

NEWER CONCEPTS OF BLOOD COAGULATION

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THE fundamental concepts of blood coagulation formulated almost 50 years ago by Morawitz¹ have not changed significantly. The theory is simple but fails to explain three important features. These are the mode of action of the various substances involved in the clotting process, the origin of thromboplastin, and the function of platelets. During the past decade knowledge pertaining to the mechanism of blood coagulation has become increasingly complex.

Theory of Morawitz

- I. Calcium + thromboplastin + prothrombin $\longrightarrow$ thrombin
- II. Thrombin + fibrinogen $\longrightarrow$ fibrin

It now appears that prothrombin is converted to thrombin with the aid of conversion and accelerator factors from plasma and platelets, and that the formation of fibrin is enhanced by an accelerator from platelets.

Origin of Thromboplastin and the Function of Platelets

Morawitz's theory presumes the liberation of free thromboplastin as a preliminary step in clotting. Quick^{2,3} states that thromboplastin still remains an undefined entity. Free thromboplastin is present in tissues. In plasma it exists as a precursor which is activated by an enzyme derived from platelets. This plasma precursor, thromboplastinogen, may be identical with the plasma fraction known as antihemophilic globulin which is lacking in hemophilia. Following the lysis of platelets, an enzyme called thromboplastinogenase is liberated, which activates thromboplastinogen in the plasma to thromboplastin. Thromboplastin then acts stoichiometrically with prothrombin complex to form thrombin. Most investigators agree that thromboplastin is of at least two varieties. One is a slower acting thermostable, fat soluble phospholipid and the other a thermolabile lipoprotein. Both have special functional differences which tend to supplement each other, resulting in a synergistic effect on the thromboplastic mechanism.

Fantl and Nance⁴ and others^{5,6} have reported an accelerator factor derived from platelet extract which hastens the formation of prothrombin to thrombin. The mechanism of action is similar to serum accelerator which is described subsequently, but the chemical characteristics of each are distinguishable.

Another platelet accelerator apparently enhances the reaction between fibrinogen and thrombin in the formation of fibrin as reported by Ware, Fahey and Seegers.⁶

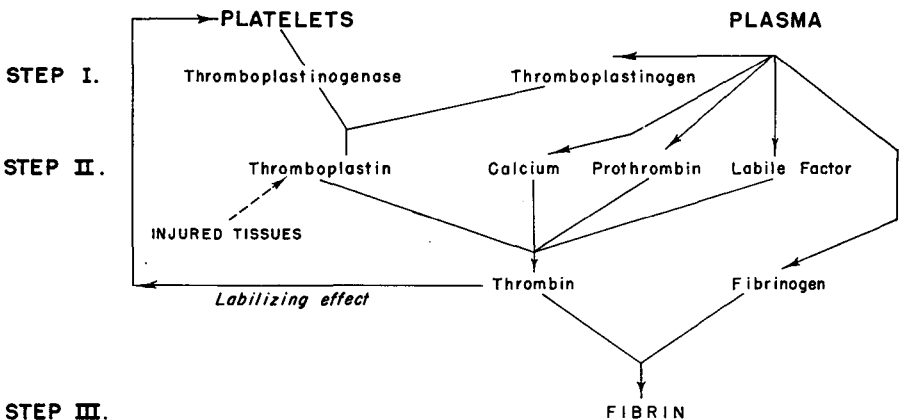
A serum vasoconstrictor agent which aids in the mechanism of hemostasis

appears to arise from platelet lysis and has been named serotonin by Rapport, Green and Page.⁷

Prothrombin Conversion and Accelerator Factors

A plasma factor responsible for variable prothrombic convertibility is not a new concept. In 1908 Nolf⁸ described a substance necessary for the formation of thrombin which he called thrombogene. Smith^{9,10,11} suggested this type of mechanism to explain deviations between the two stage and one stage tests for prothrombin. In 1943 Quick¹² proposed that prothrombin was a three component complex consisting of prothrombin A, prothrombin B and calcium. In 1947 Quick's¹³ theory was changed and the term labile factor was substituted for prothrombin A which is the substance that disappears from stored oxalated blood. Prothrombin in human plasma (stable factor) exists partly free and partly in a precursor state according to Quick and Stefanini.¹⁴ The factor responsible for activation to free prothrombin has not been identified. Calcium is apparently activated when it combines with labile factor and appears to react stoichiometrically in the formation of thrombin.

MECHANISM OF COAGULATION OF BLOOD ACCORDING TO QUICK¹⁵



In 1944 Owren¹⁶ described a patient with severe bleeding, long prothrombin times and clotting times, yet with no apparent deficiency of prothrombin. In 1947 he¹⁷ presented a classical discussion of the background for his concept of a new pair of clotting factors and was the first to show that an inactive factor becomes changed to another in the process of clotting. Factor V is described as the inactive plasma precursor of Factor VI. Its effect is to hasten the conversion of prothrombin to thrombin; it is normal in hemophilia and has no effect on the second stage of clotting. In Owren's¹⁷ original paper, he suggests that Factor V reacts with prothrombin, thromboplastin and calcium

to form Factor VI which then reacts with prothrombin in the presence of calcium to form thrombin.

MECHANISM OF BLOOD COAGULATION ACCORDING TO OWREN¹⁷

STEP I. Factor V + Prothrombin ? $\xrightarrow{Ca + Thromboplastin}$ Factor VI

STEP II. Prothrombin $\xrightarrow{Factor VI + Ca}$ Thrombin

STEP III. Fibrinogen $\xrightarrow{Thrombin}$ Fibrin

Stefanini doubts the role of prothrombin in Step I.

In 1947 Ware, Guest and Seegers^{18,19} segregated a globulin from prothrombin by differential ammonium sulfate solubility which hastened the conversion to prothrombin to thrombin and which, if sufficiently lacking, prevented the full conversion of prothrombin. The substance, believed to be a proenzyme, was designated prothrombin activator, hence the term Ac-globulin, which is probably inactive and requires only thrombin in low concentration for activation to serum Ac-globulin which acts as a catalyst in the formation of thrombin.

Ware, Fahey and Seegers⁶ have confirmed the finding that platelet extract contains only a small amount of thromboplastin and that a substance is present which hastens the conversion of prothrombin to thrombin. This substance is called platelet accelerator. A second substance was found by these investigators which hastens the formation of fibrin from thrombin and fibrinogen and is designated platelet Factor II. Although this factor shows only moderate activity, failure to recognize its existence has probably resulted in faulty interpretation of experimental results.

Platelet accelerator requires no further activation and becomes effective when platelets rupture. It serves primarily to catalyze the initial formation of thrombin. Plasma Ac-globulin is activated by the first small amount of thrombin to serum Ac-globulin which becomes the main accelerator resulting in the rapid formation of thrombin. (Refer to next chart.)

Stefanini²¹ has recently proposed a mechanism of blood coagulation in which he divides the reactions into slow and accelerated phases, thereby taking into account the common observation that normal blood shed in a glass tube remains fluid for several minutes and then quickly begins to solidify into a clot. The first phase includes a series of reactions until thrombin is formed. Once thrombin begins to form, the accelerated phase of blood coagulation begins. Thrombin autocatalytically excites its own production by labilizing more platelets resulting in the formation of more thromboplastin. Thrombin

METHOD OF BLOOD COAGULATION ACCORDING TO WARE AND SEEGRS²⁰

STEP I. Prothrombin + Thromboplastin $\xrightarrow[\text{platelet accelerator}]{Ca^{++}}$ Thrombin

STEP II. Plasma Ac-globulin $\longrightarrow$ Serum Ac-globulin

STEP III. Prothrombin + Thromboplastin $\xrightarrow[\text{Serum Ac-globulin}]{Ca^{++}}$ Thrombin

STEP IV. Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin

also activates the prothrombin conversion factor into a serum accelerator which is enhanced by a platelet accelerator in the formation of thrombin. Fibrinogen, considered a passive factor in coagulation, probably is influenced by a platelet factor and becomes quickly clotted.

HYPOTHETICAL REPRESENTATION OF THE MECHANISM OF COAGULATION OF BLOOD ACCORDING TO STEFANINI²¹

SLOW PHASE

STEP I. Thromboplastinogen (PLASMA)

Thromboplastinogenase (PLATELETS) $\longrightarrow$ Thromboplastin

STEP II. Prothrombin + Thromboplastin + Calcium +
Plasma prothrombin conversion factor $\longrightarrow$ Thrombin

ACCELERATED PHASE

STEP III. Plasma prothrombin conversion factor
(or other plasmatic precursor)

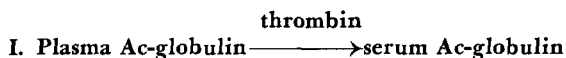
Thrombin
 $\longrightarrow$ Serum Accelerator

STEP IV Prothrombin + Thromboplastin
+ calcium $\xrightarrow[\text{platelet accelerator}]{\text{serum accelerator}}$ thrombin

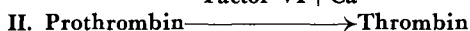
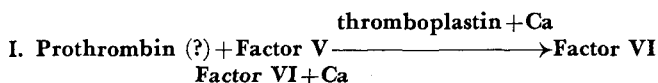
STEP V Fibrinogen $\xrightarrow[\text{platelet factor (?)}]{\text{thrombin}}$ Fibrin

Comparison of Prothrombin Conversion and Accelerator Factors

There is disagreement as to the mechanism by which conversion and accelerator factors participate in the formation of thrombin. Seegers²² assumes that prothrombin contains all the building stones for the structure of the thrombin molecule and nothing needs to be derived from calcium, thromboplastin or other activators. Purified prothrombin reacts slowly in the presence of calcium and a concentrated thromboplastin solution due to absence of Ac-globulin which apparently acts as a catalyst. Ware and Seegers²⁰ believe that thrombin is a prerequisite to serum Ac-globulin formation.



According to Owren¹⁷ Factor VI is produced by an autocatalytic reaction between prothrombin, Factor V, thromboplastin and calcium. Factor VI is believed to be the activator of prothrombin.



Owren thus considers Factor VI to be a prerequisite to thrombin formation.

Quick and Stefanini²³ believe there is a direct quantitative relationship between labile factor present and the yield of thrombin from a definite amount of prothrombin suggesting a stoichiometric type of reaction.

According to Stefanini,²¹ the various conversion factors, i.e., labile factor, Factor V and plasma Ac-globulin share similar properties and are probably identical. Controversy persists as to whether the action is simply catalytic or essential in the conversion of prothrombin to thrombin.

The function of accelerators requires further investigation. The liberation of platelet accelerator appears to set up the mechanism of blood coagulation. Serum accelerator evolves after the clotting process is under way. Evidence has accumulated that plasma contains a precursor of serum accelerator.

Seegers²⁴ states that the complexity of blood coagulation precludes the possibility of organizing a diagrammatic summary. Only controversial issues are presented herein. A discussion of physiologic anticoagulants and the fibrinolytic mechanism which inhibit intravascular thrombosis has been omitted. Seegers²⁴ has recently presented a review of this subject.

Summary

It has become increasingly apparent during the last decade that blood coagulation is a complex process. There is now general agreement that conversion and accelerator factors participate in the reaction. According to our present knowledge, thromboplastin, of tissue origin or of platelet origin or both, reacts with calcium and prothrombin to form minute amounts of thrombin. Platelet accelerator according to Seegers catalyzes the initial reaction. Plasma Ac-

globulin is activated by small amounts of thrombin to serum Ac-globulin. Rapid formation of thrombin results from the catalytic action of serum Ac-globulin and platelet accelerator. Platelet accelerator serves primarily to initiate the reaction while serum Ac-globulin becomes the major catalyst in the formation of thrombin. The reaction between thrombin and fibrinogen is probably hastened by a second platelet factor.

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