Some Recent Advances in the Study of Hemostasis

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High on the list of pathologic processes which impede the circulation with serious and often lethal consequences are thrombosis and its sequelae. Although the pathogenesis of thrombosis can vary in different situations, a major component of the usual thrombus is fibrin, the protein meshwork of blood clots. This review focuses on some of the newer information concerning the clotting process and its inhibition, the relationship between clotting and such defense mechanisms as generation of kinins and fibrinolysis, and the role of complement in blood coagulation. It attempts to present a sample of present trends rather than a compendium of newer knowledge.

A CLASSIC VIEW OF BLOOD COAGULATION

Much of the research into the nature of thrombus formation during the last few years has focused on the initial steps in the clotting process and the remarkable way in which these reactions are linked to other defense mechanisms of the body. Just when it had seemed certain that the sequence of chemical events leading to the formation of fibrin had been defined, our concepts received a number of sharp jolts. Fletcher factor, an agent thought to be deficient in the plasma of individuals with Fletcher trait, has turned out to be identical with a plasma prekallikrein, and the supposedly clear distinction between the intrinsic and extrinsic pathways of thrombin formation has become murky.

Thrombin, the enzyme responsible for the conversion of fibrinogen to fibrin, forms when plasma is studied in the test tube through either of two convergent pathways. Thrombin generation via the extrinsic pathway is initiated when blood comes into contact with injured tissue (1) (Fig. 1). The agent in tissue responsible for thrombin formation, tissue thromboplastin, has been localized subcellularly to microsomes of various cells (2) and to cell membranes of fibroblasts and vascular endothelium (3, 4). Thromboplastin is composed of at least two elements, a heat-labile protein (5, 6) and phospholipid (7, 8); both are needed for optimal activity. Thromboplastin combines, apparently in stoichiometric fashion, with a plasma protein, factor VII, to form an agent which "activates" Stuart factor (factor X); the reaction is dependent on the presence of calcium ions and the phospholipid moiety of thromboplastin (9-11). Activated Stuart factor then converts prothrombin to thrombin through an enzymatic process involving the participation of proaccelerin (factor V), phospholipid (furnished in the extrinsic pathway principally by tissue thromboplastin itself), and calcium ions (12-14). The role of proaccelerin is not understood, but this clotting factor functions optimally only after it has been altered by thrombin (15, 16). Once thrombin has been elaborated, it cleaves two pairs of peptides, fibrinopeptides A and B, from each molecule of fibrinogen (17-20). The residual fibrin monomers then polymerize to form the visible clot (21), a process accelerated by calcium ions (22), and at the same time the fibrin monomers are bonded chemically through the action of fibrin-stabilizing factor (factor XIII), a plasma transamidase (23-25) (Fig. 2).

When blood drawn from the body with minimal contamination with tissue thromboplastin is transferred to a glass tube, it will, nonetheless, clot via reactions described as the intrinsic pathway (26) (Fig. 1). Contact with glass appears to activate Hageman factor (factor XII) (27), which then converts plasma thromboplastin antecedent (PTA, factor XI) to an activated form via an enzymatic process (28). This agent, in turn, activates Christmas factor (factor IX), again enzymatically...

Activated Christmas factor next appears to form a complex with antihemophilic factor (AHF, factor VIII) on micelles of phospholipid; this process requires calcium ions (31, 32). The complex, like that evolved by tissue thromboplastin and factor VII, changes Stuart factor to its clot-promoting form. Thereafter, the steps of the intrinsic and extrinsic pathways of thrombin formation are apparently identical; phospholipid for the intrinsic pathway is furnished by platelets and by the plasma itself, since human platelet-depleted plasma readily coagulates in glass tubes (33).

The parallel between the intrinsic and the extrinsic pathway is tempting to examine. In each case, a factor which requires vitamin K for its synthesis (Christmas factor or factor VII) forms an agent which activates Stuart factor if a heat-labile protein (antihemophilic factor or the protein moiety of tissue thromboplastin), calcium ions, and phospholipid are present. Equally intriguing is the parallel between the generation of complexes of activated Christmas factor and antihemophilic factor and of activated Stuart factor and proaccelerin. Again, both complexes form only if phospholipid and calcium ions are present. Stuart factor, like Christmas factor, is synthesized only if vitamin K is available, and antihemophilic factor (16, 34), like proaccelerin (15, 16), is much more effective once it has been altered by thrombin.

How the intrinsic pathway becomes active in vivo is unclear. Certainly, nature did not anticipate the invention of glass when animals evolved the capacity to synthesize Hageman factor. Collagen (35), certain mucopolysaccharides such as chondroitin sulfate (36), sebum (37, 38), and platelets (39) have all been implicated as activators of Hageman factor. In general, activators of Hageman factor carry a negative charge, suggesting that they react with some positively charged group on the surface of Hageman factor (40). Indeed, preliminary experiments from my laboratory suggest that the various activators react with exposed arginine residues of the Hageman factor molecule (Ratnoff and Saito, unpublished observations). Earlier experiments suggested that the activation of Hageman factor was accompanied by a molecular change which rendered the factor much less soluble in aqueous media (41). More recent studies using sophisticated biophysical methods seem to demonstrate a conformational change in Hageman factor coinciding with its activation (McMillin, Saito, Ratnoff, and Walton, unpublished observations).

The plasma kallikreins are enzymes which can increase vascular permeability, contract smooth muscles, decrease blood pressure, induce pain, and provoke sticking of leukocytes to small blood vessels and migration of these cells into extravascular spaces (42). All of these responses are mediated through the liberation of peptide kinins, notably bradykinin and kallidin (lysylbradykinin), which appear to act directly on the affected tissues. The kallikreins exist in plasma as inert precursors, but they are readily activated by activated Hageman factor (43, 44) or fragments of Hageman factor (45) that are most readily obtained by incubation with such enzymes as trypsin (46, 47) or plasmin, the fibrinolytic enzyme in plasma (48, 49). Although investigators often refer to the plasma kallikreins as if they represented a single entity, in all probability two or more species of kinin-forming enzymes are present in human plasma (50, 51). Recent experiments have unexpectedly revealed that one plasma kallikrein participates not only in the liberation of kinins but in the clotting process as well.

The textbook separation of the intrinsic and extrinsic pathways of thrombin formation has the great appeal of simplicity, but nature never seems to take the hint. In 1955, Rapaport et al. (52) reported that the one-stage prothrombin time—that is, the clotting time via the extrinsic pathway of a mixture of tissue thromboplastin, plasma, and calcium ions—was shorter in plasma that had been in contact with glass than it was in untreated
plasma. This phenomenon was confirmed in various ways in other laboratories, and Soulier (53) ascribed the effect of glass to the activation of Hageman factor, thus linking the intrinsic and extrinsic pathways. Both Rapaport et al. and Soulier recognized that activation by glass ultimately affected a plasma component, but at the time a clear distinction between an effect on factor VII or Stuart factor was not possible. Recently, the role of Hageman factor in the extrinsic pathway has been reexamined by Gjønnaess in a fascinating series of experiments. Substituting Thrombotest reagent (a mixture of phospholipids, tissue thromboplastin, and bovine plasma from which the vitamin K-dependent clotting factors have been removed) for tissue thromboplastin in the procedure for determining one-stage prothrombin time, Schrogie and his associates (54) observed that the one-stage prothrombin time was dramatically shortened when the plasma of women taking oral contraceptives was stored at 0°C overnight. Gjønnaess (55) found that acceleration of the one-stage prothrombin time, as measured with Thrombotest, was associated with an elevenfold increase in the clot-promoting properties of factor VII. The effect of storage in the cold on susceptible plasmas required the presence of Hageman factor (56, 57) as well as an agent with properties resembling a plasma prekallikrein (57, 58). Activated Hageman factor and other agents which might bring about the conversion of plasma prekallikrein to kallikrein also shortened the Thrombotest prothrombin time (56) (Fig. 3). These experiments, which have been confirmed in part by Josso et al. (59), demonstrate that enhancement of thrombin formation via the extrinsic pathway can be brought about through the successive activation of Hageman factor and a plasma prekallikrein. Since plasma prekallikrein can be activated by fragments of Hageman factor induced by incubation with certain proteolytic enzymes, it is not surprising that the one-stage prothrombin time, as measured with Thrombotest, can be shortened by treating plasma with such fragments (Saito and Ratnoff, unpublished observations).

Gjønnaess (55) noted that the enhancement of the clot-promoting properties of factor VII that occurred when plasma was stored at 0°C was by no
means a universal phenomenon. Especially pertinent to our understanding of thrombosis, plasmas obtained from individuals taking oral contraceptive agents, as Schrogie et al. (64) had observed, and from pregnant women were especially susceptible to the cold-activation phenomenon, as if a subtle change in the clotting or kallikrein systems had occurred. Notably, in pregnant women the concentration of C1 inactivator in plasma is sharply decreased (60, 61). This agent is an inhibitor of C1, the esterolytic form of the first component of complement, but it also inhibits both activated Hageman factor (62) and plasma kallikrein (63). Whether this decrease in inhibitory activity is linked to the enhancement of clotting and perhaps to the increased susceptibility of pregnant women to thrombosis is, at this writing, totally speculative.

While all these studies were progressing, another line of evidence appeared which suggests that a plasma kallikrein might also participate in the intrinsic pathway of blood clotting. Some years ago, Hathaway and his associates (64) described a young girl and three of her siblings in whom the clotting time of whole blood was prolonged despite the presence in plasma of normal amounts of all recognized clotting factors. The defect was localized to the intrinsic pathway of thrombin formation. They postulated that the plasma of their patients was deficient in a "new" substance, named Fletcher factor after the surname of the affected family. Now Wuepper (65) has demonstrated that a plasma prekallikrein can correct the defect in Fletcher trait plasma. Furthermore, such plasma is deficient in antigenic material recognized by an antibody against a plasma kallikrein, which thus appears to be the proposed Fletcher factor. Consonant with this view, antiserum directed against a plasma kallikrein inhibits the intrinsic pathway of thrombin formation in normal plasma (66). The site of action of this plasma kallikrein is disputed; evidence presented previously has suggested that it acts after Hageman factor has been activated (67). Nor is it clear whether Fletcher factor must be present for activated Hageman factor to exert its clot-promoting effect. The defect in Fletcher trait plasma measured by the partial thromboplastin time disappears if the plasma is first exposed to an activator of Hageman factor such as kaolin for several minutes. Either Fletcher trait plasma contains small amounts of Fletcher factor or this agent is not an absolute requirement for the activation of plasma thromboplastin antecedent.

These several test tube observations, then, suggest that Hageman factor participates in the clotting process via both the intrinsic and extrinsic pathways and that this effect is in each case mediated by a plasma kallikrein. The dearth of hemorrhagic phenomena in patients with Hageman trait and Fletcher trait, hereditary deficiencies of Hageman factor and Fletcher factor, respectively, serves as a reminder that the role of these two clotting factors in man is not yet established. Both of these agents are present in greater or lesser concentration in the plasma of all mammals tested except members of the dolphin family (68, 69). It is intriguing to speculate about the forces that favored the evolution of cetaceans that lacked the usual devices for initiating the reactions of the intrinsic pathway of thrombin formation. These mammals are capable of prolonged submersion, during which it is believed that the circulation to nonvital tissues is sharply restricted. Perhaps, then, their deficiency of Hageman factor and prekallikrein helps to prevent inadvertent thrombosis in static blood. The implication of these observations for man, in whom stasis has long been thought to be important in the genesis of thrombosis, particularly within veins, would be more obvious had not Mr. Hageman, the index patient with Hageman trait, died of posttraumatic pulmonary embolism (70).

That Hageman trait and Fletcher trait are asymptomatic suggests that in the body the intrinsic pathway might be activated at a step subsequent to the participation of these factors. How this situation comes about is not known, but the recent demonstration that trypsin, an agent not found in normal blood, can activate plasma thromboplastin antecedent (71, 72) suggests that an as yet unidentified enzyme other than Hageman factor might carry out this step.

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RELATIONSHIP BETWEEN CLOTTING AND FIBRINOLYTIC PHENOMENA

Almost 20 years ago, I (73) reported that exposure of a fraction of plasma to glass initiated both the clotting process and the formation of plasmin, an enzyme in plasma which can digest fibrin. At the time I was not yet aware that an action of glass was to convert Hageman factor to its activated form, thus bringing into action the intrinsic pathway of clotting (25). Somewhat later, Niewiarowski and Prou-Wartelle (74) linked these two observations by demonstrating that under appropriate conditions activated Hageman factor would induce formation of plasmin from its inert precursor, plasminogen. The reaction was not direct; Iatridis and Ferguson (75) found that it required participation of a second factor, to which Ogston et al. (76) later gave the name Hageman factor-cofactor. Apparently, once Hageman factor is activated by glasslike agents, it can convert Hageman factor-cofactor from a precursor form to an agent which in turn changes plasminogen to plasmin. Alas, the reaction is still more complicated than had at first been envisioned. The effect of Hageman factor on Hageman factor-cofactor is augmented by Fletcher factor (that is, a plasma prekallikrein [67, 77]). And a second as yet undefined plasma factor might be needed for the reaction to proceed (76). The activation of plasminogen by activated Hageman factor apparently explains the long-known property of chloroform as an initiator of plasmin formation (76).

Both the precursor (78) and the activated form (76, 79) of Hageman factor-cofactor have been partially purified, but much still remains to be done to clarify these rather complex reactions. In particular, although Hageman factor-cofactor seems distinct from another Hageman factor substrate, plasma thromboplastin antecedent (76), the possibility that it is identical to a plasma prekallikrein has been raised (80, 81). Its separation from prekallikrein has been demonstrated by several techniques, however, so that the uniqueness of Hageman factor-cofactor seems probable (76, 78, 79). Further studies in this area are much in order.

Although Hageman factor-cofactor was renamed plasminogen proactivator by one group of investigators (78), it must be distinguished from the proactivator of plasminogen which appears in blood as a result of exercise (82) or the infusion of certain vasoactive substances (82, 83); Hageman factor-cofactor does not increase in the plasma of individuals undergoing exercise (76, 84). Indeed, the role of this interesting pathway is presently unknown. Its intrigue lies in the possibility that some of the mechanisms which underlie the formation of thrombi might also be involved in their dissolution, helping to reestablish blood flow to ischemic areas.

CLASSIC HEMOPHILIA AND VON WILLEBRAND'S DISEASE: A NEW ROLE FOR ANTIHEMOPHILIC FACTOR IN HEMOSTASIS

If the participation in clotting of a plasma prekallikrein, the precursor of an enzyme involved in inflammatory reactions, were not confusing enough, we now have been faced with a deluge of information concerning the complexity of antihemophilic factor, an agent functionally deficient in classic hemophilia (85) and von Willebrand's disease (86, 87). Classic hemophilia, as everyone knows, is an X chromosome-linked hereditary disorder associated, in severe cases, with a life-long propensity to serious and often apparently spontaneous bleeding. Cases vary in severity from family to family, a variation paralleled by a corresponding variation in the titer of antihemophilic factor, as measured in clotting assays. Characteristically, the partial thromboplastin time (the time elapsing until a mixture of plasma, phospholipid, and calcium ions clots), a measure of the intrinsic pathway, is abnormally long, whereas the bleeding time (the time elapsing until bleeding stops from a deliberately incised wound) is normal. Platelet function is also apparently normal, and, when hemophilic blood is passed through a column of glass beads, the platelet count of the filtrate is sharply reduced, a normal response. When preparations containing antihemophilic factor are transfused into patients with classic hemophilia, the titer of this agent, tested in clotting assays, in the recipient's plasma rises immediately to the predicted level and then rapidly decreases with an approximate half-disappearance time of about 12 hours (88).

In contrast to classic hemophilia, von Willebrand's disease is usually a relatively mild bleeding disorder, although serious and lethal bleeding can occur, particularly from the gastrointestinal tract. It is inherited as an autosomal dominant trait, although it is frequently detected less readily in men than in women, who may have menorrhagia or bleeding with or after parturition. As in classic hemophilia, the partial thromboplastin time is usually prolonged. This defect is correlated with the degree of deficiency of antihemophilic factor in clotting tests, which is ordinarily milder than it is in severe hemophilia and tends to vary among different affected individuals within the same fam-
ily. Unlike the case of classic hemophilia, the bleeding time is prolonged and the retention of platelets by a column of glass beads is often impeded (89, 90). When patients with von Willebrand’s disease are transfused with normal blood or fractions of plasma rich in antihemophilic factor, the concentration of this agent rises immediately to the anticipated level. But within the ensuing 6–8 hours, the concentration of antihemophilic factor rises still higher, as if somehow the synthesis of this agent had been induced, and thereafter its titer falls at a much slower rate than it does in patients with classic hemophilia (91–95). Even more remarkably, the transfusion of hemophilic plasma into patients with von Willebrand’s disease stimulates the rise in functional antihemophilic factor. Presumably, normal or hemophilic plasma contains an agent which stimulates the appearance of antihemophilic factor–like activity in the recipient with von Willebrand’s disease. The response occurs only in vivo. Mixing plasmas obtained from patients with classic hemophilia and von Willebrand’s disease does not induce the appearance of functional antihemophilic factor (96).

After Patek and Stetson (85) first recognized that patients with classic hemophilia lacked a clot-promoting agent, the natural assumption was made that these individuals were unable to synthesize this protein. A contrary view, that patients with hemophilia synthesized a functionally incompetent form of antihemophilic factor, was first proposed by Shanberge and Gore (97) but did not gain immediate acceptance. More recently, three groups of investigators have demonstrated that the plasma of hemophiliacs contains normal or even elevated amounts of antigenic material that can be detected by use of antiserum raised in rabbits against purified preparations of antihemophilic factor (98–100). In von Willebrand’s disease, on the other hand, the concentration of antigens recognized by such antiserum is decreased in rough proportion to the titer of antihemophilic factor measured in a clotting assay (99). Thus, it is von Willebrand’s disease rather than hemophilia that is associated with a true deficiency of antihemophilic factor.

Now all of this might seem to hold little interest for the individual concerned with the pathogenesis of thrombosis. But a number of observations derived from these studies suggest that the antigenic material related to antihemophilic factor might be concerned with the normal function of the platelets, cells that play a central role in the development of arterial thrombi; their role in venous thrombosis is less certain. Relatively purified preparations of human antihemophilic factor are readily obtained by filtration of fractions of normal plasma rich in antihemophilic factor through molecular sieves of agarose. Bouma and his associates (101) made the striking observation that the addition of such purified preparations of antihemophilic factor to the blood of patients with von Willebrand’s disease corrected the abnormality in platelet retention by columns of glass beads, a result foreshadowed by earlier experiments by Salzman and Britten (102) and Zucker (103) with whole plasma. Bouma et al. (101) obtained the same result when they added the nonfunctional antihemophilic factor–like material of hemophilic plasma to von Willebrand’s disease blood. These important experiments have been confirmed by Weiss et al. (104). Whatever the meaning of the phenomenon of retention of platelets to columns of glass bead (and it is disputed), it is apparent that this platelet function depends on the presence of something antigenically resembling antihemophilic factor.

The observations of Bouma et al. (101) reflected an in vitro phenomenon. Some years earlier, Borchgrevink (105) devised an assay which he believed measured platelet adhesiveness to vascular endothelium. He counted the platelets in venous blood and in blood oozing from a wound cut into the forearm. He believed that the difference in the two counts represented the number of platelets adherent to the wound surface. Using this technique, Borchgrevink (105) reported that platelet adhesiveness was impaired or absent in patients with von Willebrand’s disease. As far as I know, this method has not yet been applied to patients transfused with highly purified antihemophilic factor, but again we are led to the view that antigens related to antihemophilic factor are concerned with normal platelet function.

These platelet studies jibe with other data concerning antihemophilic factor and platelets, both old and new. Normal platelets have long been known to possess antihemophilic factor–like functional activity (106). Several years ago, Ristocetin, an antibiotic, was withdrawn from use because its administration was complicated by thrombocytopenia (107). Howard and Firkin (108) found that indeed Ristocetin induced aggregation of normal platelets in vitro but not of those obtained from two of three patients with von Willebrand’s disease. Extending these studies, Weiss et al. (109) found that platelet aggregation by Ristocetin in von Willebrand’s disease required the presence of anti-
The pathogenesis of the clotting defect in von Willebrand's disease, although it seems evident that something in normal or hemophilic plasma is responsible for the rise in functional antihemophilic factor seen after transfusion. Significantly, Bloom et al. (127) believe that the functional antihemophilic factor which appears after transfusion behaves as if its molecular weight is relatively small; in contrast, in one patient that we studied (Bennett and Ratnoff, unpublished observations), functional activity was associated with a molecule of high molecular weight.

The studies which I have reviewed suggest that antihemophilic factor is a molecule which dissociates into two fragments. One of these, possessing high molecular weight, is needed for the conduct of certain platelet functions, whereas the other, much smaller in size, is associated with antihemophilic factor procoagulant activity. Synthesis of the high-molecular weight fragment is apparently determined by an X-chromosomal gene but that of the low-molecular weight fragment is determined by an X-chromosomal gene. At least one other gene might be involved, since a rare disorder in which functional deficiency of antihemophilic factor and proaccelerin coexist, is inherited in an autosomal recessive manner (128, 129). The plasma of one such patient contained normal amounts of antigenic material related to antihemophilic factor as determined by precipitation with specific rabbit antiserum (99), sharply separating this disorder from von Willebrand's disease. No reason exists to assume that every molecule of antihemophilic factor normally possesses functional activity (99); indeed, the opposite is likely to be the case (124). A contrary view, however, must be recorded. Both Hougie and Sargeant (130) and Zimmerman and Edgington (131) have offered evidence that the agent in plasma corrective for von Willebrand's disease is distinct from that with procoagulant activity. Hougie and Sargeant (130) have reported that functional antihemophilic factor and antigenic material related to antihemophilic factor can be separated by cryoprecipitation of normal plasma, as if they represent different molecular species. Zimmerman and Edgington (131) have reported that a human circulating anticoagulant, rendered insoluble by binding to agarose, removes functional antihemophilic factor from normal plasma without altering its content of antigenic material related to antihemophilic factor. These investigators, then, have provided evidence that in the test tube clot-promoting and antigenic entities can be separated. Their experiments do not tell us that these fractions are unassociated in circulating plasma.
Some years ago Nilsson et al. (92) and others (91, 94, 95) reported that the transfusion of normal or hemophilic plasma temporarily shortens the bleeding time of patients with von Willebrand's disease, although the results obtained are not always impressive (93). The assumption was made that these plasmas contain a "von Willebrand's factor" needed for normal hemostasis. It would be pleasing to believe that the precipitating antigenic moiety of antihemophilic factor is the long-sought von Willebrand's factor. But the shortening of the bleeding time is even more evanescent than the plasma level of transfused antigenic material (95), and in three patients with von Willebrand's disease in whom the concentration of functional and antigenic antihemophilic factor rose to normal levels during stress the bleeding time remained prolonged (134).

I have not read much stirring debate concerning the possible role of antihemophilic factor in the pathogenesis of thrombosis. Still, evidence has been reported which suggests that the functional concentration of this clotting factor increases with age in men (133). Furthermore, its concentration rises during pregnancy (134), after exercise (135) or the injection of epinephrine (136), and in individuals undergoing stress (137). The concentration of antihemophilic factor is also elevated in patients with neoplasms (138) and in those who have sustained myocardial infarction (139). In view of the possible role of antihemophilic factor in the functioning of platelets, which form the nidus of arterial and perhaps venous thrombi, the hypothesis that this factor contributes to the pathogenesis of thrombosis demands further study. Still, the highest levels seen in my laboratory have been found in Laennec's cirrhosis (140), a condition in which thrombotic events are possibly less common than they are in other chronic diseases.

SOME PLASMA INHIBITORS OF BLOOD COAGULATION

A third area in which our understanding of the clotting process has advanced rapidly concerns the modulation of this process by inhibitory agents in plasma. Some years ago, the late Dr. Jerold Rosenblum and I (27) observed that the plasma of patients with Hageman trait and of Peking ducks, which lack Hageman factor, had the property of interfering with the clot-promoting properties of glass. Similar inhibitory properties were also found in the plasma of patients with Fletcher trait, which is lacking in a prekallikrein that, as we have seen, is essential for the normal functioning of activated Hageman factor (141). An inhibitor extracted from horse plasma was thought to interfere with early stages of blood clotting, but its site of action was not clarified nor could it be extracted from normal human plasma (142). Recently, methods of demonstrating inhibitory properties in normal human plasma have been devised, but it has not been possible to ascribe them to a single plasma constituent (143). These studies suggest that one way in which the fluidity of the blood might be maintained is through interference with the activation of Hageman factor. In all probability, however, inhibition of the activation of Hageman factor must play only a minor role in the prevention of inadvertent intravascular clotting.

Once activated, Hageman factor is rapidly inactivated in normal plasma. Thus far, the only agent that has been found which serves this function is CI inactivator (62), which also inhibits activated plasma thromboplastin antecedent (62) and plasma kallikrein (63). Presumably, this inhibitory agent serves to prevent the continued action of these agents and thus to limit the spread of intravascular clots. Activated plasma thromboplastin antecedent is also inhibited by yet another plasma agent, plasma thromboplastin antecedent inactivator, a protein which does not seem to interfere with other steps in the clotting process (144, 145). The concentration of plasma thromboplastin antecedent inactivator, like that of CI inactivator (60), decreases during pregnancy (61), raising the possibility that its deficiency might contribute to the thrombotic tendency in this state.

For many years, it has been recognized that thrombin, once formed during the clotting process, rapidly deteriorates (146, 147). At least two plasma proteins contribute to this process. The major inhibitor, designated antithrombin III, is an α1-globulin with a molecular weight of 65,000 or less (148, 149). Its action is progressive and irreversible; the inhibitor and the enzyme form an inactive complex (150, 151). A second, perhaps less important, plasma inactivator of thrombin is the fraction designated as α2-macroglobulin (152); it accounts for perhaps one-fourth of the thrombin-inhibitory properties of plasma (153). The possibility that α1-antitrypsin also contributes to the antithrombotic properties of plasma has also been suggested (154), but it is improbable that this inhibitor significantly participates in the inhibition of thrombin in normal plasma.

Years ago, Howell and Holt (155) recognized that heparin inhibited thrombin in the presence of some other agent in plasma, an observation confirmed by Brinkhous et al. (156) and Seegers et al. (157). At first, it was thought that this "heparin cofactor"
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was a distinct entity to which the name antithrombin II was attached (158). Monkhouse et al. (159), however, provided evidence that antithrombin II and antithrombin III were the same substance. Although this observation was challenged, it has been confirmed many times and the identity of antithrombin II and antithrombin III is well established (160).

In a separate series of experiments, information was gathered that plasma inhibits activated Stuart factor, retarding the formation of the prothrombin-converting principle (161). Almost from the first, it was recognized that this inhibitor was probably identical with antithrombin III (162, 163), and purified preparations of antithrombin III were potent inhibitors of activated Stuart factor (160, 164). Indeed, antithrombin III appears to be a significantly more potent inhibitor of activated Stuart factor than it is of thrombin. Since heparin functions only in the presence of antithrombin III, it now seems likely that the therapeutic effect of heparin is mediated largely by its inhibition of activated Stuart factor (165), a view anticipated by Howell and Holt (155). Since each molecule of activated Stuart factor might be responsible for elaboration of many molecules of thrombin, inhibition at this earlier step might well explain the apparent prophylactic efficacy of heparin administered in doses too small to be detected by ordinary measures of the last steps of clotting (166, 167). Additionally, heparin interferes with the functioning of activated Christmas factor (168) and probably of activated plasma thromboplastin antecedent as well (169-171).

For many years, attempts were made to correlate the occurrence of thrombosis with deficiencies of plasma antithrombin or heparin cofactor but without clear results (172). Indeed, some investigators observed that the concentration of antithrombin was normal or elevated in patients with thrombosis (173, 174). Normal adult men are said to have a higher titer of serum antithrombin than women (175, 176) and the titer might increase with age (176), although this suggestion is disputed (177). Experimentally, antithrombin III protects animals against death from intravenous infusion of tissue thromboplastin (178). That antithrombin III might be important in the prevention of thrombosis in man is supported by the study of an interesting family by Egeberg (179). Individuals of both sexes, encompassing three generations, had frequent episodes of thrombosis, particularly of the deep veins of the legs. In affected individuals, the concentration of antithrombin III was depressed, averaging about half the normal titer. Several other similar cases in which hereditary deficiency of antithrombin III or heparin cofactor appeared to be associated with a tendency to thrombosis have been observed (180, 181). Presumably, in such individuals the inadvertent intravascular elaboration of activated Stuart factor and thrombin is not checked in a normal manner. Further support for the idea that antithrombin III deficiency might increase susceptibility to thrombosis comes from the observations of von Kaulla and her colleagues (182) and those of Fagerhol and Abildgaard (176) who noted that the titer of this inhibitor was decreased in individuals taking oral contraceptives. Their findings raise the possibility that the increased tendency to thrombosis said to be present in such women is related to this change. In any event, one should keep in mind that thrombosis is an unusual event during the course of an individual's life. Presumably, then, these observations do not tell us what initiates thrombosis, but rather that under appropriate circumstances the normal mechanisms which might prevent this process are depressed.

NEW RELATIONSHIPS BETWEEN COMPLEMENT AND BLOOD COAGULATION

Some years ago, Robbins and Stetson (183) demonstrated that antigen-antibody aggregates accelerate the coagulation of rabbit blood. Among the antigens tested was E. coli endotoxin. The effect of endotoxin is complement-dependent and is probably mediated via the properdin system, more recently called the alternative pathway of complement activation (184), as suggested earlier by Robbins and Stetson (183). At the same time, Zimmerman and his colleagues (185) noted that the clotting time of whole blood was impaired in rabbits with a hereditary deficiency of the sixth component of complement (C6), a defect corrected by the addition of purified preparations of this component. The effect of complement on clotting was apparently mediated through an action on rabbit platelets. Rabbit platelets differ in many ways from those of human subjects, so that it comes as no surprise to learn that blood clotting was not defective in a patient with C6 deficiency (186). That the difference observed in human and rabbit C6 deficiency is probably in the platelets and not due to species differences in complement is supported by studies of Tomar and Kolchins (187). These authors found that the concentration of the third component of complement (C3) was depressed in 16 of 27 patients thought to have sustained disseminated intravascular coagulation.
The mechanism of depletion—whether immunologic or otherwise—was not determined, but it appeared to be unrelated to fibrinolysis.

Another possible relationship between blood coagulation and complement derives from studies of hereditary angioneurotic edema. Donaldson (188) found that the addition of ellagic acid to the plasma of patients with this disorder brought about the activation of the first component of complement (C1), generating its esterolytic form, C1e. Ellagic acid, a derivative of tannic acid, is an unusual compound, for in solution it brings about the activation of Hageman factor. The experiments of Donaldson (188), then, suggest that the initial steps of blood clotting might bring about the activation of complement. The significance of this challenging observation is not yet clear.

Still further relationships among complement, hemostasis, and thrombosis have been summarized recently (42).

This review has sampled some of the newer literature concerning the clotting process. Particular emphasis has been placed on experiments which might illuminate the pathogenesis of thrombosis. Some of the observations described provide new clues supporting the thesis that the defenses of the body against injury are intertwined and that such processes as blood coagulation, fibrinolysis, immune reactions, and inflammation are separated more readily in the laboratory than in real life (42). They serve to remind us that the investigation of thrombosis requires a broad approach; bodily reactions should be examined from diverse points of view. Recent studies have suggested that thrombosis is so prevalent an event after surgical procedures that there is an impelling need to investigate as many avenues as we can possibly imagine. As always, the list of unsolved questions seems endless.

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