they were not the first indications of the autocatalytic effect. Beginning in the nineteenth century, two complementary approaches have been made to the analysis of coagulation mechanism: (a) Separation of coagulation factors; (b) separation of individual reactions.

By 1904, this twin approach had developed far enough to engender the classic two-stage theory:

1. Prothrombin $\rightarrow$ Thrombin. In the presence of thrombokinase and calcium.
2. Fibrinogen $\rightarrow$ Fibrin. In the presence of thrombin.

But, beginning before 1904, and continuing into the present, results have been obtained with separated factors and separated reactions which show that the simple two-stage concept must be modified. And all along the line there have been repeated intimations of autocatalysis. Many studies have been made of the rate at which the coagulation products appear. A frequent finding has been a lag period followed by a period of accelerated production—characteristics of a chain reaction. This is illustrated in figure 1, which outlines the events that occur when blood clots in a glass tube. Actually, such a complete experiment has never been
done; and the diagram is a tentative, rough summary of data obtained in various ways.

During the performance of the routine test for clotting time, and when the blood is normal, the impression is gained that very little physical change takes place until the end point is imminent. Then, in a comparatively brief interval, the viscosity rapidly increases and a solid clot forms. This gross impression has been confirmed by finer methods; and the production of fibrin is accordingly represented in the diagram. At the time the solid clot appears, less than half the fibrinogen has been converted to polymerized fibrin. As this production of fibrin continues, the clot becomes firmer. After a half-hour or more, the clot retracts. Then, on standing many hours, the amount of the fibrin diminishes somewhat, as can be determined by weighing. This last effect is due to the action of one or more fibrinolytic enzymes, and represents only a partial development of the fibrinolytic potential of the blood. Normally, the enzyme is kept for the most part in an inactive state. As a result of disease or artificial manipulation, fibrinolytic activity can be developed to a surprising degree. It is a remarkable fact that blood normally contains enough potential fibrinolytic activity to liquefy its own clot. For reasons only partially understood, the fibrinolytic potential is rarely, if ever, developed to the full.

The explanation for the shape of the fibrin curve may be complex in detail, but the main reason for its late start is that, up until that time, there is not enough thrombin to clot the fibrinogen. This delay was reported in 1901 by Arthus, who further noted the accelerated production of thrombin just before the clot appeared. Soon the amount of thrombin dwindles—the antithrombin effect.

Carrying the discussion one step further, the delay in thrombin formation is due to the fact that sufficient thrombokinase activity must first be developed. Reasoning from simple experiments on whole blood, Collingwood and MacMahon concluded in 1912 that a large part of the clotting time was spent in developing thrombokinase from an inactive precursor which they called prothrombokinase. Recently, by separating the coagulation process into three stages, carried out in three successive sets of test tubes, it has been possible to show that thrombokinase activity develops as shown in figure 1. The latent period and the period of acceleration still suggested a chain reaction, an interpretation corroborated by seeding experiments. There was further noted a prompt and rapid loss of thrombokinase activity, another result foreshadowed by the work of Collingwood and MacMahon.

These are not isolated findings. Using different technical approaches, the interpretations of several recent investigators are virtually unanimous on the following broad conclusions: (a) The coagulation mechanism comprises at least three distinct reactions. (b) A chain reaction is involved in the production of a factor that can accelerate the activation of prothrombin.

Contemporary literature, however, shows that these basic findings can be elaborated with greater diversity than might be supposed. The diversity stems partly from the fact that terms like "thrombokinase" and "thromboplastin" are used in different ways and that several new terms have been introduced. More
important is the uncertainty concerning which and how many factors participate in the direct activation of prothrombin. Beyond this is the question of which factors, now thought to activate prothrombin, really do something else, such as accelerate the development of thrombokinase. How many different chain reactions occur? To date no convincing evidence has been presented either for or against the occurrence of a simple autocatalytic reaction (e.g., x accelerates the production of y). Seegers and his associates have brought forth impressive, although not quite conclusive, evidence in favor of a more complicated chain (e.g., x accelerates the production of y, which in turn accelerates the production of z).

It is possible to summarize the present situation so as to emphasize the similarities of individual viewpoints rather than their differences:

1. Prothrombokinase Complex → Thrombokinase Complex. An autocatalytic or chain reaction results in the acceleration of this conversion.

2. Prothrombin → Thrombin. In the presence of the active thrombokinase complex.

3. Fibrinogen → Fibrin. In the presence of thrombin.

This is an oversimplification, attained by neglecting details, and by grouping in the thrombokinase complex all factors, including calcium, which may be found to participate directly in the activation of prothrombin.

For the present discussion there are important differences between this formulation and the old two-stage theory. It may be emphasized that the necessary substances for these three stages of blood coagulation are present in, and obtainable from, the blood. The prothrombokinase complex is demonstrably different from the thrombokinase complex; and the conversion from the inactive form to the active form has been followed experimentally. This conversion is of further significance in that it consumes a large part of the time required for blood to clot in a glass tube. Moreover, the autocatalytic effect is concerned with the development of thrombokinase activity.* As a consequence, a small amount of coagulating blood can, when mixed with unclotted blood, accelerate the first of the three reactions, and get the clotting process off to a good start in the fresh portion of blood.

Thus we have rather detailed biochemical evidence of a chain reaction which can take place entirely within the blood, and which can propagate the clotting process.

* To avoid undue complexity, this discussion does not deal with the possibility that one or more reactions precede the three enumerated, and that the principal locus of the autocatalytic effect might be in one of them, and thence reflected in the development of thrombokinase activity. This would not change the main argument.

Also left for future elucidation is the question whether the prothrombokinase of the blood is in the platelets, the plasma (Lenggenhager 1936, 1940, cited in reference 8), or both. Any decision on this question would leave intact the essential contribution of Collingwood and MacMahon, who brought forth evidence for a prothrombokinase and a three-stage mechanism in 1912. When plasma is obtained with only ordinary precautions, prothrombokinase is found in the plasma euglobulins. Whether platelets contain prothrombokinase is uncertain.
The Role of the Thrombocytes

This advance, although not yet consolidated, is already reaching out to include the thrombocytes. On the experimental side it appears that the chain reaction will proceed in the absence of whole platelets. This does not prove that material derived from platelets is not involved, or that whole platelets are not concerned when they are present. In his review on platelets, Tocantins\textsuperscript{16} states the general impression that "... they will cause changes in the plasma, which in turn will lead neighboring platelets to alter, and so on."

The platelet lysis and fusion observed in coagulating blood are brought about not simply by contact with glass in the presence of calcium, but require particularly the presence of a heat-labile factor found in the serum globulins. The serum factor was demonstrated by Wright and Minot in 1917.\textsuperscript{17} Brinkhous,\textsuperscript{18} on the basis of different considerations, has recognized what may be the same factor, and has suggested the name "thrombocytolysin." How this is related to the other factors is not known; but it gives the means whereby the serum can change the platelets. In turn, the platelet material directly or indirectly exerts a pronounced acceleration on the production of thrombin.\textsuperscript{19, 20}

A reaction series, shuttling between platelets and plasma, could result in continuously renewed metamorphosis of platelets. Thus a white thrombus might be formed in flowing and eddying blood, where fresh plasma and platelets would be supplied continuously to a stationary nidus of metamorphosed platelets. Conceivably this might be independent of the chain concerned in the development of thrombokinase; but there is much to suggest that the two chain effects are intimately related. Exactly how they are related, and whether the platelets help or hinder the chain reaction is not known.

A further complexity cannot be ignored. The hemostatic plug, as well as the white thrombus formed in flowing blood, differ appreciably from the test tube clot, or the red thrombus formed in stagnant blood. The former have a disproportionately high content of platelets, along with some fibrin (sometimes, apparently, very little). Although the fibrin is probably a useful constituent, it is not certain that it is absolutely necessary. Various suggestions that transformed platelets may be sticky, even in the absence of fibrin, need to be corroborated. In this connection, it is curious that patients with "congenital absence of fibrinogen... are less incapacitated by their abnormality than is usually the case with haemophilia."\textsuperscript{21} Phylogenetic comparisons have suggested that alterations of the thrombocytes represent a primitive component of the hemostatic mechanism, upon which fibrin formation has been superimposed. Be that as it may, even if two adhesive materials are used, it does not necessitate two entirely different mechanisms to apply them. And as yet, the facts do not compel us to postulate a separate chain reaction for platelet metamorphosis.

The Problem of Regulation

How the chain reaction starts, or whether it is always in progress and needs only to be brought to an effective intensity or "critical concentration," is still a mystery. We are likewise ignorant of the precise mode of control. It is likely that the control
mechanism exerts not merely a static inhibition, but can also dampen the process when it is already under way. Otherwise, why would not a hemostatic plug or a fresh thrombus continue to grow until it incorporated all the blood in the cardiovascular system?

Several phenomena are known which result in the retardation of the clotting reactions or disposal of their products. Mere dispersion of coagulant products by an active circulation may be of great importance. In the test tube the rapid loss of thrombokinase activity is striking; and here the way is open for its further investigation. If, as Quick and Ware, Murphy and Seegers believe, thrombin is an important link in the chain reaction, then both the antithrombokinase and antithrombin effects offer means for breaking the chain. The fibrinolytic enzyme(s) may accomplish more than is now appreciated. Heparin augments the antithrombin effect, and in some ways delays the activation of prothrombin. Although it is still uncertain whether heparin occurs in significant quantity in normal circulating blood, it appears to reach a highly anticoagulant level after anaphylactic shock and total body irradiation. There have been suggestions that other anticoagulant secretions are discharged steadily or in response to changes in the blood.

Suggestive data on the turnover of platelets, prothrombin and fibrinogen indicate that these factors are completely renewed every few days. It has been inferred that they are consumed in the usual blood clotting reactions, slowly proceeding in the circulation. This implies a degree of dynamic balance to maintain the fluidity of circulating blood, for the converted factors must be removed fast enough to prevent gross coagulation. As illustrated in figure 1, the antithrombokinase and antithrombin effects could take part in this balance. They inactivate coagulant products which have already been formed. Depending on quantitative relations, this kind of action could slow up the clotting process when it was already under way. Such action could help to limit the growth of a hemostatic plug to the requirements of physiologic necessity. The failure, or overpowering, of such action might contribute to the excessive propagation of a thrombus.

It is quite possible that the critical juncture of the entire system is the development of thrombokinase activity. But detailed study of this has just begun.

Relation to Thrombosis

Undoubtedly, there are many who hope that few factors remain to be disclosed. Nevertheless it is clearly necessary to continue a detailed analysis of coagulation if we are to understand why a thrombus starts, how it propagates and what makes it stop. These theoretical questions are fundamental to the prognosis, prophylaxis and therapy of thrombosis. For the present, efforts are being made to estimate the likelihood of thrombosis from tests which attempt to detect a hypercoagulable state in the patient's blood. Modern anticoagulant prophylaxis and therapy are based on the prevailing view that the state of the coagulation system is a significant factor in thrombosis, and coagulation tests are extensively used as guides in the administration of heparin and dicumarol.

For the future this outline has noted only some of the prospects that beckon to the investigator. Among these is the question of the extent to which the chain
reaction furnishes the biochemical basis for the propagation of a thrombus.* In biochemical experiments the chain reaction has long been in evidence. In pathology the formation and propagation of a thrombus has been the subject of classic studies.24 One might speculate what the outcome will be as these data from different disciplines are brought together and extended. The chain reaction is so intimately a part of the coagulation mechanism that it is likely to occur whenever a thrombus forms, unless the individual’s blood is unusual. Consequently, it would usually play some part in the extension of the thrombus. The relative importance of its contribution in the genesis of various types of thrombi remains to be evaluated. There may be some conditions where a thrombus would propagate even if there were no chain reaction; in some circumstances propagation might be impossible without one. Of the latter case, the extension of a mural thrombus far into the chamber of the left ventricle might be an example.

Here the growing surface is far from the injured myocardium; and the ventricular blood could hardly be called stagnant. Is the continued deposition of platelets and fibrin sustained by diffusion of tissue factor through the thrombus? Or does it depend on repeated formation of fresh coagulant substances at the free surface, through operation of the chain reaction? Anticipation of this question is to be found in the statement of Solandt, Nassim and Best25: "It seems reasonable to suppose that, once agglutinating platelets have covered an injured region, the nature or degree of the underlying tissue damage will have little or nothing to do with subsequent growth of the thrombus." The success of their pioneer experiments in suppressing cardiac mural thrombosis by heparinization emphasized anew that the state of the clotting system is very important. This may include the chain reaction, but does not yet single it out as the sine qua non of cardiac mural thrombosis.

SUMMARY: A WORKING HYPOTHESIS

Detailed evidence has been accumulating that at least one chain reaction occurs during the coagulation of blood. Both the metamorphosis of platelets and the development of thrombokinase appear to be involved. The autocatalytic effect may serve a function in making possible the growth of a hemostatic plug. It also offers advantages in the physiologic control of the clotting mechanism. It is likely that the chain reaction occurs in most instances where a thrombus forms, and plays some part in its propagation.

The chain reaction is a potentially explosive phenomenon which demands an adequate countermechanism. With materials derived from blood, reactions have long since been demonstrated which can reduce thrombokinase activity, inactivate thrombin and liquefy fibrin. These reactions may help to maintain the fluidity of the circulating blood by removing the products of smoldering clotting reactions.

* This question has been raised independently in two articles which appeared since the present paper was submitted for publication. It was briefly mentioned by B. Alexander, A. deVries and R. Goldstein: Blood. 4: 739-746, 1949. The idea and a discussion of its implications were presented by J. H. Milstone: J. Insur. Med. 4: 5-7, July 1949. For a step toward the same idea, see reference 15, page 74.
Such effects could help to delimit the growth of a hemostatic plug, or to end the propagation of a thrombus.

While it is now possible to correlate in this way the data on blood coagulation with present knowledge of hemostasis and thrombosis, critical gaps in our understanding still remain.

REFERENCES