REVIEW ARTICLE

The Pathophysiology of the Prethrombotic State in Humans: Insights Gained From Studies Using Markers of Hemostatic System Activation

By Kenneth A. Bauer and Robert D. Rosenberg

Numerous investigators have postulated that a hypercoagulable state exists in humans for a period of time before the development of thrombotic episodes. A clear biochemical definition of the prethrombotic state, however, has proved elusive due in part to the lack of reliable techniques for monitoring pertinent changes in blood coagulability. Based on recent advances in our knowledge of the biochemistry of the coagulation system, a series of highly sensitive and specific immunochemical tools has been developed that can quantitate the activities of various steps of the hemostatic mechanism in vivo at the subnanomolar level. We have established assays for $F_{1+2}$ and the protein C activation peptide, which measure the cleavage of the prothrombin molecule by factor Xa and the scission of protein C by the thrombin-thrombomodulin complex, respectively. Nossel and coworkers had previously constructed similar assays for fibrinopeptide A (FPA) and fragment B, which monitor the cleavage of fibrinogen by thrombin and the proteolysis of fibrin I by plasmin, respectively. Substantial elevations in the levels of these markers have been found in patients with disseminated intravascular coagulation and many subjects with acute deep venous thrombosis. The $F_{1+2}$ and FPA assays have been used to demonstrate that significant increments in factor Xa activity but not thrombin activity regularly occur in the blood of nonanticoagulated individuals with congenital deficiencies of antithrombin or protein C. These two disorders are known to be correlated with the subsequent development of thrombosis. Patients with protein C deficiency have also been noted to have significantly reduced plasma levels of protein C activation peptide. By using the immunosassays for FPA and B, in studies of postoperative patients, it has been shown that an imbalance between the procoagulant action of thrombin and the anticoagulant action of plasmin on fibrin I polymer may induce an acquired thrombotic diathesis. Finally, we have recently demonstrated that prothrombin activation as measured by the $F_{1+2}$ assay is suppressed by oral anticoagulants in the blood of patients with thrombotic diatheses. These investigations suggest that these assay techniques can be used to improve our understanding of the hypercoagulable state as well as to detect more effective treatment strategies for the prevention of thromboembolic events.

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THE COAGULATION CASCADE is composed of a series of linked proteolytic reactions. At each stage of the mechanism, a parent zymogen such as prothrombin or factor X is converted to a corresponding serine protease that is responsible for a subsequent zymogen–serine protease transition. In most instances, protein cofactors such as factor V or factor VIII are present and can be activated by serine proteases and then possess the ability to bind the aforementioned reactants to specific cell surfaces. These natural surfaces include platelets, WBC, or endothelial cells. This process usually leads to a dramatic acceleration as well as a partial localization of the reactions. The result of these transformations is the generation of thrombin, which is able to act upon fibrinogen and platelets to produce the hemostatic plug.

For coagulation system enzymes to be generated at any significant rate, a zymogen, a cofactor, and a converting enzyme must form a multimolecular complex on a natural surface. These transformations are suppressed if the converting enzyme is inhibited, the protein cofactor destroyed, or the surface receptors that are essential for the assembly of the macromolecular complex sequestered. Previous methods for monitoring these processes in the clinic have been directed at measuring the levels of zymogens (ie, factor X, prothrombin, protein C), inhibitors (ie, antithrombin), or substrates (ie, factor VIII, factor V, fibrinogen) of the hemostatic system. These molecular species are present in large excess within the blood, and only a small percentage of the zymogens are converted to active enzymes under in vivo conditions. Thus, attempts to monitor thrombotic or prethrombotic states by measuring the ambient levels of zymogens or inhibitors by
determining their catabolic rates within the circulatory system are unlikely to be successful in most instances. Similarly, clotting assays (ie, partial thromboplastin time) that are carried out under arbitrary in vitro conditions have not proved to be particularly useful in the assessment of hemostatic system hyperactivity. In addition, neither of these methodologies reflects the endogenous anticoagulant properties of the vascular endothelium.

The enzymes generated by the coagulation cascade are not directly available for quantitation because they are evanescent species that are rapidly neutralized by naturally occurring protease inhibitors. Faced with these obstacles, we have focused our attention on the development of a series of highly sensitive and specific radioimmunoassays for peptides that are liberated with the activation of hemostatic system zymogens in vivo. These by-products of zymogen transformation are stable entities with finite half-lives in the circulation. Assays for the F1+2 fragment and the protein C activation peptide have been established that measure the cleavage of the prothrombin molecule by factor Xa (Fig 1) and the scission of protein C by the thrombin-thrombomodulin complex (Fig 2) respectively. Nossel and coworkers had previously constructed immunoassays for fibrinopeptide A and the B3 1-42 fragment that monitor the proteolysis of fibrinogen by thrombin (Fig 1) and fibrin I by plasmin, respectively. All individuals exhibit measurable amounts of these markers under normal conditions, and substantial elevations have been noted in patients with disseminated intravascular coagulation, deep venous thrombosis, pulmonary emboli, etc. In the following sections we present the data from several clinical studies in which the aforementioned assay techniques have been used to biochemically define the prethrombotic state in patient populations known to suffer from disorders characterized by defective regulation of thrombin generation. We will also present results supporting the view that an imbalance between the procoagulant action of thrombin and the anticoagulant effect of plasmin on fibrin I polymer may lead to a thrombotic diathesis in situations where substantial thrombin-mediated proteolysis of fibrinogen has already occurred. The knowledge obtained can then be used to provide a conceptual basis for understanding how these immunochemical tools might be used in more complex situations where it may not be possible to readily define the major molecular defect responsible for thrombotic phenomena in a given individual. No attempt has been made to consider methods for the in vivo assessment of platelet activation in this review.

A MODEL FOR THE REGULATION OF THROMBIN GENERATION

Based upon recent advances in our knowledge of the hemostatic mechanism, we will first construct a tentative

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**Fig 1.** Radioimmunoassays (RIAs) for F1+2 and fibrinopeptide A (FPA). The conversion of prothrombin to thrombin takes place under physiological conditions in the presence of factor Xa, factor Va, calcium ions, and platelets. During this process, two peptide bonds in the zymogen are scissed. Cleavage at Arg27-Thr274 results in the release of the inactive F1+2 fragment from the amino terminus of prothrombin, and a second bond scission at Arg927-Leu928 results in the generation of the two-chain serine protease thrombin. Once this enzyme is evolved, the initial step in the conversion of fibrinogen to fibrin results in the liberation of FPA.
FACTOR 4

THROMBIN

ACTIVATED

PROTEIN C

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OTHELIAL ACE

model for the regulation of thrombin generation under in vivo conditions (Fig 3) with which to interpret the results of our clinical investigations. We hypothesize that small amounts of factor Xa as well as factor Va are continuously formed within the vascular system. These components are able to bind to specific platelet receptors to form the prothrombinase complex and produce small amounts of thrombin. Natural anticoagulant mechanisms act to oppose the generation of the latter enzyme.8-9 The endogenous heparan sulfate–antithrombin and protein C–thrombomodulin mechanisms function to inhibit serine proteases (ie, factor Xa, thrombin) and inactivate activated cofactors of the coagulation cascade (ie, factor VIIIa, factor Va), respectively. Hence, these processes oppose the formation of the prothrombinase complex as well as the action of thrombin. The ability of these two regulatory systems to limit the production of thrombin on the surface of platelets and endothelial cells should determine whether activation of platelets takes place within the circulatory system. If platelet activation occurs, prothrombin is brought into close proximity with the factor Va–factor Xa complex on the platelet surface, and significant amounts of thrombin can be generated that might gradually overwhelm the natural anticoagulant mechanisms.

CLINICAL STUDIES OF THE INHERITED THROMBOTIC DISORDERS

The aforementioned model for the regulation of thrombin generation predicts that the prethrombotic state can be defined as an imbalance between the procoagulant and natural anticoagulant mechanisms that eventually leads to the development of overt thrombotic phenomena. To examine this hypothesis, we have studied the biochemical alterations in hemostatic system activity that arise in patients with congenital reductions in the levels of antithrombin and protein C. These relatively homogenous inherited disorders should cause a prolonged decrease in the activity of the endogenous heparan sulfate–antithrombin or protein C–thrombomodulin mechanisms before the occurrence of thrombotic disease. Indeed, these patients often do not become symptomatic until their early twenties, and increasing numbers of individuals experience venous thrombotic events with advancing age.10-11

Radioimmunoassays for the prothrombin activation fragment F1+2 and fibrinopeptide A were used to quantitate the levels of free factor Xa and thrombin, respectively, within the blood of 22 asymptomatic individuals not receiving anticoagulants from eight separate kindreds with congenital antithrombin deficiency.12 The median age of this cohort was 22. Only seven of these patients had a prior history of an overt thrombotic episode, and at least two other members of each family had exhibited deep vein thrombi or pulmonary emboli. Our studies demonstrated that the plasma F1+2 levels were significantly elevated in almost all patients with this disorder (P < .001) (Table 1) whereas the concentrations of fibrinopeptide A were not substantially altered in these individuals as compared with normal controls. Clearance studies with 131I-F1+2 revealed that the metabolic behavior of this component was identical in antithrombin-deficient subjects and normal individuals. These findings suggest that patients with this inherited thrombotic disorder exhibit
increased concentrations of factor Xa that lead to excessive activation of prothrombin but that the resulting thrombin is neutralized before it can cleave fibrinogen to any significant extent. We also infused purified antithrombin concentrate into several affected individuals and monitored the response of their hemostatic mechanisms. The data obtained showed that elevations in the levels of protease inhibitor led to a decrease in the abnormally high concentrations of F1+2. It was also observed that the levels of this marker remained at a relatively constant normal value as antithrombin levels steadily declined from their peak concentrations postinfusion but started to rise toward the high preinfusion value when the plasma concentrations of the inhibitor dropped below ~70% of normal.

Marcum et al. have provided biochemical, cell biologic, as well as physiological evidence that heparinlike proteoglycans intimately associated with the vascular endothelium can accelerate hemostatic enzyme–antithrombin complex formation via a process that is identical to that of commercial heparin. In addition, Carlson and coworkers have noted that metabolic turnover studies in humans with radiolabeled antithrombin are consistent with a model in which ~10% of the total pool of antithrombin is associated with a noncirculating vascular compartment that probably represents the heparinlike proteoglycans of the vessel wall. It therefore appears likely that a small fraction of plasma antithrombin is normally bound to the unique heparinlike proteoglycans of the vessel wall in an activated conformation. This pool of protein probably represents the physiologically relevant protease inhibitor population, whereas the same component is of minimal functional relevance when free within the blood. Therefore, we surmise that antithrombin-deficient patients have an insufficient plasma level of protease inhibitor to saturate the heparinlike proteoglycans of the vessel wall and hence cannot generate sufficient activated protein to suppress factor Xa formed within the circulatory system. This supposition would provide an explanation for the decrease in F1+2 values that occurs when the concentrations of antithrombin are ~70% of normal and for the subsequent plateau of this parameter despite ambient levels of protease inhibitor that ranged between 70% and 250% of normal.12

We have also used the specific radioimmunoassays for F1+2, fibrinopeptide A, and protein C activation peptide to quantitate the production and action of thrombin as well as the activation of protein C within the blood of 23 asymptomatic individuals not receiving anticoagulants from 13 kindreds with an isolated congenital deficiency of protein C. The median age of this cohort was 37, and nine of these patients had previously sustained at least one thrombotic event. The mean concentrations of both protein C and protein C activation peptide were reduced to about 50% of normal in the members of this population. The mean level of F1+2 was significantly elevated by almost twofold in subjects with protein C deficiency (P < .0005) (Table 1) whereas the mean concentration of fibrinopeptide A was only minimally raised as compared with an age-matched control group. The metabolic behavior of 131I-F1+2 was found to be similar in protein C–deficient patients with elevated F1+2 measurements and normal individuals. Several of the 23 subjects, however, had normal plasma F1+2 concentrations, and our results suggest that the extent of hemostatic system activation as measured by this assay in heterozygotes with protein C deficiency may be much more variable than that in patients with congenital antithrombin deficiency. Data similar to that outlined for the antithrombin-deficient patients have been obtained in a more limited number of kindreds with protein S deficiency. Based upon these data, it would appear that malfunction of the endogenous heparan sulfate–antithrombin or protein C–thrombomodulin mechanisms will result in excessive generation of thrombin well before the development of overt thrombotic disease.

In this regard, it is critical to emphasize that many asymptomatic patients with the aforementioned deficiency states exhibit hemostatic system hyperactivity for prolonged periods of time but the documented occurrence of thrombotic complications within this population is episodic. The biochemical processes that are responsible for the conversion of this hypercoagulable state into an actively thrombotic one are poorly understood at the present time. A variety of events could acutely depress the effective functioning of the endogenous heparan sulfate–antithrombin or protein C–thrombomodulin mechanisms. These would include minor alterations in the plasma concentrations of antithrombin or protein C and transient reductions in the levels of thrombomodulin or heparinlike proteoglycans on the surface of endothelial cells. In addition, a decrease in the free plasma protein S concentration via changes in the interaction of this component with the C4b-binding protein may reduce the ability of activated protein C to inactivate factors VIIIa and Va.18,19

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**Table 1. Levels of F1+2 in Patients With Hereditary Deficiencies of Antithrombin or Protein C**

<table>
<thead>
<tr>
<th>Patient Population</th>
<th>F1+2 (nmol/L) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not receiving anticoagulants</td>
<td></td>
</tr>
<tr>
<td>Antithrombin-deficient subjects (22)</td>
<td>3.91 ± 1.13†</td>
</tr>
<tr>
<td>Protein C-deficient subjects (23)</td>
<td>2.54 ± 1.11‡</td>
</tr>
<tr>
<td>Age-matched normal subjects without an inherited or acquired thrombotic diathesis (31)</td>
<td>1.51 ± 0.68</td>
</tr>
<tr>
<td>Receiving oral anticoagulants</td>
<td></td>
</tr>
<tr>
<td>Antithrombin-deficient subjects (12) (PT ratio* = 1.85 ± 0.41)</td>
<td>0.714 ± 0.26§</td>
</tr>
<tr>
<td>Protein C-deficient subjects (22) (PT ratio* = 1.78 ± 0.32)</td>
<td>0.205 ± 0.25</td>
</tr>
<tr>
<td>Subjects without an inherited thrombotic diathesis (23) (PT ratio* = 1.74 ± 0.32)</td>
<td>0.231 ± 0.24</td>
</tr>
</tbody>
</table>

*PT (prothrombin time) ratio = PT of patient/PT of normal plasma pool and is expressed as the mean ± SD.
†P < .0001. Antithrombin deficient subjects not receiving oral anticoagulants v age-matched normal subjects.
‡P < .0005. Protein C deficient subjects not receiving oral anticoagulants v age-matched normal subjects.
§P < .01. Antithrombin deficient subjects receiving oral anticoagulants v anticoagulated subjects without an inherited thrombotic diathesis or protein C deficient subjects on oral anticoagulants.
∥P < .0001. Subjects without an inherited thrombotic diathesis receiving oral anticoagulants v age-matched normal subjects.
POSTULATED MOLECULAR DEFECTS IN COMPLEX THROMBOTIC DISORDERS

Despite our uncertainties about various aspects of the pathophysiology of congenital deficiencies of antithrombin, protein C, and protein S, it is clear that many patients with these disorders biochemically exhibit a true prethrombotic state, i.e., they have excessive production of factor Xa enzymatic activity with insufficient generation of thrombin to convert significant amounts of fibrinogen to fibrin. It remains to be determined whether elevations in the concentration of $F_{1+2}$ will have predictive value in determining the subsequent development of a thrombotic event in these populations. The vast majority of thrombotic disorders, however, are not associated with inherited risk factors and are oftentimes associated with acquired conditions, i.e., lupus anticoagulant, neoplasia, advancing age, postoperative state, estrogen therapy, obesity, etc. Due to their complex pathophysiology, it seems unlikely that one will be able to implicate isolated abnormalities in the levels of antithrombin, protein C, or protein S as primary etiologic factors for the hypercoagulable syndromes associated with these conditions. The application of immunochemical assays such as that for prothrombin fragment $F_{1+2}$ to such populations might potentially identify those individuals at highest risk for the subsequent development of thrombotic phenomena.

In view of the known association of vascular disease with increasing age, we have conducted a detailed analysis of hemostatic system activity with respect to perturbations induced by aging phenomena. None of these individuals exhibited an immunological deficiency of either antithrombin or protein C. The levels of $F_{1+2}$ were quantitated in the plasma of 199 healthy males between the ages of 42 and 80 and demonstrated a highly significant positive correlation with increasing age ($P < .0001$). The elevations in the concentration of this component in aged patients were not due to diminished clearance of the fragment. Significant elevations in the levels of fibrinopeptide A ($P < .01$) and protein C activation peptide ($P < .002$) were also observed with advancing age. These results indicate that many apparently normal males of increasing age exhibit a biochemical defect that denotes the presence of an acquired prethrombotic state.

One can use the aforementioned model (Fig 1) to suggest a variety of potential molecular defects in the natural anticoagulant mechanisms that might lead to elevated $F_{1+2}$ levels in populations with acquired risk factors for the eventual development of thrombotic disease. For example, decreased synthesis of heparinlike molecules by endothelial cells or increased release of platelet factor 4 by platelets are likely to significantly reduce the activity of the endogenous heparan sulfate–antithrombin mechanism without altering the levels of the protease inhibitor. In addition, decreased production of thrombomodulin by endothelial cells, decreased activity of the postulated receptor for activated protein C on platelets, decreased levels of functional protein S, or increased levels of the protein C inhibitor might also dramatically reduce the activity of the protein C–thrombomodulin mechanism without altering the concentrations of protein C. Furthermore, decreased generation of prostaglandin 1$\alpha$ by endothelial cells, decreased numbers of receptors for this prostaglandin on platelets, or decreased ability of platelets to respond to the suppressive action of this prostaglandin would allow the bound prothrombinase complex to activate increasing amounts of thrombin.

Other patients who experience multiple thrombotic episodes may exhibit an increased production of thrombin within the circulatory system whose actions cannot be suppressed by the normal functioning of the natural anticoagulant mechanisms. The potential molecular defects that could lead to this situation include excessive exposure of tissue factor by endothelial cells or WBC, increased numbers of receptors for factor IX/Xa or factor X/Xa on endothelial cells, augmented numbers of sites for factor Va on platelets or endothelial cells, or increased capacity of platelets or endothelial cells to bring prothrombin into close approximation with the prothrombinase complex, etc. At present, we have little real knowledge about this set of potential abnormalities.

A MODEL FOR THE REGULATION OF THROMBIN ACTION ON FIBRIN I POLYMER

The conversion of fibrinogen to fibrin by thrombin initially results in the formation of fibrin I monomer and the release of fibrinopeptide A (Fig 4). Fibrin I monomer is able to polymerize, and thrombin can then cleave this polymer, thereby resulting in the generation of fibrin II and the release of fibrinopeptide B. Tissue-type plasminogen activator is able to bind to fibrin, which allows plasminogen to be activated to plasmin at an increased rate. The action of the serine protease plasmin on fibrin I polymer results in the production of fragment X along with fragments from the $\alpha$ and $\beta$ chains ($B\beta$ 1-42). Plasmin can subsequently convert fragment X to soluble degradation products.

MOLECULAR DEFECTS IN THE FIBRINOLYTIC MECHANISM

Dysfunction of the fibrinolytic system may also play an important role in thrombus formation. Although investigators have identified a few individuals with inherited abnormalities of the fibrinolytic mechanism and recurrent venous thromboembolism, we consider the clinical association between these two conditions to be less convincing than what has been observed in many kindreds with deficiencies of antithrombin, protein C, or protein S. This relative lack of success in the identification of fibrinolytic mechanism defects may be related to inadequacies in the laboratory methodologies that have been available for the detection of clinically relevant abnormalities.

It has been tacitly assumed that thrombotic phenomena observed in patients with fibrinolytic system defects are due to their reduced ability to lyse small fibrin clots and prevent proximal extension. Nossel proposed an alternative view that an imbalance between the relative rates at which thrombin and plasmin split the $B\beta$ chain of fibrin I polymer could determine the occurrence of venous thrombosis. Owen et al have used radioimmunoassays for fibrinopeptide A
Fig 4. A model for the regulation of thrombin action on fibrin I polymer in vivo. (A) The conversion of fibrinogen to fibrin II by thrombin. (B) The fibrinolytic activity of human plasma is determined primarily by the presence of tissue-type plasminogen activator (TPA) and its inhibitor (PAI), which are released from vascular endothelial cells. TPA is able to bind to fibrin I polymer, which allows plasminogen to be activated to plasmin at an increased rate.

and Bβ 1-42 (measured as thrombin-increaseable fibrinopeptide B) to quantitate thrombin and plasmin activity, respectively, in patients under craniotomy. Venous thrombosis was documented by 125I-fibrinogen leg scanning. The results obtained indicated that individuals in whom thrombi developed, when compared with those who did not suffer from this complication, exhibited levels of fibrinopeptide A that were considerably greater than the concentrations of Bβ 1-42 during the four days preceding the onset of this disorder. These observations suggest that thrombosis occurs at a time when thrombin action is enhanced relative to that of plasmin.

The precise molecular defects that are responsible for these phenomena are not currently well understood. Fibrinolysis is initiated by plasminogen activators that convert plasminogen to the active enzyme plasmin by limited proteolysis. Plasmin is able to degrade fibrin to soluble products or alternately may be inactivated by forming 1:1 stoichiometric complexes with its major physiological inhibitor α2-antiplasmin.33 The fibrinolytic activity of human plasma is determined primarily by the presence of tissue-type plasminogen activator and its inhibitor, which are released from vascular endothelial cells.33,34 Immunochemical methods for the quantitation of tissue-type plasminogen activator and functional assays for the inhibitor have recently been applied to the study of patients with documented venous thrombembolism. These studies have suggested that defective synthesis or release of tissue-type plasminogen activator as well as an increased concentration of the inhibitor of this serine protease may be important pathogenetic factors in some of these individuals.35,36 It should also be noted that reduced fibrinolytic activity due to increased plasma levels of a rapid inhibitor of tissue-type plasminogen activator has been found in young survivors of myocardial infarction.37

SUPPRESSION OF PROTHROMBIN ACTIVATION BY ORAL ANTICOAGULANTS

The radioimmunoassay for F1+2 has recently been used to study patients without an inherited thrombotic diathesis who were stably anticoagulated with sodium warfarin.38 Our results demonstrate that prothrombin activation as measured by this assay is markedly suppressed in individuals with prothrombin time ratios (prothrombin time of patient/prothrombin time of normal plasma pool) greater than 1.5 (Table I). The reduction in the plasma concentration of this component was not due to decreased immunoreactivity or rapid clearance of partially γ-carboxylated forms of the fragment. Thus, measurement of F1+2 levels should serve as an accurate marker of factor Xa activity upon prothrombin in this patient population. The substantial suppressive effect of standard warfarin schedules suggests that a less intense
anticoagulation regimen might lead to a significant reduction of elevated F\(_{1+2}\) levels into the normal range rather than to subnormal concentrations. We therefore carried out studies of the response of the hemostatic mechanism to a very low intensity anticoagulation program (prothrombin time ratio less than 1.3) in five patients with a prior history of thrombotic disease. All of these individuals had elevated plasma F\(_{1+2}\) levels before the institution of warfarin therapy. The administration of low doses of the drug led to a decrease in the concentration of this marker into the normal range at a mean prothrombin time ratio that was essentially indistinguishable from untreated subjects. A schematic diagram of the results of this investigation is shown in Fig 5.

We also evaluated the effects of warfarin on factor Xa enzymatic activity in individuals with hereditary deficiencies of antithrombin or protein C who were chronically receiving warfarin. At equivalent levels of intensity of oral anticoagulation, the mean plasma F\(_{1+2}\) level in patients with anti-thrombin deficiency was significantly elevated as compared with the subjects with protein C deficiency or with the group of anticoagulated persons without an inherited thrombotic disorder (P < .01) (Table 1). We therefore conclude that the effect of warfarin on hemostatic system activation is modulated by the endogenous heparan sulfate–antithrombin mechanism. These data also imply that the chronic administration of warfarin leads to a dominant anticoagulant effect on the vitamin K–dependent procoagulant factors of the hemostatic system as compared with the antagonistic effect of the drug on the protein C anticoagulant mechanism when the endogenous heparan sulfate–antithrombin anticoagulant pathway is functioning normally.

Recent clinical studies have demonstrated that low-intensity regimens of warfarin provide prophylaxis against venous thrombosis, but the minimal level of oral anticoagulants required in a given patient remains to be determined. The F\(_{1+2}\) assay may therefore be useful in monitoring the in vivo action of low-intensity warfarin therapy in clinical trials designed to establish the lowest amount of medication needed to protect against a thrombotic event.

**CONCLUSION**

This review has summarized the results of several clinical studies that indicate that a biochemical imbalance between procoagulant and anticoagulant mechanisms can be detected in the blood of humans before the appearance of thrombotic phenomena. The continuing application of biochemical, molecular biologic, and clinical approaches to investigations of the hemostatic system should lead to a greater appreciation of the molecular defects that lead to venous and arterial vascular disorders in humans. It is to be hoped that this new information will allow us to more precisely identify individuals who are entering a clinically relevant hypercoagulable state and intervene with appropriate therapy before the onset of overt thrombotic disease.

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The pathophysiology of the prethrombotic state in humans: insights gained from studies using markers of hemostatic system activation

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