Coagulation Components and Sequence

NOTABLE ADVANCES in our knowledge of coagulation during the past decade, arising largely from studies of hemorrhagic diseases, have served to focus renewed attention on the role of clotting in the pathogenesis and management of atheromatous disease and thromboembolism. More than a century ago v. Rokitansky postulated that atherosclerosis follows initial fibrin deposition on the vessel wall. This concept has recently been taken up anew by Duguid and others who studied morphologic sequences in pathologic, or experimentally introduced, intravascular whole-blood clots or fibrin. The theory that localized coagulation on the arterial wall with subsequent organization is, at least in some instances, the initiating factor in atherosclerosis has been extended by Astrup. This investigator views coagulation as being normally in a state of delicate balance, proceeding throughout the vascular system at a very slow rate, and restrained by opposing anticoagulant forces, particularly the fibrinolytic system, which is also ubiquitous in blood and tissues. Fibrinolysins are thought to be constantly removing the end product, fibrin, as it accumulates on the vessel walls. Their failure to function adequately is postulated as contributing to atherogenesis under certain circumstances.

This "encrustation" theory merits vigorous study in an attempt to elucidate the pathogenic scheme divided arbitrarily into four sequential phases of atherosclerosis. Some idea of the magnitude of the problem is revealed in table 1, which depicts the current coagulation phases. More than 20 distinct factors, both procoagulant and anticoagulant, are now recognized (table 2). Several more are suspected but are as yet not clearly defined. These factors interact in an extremely complex manner involving protein-protein and protein-ion reactions, which include positive as well as negative feed-back mechanisms controlling the kinetics of clotting. The four phases of coagulation are shown more simply in table 3.

Many questions immediately arise. Since the factors are all present in the circulation, except perhaps for the "foreign" surface, does coagulation actually proceed constantly in the normal subject, although at a very slow smoldering rate? Or is it held in complete abeyance ready to be triggered only when called upon to serve the needs of stasis? If so, which factors are responsible for triggering and speeding up this mechanism in the presence of disease? Are these factors any of the known clotting constituents, or does pathologic triggering or acceleration occur through a different pathway, as yet unknown? Thus each new clotting factor that is discovered becomes suspect in the pathogenesis of thrombotic disease.

Why, when, and how is the delicate equilibrium of normal blood fluidity tipped in the
BLOOD COAGULATION AND THROMBOTIC DISEASE

Table 1

**Current Coagulation Scheme**

<table>
<thead>
<tr>
<th>Phase I: Formation of thromboplastic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IA:</strong> Elaboration of intrinsic thromboplastic activity</td>
</tr>
<tr>
<td>(1) Factor XII (Hageman &quot;complex&quot;) → &quot;Foreign&quot; → Active Hageman factor (HF)</td>
</tr>
<tr>
<td>(2) HF + factor XI (PTA) + factor IV (Ca++) → (HF + PTA) &quot;complex&quot;</td>
</tr>
<tr>
<td>(3) (HF + PTA) &quot;complex&quot; + factor IX (PTC) + IV → Active IX</td>
</tr>
<tr>
<td>(4) IX + factor VIII (AHF) + factor X (Stuart) + IV → Intermediate product 1</td>
</tr>
<tr>
<td>(5) Intermediate product 1 + platelet &quot;factor 3&quot; + IV → Intermediate product 2</td>
</tr>
<tr>
<td>(6) Intermediate product 2 + factor V (Ac-globulin) + IV → &quot;Intrinsic&quot; thromboplastin (&quot;Intrinsic prothrombinase&quot;)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase II: Formation of thrombin from factor II (prothrombin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IIA:</strong> Intrinsic system</td>
</tr>
<tr>
<td>&quot;Intrinsic&quot; thromboplastin + II (prothrombin) + IV → Thrombin</td>
</tr>
<tr>
<td><strong>IIB:</strong> &quot;Extrinsic&quot; thromboplastin + II + IV → Thrombin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase III: Formation of fibrin from factor I (fibrinogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin + I → Fibrin + fibrinopeptides + Metathrombin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase IV: Dissolution of fibrin by fibrinolysin (plasmin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Activator + profibrinolysin (plasminogen) (Blood kinase)</td>
</tr>
<tr>
<td>(Tissue kinase)</td>
</tr>
<tr>
<td>(Urokinase)</td>
</tr>
<tr>
<td>(Streptokinase)</td>
</tr>
<tr>
<td>(2) Fibrinolysin + I + Fibrin</td>
</tr>
<tr>
<td>+ Antifibrinolysin (antiplasmin)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*Nomenclature of clotting factors designated by roman numerals is in accordance with recommendations of the International Committee on Nomenclature of Blood Clotting Factors (table 2).

Not included in the scheme are several of the known, or postulated, inhibitors and certain negative feedback mechanisms. In the formulation of the equations no consideration is given to whether the components are involved stoichiometrically or enzymatically.

Heparin inhibits "intrinsic" thromboplastin formation and also inhibits the action of thrombin.

Coumarin-type compounds depress factors II, VII, IX, and X.

direction of laying down of a clot? Can such imminent or actual imbalance be detected? How much of the widely and indiscriminately used concept of hypercoagulability (the first of the Virchow triad) is fact, how much fancy? Which of the clotting constituents contain lipid, and what relationship is there, if any, between other blood lipids and physiologic or pathologic coagulation?

What constitutes the nature of surface "foreignness," and is this, the second of Virchow's triad, the crucial factor that initiates intravascular clotting at a particular site? To what extent do other local factors such as velocity of blood flow, anatomic blood supply to the vessel wall itself, local tissue kinases—thromboplastic as procoagulant, on the one hand, or the kinase activator of fibrinolysis on the other—operate in development of the disease process itself? And finally, how can any one of these components be controlled therapeutically to correct any suspected imbalance, or to remove the end products of clotting, once they have developed?

Because coagulation is so frequently the
ultimate cause of obstruction of a vessel with atheromatous involvement, and since it is the physiologic mechanism at which therapy is aimed, this paper is intended to include certain basic aspects with particular reference to initiation of the clotting sequence, subsequent elaboration of thrombin, and consideration of the so-called "hypercoagulable" state in the pathogenesis of thrombotic disease.

**Initiating Factors**

Students of coagulation have long been concerned with the riddle of why blood does not clot intravascularly despite the fact that all the necessary clotting factors are present except the "foreign" surface. This blood fluidity has been variously attributed to the fact that several first-phase clotting components, e.g., factor V (Ac-globulin), factor IX (PTC), exist in circulating blood in relatively inert precursor form; to the presence of inhibitor-factor combination, e.g., Hageman inhibitor; or to natural circulating anticoagulants such as heparin.

That the nature of the surface to which blood is exposed is of fundamental importance in initiating or accelerating the clotting sequence has long been known. This is elegantly demonstrable in the "coagulation" of incoagulable congenital afibrinogenemic blood. The sequential changes were essentially normal when the blood was exposed to ordinary glass in contrast to blood exposed to silicone-coated vessels. Everything is retarded in the latter. In this patient, the third clotting phase—the thrombin-fibrinogen interaction—had been deleted by the grace of God, and thus could be eliminated as of possible influence. Under more physiologic conditions, as in an isolated venous segment in the dog, coagulation is also relatively slow and indeed incomplete for as long as 8 hours, as judged from prothrombin disappearance. Yet very little prothrombin need be consumed to yield a visible clot, in harmony with observations of the coagulation of hemophilic blood, which will give voluminous clots despite negligible prothrombin conversion to thrombin. It is also well known that the introduction of foreign materials into the vascular channels in-

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**Table 2**

**Synonymy of Blood Clotting Factors**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Other names</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Thromboplastin</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>V</td>
<td>Ac-globulin, labile factor, proaccelerin, accelerator factor, plasma prothrombin converting factor</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin → Convertin, Stable factor, serum prothrombin conversion accelerator (SPCA), prothrombinogen, autoprotrombin I, prothrombokinase</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor (AHF), antihemophilic globulin (AHG), hemophilia factor A, platelet cofactor I, thromboplastinogen, thrombocyte lysin</td>
</tr>
<tr>
<td>IX</td>
<td>Plasma thromboplastin component (PTC), Christmas factor, hemophilic factor B, autoprotrombin II</td>
</tr>
<tr>
<td>X</td>
<td>Stuart factor, Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent (PTA)</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor (HF)</td>
</tr>
<tr>
<td>Profibrinolysin</td>
<td>Plasminogen</td>
</tr>
<tr>
<td>Fibrinolysin</td>
<td>Plasmin</td>
</tr>
</tbody>
</table>

*Nomenclature adopted by the International Committee for the Nomenclature of Blood Clotting Factors.
BLOOD COAGULATION AND THROMBOTIC DISEASE

Table 3
The Four Basic Phases of Coagulation

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Clot formation</td>
<td>- Evolution of thromboplastic activity</td>
</tr>
<tr>
<td>II. Phase II</td>
<td>- Conversion of prothrombin to thrombin</td>
</tr>
<tr>
<td>III. Phase III</td>
<td>- Conversion of fibrinogen to fibrin by thrombin</td>
</tr>
<tr>
<td>IV. Clot dissolution (lysis)</td>
<td>1. Evolution of plasmin from plasminogen</td>
</tr>
<tr>
<td></td>
<td>2. Destruction of fibrin and fibrinogen by plasmin</td>
</tr>
</tbody>
</table>

Table 4
In Vivo Turnover of Plasma Proteins

<table>
<thead>
<tr>
<th>Factors</th>
<th>Time* (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation components</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>1.2</td>
</tr>
<tr>
<td>I (Fibrinogen)</td>
<td>5.8</td>
</tr>
<tr>
<td>II (Prothrombin)</td>
<td>2.3</td>
</tr>
<tr>
<td>V (Ac-G)</td>
<td>2.3</td>
</tr>
<tr>
<td>VII (Proc.)</td>
<td>1</td>
</tr>
<tr>
<td>VIII (AHF)</td>
<td>1.2</td>
</tr>
<tr>
<td>IX (PTC)</td>
<td>1.2</td>
</tr>
<tr>
<td>X (Stuart)</td>
<td>2.3</td>
</tr>
<tr>
<td>XI (PTA)</td>
<td>2.3</td>
</tr>
<tr>
<td>Components not involved in clotting</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>14-23</td>
</tr>
<tr>
<td>Gamma globulin</td>
<td>20-30</td>
</tr>
<tr>
<td>B: lipoprotein</td>
<td>4-7</td>
</tr>
</tbody>
</table>

*Estimated turnover time of total pool: for clotting factors, based on clinico-laboratory observations on correction of defects of patients (heredo-familial or acquired) following transfusion with blood, plasma, or specific factor; for non-clotting factors, based on data obtained with labeled purified fractions administered to normal and pathologic subjects.

Reduces local clotting more or less rapidly, depending upon the nature of the surface and the velocity of blood flow.

Besides the surface, certain clotting components play important roles in this initial phase of clotting. Factor VIII (antihemophilic factor) is needed early in the sequence, since, when absent, clotting is considerably retarded. More recent work by Shafrir and de Vries, Margolis, Ratnoff, Soulier and colleagues, and Waaler shows that the accelerating effect of certain surfaces such as glass operates via a glass-activated factor, which induces release of Hageman factor from its combination with inhibitor.

What is it in blood that distinguishes between "foreign" and "non-foreign" surfaces? Probably related to complex physiochemical alterations of the molecule of an as yet obscure factor, this mechanism is one of the most important problems yet to be resolved; it is not only pertinent to our problem of thrombotic disease, but is also of fundamental biologic significance.

The Clotting Equilibrium

The still unexplained riddle of why blood under normal circumstances does not clot intravascularly, in contrast to shed blood, requires re-examination of the fundamental premise, namely, that intravascular clotting does not take place normally. It is more reasonable to postulate that it proceeds continuously, although extremely slowly. In support of this hypothesis are observations on the rate of turnover in vivo of certain clotting components compared with other plasma proteins (table 4). In hemophilic patients who receive fresh factor VIII (in normal citrated plasma), the clot-promoting effect disappears essentially within 24 to 48 hours; this depends to some extent on the amount administered. Factors V, II (prothrombin), and VII are similarly short-lived. Moreover, factors II and VII virtually disappear within two days, when their synthesis is completely blocked by coumarin drugs. Comparable observations have been reported by others regarding platelets, factor IX, PTA, and fibrinogen. Since these constituents are generally far more stable in vitro, this evidence may be construed as reflecting their rapid consumption in the body rather than their inherent lability, or their inordinately rapid destruction by the patients with specific clotting deficiencies to whom they have been administered.

Thus, our present working concept, in agreement with that of Astrup, is that intravascular coagulation constantly proceeds even under physiologic circumstances, and that normally it is in a state of delicate dynamic balance that is controlled by pro-coagulant forces counterbalanced by anticoagulant
forces. This equilibrium may be tipped in either direction by induced physiologic or pathologic stresses, such as exercise, trauma, shock, asphyxia, and infection; by local abnormality of vessel wall; by altered hemodynamics; or by heredo-familial, congenital, or acquired defects of any of the coagulation components.

If the balance is "stable,"* any induced disequilibrium will most likely be episodic or fleeting (e.g., thrombotic) with early restoration to the original state of equilibrium. If, on the other hand, it is an "unstable"* balance, the resulting imbalance will lead to progressive collapse of the primary physiologic function (hemorrhage, or extensive thrombosis as the case may be). Or, if the balance is "neutral,"* the disequilibrating forces may eventually result in a state of equilibrium operating at a level differing from the original (e.g., excessive continuous fibrin deposition).

Aside from the possible significance of coagulation in the origin as well as in the complications of atherosclerotic disease, adequate understanding of this mechanism is essential to the rational use of anticoagulants and to other aspects of management of thromboembolic disorders. The physician who employs these agents must realize clearly that he may be tampering with a most important and intricate homeostatic mechanism.

The early sequential changes in clotting blood have been considerably clarified by studies on patients with rare and unique coagulation defects. As already mentioned, from observations in incoagulable congenital afibrinogenemic blood, it is clearly evident that fibrinogen-fibrin plays no significant role in the early activation of certain clotting factors, in platelet agglutination and disintegration, and in the consumption of factor VIII (AHF), factor V, and factor II (prothrombin) following the shedding of blood and its exposure to glass. It should be emphasized that the afibrinogenemic blood in these studies remained fluid throughout; no clot formed, yet "chemical" coagulation proceeded quite normally, i.e., sequential interactions of various clotting components took place despite no evident gelation of the blood.

Moreover, the afibrinogenemic blood could inactivate added thrombin in a normal manner. This antithrombin activity is one of several important protective negative feedback mechanisms; it disposes of thrombin when, for any reason, it forms to an excessive extent intravascularly, and thus threatens vascular patency. Conceivably, an aberration in this mechanism also may underlie intravascular clotting.

Another example of feed-back mechanisms serving to maintain coagulation in dynamic equilibrium, is factor V. Under the influence of a minute amount of formed thrombin, factor V is rapidly activated—thus accelerating coagulation, while at the same time the activated form becomes very labile and deteriorates. This results in deceleration of clotting kinetics, since this clotting factor is essential in both the first phase of coagulation (thromboplastin generation) and in the second (thrombin elaboration from prothrombin).

A third example, one currently of practical clinical significance, is the antifibrinolysin in circulating blood. Clearly this balanced inhibitor of the physiologic clot-dissolving system must be overcome if therapeutic thrombolysis is to be effected by administration of active fibrinolysin (plasmin).

**Intrinsic Thromboplastin Generation**

Let us now return to the first stage of coagulation. Our knowledge regarding the essential components (table 1) derives largely from study of thromboplastin generation
Coagulation, Proteolysis, and Thrombosis

Just how intrinsically evolved thromboplastin activates prothrombin, leading to thrombin formation, is also pertinent to the problem of intravascular clotting. Another pathway by which prothrombin can be activated is with certain proteolytic enzymes. As seen in table 5, of many such enzymes explored only trypsin and papain can convert prothrombin to thrombin. Trypsin can convert prothrombin directly, requiring no other ingredients, whereas papain requires another entity, presumably factor VII. Both can be blocked by soy bean trypsin inhibitor. From the known action of trypsin, it is postulated that thrombin is formed by cleavage of the prothrombin molecule at a specific arginyl- or lysyl-peptide bond. In accordance with this deduction, intrinsic evolved thromboplastin may actually be, or potentiate, a protease in plasma with such specific action (prothrombinase). These possibilities are being explored in our laboratories.

More recently investigation of proteolytic activation has been extended to include studies on other clotting factors. Of many proteases investigated, only trypsin, papain, and fecin could activate highly purified factor VII (proconvertin) and factor X (Stuart). Activation was extremely prompt, progressive, and profound. No thrombin evolved, and calcium was not required. In contrast to the activating effects of these proteases on factors VII and X, plasmin (fibrinolysin) and thrombin were inert, whereas they could activate factor V (Ac-globulin). The activating effect of plasmin was promptly followed by deterioration of factor V.

It is also of interest that tissue thromboplastin resembles trypsin and papain in activation of factors VII and X. These observations suggest that the biologic mechanism of thrombin formation, either through intrinsic (blood) thromboplastin or via extrinsic (tissue) thromboplastin, is one of proteolysis.

The possible significance of the proteolytic pathway in the initiation of thrombin formation under pathologic conditions is evident
Table 5
The Effect of Proteolytic Enzymes on Prothrombin, Thrombin, and Fibrinogen

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effect on prothrombin</th>
<th>Thrombin formation†</th>
<th>Effect on thrombin</th>
<th>Thrombin-fibrinogen interaction</th>
<th>Effect on fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F. VII &amp; X- Rich</td>
<td>F. VII &amp; X- Poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminotripeptidase</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>Bromelin</td>
<td>No effect</td>
<td>+</td>
<td>0</td>
<td>No effect for 48 hrs.</td>
<td>---</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Progr. slow inact.</td>
<td>0</td>
<td>0</td>
<td>No effect for 2 hrs.</td>
<td>0</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td>Progr. rapid inact.</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>---</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Progr. rapid inact.</td>
<td>0</td>
<td>0</td>
<td>Progr. rapid inact.</td>
<td>0</td>
</tr>
<tr>
<td>Collagenase</td>
<td>Progr. inact.</td>
<td>0</td>
<td>0</td>
<td>Moderate inact.</td>
<td>---</td>
</tr>
<tr>
<td>Elastase</td>
<td>Progr. rapid inact.</td>
<td>0</td>
<td>0</td>
<td>Progr. rapid inact.</td>
<td>0</td>
</tr>
<tr>
<td>Flein</td>
<td>Progr. very rapid inact.</td>
<td>0</td>
<td>0</td>
<td>Precipitation</td>
<td>0</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>---</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>0</td>
</tr>
<tr>
<td>Oranuicoid trypsin</td>
<td>No effect for 24 hrs.</td>
<td>0</td>
<td>0</td>
<td>Slow progr. inact.</td>
<td>0</td>
</tr>
<tr>
<td>inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>Prompt moderate inact.</td>
<td>(50%), not progr.</td>
<td>0</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>Papain</td>
<td>Immed. &amp; progr. inact.</td>
<td>++ +</td>
<td>0 +</td>
<td>Moder. inact. 24 hrs</td>
<td>Enhanced</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Slow progr. inact.</td>
<td>0</td>
<td>0</td>
<td>Rapid inact.</td>
<td>---</td>
</tr>
<tr>
<td>Plasmin</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>? 0</td>
</tr>
<tr>
<td>Rennin</td>
<td>Progr. inact.</td>
<td>0</td>
<td>0</td>
<td>Modern inact.</td>
<td>---</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>0</td>
</tr>
<tr>
<td>Soy bean trypsin</td>
<td>No effect for 24 hrs.</td>
<td>0</td>
<td>0</td>
<td>---</td>
<td>0</td>
</tr>
<tr>
<td>inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylo-coagulase</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>Moder. inact. not progr.</td>
<td>---</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>No effect</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>followed by recovery in some experiments</td>
<td>+ + + +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsinogen</td>
<td>—</td>
<td>+ + + +</td>
<td>No effect 4 hrs. Slight deter. in 24 hrs.</td>
<td>---</td>
<td>Slight inact. after 30'</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Progr. inact.</td>
<td>0</td>
<td>0</td>
<td>Progr. inact.</td>
<td>---</td>
</tr>
<tr>
<td>Urease</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>No effect for 24 hrs.</td>
<td>---</td>
</tr>
</tbody>
</table>

*Expressed semiquantitatively as 0—+, +, ++. Symbol ‘‘0’’ indicates that no effect was found. The two columns indicate thrombin formation from two types of purified prothrombin: one deliberately prepared so as to contain factors VII and X as contaminants; the other, devoid of these factors.

†Indicating the immediate effect of the enzyme on the clot ability of a standard fibrinogen solution by thrombin. In the instance of plasmin, it progressively (within minutes) blocks the thrombin-fibrinogen interaction as fibrinogen exposure to the enzyme continues. With relatively large concentrations of plasmin fibrinogen clotability is blocked promptly.
when one realizes that normal and certain pathologic plasmas contain enzymes with trypsin-like activity (tryptases) as well as antitryptases. ⁴⁰⁻⁴² Whether such an alternate pathway of prothrombin activation plays a role in the pathogenesis of intravascular clotting deserves extensive exploration. Highly suggestive are recent observations ⁴⁵ that the blood of some patients with thrombotic disease shows significantly increased non-plasmin proteolytic activity. Perhaps of more practical clinical significance in this connection is the current interest in thrombolysis by means of proteolytic enzymes. On the basis of the observations with trypsin described above, the use of this proteolytic enzyme for thrombolysis is deplored. From the point of view of thrombolytic therapy or acquired fibrinolytic states, moreover, the untoward effect of plasmin on factor V has special pertinence.

Hypocoagulability and Hypercoagulability

The possibility of increased thromboplastin generation or excessive trypsin activity in certain thrombotic states again brings the concept of "hypocoagulability" into consideration. It would seem a priori that thrombosis cannot occur if the blood is incoagulable. This sine qua non may not be as definite, however, as appears at first glance. For example, platelets agglutinate even in incoagulable a fibrinogenemic blood. ¹³ This is important in hemostasis. Indeed, this is the sole method of "thrombus" formation in certain invertebrates. It is well known, ⁵⁰⁻⁵⁴ furthermore, that one of the first visible changes in vivo in mammalian blood exposed to a foreign surface, such as a traumatized blood vessel, is the local accumulation of platelets in aggregates, both within and without the vessel. The term "coagulability," "hypocoagulability," "incoagulability," and presumably "hypercoagulability" must therefore be defined more specifically, with special reference to the component or phase of coagulation implicated. This is particularly pertinent to the rationale in the use of anticoagulant drugs, in their aim, their action, and prevention of hemorrhage consequent to their use. Conceivably, agents that induce hypocoagulability by defibrination, for example, might be far less effective than substances that prevent platelet agglutination. Moreover, anticoagulants that affect more than one phase of coagulation might be expected to endanger hemostasis far more than drugs that influence only a single phase. Converse considerations might be reasonably applied to the matter of "hypercoagulability."

Little would be gained by a detailed account of the many claims and counterclaims regarding blood clottability in conditions known to be associated with a high incidence of thrombotic disease. ⁵⁵⁻⁶⁸ Probably much of the controversy is attributable to differences in technics of study, to failure to consider the limitations and inaccuracies of the rough tests employed, the subtleties and complex interrelation of the variables involved, etc. One group of investigators ⁶⁹ even reported that in the Bantu race, in which coronary artery disease is rare, the blood is in certain respects more coagulable than normal.

It is advantageous to define our terms more precisely. What is meant by the word "hypercoagulable"? Experience in hemorrhagic disorders has led us to think of hypocoagulability, which becomes manifest by retarded coagulation; this delayed clotting is associated with deficiency, quantitative or qualitative, in one or more clotting factors, or with circulating anticoagulants arising de novo or administered for therapeutic purposes. It would be natural to view thrombotic disease as the other side of the coin, showing itself in a similar topographical manner. Thus, in some instances at least, intravascular clotting might be due to excessive amounts or activity of one or more of the procoagulant factors. Similarly, a decrease in anticoagulant factors is also possible. The net result in either case might be to shift the delicately balanced equilibrium toward faster deposition and relatively slower removal of a clot.

At present the profile for a disturbance in the dynamics of clotting can be ascertained in the laboratory only by measuring the rela-
tive concentrations of the known clotting constituents. This technic has been most rewarding in diagnosis and treatment in the instance of the hemorrhagic diseases. The same approach applied to the study of thrombosis has not yet produced a reliable procedure for detection of the imminent, incipient, or overt thrombotic state.

Notable among the many tests explored has been the over-all whole-blood clotting time, both with and without the use of heparin in vitro and in vivo, and in both ordinary and silicone-coated glass tubes. Those experienced with this test would not expect it to prove reliable. Even in studies of hemorrhagic disorders, the clotting time is an arbitrary, empiric value, which although reflecting much of the kinetics of coagulation is difficult to perform meticulously. Furthermore, it varies widely even in normal subjects. Moreover, it is well known that coagulation (and hemostasis) can be seriously compromised without the clotting time being abnormal, e.g., in thrombocytopenia, following coumarin drugs, and in other hemorrhagic clotting disorders. Curiously enough, as already mentioned, the converse is also true: in Hageman factor deficiency \(^{70-72}\) the clotting time is markedly elevated yet hemorrhagic phenomena are so rare as to cast doubt on the diagnosis when present.

In general, individual clotting factors can be reduced to as little as 20 per cent of normal before clotting kinetics become significantly impaired. Conversely, it would require relatively enormous increases above normal in a particular component to lower the clotting time significantly below normal (accelerated clotting) because in this range of the curve the kinetics are least sensitive to changes in concentration. This important relationship holds generally for all clotting components.

It should be further emphasized that clotting kinetics in vitro do not necessarily reflect what actually goes on in vivo. The diagnostic value of the clotting time in hemorrhagic diseases derives from the recognized empiric association of an elevated clotting time with certain hemorrhagic disorders, but, as already noted, this correlation is by no means absolute.

It is well known that certain conditions are associated with a high incidence of thromboembolic disease, particularly malignancy, and postoperative and postpartum states. Recently we studied certain clotting parameters in pregnancy. \(^{73,74}\) This was especially selected because it constitutes a common, temporary, reversible physiologic state that is so frequently complicated by thrombosis. Furthermore, coagulation abnormalities reflecting the other side of the coin—hemorrhagic phenomena—are also frequent in the gravid. Moreover, retarded venous flow, constituting the third of Virchow's triad, is well documented in pregnancy. \(^{76-77}\)

Specifically, factor VII (proconvertin) or factor X (Stuart), or both, were found to be strikingly elevated in the gravid. \(^{74}\) There was a significant correlation with the duration of pregnancy; higher values occurring preponderantly in the last trimester. Plasma prothrombin was less significantly altered, although in some subjects the prothrombin also was somewhat elevated.

Following delivery the elevated factor VII level dropped noticeably, usually within 3 to 7 days post partum. Striking elevations were, however, still present in some subjects on the fourth or fifth day following delivery, but in all subjects normal values were attained within 6 weeks. As far as factor VII was concerned, the high levels were in actual proconvertin rather than its activated form, convertin. The relatively inert plasma precursor, present in excessive amounts, could still be highly activated by thromboplastin and calcium.

These observations indicate progressive elevation in factor VII, or in factor X, and in some instances prothrombin, as pregnancy advances. Fibrinogen is also increased in the gravid state; \(^{78}\) and, in the early puerperium, a rise in platelet count and increased platelet stickiness have been reported. \(^{76,77}\) Taken altogether, these changes may be construed as reflecting a truly "hypercoagulable" state, ready to be triggered into pathologic
intravascular clotting. This could be actuated by failure of anticoagulant forces or by the liberation into the circulation of thromboplastic tissue elements from the gravid uterine wall shortly before term, during labor, or in the early puerperium. Such embolization is known to occur in pregnancy, occasionally to the point of complete defibrination of the blood.79, 80 Moreover, the possibility of thromboembolism is considerably enhanced by the progressive slowing in venous blood flow in the leg and pelvic veins as pregnancy advances. Thus, the setting for thromboembolism is ominous, merely awaiting further imbalance by procoagulant excesses or anticoagulant inadequacies.

Question immediately arises as to whether a similar stage exists in coronary or other thrombotic disease. In ischemic heart disease, both acute and chronic, plasma fibrinogen is often elevated. Also, McDonald and Edgill26 claim increased platelet adhesiveness, greater thromboplastic generation, and a faster prothrombin time. Poller,27 moreover, has recently reported increased factor VII activity in 20 patients with 2-day-old thrombotic disease: 18 with coronary thrombosis and two with thrombophlebitis. Many patients also showed increased thromboplastin generation. Changes in the kinetics of thromboplastin elaboration in occlusive arterial and venous disease have also been reported by Spittel et al.,81, 82 attributed to a hitherto unrecognized clotting entity. These authors suggested that a modified thromboplastin generation test may therefore be of diagnostic value. de Nicola83 had similarly reported elevated factor VII in thromboembolic disease, and Moolten et al. have found increased platelet adhesiveness following trauma, surgery, or myocardial infarction.84, 85 The resemblance in these respects to what obtains in the gravid individual is thus striking.

A few words of caution are nevertheless indicated. As was rightfully pointed out by McDonald and Edgill,26 the postulated "hypercoagulability" may be consequent to the thrombotic disease rather than pathogenetic. The observations in pregnancy, however, amply confirmed by others,86-89 cannot be construed as originating from a disease process. Indeed, one is tempted to interpret them teleologically as a mechanism designed to assure prompt hemostasis after parturition, but unfortunately subject to pathologic influences from other factors.

Serum Factors in Experimental Thrombosis

This concept of a "hypercoagulable" state, which in conjunction with venous stasis may be pathogenetic in thromboembolic disease, is supported by exciting experimental work by Wessler and colleagues.14, 90-95 These investigators use a technic far more physiologic than older methods. Instead of depending upon intimal damage induced by mechanical, chemical, or electrical injury,50, 53, 96-106 or upon injections of tissue thromboplastin,107-110 thrombin,101, 111, 112 whole-blood clot,113-119 fibrin,120, 121 or foreign particulate matter,122-131 they infuse serum or purified fractions thereof, and induce local vascular obstruction. Serum had been used earlier also by Hayem132 and Feissly133 to induce in vivo coagulation. The technic is simple and reproducible. Clots of uniform composition and predetermined size can be produced in one or more vessels, arterial or venous, or intracardiac, without systemic disturbance or significant local intimal damage. Their subsequent release permits study of thromboembolic phenomena. The morphology and organization of the serum-induced thrombus is remarkably similar to what is found in spontaneously arising thrombi in man.

The thrombus forms in the partially occluded or completely clamped vascular segment, often also distal to the clamps, and it may fill the entire venous tree distal to the single occluding point. The phenomenon shows no species specificity; homologous or heterologous serum or serum fractions from many species, including human, are effective. Thus, by using a "product" of the coagulation mechanism, Wessler et al. induce a transitory "hypercoagulable" state, which, together with retarded blood flow, results in local clot formation.

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One additional cardinal point emerges from their work: clot begets clot, thus providing a mechanism for in vivo clot propagation, if not initiation, and creation of a vicious circle.

Of considerable interest is the effect of anticoagulation. Prior heparinization prevents the serum-induced clot, whereas short-term dicumarolization to the extent of 10 per cent prothrombin and 1 per cent factor VII activity does not. The degree of heparinization required for protection is an amount sufficient to double the clotting time. This is fully protective although lower levels may afford some protection.

In the light of our work on the profound activation of purified factors II (prothrombin), VII, and X by trypsin, other observations of Wessler are intriguing, if not frightening, since this protease has been advocated therapeutically as a thrombolytic agent. Trypsin administered to dogs with serum-induced venous clots, not only failed to dissolve them, but also resulted in extensive additional thrombi which formed along the stream of blood flow, attaching themselves to the initial clots. The hazard of trypsin therapy is thus clear, in agreement with observations of Taylor et al., whereas no such danger exists with plasmin, since it is inert on factors II, VII, and X. From this point of view plasmin would thus appear safe as a therapeutic agent.

The capacity of the animal to handle substantial amounts of serum-induced clot has also been studied. Massive pulmonary emboli resulting from release of multiple serum-induced thrombi produce only transient physiologic disturbance. Apparently the inherent normal fibrinolytic mechanism can combat this gross insult readily.

If the serum-induced experimental clot has its counterpart in man, as we believe likely, the following crucial question is raised: what is (are) the substance(s) in serum, which, arising during the clotting process itself, produces this "hypercoagulability" state, and which, when blood flow is locally impeded, can cause intravascular thrombosis? Substantial progress has been made in answering this question, although a definitive answer is still not at hand. Some important clues arise from study of the clot-inducing activity of sera derived from patients with specific clotting abnormalities. Sera deficient in factors V (Ae-G), VIII (AIF), and X (Stuart) were found fully active. The essentiality of these factors can thus be excluded. Similarly the data with factor VII deficiency indicated that it, too, is not required. Apparently the presence of factor IX and Hageman factor, or some related entity, is however essential for the thrombus-inducing phenomenon. It will be recalled that these substances function in the earliest phase of coagulation, namely, thromboplastin generation, yet other factors similarly necessary for this phase—factors V, VIII, and X—are apparently not necessary for elaboration of the serum clot-inducing activity.

Morphologic studies by Wessler et al. of serum-induced thrombi and their subsequent embolization suggest that under certain circumstances a comparable mechanism may occur in man. Such clots might be difficult to distinguish from those originating as a platelet nidus at a local site of endothelial injury, followed by continued clot accretion. This concept is supported by examination of serum-induced experimental thrombi permitted to embolize 2 weeks after their formation. Months later the emboli are represented only by minute yellow flecks, some flat and plaque-like, adherent to the intima, and undergoing organization. Foam cells in their base complete a picture that closely resembles the atheromatous plaque described by Duguid.

We thus have a valid, and vicious circle: "hypercoagulability," inducible as in the experimental model described above, or acquired under pathologic circumstances. Aided and abetted by local factors, a thrombus is formed; clot then begets clot; clot also begets atheromatous vascular degeneration; atheroma begets clot, and so on. Rational therapy would necessitate breaking the circle at any one of several points. Prevention would be predicated on early recognition of the ingre-
dients in the intricate stage-setting, and holding them in abeyance by maintaining the normal coagulation balance.

**Lipids, Coagulation, and Atherosclerosis**

Let us now consider the role of lipids in coagulation, in atherogenesis, or in both. It is now generally accepted that some of the unsaturated lipids perhaps in their ratio to their saturated counterparts, influence lipoprotein composition and plasma cholesterol concentration. The relationship between these and atheromatous disease is still uncertain and controversial.140,141

Be that as it may, there is now little doubt that the lipids affect coagulation.142 Crude lecithin and cephalin have thromboplastic activity143 which is probably referable to their content of phosphatidyl ethanolamine, phosphatidyl serine, or both.142-146 Numerous investigators have also been concerned with the clot-accelerating effect of neutral fat ingestion.147-157 Although there is still considerable controversy, most agree that either saturated or unsaturated fats accelerate both spontaneous coagulation in collodion or silicone-coated tubes, or clotting induced by the thromboplastic activity of Russel’s viper venom (the “Stypven” time).142 Conversely, removal of fat chylomicra from plasma by high-speed centrifugation retards clotting. This can be corrected by their restoration or by addition of crude lecithin.158 The active agent is thought by most investigators to be phosphatidyl ethanolamine,142 which is also present in platelets and which is postulated to be essential for physiologic coagulation. Whether the clot-accelerating effect of a fatty meal is directly referable to increase in the plasma concentration of neutral fat per se, of fatty acids, or of important phosphatides, is however still obscure in view of the complex composition of ingested fats.157-159

At first glance it would appear that the only significance of the “hypercoagulability” induced by lipids is that a person who ingests a fat-rich meal should avoid being bitten by the Russel viper, as intimated by O’Brien.153 Likely to be more significant are recent observations that the fats may influence the clotting balance through their inhibitory effects on fibrinolysin,160,161 as well as by their enhancement of platelet count and adhesiveness.85 Greig and Runde,161 and others162,163 showed that meals, particularly rich in fats of low iodine number, **reduce** plasma fibrinolytic activity. This is said to be reversible by removal of the plasma chylomicra and beta lipoprotein.

Of further interest is the finding that cholesterol oleate inhibits fibrinolytic activity.164,165 Also pertinent are the findings of Kwaan et al.164,166 that experimentally induced thrombi took four times longer to lyse in cholesterol-fed rabbits than in normal ones, and the recent report of Heptinstall167 who produced rapid lipid deposition and atheroma in the pulmonary arteries of rabbits injected with fibrin emboli following a large infusion of hyperlipemic serum, or after 6 days of high-cholesterol feeding. Furthermore, by incorporating large amounts of various fats including butter and lard into the diet, Hartroft168 could induce in rats an extremely high incidence of coronary and renal artery thrombosis, the animals having first developed arterial lipoidosis consequent to the administration of cholesterol, cholate, and thiouracil by the technic of Fillios et al.169

All these observations provide support for an intriguing and challenging theory, originally postulated by Greig,160 which now embraces both current concepts concerning coagulation, the respective roles of fibrin-fibrinolysin, and fat metabolism, not only in atherogenesis but also in thrombotic disease. Indeed, the relationship between diet and thromboembolism in general, as discussed by Keys,170 may have broader significance than simply the possible role of diet in atherogenesis per se. It should be emphasized, however, that the above theory does not exclude other pathogenetic mechanisms, postulated or proved.

In our presentation we have thus turned complete cycle, returning to our starting point—the v. Rokitansky-Duguid-Astrup hypothesis. In our course, encompassing athero-
sclerosis and intravascular thrombosis, we have delineated some pertinent concepts and aspects of the coagulation sequence, with particular reference to its early phase, and have considered the normal coagulation balance and the "hypercoagulable" state, wherein the lipids also may be operative.

How can these concepts be further tested? In the light of the theory of continuous intravascular coagulation and the clotting balance, it is suggested that a fruitful approach might be study of the turnover rate of certain labeled clotting constituents under experimental conditions designed to shift the coagulation equilibrium, and in those clinical states in which hypercoagulability is suspected. The "enercystation" concept might be further elucidated by ascertaining with meticulous clinicopathologic study, the degree of atheromatous disease in patients with long-term heredo-familial hypercoagulability due to coagulation defects, e.g., hemophilia, PTA deficiency, and Hageman factor deficiency. A relatively normal amount instead of less atherosclerosis would tend to discredit the concept. Of perhaps even greater significance would be the findings, however meager, in all available cases of congenital asfibrinogenemia. Similar thoughts have been entertained by Astrup as well as Ratnoff. In this way we would, to paraphrase the words of Osler, be making worthy use of the unique experiments of Nature.

Anticoagulation

Finally, a few words of practical value regarding the effects of anticoagulants on the basic clotting mechanism. Heparin acts both as an antithromboplastin in the earliest phase of clotting and, together with an as yet unidentified co-factor in plasma, as an antithrombin. It can be rapidly counteracted by protamine. In contrast, the coumarin drugs and related compounds act by depressing first factors VII and X; second, factor II (prothrombin); third, factor IX (PTC) —in that order.172, 173* Their action is in equilibrium with the body stores of vitamin K, and can be reversed by supplements of this nutrient.

Which or what combination of, anticoagulants will prove most effective, and how they should be applied on a broad scale in prevention and long-term treatment, awaits longer experience. It would be expected a priori that any agent that produces a deficit in components functioning in more than one phase of clotting would in general compromise coagulation (as well as hemostasis) more profoundly than drugs that reduce single-phase components. At present, not enough is known to permit a definitive statement as to which clotting factor, or combination of factors, is crucial to the pathogenesis or prevention of thrombotic disease. It is generally accepted that of the coumarin-vulnerable entities (factors II, VII, IX, and X), factor II (prothrombin) appears to be least important. As to hemorrhagic complications of coumarin therapy, however, considerable disagreement exists; according to some observers172 prothrombin is most significant. Others173 claim that factor X is the more important, while some believe measurement of the total activities of all these four factors is the best guide to anticoagulant therapy and its hemorrhagic complications.174 Many of these aspects of anticoagulant therapy have recently been reviewed.175

Summary

The role of blood coagulation in the pathogenesis of thrombotic disease as well as atherosclerosis is receiving renewed attention, and has been greatly extended into the area of therapy. The clotting mechanism has been delineated in accordance with most recent information, with particular reference to the early phases and the coagulation balance. The close relationship between clotting and proteolytic phenomena is considered, and the concept of hypercoagulability is critically scrutinized. Experimentally induced thrombosis by means of serum factors is reviewed in support of this concept. Also discussed is

*Some observers believe that PTC is depressed earlier. The discrepancies may be related to the use of different drugs, dosage schedules, and assay technics.
the place of lipids in coagulation and atherosclerosis in an attempt to embrace the lipid theory of the pathogenesis of atherosclerosis with current concepts of clotting. Finally, certain aspects of anticoagulant action are covered.

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Erratum

In the article by Jolliffe et al., December 1961 issue, the graphs for figures 1 and 2 on pages 1417 and 1419 are interchanged. The legends for these figures are correct as labeled.
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