Conversion Factors and Accelerators in the Formation of Thrombin

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Even to the reader intimately acquainted with the field, several of the recent developments in the study of the mechanism by which blood clots have often proved extremely puzzling. The number of communications which have appeared on the subject, especially during the past ten years, is indeed amazing, and their quantity alone may well preclude the possibility of fair critical appraisal.

More than any other subject, the mechanisms by which prothrombin is converted to thrombin have become the center of conflicting interpretations. In his classical hypothesis of the coagulation of blood, Morawitz considered that the formation of thrombin was due simply to the interaction of prothrombin, thrombokinase (thromboplastin) and calcium. The results of investigations published during the last decade have complicated this rather simple scheme considerably and, in turn, have vindicated several forgotten observations of the past. Other factors, besides those considered by Morawitz, have been demonstrated either to take part in the conversion of prothrombin to thrombin, or to accelerate the progress of this reaction. These agents, described under different names, have been found in plasma, serum and platelets.

Prothrombin and fibrinogen should be considered the fundamental materials for the formation of fibrin. Prothrombin is converted to thrombin and this enzyme, in turn, clots fibrinogen to fibrin. At least five other agents, however, have a role in the formation of thrombin. They may be divided into two groups: a) conversion factors, i.e., those which appear indispensable for the reactions leading to the formation of thrombin, and in which they participate according to definite quantitative proportions; b) accelerators, i.e., those which simply accelerate the formation of thrombin, but do not appear indispensable for the formation of this agent. The conversion factors include thromboplastin, calcium and another agent (labile factor of Quick, factor V of Owren, plasma Ac-globulin of Ware and Seegers) present in plasma; the accelerators include two agents present in serum and platelets respectively. It should not be forgotten, however, that other agents of less specific nature may actively convert prothrombin to thrombin in plasma. Thus foreign surfaces, such as glass, by lysing platelets, and therefore determining the formation of thromboplastin, cause the conversion of prothrombin to thrombin; and thrombin itself, as will be discussed later, accelerates its own formation powerfully.

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Prior to the conversion of prothrombin to thrombin, other reactions which have only recently been investigated probably take place. They involve thromboplastin and calcium, two agents long known to be active in the conversion of prothrombin to thrombin. Calcium is apparently active only if first combined with some other coagulation factor, probably labile factor or plasma prothrombin conversion factor. Thromboplastin, present as such in the tissues, is not to be found in any appreciable amount in the platelets or free in the circulation, but apparently is formed during a preliminary step in the coagulation process from the interaction of an agent supplied by the platelets when lysed and another agent present in plasma. The first factor is probably of enzymatic nature and has been defined by Quick as thromboplastinogenase; the second, defined by Quick as thromboplastinogen, may be identical with the plasma fraction known as antihemophilic globulin and also described by Laki as "plasma kinin."

Until recently, Brinkhous had maintained a slightly different point of view stating that plasma supplied only a factor necessary for the lysis of platelets (thrombocytolysin). Other possibilities have also been mentioned by Ferguson who postulated that a thromboplastic enzyme is present in plasma which in some way mobilizes thromboplastin material; MacFarlane has suggested that a thromboplastic factor in platelets might be potentiated by a plasma enzyme. All these hypotheses are of great importance because they establish platelet lysis

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Diagram 1—Mechanism of the coagulation of blood according to Quick.

1. Antihemophilic globulin (?); “Plasma kinin, 7” (?)
2. Thrombokinase
3. Plasma prothrombin conversion factor, factor V, plasma Ac-globulin, etc.

* In this, as well as in the following diagrams, physiologic anti-coagulants and fibrinolytic mechanism are omitted for simplicity.
as the first step in the coagulation of blood (diagram 1). The role of this mechanism in initiating the coagulation of blood both in vivo and vitro seems reasonable, since aggregation and lysis of platelets can easily follow the injury of the vessel wall and the contact of blood with foreign surfaces. Quick's concept has also proved of value in the understanding of the clotting defect underlying hemophilia\(^5\) and thrombocytopenic purpura.\(^6\)

Mention has already been made of both the recent recognition of a previously unknown conversion factor in plasma and of two accelerator agents in serum and platelets respectively. The present communication is directed mostly at the illustration and the discussion of the importance and mechanism of action of these agents in the coagulation of blood. Throughout the course of this review the definition of “plasma prothrombin conversion factor (PPCF)” will refer to the agent demonstrable in plasma and that of “platelet accelerator” and of “serum accelerator” to the agents present in platelets and serum respectively. The fundamental difference in the physiologic action between the plasma and the platelet and serum agents has previously been mentioned.

The designation of the plasma factor as “plasma prothrombin conversion factor (PPCF)” was selected in preference to those previously adopted in the literature since all of them appeared unsatisfactory. To mention only those most commonly used, “labile factor” stresses only one characteristic of an agent which is quite variable from species to species, and which depends to some extent on the technic of preservation of the blood. “Factor V” does not specify or indicate any special characteristics or the mechanism of action of the agent. The definition of “plasma Ac-globulin” cannot be accepted at present until more definite evidence is given that the agent possesses only accelerator activity. Although it adds a new term to the already encumbered literature, “plasma prothrombin conversion factor” indicates mainly the fundamental mechanism of activity of the agent and should be compatible with all the different concepts as to the nature of this factor.

**Plasma prothrombin conversion factor (PPCF)**

PPCF was described by Nolf\(^10\) under the name of “thrombogène” as an agent necessary for the formation of thrombin. Quick\(^1\) described such a factor as “component A of prothrombin” and later renamed it “labile factor.”\(^12\) Independently, Owren\(^13,\)\(^1\) described a similar agent (“factor V”) and presented a patient in whom its deficiency was responsible for severe hemorrhagic symptoms.\(^14\) Agents capable of influencing the conversion of prothrombin to thrombin were later described by Fantl and Nance (“accelerator factor”);\(^15\) Honorato (“co-factor of thromboplastin”);\(^16\) Munro and Munro;\(^17\) Ware, Guest and Seegers (“plasma Ac-globulin”);\(^18,\)\(^1\) and others.

It may now be stated without too much fear of contradiction that all these different factors share similar general properties and are probably identical. Most of the discrepancies as found in the earlier literature on this subject are probably due to incomplete characterization and to different degrees of purification of the various factors, as well as to different methods for their assay and determination. The plasma prothrombin conversion factor is less resistant than
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prothrombin to heat and chemical agents; it is characteristically labile during storage, especially in decalcified plasma; it is not adsorbed on BaCO₃, BaSO₄ and Ca₃(PO₄)₂ gel. The inability of Ca₃(PO₄)₂ gel and BaSO₄ to adsorb the plasma prothrombin conversion factor while avidly adsorbing prothrombin (provided definite proportions between adsorbant and plasma are maintained) is the basis of a simple procedure for the separation of the two agents.¹⁹ Many biochemical properties of the plasma prothrombin conversion factor have furthermore been described by Owren²² and by Ware and Seegers,²³ who have obtained purified preparations. The “prothrombinokinase” described by Milstone²⁴ shares most of the properties of the plasma prothrombin conversion factor.

The mechanism by which PPCF participates in the formation of thrombin is the subject of much discussion. The main controversy centers on the question as to whether this agent acts simply as a catalyst in the formation of thrombin or is an essential participant in the conversion of prothrombin, reacting with this factor and the others (calcium, thromboplastin) involved in definite quantitative proportions. What is said here about PPCF also closely applies to calcium and thromboplastin. From the physiologic standpoint there is no doubt that adequate amounts of plasma prothrombin conversion factor must be available if thrombin is to be formed in the concentration and at the speed required to maintain an effective hemostatic mechanism. When the plasma prothrombin conversion factor is depleted, the formation of thrombin from prothrombin becomes inadequate; the plasma prothrombin activity then becomes strikingly affected,²⁵ the hemostatic process is abnormal and a serious hemorrhagic condition may develop.¹⁴ On the other hand, it is known that thrombin may evolve from purified prothrombin even in the absence of calcium, thromboplastin and PPCF.²⁶⁻²⁸

Thus Seegers has shown that purified prothrombin in 25 per cent solution of sodium citrate will be slowly activated to thrombin. Logically enough, he concludes that prothrombin contains all the materials necessary for the formation of thrombin and that thromboplastin, calcium and PPCF should therefore be considered as catalytic agents. Little consideration, however, seems to be given to the fact that the self-activation of prothrombin is not equally effective at lower concentrations of sodium citrate. Moreover, the possible role of minute traces of contaminating substances²⁶, † and that of protein-bound calcium should be kept in mind. Finally, equally strong experimental evidence supports the contention that PPCF is indispensable for the formation of thrombin and

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* The prothrombin adsorbed may be subsequently eluted from the various adsorbents (but not from Al(OH)₃ and Mg(OH)₂ gels) by means of sodium citrate.² This observation has been the basis of a simple procedure for the preparation of concentrates of prothrombin²⁹ and has also been utilized in a one-stage technic for the quantitative determination of plasma prothrombin.³¹

† As very little thrombin is necessary to activate PPCF to serum accelerator,²¹, ²² which greatly accelerates the production of thrombin, a large amount of thrombin may, under certain experimental conditions (absence of antithrombin) be formed with very little PPCF. In such a situation the mechanism of action of PPCF could be erroneously considered of catalytic nature.
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reacts with prothrombin, thromboplastin and calcium in definite quantitative relationships. Owren\(^2\) and Lewis and Ferguson\(^3\) agree that thrombin cannot be formed in the total absence of PPCF. Quick and Stefanini\(^3\) demonstrate that a direct quantitative relationship exists between amount of PPCF ("labile factor") present and the yield of thrombin from a definite amount of prothrombin. Stefanini and Crosby\(^2\) and Alexander et al.\(^3\) give evidence that plasma prothrombin conversion factor is consumed during the formation of thrombin; the significance of this observation is, however, dubious as it is known that PPCF might be the precursor of serum accelerator\(^4,5\) and is consumed while accelerator appears in the serum.\(^5\) Its utilization during the formation of thrombin could, therefore, be equally explained by the reaction of the factor with prothrombin, thromboplastin and calcium to form thrombin, as well as by its conversion to serum accelerator. It is evident that the problem of the role of PPCF during the coagulation of blood is far from settled, but the significant point may be made that in vivo at least, the presence of this factor in adequate amounts is indispensable for the adequate formation of thrombin from prothrombin.

Platelet accelerator

An accelerator of the conversion of prothrombin to thrombin has been found in variously prepared platelet extracts. A factor with this function was first described and obtained as a thermostable impure lipid material (cytozime) by Bordet.\(^4\) An agent with similar activity has been reported to be present in saline extracts of platelets by Mann et al.\(^3\) and by Ware, Fahey and Seegers.\(^3\) The "platelet accelerator" has chemical characteristics clearly distinguishable from those of serum accelerator, but apparently the two agents have a similar mechanism of action. Both lose their activity when heated at 60 C. for thirty minutes or when stored for twenty-four hours; both increase the prothrombin activity of stored plasma.

Serum accelerator

The existence of a serum accelerator capable of hastening the formation of thrombin was also described early in the century by Bordet (propriété excito-productrice de la thrombine du sérum).\(^5\) The findings of this author, one of the men who contributed most with his highly original observations to our present knowledge of blood coagulation, have been extended by Owren\(^5\) and by Ware and Seegers\(^5\) who described serum accelerator agents as factor VI and serum Ac-globulin, respectively. These factors are admittedly identical. More recently, Alexander et al.\(^6\) have described as "serum prothrombin conversion accelerator (spca)" an agent present in serum which is also capable of accelerating the conversion of prothrombin to thrombin. They believe, however, that this agent is somewhat different from serum Ac-globulin, which they claim represents a more impure material consisting of a mixture of PPCF and serum accelerator.\(^6\) Labile factor (PPCF) would be necessary for the activity of spca. The agent described by Jacox\(^7\) under the name of "prothrombin conversion factor" requires further evaluation, since its properties and mechanism of action are different from those
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of any known agents and might well represent a new system necessary for the conversion of prothrombin to thrombin. At the present time, not enough data are available to judge the nature and the significance in the coagulation of blood of such rather poorly characterized factors as the heat stable plasma co-thromboplastin and the inactive prothrombin.

The function of the accelerators in the formation of thrombin has not been sufficiently investigated. They apparently require the presence of calcium and thromplastin for their action. The liberation of platelet accelerator probably

- DIAGRAM 2—Mechanism of blood coagulation according to Owren.

  **Step 1**  
  Factor V(1) + Prothrombin* (?)+ Ca + Thrombokinase (2) → factor VI(3)

  **Step 2**  
  Prothrombin → Factor VI + Ca → Thrombin

  **Step 3**  
  Fibrinogen → Thrombin → Fibrin

  (1) Plasma prothrombin conversion factor; labile factor; plasma Ac-globulin, etc.
  (2) Thromboplastin
  (3) Serum accelerator; serum Ac-globulin

  *Present in Owren's original paper. The author is doubtful regarding the role of prothrombin in this step.

- DIAGRAM 3—Mechanism of blood coagulation according to Ware and Seegers.

  **Step 1**  
  Prothrombin + Thromboplastin (1) → Platelet accelerator → Ca++ → Thrombin

  **Step 2**  
  Plasma Ac-globulin (2) → Thrombin → Serum Ac-globulin (3)

  **Step 3**  
  Prothrombin + Thromboplastin → Ca++ → Platelet accelerator → Thrombin

  **Step 4**  
  Fibrinogen → Thrombin → Fibrin

  (1) Thrombokinase
  (2) Plasma prothrombin conversion factor; labile factor; factor V, etc.
  (3) Serum accelerator, factor VI

follows the agglutination and lysis of platelets which sets up the mechanism of blood coagulation. On the other hand, the serum accelerator evolves only after the coagulation process is under way, and strongly suggestive evidence has accumulated that plasma contains a precursor of serum accelerator. This could be represented by PPCF. Owren is inclined to think that factor V (PPCF) reacts with prothrombin (?), thromboplastin and calcium to form factor VI (serum accelerator). This, in turn, would react with prothrombin to form thrombin in the presence of calcium (diagram 2). Ware and Seegers believe that minute amounts of thrombin develop from the reaction of prothrombin, thromboplastin and
calcium, and induce the activation of plasma Ac-globulin (PPCF) to serum Ac-globulin (serum accelerator). This agent, reacting with thromboplastin, calcium and prothrombin would determine an accelerated formation of thrombin (diagram 3). That PPCF is converted into a powerful serum accelerator is also admitted by Carter and Warner. These authors advance the interesting speculation that the action of thrombin is simply that of eliminating fibrinogen, which would possess a protective action on the PPCF. Alexander et al. also claim that their “spea” evolves from a plasmatic precursor which remains to be identified.

The discovery of the conversion factor and of the accelerators of the formation of thrombin has had a considerable influence on the theory and understanding of blood coagulation. As a result, important information has been added to the pathogenetic mechanism of hemorrhagic diseases and thrombotic tendencies, and to the mechanism of action of anticoagulants. The advances of the past five years have also greatly elucidated a problem which had puzzled many investigators since Arthus. The problem is the autocatalytic mechanism of the coagulation of blood. It is common observation that blood from a healthy individual shed in a glass test tube will remain fluid for several minutes (“lag period”) and then quickly begin to solidify and form a massive clot in a matter of seconds. It is logical to assume that the lag period represents the time during which sufficient thrombin is formed to clot fibrinogen. From the moment in which thrombin first appears, this enzyme is produced in ever increasing quantities and speed. The mechanism of this effect has been the subject of much speculation. Accumulating evidence has been brought forward to indicate that thrombin itself is capable of accelerating its own production, probably by at least two mechanisms. Much indirect evidence, and more recently some direct evidence, has been collected to show that thrombin can labilize platelets, and by liberating their enzymatic products, cause increased formation of thromboplastin, which, in turn, results in the increased formation of thrombin. On the other hand, many experimental results suggest that thrombin itself may convert PPCF or other plasma precursors into serum accelerator, thus greatly enhancing its own production from prothrombin. With these facts in mind a hypothesis of the mechanism of the coagulation of blood is presented in diagram 4, which is a modification of a schema previously published.

A more detailed account of the autocatalytic mechanisms in the coagulation of blood will be found in a classical presentation by Milstone (“The chain reaction of the blood clotting mechanism in relation to the theory of hemostasis and thrombosis”). In this article are discussed the historical aspects, the experimental evidence, and the bearing of the autocatalytic mechanism of blood coagulation on the pathogenesis of hemorrhagic diseases and of thrombotic tendencies.

This short review is presented with considerable trepidation and in the hope that this will help the uninitiated reader in the understanding of a highly controversial field. We have presented the available facts and have interpreted them
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Diagram 4—Hypothetical representation of the mechanism of coagulation of blood.*

Only the positive forces are considered, and both the anticoagulants physiologically present and the fibrinolytic mechanism are not included.

**SLOW PHASE**

1. Thromboplastinogen (Plasma) (1) + (?)
   Thromboplastinogenase (Platelets) → Thromboplastin (2)

2. Prothrombin + Thromboplastin + Calcium + Plasma Prothrombin Conversion Factor (3) → Thrombin

**ACCELERATED PHASE**

3. Plasma Prothrombin Conversion Factor
   (or other plasmatic precursor)
   → Thrombin → Serum Accelerator (4)

4. Prothrombin + Thromboplastin + Calcium → Thrombin

5. Fibrinogen + Thrombin
   → Platelet Factor (?) → Fibrin

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(1) Antihemophilic globulin (?); Plasma kinin (?)
(2) Thrombokinase
(3) Thrombogène[^11]; component A of prothrombin[^11]; factor V[^12]; labile factor[^13]; accelerator factor[^14]; co-factor of thromboplastin[^14]; plasma Ac-globulin[^15]; etc.
(4) Factor VI[^16]; serum Ac-globulin[^16]; prothrombin conversion factor[^16], possibly, serum prothrombin conversion accelerator[^16]

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* The blood coagulation process in this hypothetical representation is divided into two major phases, slow and accelerated. The first phase includes the series of reactions which take place from the moment when the blood is shed to the moment when the first thrombin is formed. In a first step, active thromboplastin is formed by the interaction of a plasmatic and platelet factor; in a second, thromboplastin, calcium, prothrombin and plasma prothrombin conversion factor react together to form thrombin, probably in definite quantitative proportions. Once thrombin has been formed, the accelerated phase of blood coagulation sets in. Thrombin autocatalytically excites its own production, by labilizing the platelets and, therefore producing more thromboplastin (see broken arrow). It also converts the plasma prothrombin conversion factor or, perhaps, another plasmatic precursor into a serum accelerator. This is capable of greatly accelerating the conversion of prothrombin to thrombin, in the presence of calcium and thromboplastin. An accelerator supplied by the platelets also enhances the production of thrombin at this point. Once enough thrombin has been formed, fibrinogen is quickly clotted. A factor, also supplied by the platelets, seems to cooperate in accelerating this reaction.

mostly in the light of their physiologic significance, which is uppermost in the mind of the clinician. No complete agreement and approval are expected, since it is realized fully how difficult it is to remain objective in the presentation of a subject which seethes with so many often violently expressed concepts. It is also
realized that many omissions have occurred. They are due to the great difficulty in fully acknowledging the tremendously large number of contributions of the past decade, and not to personal inclination or choice.

Summary

Prothrombin and fibrinogen represent the fundamental materials in the formation of the solid clot. During the process of blood coagulation, prothrombin is converted to thrombin, and this enzyme, in turn, clots fibrinogen.

The formation of thrombin is the essential step in the clotting of blood. Besides prothrombin, at least five other agents participate in the reaction. They may be divided into: a) conversion factors, agents actively participating and indispensable for the formation of thrombin, at least for physiologic needs; and b) accelerators, which increase the speed of the conversion of prothrombin to thrombin and the yield of this enzyme once the process has been initiated. The first group includes thromboplastin, calcium and a plasma prothrombin conversion factor (PPCF); the second, a serum accelerator and a platelet accelerator. Platelets may also supply a factor which accelerates the formation of fibrin from fibrinogen.

Thrombin, once formed, is able to accelerate its own production (autocatalysis), thus determining an “explosive” formation of fibrin. This mechanism is of great importance in assuring efficient hemostasis. Adsorption of the thrombin on the fibrin clot and neutralization of the enzyme by the natural plasma antithrombin when it is slowly released during the retraction of the clot are two known mechanisms by which the organism protects itself from any undue extension of the intravascular thrombus.

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