Platelet Coagulant Activities and Hemostasis: A Hypothesis

By Peter N. Walsh

Well, yes: he may have done all that, but there’s no proof that he did. I am beginning to believe that nothing can ever be proved. These are reasonable hypotheses which take the facts into account: but I am only too well aware that they come from me, that they are simply a way of unifying my own knowledge. Not a single naturally occurring inhibitors. Based on these observations, an hypothesis is presented in which the events of primary hemostasis (platelet adhesion, aggregation, and release) and blood coagulation are linked. As platelets aggregate to form a hemostatic plug, they provide a protective and catalytic surface for activation of the clotting mechanism and fibrin formation. Localized hemostasis is promoted and circulating blood kept fluid by means of a number of control mechanisms, some of which are mediated by autocatalytic effects of thrombin.

Recent evidence indicates that platelets can initiate blood coagulation by two alternative pathways and that they subsequently catalyze the reactions of intrinsic clotting factors, first in the formation of factor Xa and thereafter in prothrombin activation. These platelet coagulant activities may provide a theoretical basis for better understanding the hemostatic mechanism. The purpose of this communication is to present an hypothesis concerning (1) the relationship between platelet plug formation and blood coagulation, and (2) the possible role of platelets in effecting control mechanisms that may concurrently help to maintain circulating blood in a fluid state while promoting local hemostasis.

INITIATION OF BLOOD COAGULATION

Macfarlane has identified a number of unanswered questions concerning how the hemostatic process might be triggered and how the formation of platelet plugs might be related to the coagulation mechanism. The following...
brief summary of some of these enigmas is an oversimplification of an elegant and closely reasoned argument concerning an exceedingly complex matter.

(1) Experimental evidence suggests that "contact activation" is a necessary primary stimulus for intrinsic clotting. However, the coagulation factor that is the sine qua non of the contact phase of clotting (i.e., factor XII) is the only known factor whose presence is apparently not necessary for normal hemostasis.

(2) The view that blood coagulation can be effected via the extrinsic mechanism of factor X activation by the release of tissue factor at sites of vascular injury, though supported by in vitro data, is not consistent with the observed fact that patients with deficiencies in the activities of intrinsic clotting factors (especially factors VIII and IX) are generally much more severely affected hemostatically than are patients with defects of the extrinsic system (factor VII deficiency). Nemerson7 has suggested that the extrinsic system might function to generate minute quantities of thrombin that can activate factor VIII8 and thus permit the intrinsic system to operate.

(3) The possibility that the events of primary hemostasis (platelet adhesion, aggregation, and the release reaction) might somehow activate the coagulation mechanism has been attractive.

A number of lines of evidence now support this third possibility,1,2,9 i.e., that the platelets themselves can do more than simply accelerate reactions concerned with the final stages of coagulation by providing platelet factor 3 (PF3). They have the capacity, when stimulated by adenosine diphosphate (ADP), to trigger the contact phase of coagulation by activating factor XII.1 This physicochemical property of the normal platelet surface, termed contact product-forming activity (CPFA), is specifically and rapidly stimulated by physiologic concentrations of ADP in platelet suspensions not stirred or undergoing aggregation, in which PF3 availability is not observed. Unfortunately, this observation does little more than compound the enigma of the initiation of clotting, since it assigns a crucial role to the one clotting factor that is apparently unnecessary for normal hemostasis.

In contrast, another set of observations might explain not only how blood coagulation is triggered in vivo in normal individuals but also how individuals with Hageman trait do so well hemostatically without factor XII. When platelets from factor XII, XI, IX, and VIII deficient and normal subjects were washed free of loosely adsorbed coagulation factors by albumin density gradient separation,11 incubated with collagen, and returned to the autologous plasmas for clotting-time determination, it was found that the factor XII deficient platelet-plasma mixture, but not the factor XI, IX or VIII deficient mixtures, was made indistinguishable from the normal by collagen incubation.2 It has been inferred from this experiment and others that collagen can activate an alternative mechanism that does not require factor XII, provided factor XI and platelets are present; thus, activation of factor XII can be bypassed in the initiation of the intrinsic coagulation system. This property of collagen-stimulated platelets, referred to descriptively as collagen-induced coagulant activity (CICA), develops rapidly in unstirred platelet suspensions that are not undergoing aggregation. In contrast, PF3 activity is made available only when platelet
suspensions are stirred with collagen and thereby aggregated.\textsuperscript{2,12,13} It seems reasonable to postulate that collagen-induced coagulant activity is an important physiologic mechanism for the initiation of blood coagulation.

\textbf{ROLE OF PLATELETS IN SUBSEQUENT COAGULATION REACTIONS}

After factor XIa is formed in association with platelets, either by activation of platelet-associated factors XII and XI in response to ADP or by activation of platelet-associated factor XI in response to collagen, it would appear that the platelets have the capacity to “protect” factor XIa from inactivation by its natural inhibitor, antifactor XIa. This conclusion has been drawn from the demonstration that factor XIa was much less rapidly inactivated by antifactor XIa when platelets were present than when a phospholipid platelet substitute replaced the platelets.\textsuperscript{4} It has further been shown that factor XI, in contrast to many other clotting factors, is not removed from platelets after many washes\textsuperscript{11,14} and that the factor XIa formed via the two alternative pathways remains associated with the platelets and not the surrounding plasma.\textsuperscript{4} It can be inferred that the platelets provide a protective nidus for the interaction of the contact factors and the protection from inactivation of their reaction products.

Milestone\textsuperscript{5} has presented evidence that phospholipids catalyze the reaction of factors Xa, V, and II to form thrombin in the presence of calcium, and Lundblad and Davie\textsuperscript{3} and Schiffman et al.\textsuperscript{15} have demonstrated a requirement for phospholipid in the reaction of factor IXa with factor VIII to form factor X activator. These observations stimulated an investigation of the possible catalytic function of intact platelets in these intrinsic coagulation reactions. Not only do intact platelets catalyze the reactions of factors XIa, VIII, IX, and X in the formation of factor Xa, but in contrast to exogenously added phospholipid, they can be shown to retain this catalytic activity in the presence of the natural inhibitors to active clotting factors, antithrombin III, antifactor Xa, and antifactor XIa.\textsuperscript{4} This complex function has been termed intrinsic factor Xa-forming activity (XaFA) to distinguish it from the latent capacity of platelets\textsuperscript{16} aggregated with collagen,\textsuperscript{17} treated with kaolin,\textsuperscript{18} or frozen and thawed\textsuperscript{16} to catalyze the reactions of factors Xa, V, and calcium to activate prothrombin. This latter activity is also supplied by phospholipid and has been referred to as platelet factor 3 activity (PF3A) by long usage\textsuperscript{19} and to differentiate it from the other platelet coagulant activities referred to above.\textsuperscript{20}

Thus it would appear that the platelets are involved in providing a protective and catalytic surface for the interaction of intrinsic coagulation factors at every stage from contact activation to fibrin formation. Although it seems likely that the biochemical determinants of XaFA and PF3A are phospholipoproteins located in the platelet membrane,\textsuperscript{21} neither the subcellular localization nor the biochemical basis for CPFA or CICA are yet known. The concept of an interaction of protein clotting factors occurring on a catalytic surface provided by phospholipoproteins is supported by the work of a number of investigators,\textsuperscript{3,5,15,22-31} who have proposed similar mechanisms for factor-X and prothrombin activation. Though these catalytic functions of platelets in coagulation are perceived as a continuum of sequential factor activation, there is
evidence that each of the coagulant activities provided by platelets is separate and distinct from the others. Additional evidence indicates that these platelet coagulant activities are important determinants of hemostatic efficiency, at least in thrombasthenia and hemophilia.

PRIMARY HEMOSTASIS, BLOOD COAGULATION, AND HEMOSTATIC CONTROL MECHANISMS

The most significant conceptual advance in blood coagulation research was the appearance in 1964 of the cascade and waterfall theories, which independently suggested that clotting factors are sequentially activated, so that the coagulation mechanism acts as an amplifier and thereby promotes local fibrin formation once the system has been triggered. Fully 122 years previously, Gulliver and Addison had independently seen platelets in close association with fibrin, an observation subsequently extended, which eventuated in the concept originally proposed by Roskam that platelets have a "plasmatic atmosphere" containing blood coagulation factors that participate in reactions on the platelet surface to form fibrin. Now it is possible to substantiate and extend this concept by proposing a mechanism which links the events of platelet plug formation and blood coagulation (Fig. 1).

The platelets are seen as providing a protective and catalytic surface, perhaps with appropriate binding sites for the various coagulation proteins, where the events of blood coagulation are initiated and sustained as the platelets first adhere to the vessel and then cohere to form a hemostatic plug. The full hemostatic sequence does not occur after minimal vascular injury or when ADP is applied locally to small vessels. Therefore, it seems reasonable to suppose that an interaction is required between platelets and some subendothelial component of the vessel wall, such as collagen, to which platelets can stick and initiate intrinsic coagulation by activating platelet-associated factor XI. Collagen-induced coagulant activity is probably a much more important trigger mechanism for clotting than is contact product-forming activity, since the latter requires a clotting factor (XII) that is unnecessary for normal hemostasis and a primary stimulus (ADP) that does not lead to fibrin formation in animal experiments.

As the platelets are aggregating in response to ADP, which may be released from injured cells present in blood vessels, a sequential activation of intrinsic clotting factors occurs on the platelet surface, as shown schematically in Fig. 1. To promote this dual process of localized platelet plug and fibrin formation and to maintain the fluidity of the circulating blood, a number of control mechanisms must operate. The first of these is the amplifier function of the coagulation cascade, proposed by Macfarlane, by which a minute initial stimulus, progressively magnified by sequential enzyme-substrate interactions, results in the local explosive generation of fibrin.

The second type of control mechanism is a consequence of the fact that coagulation reactions are initiated and supported only in relation to aggregating platelets and not in circulating plasma. Once formed on the platelet surface, factor Xla is protected from inactivation by antifactor Xla and allowed to participate in reactions with factors IX, VIII, and X to form factor Xa. Factor
Hemostasis

Xa formation has also been shown to occur on the platelet surface and not in platelet-free plasma containing natural inhibitors to active clotting factors. It has further been shown that factor Xa on the platelet surface or in the presence of phospholipid is protected from inactivation by antifactor Xa. Furthermore, Marciniak has recently shown a protective effect of a factor V-phospholipid complex on factor Xa inactivation by antifactor Xa, and Yin and Wessler have presented evidence that factor Xa inactivation by antifactor Xa is delayed in the presence of a mixture of phosphatidyserine and phosphatidyl choline. Thus it would appear that factor Xa formed on the platelet surface is relatively stabilized, but not entirely protected from inactivation.
Another possible mechanism for the protection of factor Xa from inactivation has been proposed and is shown in Fig. 2: (1) The inactivation of factor Xa by antifactor Xa is greatly potentiated by the presence of minute quantities (0.0005 U/ml) of heparin. Large quantities of heparin are present in human liver, lung, and mast cells, and there is some evidence to suggest the presence of heparin in circulating blood. (2) When the platelet release reaction is induced in response to thrombin or collagen, heparin neutralizing activity (HNA) is released from platelets in soluble form. Heparin is thus complexed, its potentiating effect on the action of antifactor Xa is removed, and factor Xa is thereby further protected from inactivation. (3) Factor Xa can then participate in reactions with factor V and calcium on the platelet surface to activate prothrombin.

It would therefore appear that two properties of platelets account for the observation that coagulation reactions occur selectively at sites of platelet plug formation: the first is that they provide a catalytic surface for the interaction of clotting factors at every stage, and the second is that they appear to protect active clotting factors from inactivation by no fewer than two separate mechanisms.

The third type of control exercised over hemostasis is a consequence of the autocatalytic or positive feedback mechanisms mediated by the last enzyme generated during clotting, i.e., thrombin. Thereby, factors V and VIII are activated, and the platelet release reaction is induced. The release of ADP from platelets potentiates platelet plug formation (by stimulating platelet aggregation) and blood coagulation (by stimulating contact product-forming activity). The release of heparin-neutralizing activity, also mediated by a thrombin-induced release reaction, might likewise potentiate blood coagulation by binding heparin and protecting platelet-associated factor Xa from inactivation. Finally, the availability of platelet phospholipids might be enhanced by exposure of platelets to thrombin, so that intrinsic factor Xa-forming
activity and platelet factor 3 activity are made available and coagulation thereby potentiated locally.

The concept presented here, in which the initiation and subsequent events of clotting are confined to the platelet surface, may overcome many of the enigmas of hemostasis and provide for a more intimate interrelationship between primary hemostasis (platelet plug formation) and blood coagulation than previously considered. Although unproven, it is offered as an hypothesis worth testing.

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