Blood Coagulation and the Practical Significance of Recent Advances in Knowledge of Prothrombin and Ac-Globulin

By Walter H. Seegers, Ph.D.

An attempt is made to describe the nature of the chemical interactions participating in blood clotting. In the laboratory, thrombin can be derived directly from prothrombin without the physiologic activators calcium, thromboplastin, Ac-globulin, and platelet derivatives. The newly recognized clotting factor, Ac-globulin, is essential for normal physiologic hemostasis. Plasma Ac-globulin concentration decreases in experimental liver damage, temporarily with dicumarol administration and may increase from high doses of aminophylline. In citrated storage plasma it is stable for a week but not in oxalated human plasma. Compared with other species, human plasma contains a low concentration of Ac-globulin. Methods for Ac-globulin analysis have been developed; perhaps more important is the fact that the two-stage method for prothrombin analysis need no longer be considered a complicated procedure.

It is a simple matter to arouse curiosity concerning the problem of blood coagulation. If one begins a conversation with someone who has had only limited experience in biology or for that matter with any scientific subject, it may be suggested that blood will clot a few minutes after it is shed, and that this must be something very special, because that same blood remained fluid as long as it was contained in the vessels. It is at once evident that this clotting mechanism is a powerful defense against fatal hemorrhage. Furthermore, it is logical that the problem of thrombosis is largely a matter involving the clotting of blood within the vessels themselves. It is more difficult to convey the idea that the problem of blood coagulation encompasses fundamental problems in biology. This broader aspect even now escapes the attention of most scientists. We are dealing with the interactions of marked molecules. Each one of them is marked as distinctively with respect to the other as if it contained a radioactive tracer element. Just as the radioactive materials can be traced through complex metabolic phenomena so the marked protein molecules can be traced through many complex interactions in the metabolic machinery and in the test tube. By understanding the nature of protein reactions, we get closer and closer to understanding the nature of living processes. And we can think of the complex act of blood coagulation as being an operation of life.

I would like to be as practical as possible in this discussion. First, an attempt will be made to describe in the simplest manner possible the nature of the chemical interactions which participate in blood clotting. This is appropriate because a clear concept cannot be obtained from the literature. Second, a new clotting factor, accelerator globulin, has been recognized. In order to understand the relationship of this factor to prothrombin it is of prime importance to know certain basic facts about prothrombin itself. For that reason some of the new information on prothrombin will be described. Third, important information on Ac-globulin is already available and the following topics can be discussed briefly: (a) Ac-globulin changes in storage plasma, (b) effect of experimental liver damage, (c) Ac-globulin concentration in human plasma as compared with other species, (d) changes produced by aminophylline, and (e) the effect of dicumarol administration.

Figure 1 symbolizes my first assignment; namely, to present rapidly our current knowledge of the fundamental nature of blood coagulation. It requires a brief summarization of the main information in approximately 10,000 pa-
pers. In a large proportion of these papers the conclusions are pushed beyond the just limits of the data. The result is great confusion, and, for want of principles to direct his choice, even the critical reader can adopt mistaken viewpoints. Eventually the true pattern must become clear and it seems likely that the main paths are already well outlined.

It is advantageous to consider the blood clotting mechanism as though two mechanisms had been provided: one involving the interaction of the proteins which are concerned with clot formation; and, second, a mechanism provided for the removal of a clot. The latter mechanism is not primarily an inhibitor of clot formation but can be regarded as a separate system. On the basis of that general viewpoint the schematic presentation of the clotting mechanism of figure 1 was arranged.

Prothrombin is constantly present in the blood, provided there is an adequate amount of vitamin K available for its synthesis by the liver. Thrombin is derived from the prothrombin and it is the substance which reacts with the fibrinogen of the plasma to form fibrin, which is the clot.

From purification work we know that prothrombin is a glycoprotein. Its normal concentration is about 15 to 20 mg. per 100 cc. of plasma. This means that the average individual on dicumarol therapy manages to get along with approximately 20 mg. of prothrombin. Purified material has powerful clotting activity when fully activated. In fact, a milligram is almost sufficient to clot 2.0 liters of standardized fibrinogen solution in fifteen seconds.

In the clotting of fibrinogen by thrombin, calcium tends to have a favorable influence but is not essential. A variety of artificial sub-

![Diagram of blood clotting mechanisms](image)
needed and although potent concentrates of thromboplastin can be derived from almost any tissue the exact chemical nature of the reactive groups in thromboplastin is still not known. Lately there has been some interest in thromboplastin as related to toxemia of pregnancy. There is evidence to indicate that it gets into the maternal blood circulation and is in part responsible for toxemia of pregnancy. Actual proof is lacking but the possibility is so real that the problem is being studied thoroughly.

Accelerator globulin is a plasma protein which is essential for the rapid activation of prothrombin. A deficiency of this globulin may result in a bleeding tendency. Dr. Owren, in Norway, described the deficiency of his patient, a 29 year old woman. At the age of 3½ years she had a severe nose bleed which stopped spontaneously in a few hours. During adolescence she had frequent nosebleeds. There was difficulty with bleeding during menstruation. At the age of 29 years the clotting time by capillary method was fifteen minutes, and in test tubes twenty-five minutes. Clot retraction was normal. The prothrombin time was seventy to eighty seconds. Vitamin K was of no value, and it could be shown that this patient was not deficient in prothrombin. More will be said about accelerator globulin later.

The platelets furnish material which participates in the activation of prothrombin but contrary to the view held for many years they very likely do not furnish very much thromboplastin activity. The activity which they supply is more nearly like that supplied by Ac-globulin. In other words, it is material which acts in conjunction with thromboplastin.

It has been indicated in figure 1 that there are other factors which participate in the activation of prothrombin. Some of these are as yet not well characterized. It is known that the deficiency in hemophilia is due to a lack of essential material required for the activation of prothrombin. Probably the hemophilic lacks a plasma factor which acts in conjunction with platelets to furnish the equivalent of thromboplastin activity.

Calcium, thromboplastin, Ac-globulin, platelet derivatives and other factors all contribute to the activation of prothrombin, and under appropriate conditions they are capable of being prevented from doing so by a number of inhibitors of prothrombin activation. The best known inhibitor is heparin. From experiments involving total body irradiation it seems probable that substances quite similar to heparin may also exist. Another inhibitor has recently been recognized and is referred to by the term "antithromboplastin." Whether it acts specifically against thromboplastin remains to be proved. All that is known is that it inhibits the first phase activation process. Purification work done in two different laboratories shows that this inhibitor may be a specific kind of lipid. Dr. Tocantins in Philadelphia and Dr. Overman and Dr. Wright in New York have independently obtained materials possessing interesting physiologic properties, and Dr. Tocantins believes that this inhibitor is one of the important factors in hemophilia. It seems likely that this substance may be a useful anticoagulant.

It has long been known that plasma will inactivate thrombin. This is the antithrombic activity of plasma. Our recent studies of the problem indicate that there is only one antithrombin and that its activity is not influenced by heparin. It is capable of destroying large quantities of thrombin. An effect which has probably been mistaken for antithrombic activity of plasma can be ascribed to an odd phenomenon which has to do with adsorption. For example, when thrombin clots fibrinogen, much of the thrombin is physically adsorbed on the clot. The amount of thrombin adsorbed is proportional to the concentration of the thrombin used for producing the clot. Furthermore, the amount adsorbed will be increased if heparin and crystalline albumin are in the reaction mixture. Heparin alone or crystalline albumin alone are not sufficient. The two must be present simultaneously. The supposed antithrombin effect of heparin is concerned with thrombin adsorption and interference with the thrombin-fibrinogen reaction. It is not properly regarded as an antithrombin effect because the adsorbed thrombin can be recovered by dissolving the clot with fibrinolysin.

There is also a mechanism which is concerned with the lysis of a clot and this mechanism
bears a striking resemblance to the clotting system. A plasma substance called profibrinolysin becomes activated to fibrinolysin and this enzyme can dissolve a fibrin clot.

Like prothrombin this profibrinolysin must first be activated and an activator has been discovered in many tissues and is referred to by the term "fibrinokinase." So far it has not been possible to make potent preparations of this activator, but artificial substances have been employed. For example, profibrinolysin is activated by shaking with chloroform. It can also be activated by an enzyme found in certain bacteria. For example, staphylokinase or streptokinase are potent activators. When bacteria cause fibrin to lyse it is thought to be the result of their capacity to activate plasma profibrinolysin.

Presumably there are also inhibitors of profibrinolysin but so far none have been described in the literature.

Fibrinolysin can be inactivated by plasma antifibrinolysin. This antifibrinolysin of plasma is a powerful inhibitor found in the plasma of almost all species. Antifibrinolysin concentration in plasma is considerably increased in folic acid deficiency. It is also increased in concentration in a variety of pathologic states; for example, pernicious anemia, cirrhosis of the liver, coronary thrombosis, pneumonia, and intestinal obstruction.

A review of the two summaries in figure 1 shows that the two systems bear a striking resemblance to each other. This outline may constitute oversimplification, but it has the advantage of being easy to comprehend and is easily remembered. Further details can be developed as subtopics of these main fundamentals.

It can be seen that prothrombin occupies a central and dominant position in the clotting mechanism. It is indicated on the outline of figure 1 that thrombin can be derived directly from prothrombin; and that calcium, thromboplastin, Ac-globulin, platelet derivatives and other factors are accessory factors. The inhibitors are also accessory factors and may counterbalance or overpower the activators.

How do we know that this view is correct? It is generally conceded that the answer to such a question can be supplied only by working with purified clotting factors. There are altogether too many variables when all of them are present and acting simultaneously, and being measured by only one indicator; namely, the rate of clot formation.

There are two main philosophies or approaches to the problem of plasma fractionation. One maintains that plasma should be fractionated systematically into its several components and when more and more subfractions are made we will eventually obtain all of the plasma entities in purified form and in substantial yield. There are several fallacies in this philosophy which make it untenable at times. For example, many prothrombin fractions have been described. Some were products of systematic fractionation procedures, but the yield and degree of purification was unsatisfactory. The difficulty arises in that a reagent which may not be damaging to albumin or some other component may be very damaging to prothrombin. Another consideration which has a bearing on the problem is the relative concentration of the several proteins. The concentration of albumin in the blood is roughly 2 per cent whereas the concentration of prothrombin is less than 0.01 per cent. The concentration of prothrombin is thus a special purification problem.

The single objective route is the other approach to the problem of plasma fractionation. One centers all attention on one of the plasma constituents and plans all kinds of fractionation procedures with the aim of getting that component in good yield and purity. It gives plenty of opportunity for trial-and-error probing. This approach may be regarded as the poor man's approach and is the more common way in which plasma proteins are obtained for scientific study. In this way we have been able to obtain prothrombin in good yield and a reasonable degree of purity, but only after ten years of work. That may be stated in another way. About 1,800 fractionation procedures were tried before satisfactory products were obtained.

It has been possible to do a number of experiments with the purified material. One is of special importance. When the purified prothrombin is dissolved in a 30 per cent solution
of sodium citrate it becomes activated to thrombin. It is essential that sodium citrate be in high concentration although some activation takes place in 5, 10, and 15 per cent solutions.

The curve in figure 2 represents the thrombin activity found when purified prothrombin was activated with 30 per cent sodium citrate solution. Thrombin began to appear at the end of three hours. At the end of five hours the concentration began to increase rapidly. This rapid increase continued through the next four hours. Thereafter thrombin production was slow again. The curve, as drawn, represents the mathematical curve for autocatalysis and it can be seen that the experimental points fall reasonably well on the curve. There are

many references in the literature to autocatalytic formation of thrombin but I believe this is the first unequivocal demonstration of such a phenomenon. To obtain additional evidence of autocatalysis, thrombin was added to the prothrombin solution at various times during and before activation had begun. Such additions of thrombin always caused thrombin to form more rapidly.

Now we can go back for a brief review. It was indicated that prothrombin occupies a dominant position in the clotting mechanism. The outline of the clotting mechanism indicates that thrombin can be derived directly from prothrombin, and that calcium, thromboplastin, Ac-globulin, platelet derivatives, and other factors are catalysts of prothrombin activation. It was asked, how do we know that this is correct? The answer has been supplied. Prothrombin has been isolated. Evidence for purity has been obtained. The prothrombin was activated autocatalytically in concentrated sodium citrate solution, thus showing that prothrombin itself contains all the structural material needed for thrombin and nothing needs to be added by the activators of prothrombin.

In figure 2 it can be seen that it took about sixteen hours for the activation of the prothrombin. Obviously this is far too slow to be of any value for avoiding a fatal hemorrhage. What is required for rapid activation of prothrombin? It has been shown that calcium and thromboplastin are not sufficient. Another substance is essential; namely, plasma accelerator globulin. It functions as a co-factor of thromboplastin, so that calcium, thromboplastin, and Ac-globulin together will activate purified prothrombin rapidly.

Ac-globulin is a normal plasma constituent and has already been obtained in concentrated form. It is less soluble in ammonium sulfate solution than prothrombin. It is more sensitive to acids and alkali than prothrombin. It is not adsorbed on barium carbonate whereas prothrombin is. Like prothrombin it is found in plasma in trace quantities.

Its exact position in the clotting mechanism has not been established as securely as it eventually must be. A number of sound experiments and lines of evidence indicate that the following diagram may represent how Ac-globulin participates in prothrombin activation.

prothrombin + thromboplastin + calcium ^+ + platelet accelerator \rightarrow thrombin

Plasma Ac-globulin \rightarrow thrombin \rightarrow serum Ac-globulin

prothrombin + thromboplastin + calcium ^+ + platelet accelerator \rightarrow thrombin

Fibrinogen \rightarrow thrombin \rightarrow fibrin

First, a small amount of prothrombin is activated by calcium, platelet accelerator, and thromboplastin. The small amount of thrombin formed can then activate plasma accelerator globulin. Then the interaction of prothrombin, thromboplastin, calcium, platelet accelerator, and active accelerator globulin is rapid. Throm-
bin is thus formed rapidly after a slow beginning.

Quantitative methods have been developed for measuring its activity in plasma. We believe that these methods are reliable and give a reliable measure of Ac-globulin activity. These methods have been applied to the following studies: (a) Ac-globulin concentration in storage plasma. (b) Ac-globulin concentration in experimental liver damage, (c) Ac-globulin concentration in the plasma of various species, (d) Ac-globulin concentration during aminophylline administration, and (e) Ac-globulin concentration during dicumarol therapy. Simultaneously, the prothrombin concentration was measured and it was possible to get an indication of the variation of both of these plasma constituents in the same experiment. The finding in these experiments will be reviewed briefly.

In stored citrated human plasma the prothrombin concentration remains normal for long periods of time (fig. 3). A number of years ago there was much discussion about the disappearance of prothrombin in stored plasma. At that time we were shipping plasma by surface carrier and using the plasma for the industrial production of thrombin for use as a hemostatic agent. According to the literature of that period we should never have been able to obtain any thrombin from such plasma.

Ac-globulin is also stable for a period of about a week, but thereafter its activity begins to disappear fairly rapidly. It is an interesting fact that Ac-globulin is not stable in oxalated human plasma. As a simple guide to the physician it is satisfactory to rely on storage plasma as a source of Ac-globulin if the plasma is not unduly old. There are many interesting problems regarding Ac-globulin stability which, however, have a primary bearing on research work.

In experimental liver damage both the prothrombin concentration and Ac-globulin concentration drop. In this experiment with dogs given deep chloroform anesthesia, the activity of both plasma factors decreased. The Ac-globulin recovery was perhaps a bit more rapid than that of the prothrombin. We do not know that an acute experiment of this kind on animals can be taken as any indication as to what would happen in chronic diseases in man, but presumably one could expect a drop in Ac-globulin concentration. We believe Ac-globulin and Owren's Factor V to be one and the same substance, and Dr. Owren has reported that
in Ac-globulin might not be of considerable consequence. The difficulty, of course, resides in the fact that the significance of the prothrombin/Ac-globulin ratio is not understood as yet.

Some time ago it was reported by Link and his associates that the methyl xanthines, including aminophylline, can alter the extent and duration of hypoprothrombinemia caused by dicumarol. It was their view that the methyl xanthines, including aminophylline, are capable of producing a hyperprothrombinemia. Their original work became involved in contradictions. Being in the possession of a method which would measure both prothrombin concentration and Ac-globulin concentration quantitatively, we were prompted to do some experiments with aminophylline. In the experimental result shown by figure 5 it is seen that aminophylline administered in the amount of 100 mg. per kilogram of body weight in the dog caused a very prompt increase in Ac-globulin concentration. There was also a temporary increase in prothrombin concentration. Later this tended to drop somewhat below normal and definitely returned to normal in thirty days. The high Ac-globulin concentration persisted for awhile and then declined to normal over a period of about thirty days. This experiment has been repeated in several animals with the same results. One thing which was most impressive about these experiments was the high dose required to obtain the response. This work was done on animals and further information is needed on the effect which may be produced in human beings. Preliminary indications come from the work of Dr. John Olwin of Chicago who has commonly found it to be the case that far more dicumarol is required when aminophylline is also given. His work shows this to be true in about one-half of the human patients on which he has data.

The administration of dicumarol may also effect the plasma Ac-globulin concentration. In experiments performed with dogs, prothrombin concentration decreased in a manner typical of many experiments recorded in the literature. The Ac-globulin concentration also decreased, but only slightly. When the prothrombin began to reappear in the plasma the Ac-globulin concentration not only returned to normal levels but in addition it increased above normal for a period of time.

There was also opportunity to study human patients on dicumarol therapy. This was in cooperation with Dr. John Olwin of Chicago. As in the experiments on dogs, it was found that Ac-globulin may decrease when the drug is first given. When the maintenance dose is being administered this does not further depress the Ac-globulin concentration. On the contrary, the Ac-globulin concentration returns to normal while the prothrombin is being maintained at the desired low levels.

Ac-globulin concentration may quite definitely be an important factor in dicumarol therapy. There is an interesting theoretic speculation in regard to the safety of the drug. If Ac-globulin concentration does not decrease appreciably but the prothrombin concentration drops to a low level, the Ac-globulin may compensate for a low prothrombin level. This implies that when the physician progressively lowers the prothrombin by administering dicumarol the Ac-globulin becomes a safety factor which acts as a buffer, causing the remaining prothrombin to be utilized very efficiently. That may be premature speculation; but it is logical, and, I hope, a comforting thought.

It must be evident that methods which are capable of giving reliable quantitative values for prothrombin concentration and reliable values for plasma Ac-globulin concentration are practically essential in the research labora-

![Graph](image-url)
tory, and perhaps no one would dispute their desirability for general use. At the present time it must be admitted that the analysis for Ac-globulin activity which was used in the experiments just described is somewhat cumbersome.

What is the status with respect to prothrombin analysis? The two-stage procedure for prothrombin has long had the reputation of being impractical for general use. Often reviews will contain a statement such as the following: “Practically all investigators agree that the two-stage is much more laborious than the one-stage procedure. This precludes its routine use in most clinical laboratories.” Such statements are becoming obsolete. First, the two-stage procedure for prothrombin analysis is a quantitative analysis for prothrombin whereas the one-stage procedure is not. It measures a variety of factors. Second, an effort has been made to simplify the two-stage method and to make the required reagents generally available. There has been considerable success in that connection. A detailed description of the two-stage method, recently published (Am. J. Clin. Path. 19: 41, 1949), gives sufficient information for anyone to master the technic. Furthermore, the necessary reagents are being made available commercially. As a consequence, the clinical laboratory has at its disposal a very excellent method for measuring quantitatively the prothrombin concentration of plasma.

SUMMARY

The main points of this discussion can be reviewed briefly. It is advantageous to consider blood clotting as though two mechanisms had been provided, one for clot formation and one for clot removal. Prothrombin is the substance which must be activated to form thrombin. There are many activators of prothrombin and their action may be counterbalanced by inhibitors. One of the activators is Ac-globulin, which is a plasma protein. It acts as a co-factor of thromboplastin and calcium. This newly recognized clotting factor must be considered in methods for prothrombin analysis. Relative to other species, man possesses a low concentration of Ac-globulin. It decreases in experimental liver damage, also temporarily in dicumarol therapy, and slowly in citrated human plasma after ten days of storage. With large doses of aminophylline the plasma concentrations may double.

Physicians are constantly expected to prognosticate. In contrast, this is not required of those who work in the laboratory. We are supposed to describe our experiments and be careful not to push conclusions beyond the just limits of the data. Now and then it is permissible to extrapolate. On that ground I am departing from data, but only for a moment.

Rapid progress has been made in our theoretical knowledge of the blood-clotting mechanism. Progress has been made in the control of hemorrhage, and in the control and prevention of thrombosis. There must be further endeavor not only to add to this knowledge but also to apply it effectively. One person has the following to say, “The truth is that fruitful progress occurs only when a large number of people are thinking together about the same sort of thing.”

It is my understanding that this is the first time someone who is not himself a physician has presented the George Brown Memorial Lecture. I am very grateful for that, and if it was a bit abstract in spots, won’t you please recall this sentence: Fruitful progress occurs only when all sorts of people are thinking together about the same sort of thing.
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