78.

HEMOSTASIS MANUAL

1968

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The hemostatic mechanism is so designed as to maintain fluidity of the blood, yet with the capacity to rapidly repair any break in the vascular endothelium. The potential for rapid localized hemostasis within a fluid media is not without risk. Imbalance in one direction leads to excessive bleeding, and in the other to thrombosis. Considered to be part of the hemostatic apparatus are the blood vessels, platelets, coagulant, anticoagulant, and lytic plasma factors. The active phase of hemostasis consists of a series of reactions leading to a mechanical seal (the platelet plug and clot) followed by slow lysis and final repair. The chemical nature of clotting factors and their interactions are only partially understood. However, sufficient knowledge is available for a practical understanding of hemostasis and an orderly approach to its disorders.

I. BLOOD VESSELS

a. Vessel structure and function

The arterial and venous systems are durably built for the bulk transport of blood rather than for membrane exchange of oxygen and carbon dioxide. Arteries, because of their heavy muscular coats, are the most resistant of all vessels to bleeding so that severe trauma or erosive disease is required for their rupture. When bleeding does occur, it is under considerable pressure ("blow-out" hemorrhage) and constitutes the most severe test of the hemostatic mechanism. Arterial hemostasis is primarily accomplished by vascular contraction, although fibrin formation and platelet aggregation are also required. Veins which contain some 70% of the blood volume are thin-walled and relatively non-elastic. They may rupture with only slight trauma, particularly in the legs. Large, ill-defined soft tissue hemorrhages (ecchymoses) result. The control of bleeding from large veins depends on vascular contraction as well as both extra- and intravascular clotting factors.

The capillaries constitute a mass more than twice the size of the liver. Anatomically they are delicate tubes of tightly adherent endothelial cells surrounded by a pericapillary sheath with an inner layer of ground substance and an outer layer of connective tissue fibrils. Capillaries have extremely thin walls, low pressure, and slow blood flow to facilitate exchange between plasma and extravascular fluids. The capillaries, small arteries, and small veins constitute together the microcirculation. Maintenance of the microcirculation depends on the integrity of the pericapillary sheath externally and on the internal action of platelets in repairing endothelial breaks. Increased capillary permeability (leakage of plasma proteins) is seen in inflammatory lesions of the capillary walls, but is not part of hemostatic disorders. Increased capillary fragility (leakage of red cells) is reflected by tiny hemorrhages or petechiae, usually from the arteriolar end of capillaries. Capillary lesions are mainly sealed by endothelial adhesions, and lesions of small veins and arteries are capped by platelet plugs. Coagulation becomes progressively more important as the vascular size increases. From a clinical standpoint it is not possible to make a clear distinction between pure capillary bleeding and bleeding from small arterioles and venules. In general, the larger the area of bleeding, the larger the vessel involved.

However, hemorrhages are also proportionate to the severity of the hemostatic abnormality.

b. Vascular disorders

It is often difficult to demonstrate the cause of vascular bleeding, but there are a number of recognized causes of vascular damage. The most important of these is immunologic damage. Hemorrhagic rashes occur associated with drug reactions, as part of collagen diseases, and serum sickness. So-called allergic or anaphylactoid purpura, assumed to be immunologic, is a serious clinical condition because of the extent of involvement of the microvasculature. Small venules of the lower extremities may be particularly affected and may be accompanied by arthritis (Schonlein's purpura) or damage to the submucosal vessels of the intestinal tract (Henoch's purpura) which may lead to extensive gastrointestinal bleeding. Extensive pulmonary bleeding (Goodpasture's syndrome) and glomerulonephritis are also seen as manifestations of anaphylactoid purpura. If the vascular lesions involve small arterioles, a clinical picture is quite different (thrombotic thrombocytopenic purpura), characterized by hemorrhagic infarction especially in kidney and brain and dysfunction of these two organs.

Bleeding may also occur from obstruction to blood flow. Abnormal proteins of high viscosity such as cryoglobulins and/or macroglobulins may block flow in small vessels and produce vascular rupture. Embolism by fat, tumor, bacteria, or atheroma may similarly produce purpuric lesions. Widespread small vessel thrombosis by fibrin formation during intravascular clotting may result in local necrosis and bleeding. Skin purpura is also seen in severe bacterial infections, perhaps due to damage of vascular walls by bacterial toxins (Waterhouse-Friderichsen syndrome of meningococcemia). Direct damage by the offending organism to the vascular wall is also seen in certain bacterial and rickettsial diseases.

The congenital vascular anomalies of familial telangiectasia produce focal bleeding while Ehlers-Danlos syndrome, associated with a congenital defect in elastic tissue of vessels, may produce more general bleeding. The purpura of senility or Cushing's disease, involves larger vessels and is considered to be due to a decrease in supporting tissues of venous adventitia. In vitamin C deficiency, the occurrence of purpura is ascribed to a lack of cementing substance. Abnormal purpura may also occur as the result of amyloid in intestinal vessels and of hyaline deposits in small vessels in diabetes.

II. PLATELETS

A continuous function of platelets is the maintenance of integrity of small vessels. This is illustrated by the general migration of red cells through vascular walls in severe thrombocytopenia. A second essential function of platelets is the formation of a plug at the site

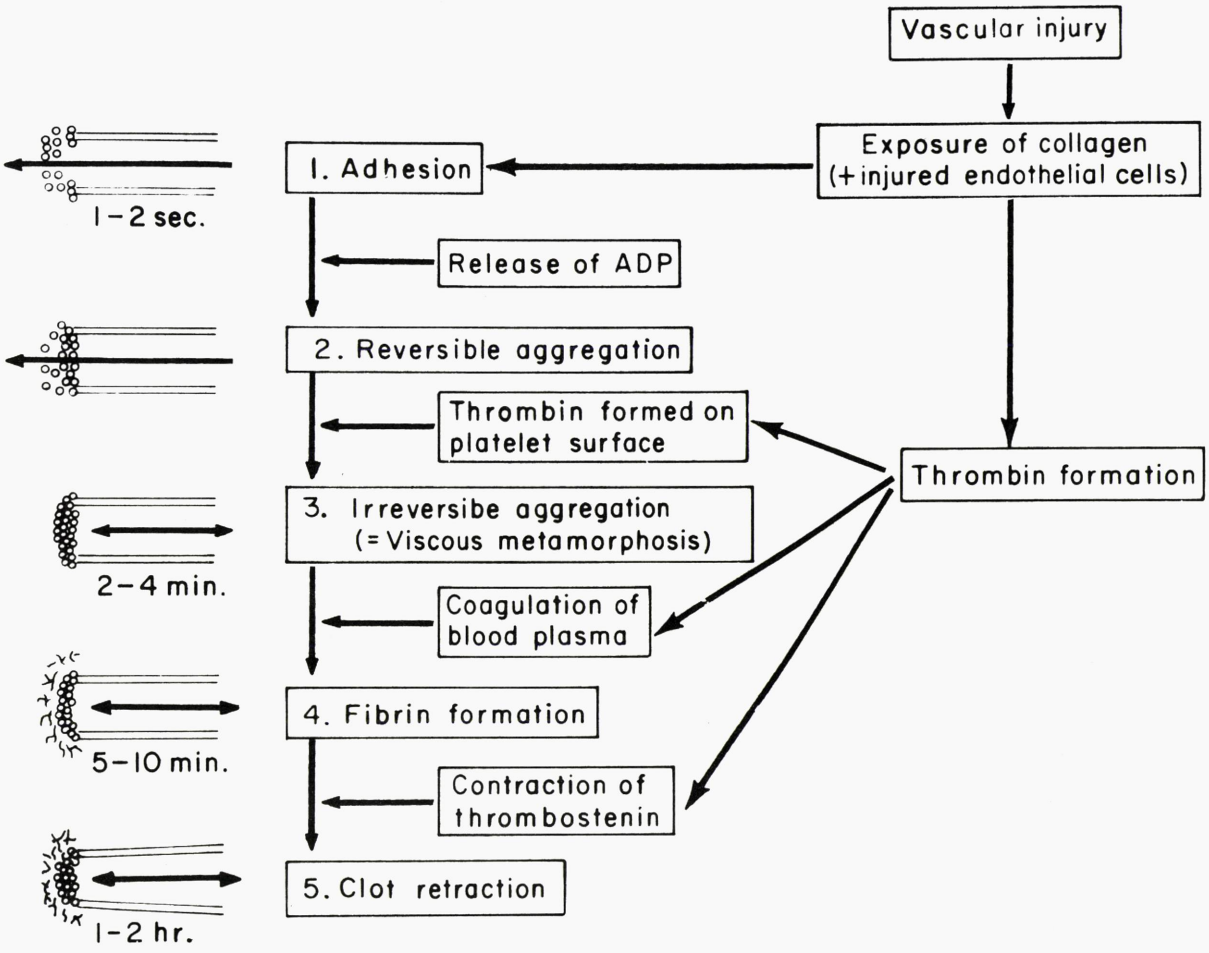


Fig. 1. Stages in the formation of a platelet plug (white thrombus).

of vascular injury, which also acts as a nidus for activation of the coagulation system at the point of damage.

a. Platelet structure and function

Platelets circulate as cytoplasmic discs about 2 μ in diameter and about 5 u^3 in volume. With Wright's stain they have a clear blue outer zone and azure-colored central granules. These granules contain a high molecular weight phospholipid (platelet factor 3). Within the cytoplasm there are also mitochondria which generate metabolic energy and microtubules which are involved in the physical structure of platelets and in clot retraction (Figure 1).

The in vivo function of platelets involves sequential steps which are initiated by vascular injury. Platelets first adhere to the injured vessel wall, especially to exposed collagen fibers. The adherent platelets induce aggregation of adjacent circulating platelets by the release of substances of which adenosine diphosphate is thought

to be the active component. This reversible aggregation does not depend on coagulation but does require intact platelet glycolysis and perhaps the platelet factor which is lacking in von Willebrand's disease. The resulting mass of platelets forms the provisional platelet plug which abruptly undergoes an irreversible change into a solid mass impervious to blood flow (referred to as "viscous metamorphosis"). In this process thrombin triggers the formation of fibrin as well as the liberation of the phospholipid platelet factor 3. The resultant fibrin seals the platelet membranes together and firmly anchors the solidified platelet plug to the vascular wall. The release of phospholipid which is morphologically characterized by platelet degranulation further accelerates coagulation. In addition, the plug is strengthened by the gradual process of clot retraction which consists of orientation of fibrin strands by the structural change of the contractile protein thrombosthenin within the platelet. Some of the essential processes involved in the formation and consolidation of the platelet plug have been high-lighted by disease states in which a specific abnormality exists. Thus, aggregation of platelets may be impaired with defective platelet glycolysis (thrombasthenia or Glanzmann's disease), with coating of the platelet membrane by dextran and perhaps macroglobulin. In von Willebrand's disease, the prolonged bleeding time has been interpreted as an abnormality in platelet plug formation, and this is ascribed to a plasma factor deficiency. Consolidation of the platelet plug ("viscous metamorphosis") requires fibrinogen, since with total afibrinogenemia it does not occur. Failure of clot retraction also occurs with glycolytic abnormalities of the platelets.

b. Platelet kinetics

Platelets are produced in the bone marrow from mature megakaryocytes by the fragmentation of their cytoplasm into the circulation. The megakaryocyte, which has a lifespan of about 10 days, arises from an undifferentiated precursor cell and undergoes repeated nuclear replication within a common cytoplasm (endomitosis). Because intracellular nuclear replication occurs synchronously, the number of nuclei within a megakaryocyte is 2, 4, 8, 16, or 32. Normally, 10 - 15% of the cells have 4 nuclei, 60 - 65% have 8 nuclei, and 20 - 25% have 16 nuclei. The initial phase of nuclear multiplication takes about three days. Cytoplasmic volume is directly related to the number of nuclei within the cell. Cytoplasmic maturation is morphologically characterized by the appearance of granulation; and in the normal marrow about a fourth of the megakaryocytes show no granulation, a fourth have partial granulation, and one-half are fully granulated. There is approximately one megakaryocyte for every 500 nucleated red cells in the normal marrow.

The production of platelets is under the regulation of a humoral factor, thrombopoietin, much as erythropoiesis is regulated by erythropoietin. When thrombocytosis is artificially produced by platelet transfusion, platelet production is decreased; whereas platelet production is augmented by thrombocytopenia. One mechanism for changing platelet production is through the regulation of endomitosis. In stimulated states the number of nuclei within the megakaryocyte increases with a parallel increase in cytoplasmic volume and,

consequently, in platelet production per megakaryocyte. A second mechanism for changing platelet production is by altering the number of megakaryocytes produced. By these two devices platelet production may increase 3 - 4 times acutely, and 6 - 8 times in chronic thrombocytopenic states. The initial change in platelet output after stimulation is about 4 days.

Circulating platelets are normally present in a concentration of 250,000 ± 75,000 per cubic millimeter of whole blood. Survival of transfused platelets is nearly linear, suggesting that most platelets have a finite lifespan of about 10 days. There is no significant rapidly mobilizable reserve pool of platelets.

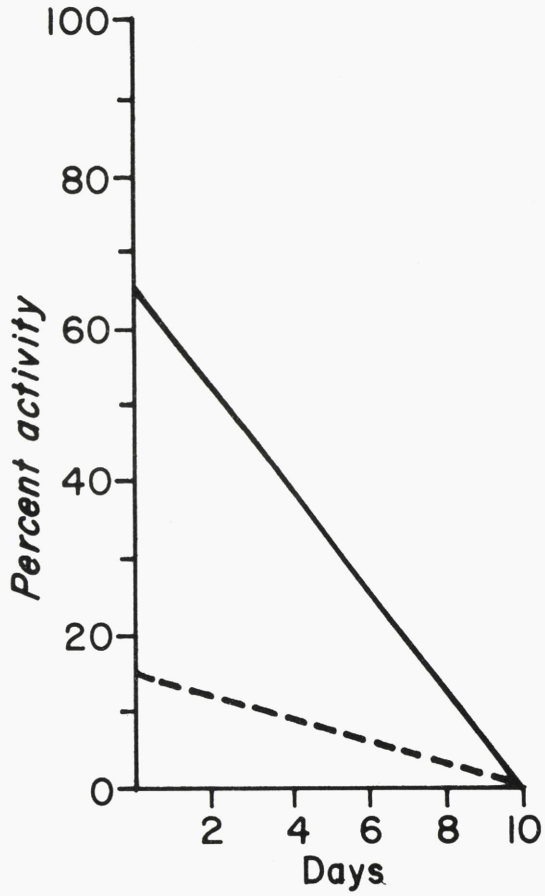


Fig. 2. Platelet survival. The normal survival curve obtained by ^{51}Cr-tagging is shown. Most of the initial "loss" is due to platelet sequestration in the spleen, since 90% of activity is found in circulation in splenectomized subjects. Splenic localization is much greater in patients with splenomegaly (shown in dashed line).

Platelets are concentrated in the spleen, and this splenic platelet pool is maintained by dynamic equilibrium between the splenic and the general vascular system. The amount of platelet sequestration in the spleen appears to be proportional to the amount of splenic red pulp and normally consists of about 25 - 30% of all the platelets in the body. With marked congestive splenomegaly, for example, the equilibrium favors the splenic vasculature, and splenic pooling may amount to 90% or more of the total platelets. The distribution between the general and splenic vasculature can be estimated by determining the proportion of ^{51}Cr-tagged platelets that remains in the general circulation after infusion (Fig. 2).

Although the pattern of platelet size is usually constant, changes do occur. A 15 - 25% decrease in average platelet volume is present in marked iron deficiency. In contrast, platelets released under the stress of severe thrombocytopenia are increased in volume 25 - 50% ("shift" or "stress" platelets).

c. Quantitative disorders of platelets

Thrombocytosis (2 - 3 times normal) may be seen as part of an inflammatory response (infection, neoplasm, postoperative). The platelet count is also elevated for weeks or months after splenectomy, and the degree of thrombocytosis is roughly proportional to the pre-operative degree of splenic sequestration. Thrombocytosis, usually of a much greater degree, occurs in myeloproliferative disorders (polycythemia vera, chronic granulocytic leukemia, and so-called thrombocythemia). Such very high platelet levels, usually over one million, may result in bleeding, especially from the gastrointestinal tract, and this bleeding will remit when the platelet count is reduced by treatment.

The pattern of megakaryocyte nuclear number differs in these states and may be of diagnostic assistance. In thrombocytosis due to inflammation, the pattern of nuclear number is shifted towards smaller nuclear numbers, indicating a decrease in nuclear replication, apparently in response to the increased platelet count. The number of megakaryocytes, of course, is greatly increased and overwhelms the decreased production of each megakaryocyte, producing the resultant thrombocytosis. In contrast, thrombocythemia is characterized by a greatly increased average number of megakaryocyte nuclei, which is inappropriate for the high peripheral platelet count, suggesting autonomous proliferation.

Thrombocytopenia is probably the most common cause of major bleeding in man. Hemostasis may be well maintained with platelet levels of only 5,000 per cubic millimeter in the absence of trauma or other hemostatic abnormalities and with normal function of the residual platelets. However, levels of 5,000 - 60,000 may be associated with excessive bleeding even though other abnormalities are not demonstrable. Thrombocytopenia stimulates an increase in endomitotic nuclear replication to achieve greater platelet production per megakaryocyte. The number of cells differentiating from the precursors is also increased.

The amount of cytoplasm of the megakaryocyte determines its platelet output. Estimates of total production represent the product of megakaryocyte number and volume compared with normal. Platelet turnover, $\frac{\text{platelet count}}{\text{platelet survival}}$, reflects effective platelet production. When such measurements are compared with normal, total and effective thrombopoiesis can be evaluated. The difference represents ineffective production. For example, in idiopathic thrombocytopenic purpura, no ineffective thrombopoiesis is present, i.e., total production equals effective production. Similarly, production in thrombocytosis of inflammation is effective. However, significant disparity between

total and effective production, i.e., ineffective thrombopoiesis, is characteristic of maturation disorder (B_{12} or folate deficiency) and the autonomous production of thrombocythemia. Occasionally a patient with thrombocytopenia and active megakaryocyte production in the marrow has a normal platelet survival indicating ineffective thrombopoiesis as the functional disorder. Before platelet survival measurements were available, such a patient would be diagnosed as ITP and subjected to an unsuccessful splenectomy. Typical examples of such production estimates in Table I illustrate these findings.

TABLE I

	Thrombopoiesis			
	Effective Platelet Production			Total Platelet Production
Disorder	Platelet Count/µl	Platelet Survival	Proportion of Normal*	Proportion of Normal[x]
ITP	15,000	2.4 hrs.	6.0	6.0
Hypersplenism	100,000	10 days	2.0[‡]	2.0
B_{12} deficiency	50,000	10 days	0.2	4.5
Secondary Thrombocytosis	500,000	10 days	2.0	2.0
Thrombocythemia	1,500,000	10 days	6.0	15.0

* Expressed as the number of times increased over normal platelet turnover, which is $\frac{250{,}000 \text{ plat/ul}}{10 \text{ days}}$ = 25,000 platelets/ul/day.

[x] Expressed as the number of times increased over normal total megakaryocyte volume, which is 6.0×10^6 kg $\times$ 4700 u^3 = 2.8×10^{10} u^3/kg.

‡ Corrected for the amount of splenic pooling over normal.

When thrombocytopenia is detected by blood film or platelet count, the cause is in one of the following categories.

1. Disorders of production

(a) Hypoproliferative disorders in which the total number of megakaryocytes is either reduced (absolute) or is not appropriately increased in the presence of thrombocytopenia (relative). Endomitosis in those megakaryocytes present is, however, increased. Characteristic disorders include those involving total marrow damage (irradiation or drugs), leukemic invasion of the marrow or marrow replacement (fibrosis, metastases).

(b) Maturation disorders in which the number of megakaryocytes is increased but production of viable platelets is reduced (ineffective thrombopoiesis), as is endomitotic replication. Platelets

are often variable in size and are of bizarre shapes. This condition is seen in vitamin B_{12} deficiency and folate deficiency.

2. Disorders in distribution of platelets

In patients whose splenic red cell pulp is increased in size (congestive splenomegaly or myeloid metaplasia), there may be localization of the majority of platelets within the spleen.

3. Disorders of destruction and loss

Increased destruction of platelets may be considered under two headings:

(a) Immunological injury involves direct reaction of an antibody with antigens intrinsic to the platelet. With milder injury, the spleen is often the main organ of destruction; whereas with more severe injury, platelets are disposed of largely in the liver. There are three major kinds of immunologic mechanisms.

(i) Auto-antibody formation, which is usually idiopathic (ITP), a complication of lymphatic leukemia, or of disseminated lupus erythematosus. Unfortunately, such auto-antibodies are usually not demonstrable by in vitro tests. Indirect evidence of immunologic mechansims may be obtained by measuring either the lifespan of transfused platelets or the remedial effect of large doses of steroids.

(ii) Iso-antibodies which occur as a result of sensitization by either transfusion or pregnancy, are often demonstrable by complement fixation tests.

(iii) Drug-induced antibody mechanisms involve an antibody, a drug (such as quinidine, quinine, or sulfonamides), and platelets.

(b) Intravascular coagulation produces depression of the platelet count associated with depletion of those clotting factors which are consumed during clotting. In anomalies of vascular structure, such as hemangiomas, intravascular clotting may be a continuous process. Platelets are localized in such vascular lesions, and the entire process may be reversed by the anticoagulant effect of heparin. Intravascular clotting may also occur as a sudden fulminating, life-threatening process with virtual disappearance of platelets and clotting factors within minutes or hours. Usually, however, the thrombocytopenia with intravascular coagulation is not as severe as is seen with immunologic destruction. So-called intravascular clotting may be activated by extensive endothelial damage or from a variety of disease processes (bacterial toxins, antigen-antibody reactions, shock, neoplasm, fat embolism, extracorporeal circulation, severe hemolysis, amniotic fluid embolism, and in polycythemia).

III. COAGULATION

The coagulation system (1) converts the platelet plug through the action of thrombin into a cohesive mass anchored by fibrin, and (2) produces clots in large vessels by the massive conversion of fibrinogen to fibrin much as a clot forms in a test tube in vitro. The end point of normal coagulation is the conversion of fibrinogen into a fibrin clot. This is preceded by a complex reaction sequence which acts as an

amplifier, building up a large amount of enzymatic activity for fibrinogen conversion to fibrin. The understanding of coagulation is simplified by examining the various steps in this sequence of reactions that lead to thrombin formation.

a. The coagulation factors

Earlier ideas of blood coagulation visualized the activation of thrombin from an inactive precursor, prothrombin. In the search for the means of activation of prothrombin, a number of other clotting factors have been uncovered. The identification of these factors has largely come from studies on patients with bleeding due to an inherited deficiency of a specific clotting factor. As new patients were found, the identity or lack of identiy of their clotting factors with previously recognized deficiencies was established by mixing experiments, determining whether or not the two abnormal plasmas corrected each other in in vitro tests. In order to avoid confusion on terminology, an International Committee on Nomenclature of Blood Clotting Factors was established. The minimum criteria for clotting factors were defined by this committee as, (1) reliable data on stability, absorbability, and inactivation, (2) a clinical identifiable state, usually a bleeding disorder caused by a deficiency of the factor, and (3) reliable assay methods. On the basis of these criteria, twelve factors have so far been accepted, and each has been assigned a Roman numeral. These factors are tabulated in the following table.

TABLE II

Factor	Name	Clinical Syndrome due to Deficiency
I	Fibrinogen	+
II	Prothrombin	+
III	Tissue thromboplastin, thrombokinase	0
IV	Calcium	0
V	Proaccelerin, Ac-globulin, labile factor	+
VII	Proconvertin, stable factor	+
VIII	Antihemophilic A factor or AHF, Antihemophilic globulin or AHG	+
IX	Antihemophilic B factor or AHB, plasma component or PTC, Christmas factor	+
X	Stuart or Stuart-Prower factor	+
XI	Plasma thromboplastin antecedent or PTA	±
XII	Hageman factor, contact factor	0
XIII	Fibrin stabilizing factor, Laki-Lorand factor, fibrinase	+

It will be noted that factors III and IV do not fulfill the previous criteria for a coagulation factor. Tissue thromboplastin (factor III) is not found at all in the blood, but is found in most body tissues and can be isolated in large amounts from brain or lungs. All other coagulation proteins are present in trace amounts except for fibrinogen, the normal level of which is 200 - 400 mg percent. There was originally a factor VI, but this proved to be an intermediate product and not a clotting factor and, therefore, is not currently used. Additional factors, such as "Dynia factor", have recently been described but their exact role in coagulation has not yet been defined.

b. Interaction of clotting factors

It is useful to construct a conceptual model expressing the interrelationships of these various factors. The model generally accepted divides coagulation into two overlapping systems, the intrinsic and the extrinsic systems, as shown in the following figure.

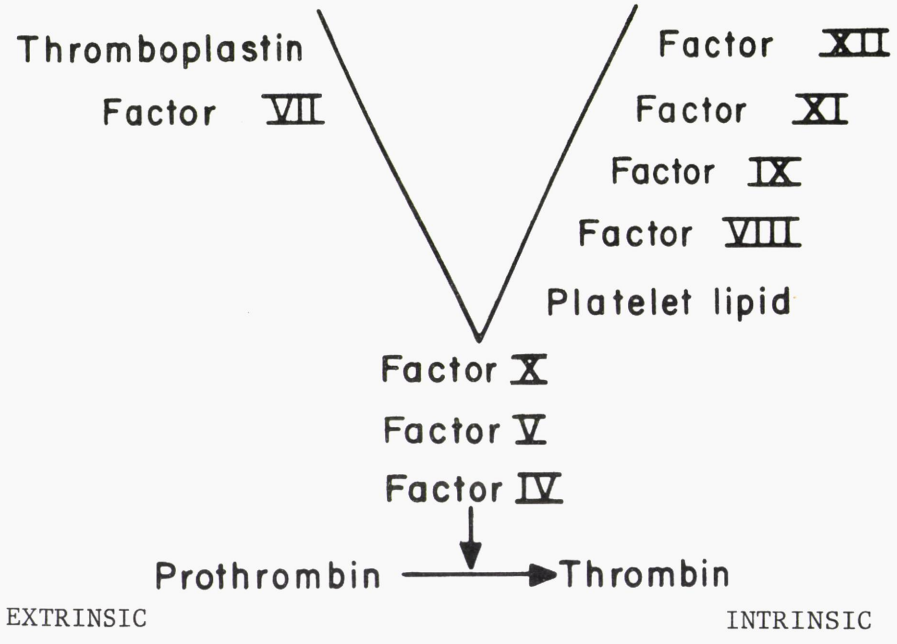


Fig. 3. Prothrombin activation.

All factors required for the intrinsic system are present in the circulating blood, while an extrinsic system depends on a tissue lipoprotein, thromboplastin, or factor III which is released from damaged cells and requires only a portion of the clotting factors of the intrinsic system. Tissue thromboplastin appears to have two active components, a protein (enzyme) which activates factor VII, and a phospholipid. The extrinsic clotting system may well operate intravascularly also, since the vascular endothelium contains tissue thromboplastin.

The intrinsic system (Fig. 4) is triggered in the test tube by contact with a foreign surface. In the body, it is activated in a different but still unknown way, possibly from the tissues by very small amounts of tissue thromboplastin or by a factor in platelets. In the test tube, factor XII is activated by surface contact, such as with glass. Plasma collected in glassware lined with paraffin or silicone does not clot and may stay fluid for 24 hours or longer. Once factor XII is activated, it converts factor XI to an activated form.

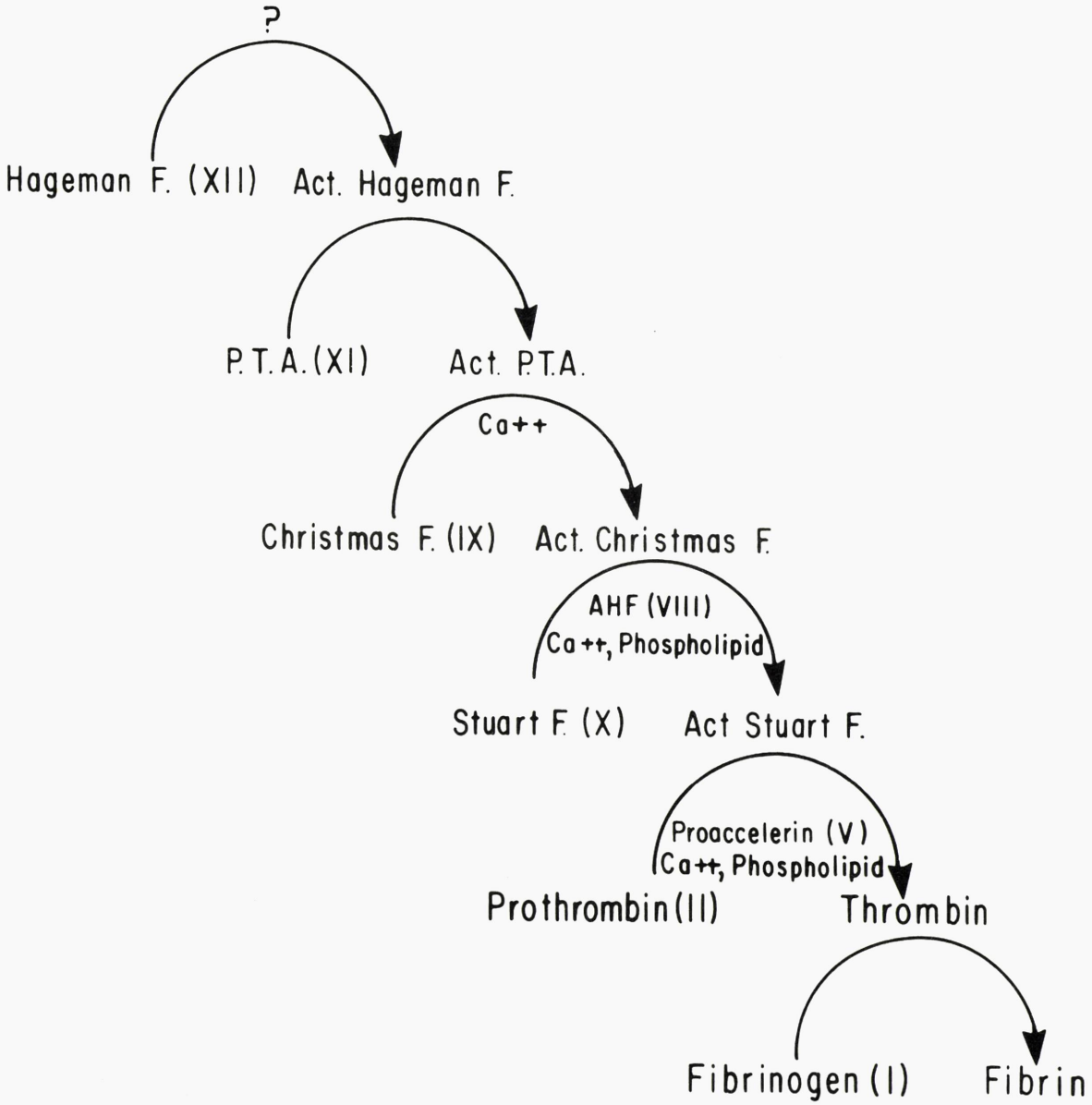
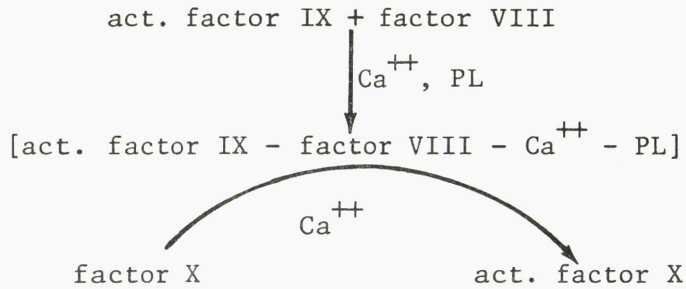


Fig. 4. Tentative mechanism for the initiation of blood clotting in mammalian plasma in the intrinsic system.

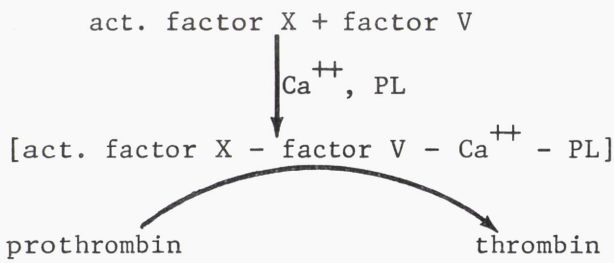
The next reaction in this sequence is the activation of factor IX by activated factor XI. In this reaction, activated factor XI participates as an enzyme, converting its substrate, factor IX, to an activated form. The reaction has an absolute requirement for divalent metal ions such as calcium. The activation of factor IX is blocked by a number of inhibitors. Agents such as citrate and oxalate are effective inhibitors by virtue of their calcium-binding capacity. The chemical events which occur during the activation of factor IX are unknown. It probably involves partial proteolysis of the factor IX molecule.

Once factor IX becomes activated, it interacts with factor VIII (AHF) in the presence of calcium ions and phospholipid (PL) to form a complex capable of activating factor X (Stuart factor). These reactions are as follows:



The phospholipids required in the interaction of activated factor IX and VIII are supplied by the platelets. Mixtures of various phospholipids such as equal amounts of phosphatidyl serine and phosphatidyl choline are very active in this reaction. This reaction is also blocked by heparin, a sulfonated polysaccharide which apparently forms an inactive complex with activated factor IX.

Activated factor X then interacts with factor V (proaccelerin) in the presence of calcium ions and phospholipid (PL) to form a complex capable of activating prothrombin.



These reactions are analogous in many respects to those involving activated factor IX.

c. Fibrin formation

In the final series of reactions, thrombin converts fibrinogen (factor I) to fibrin by partial proteolysis (Fig. 5). Fibrinogen is

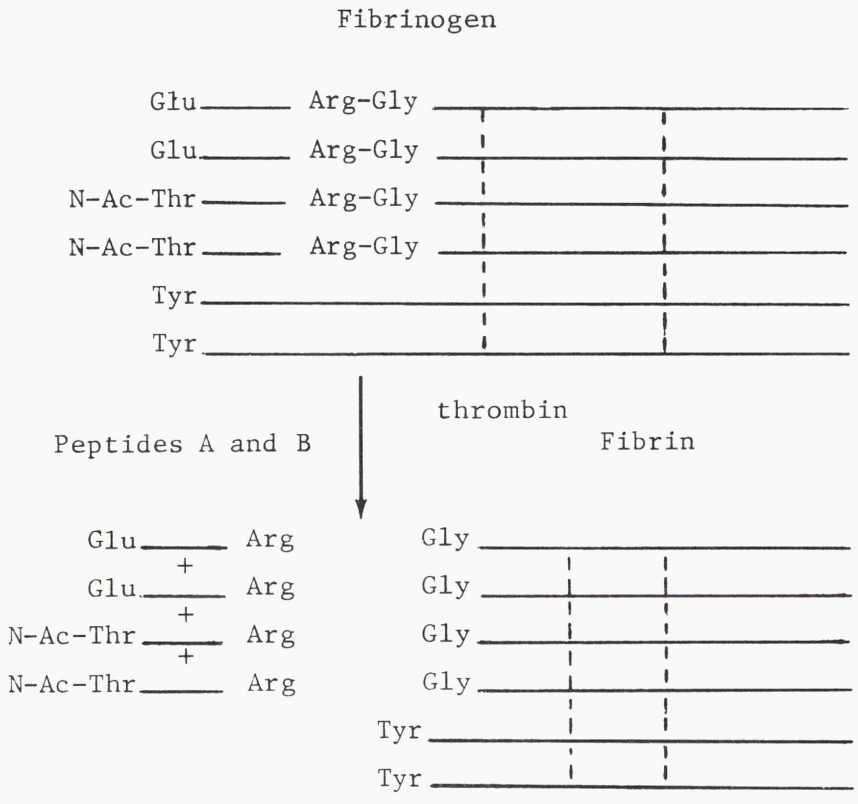


Fig. 5. Conversion of bovine fibrinogen to fibrin monomer by thrombin.

one of the larger proteins in the plasma with a molecular weight of about 350,000. Thrombin specifically attacks the arginine-glycine bonds in the fibrinogen molecule, splitting off two different peptides constituting about 3% of the fibrinogen molecule. Of these, peptide A from the end of the molecule contains 19 amino acids and is released early in the reaction; polypeptide B from the center contains 21 amino acids and is released later in the reaction. Peptide B has an interesting property in that it stimulates vascular contraction and thus diminishes blood flow.

The next step is the spontaneous polymerization of fibrinogen molecules into the fibrin net (Fig. 6). Both end-to-end and side-to side aggregation occur by hydrogen bonding. A final step is required before the clot acquires full strength. The plasma contains a proenzyme, fibrin stabilizing factor (factor XIII), which has a molecular weight of about 350,000 and is also activated by thrombin. In its activated form and in the presence of calcium, this enzyme leads to firmer bonding between fibrin molecules; and fibrin formed in the presence of factor XIII is insoluble in 5 M urea or 1 M monochloracetic acid as opposed to fibrin formed in the absence of factor XIII. Fibrin stabilizing factor appears to function as a transaminase or transglutaminase. The ultimate orientation and tensile strength of fibrin depends also on interaction with the platelet contractile protein, thrombosthenin.

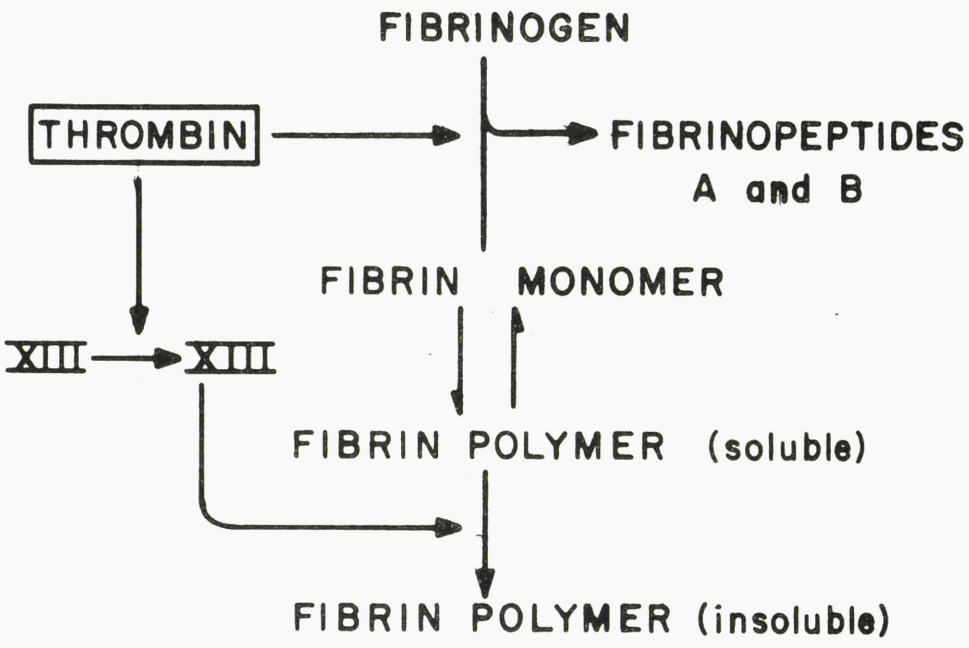


Fig. 6. Fibrin formation. The last step in coagulation.

d. Extrinsic blood coagulation

In the extrinsic system tissue thromboplastin (factor III) plays an important role, and only four additional factors are required, i.e., factors IV, V, VII, and X. In this pathway of coagulation, there is no participation of factors XII, XI, IX, and VIII. Factor X is activated directly by factors III and VII yielding activated factor X.

$$\text{factor X} \xrightarrow[\text{factor VII}]{\text{tissue thromboplastin (III)}} \text{act. factor X}$$

Activated factor X then participates in the same sequence of reactions as found in the intrinsic system leading to fibrin formation.

The functional capacity of the intrinsic versus the extrinsic pathways may be evaluated by in vitro tests. In the extrinsic test (the prothrombin time), tissue thromboplastin and calcium are added to plasma; whereas the intrinsic system (partial thromboplastin time) is evaluated by the addition of calcium and a partial thromboplastin (the lipid is extracted from the tissue thromboplastin). Whereas clotting occurs in 10 - 20 seconds in the extrinsic system, about 2 - 3 minutes is required for generation of thrombin along the intrinsic pathways.

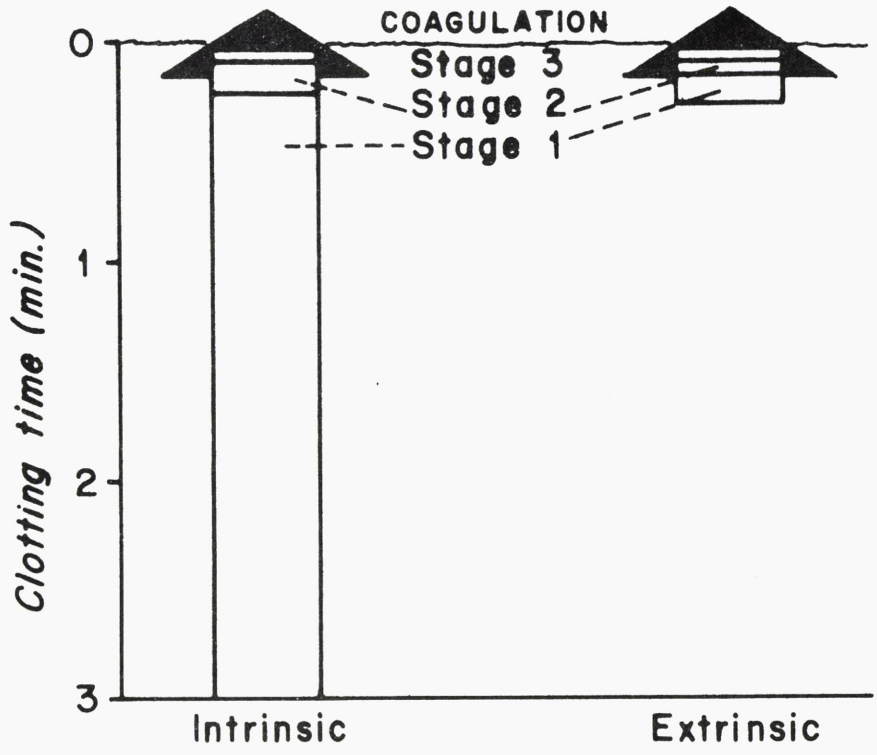


Fig. 7. Extrinsic and intrinsic clotting systems. An illustration of the time relations of the three successive stages of prothrombinase, thrombin, and fibrin formation.

However, once thrombin appears, both pathways become more reactive. The rate of thrombin formation thus follows an S-shaped curve.

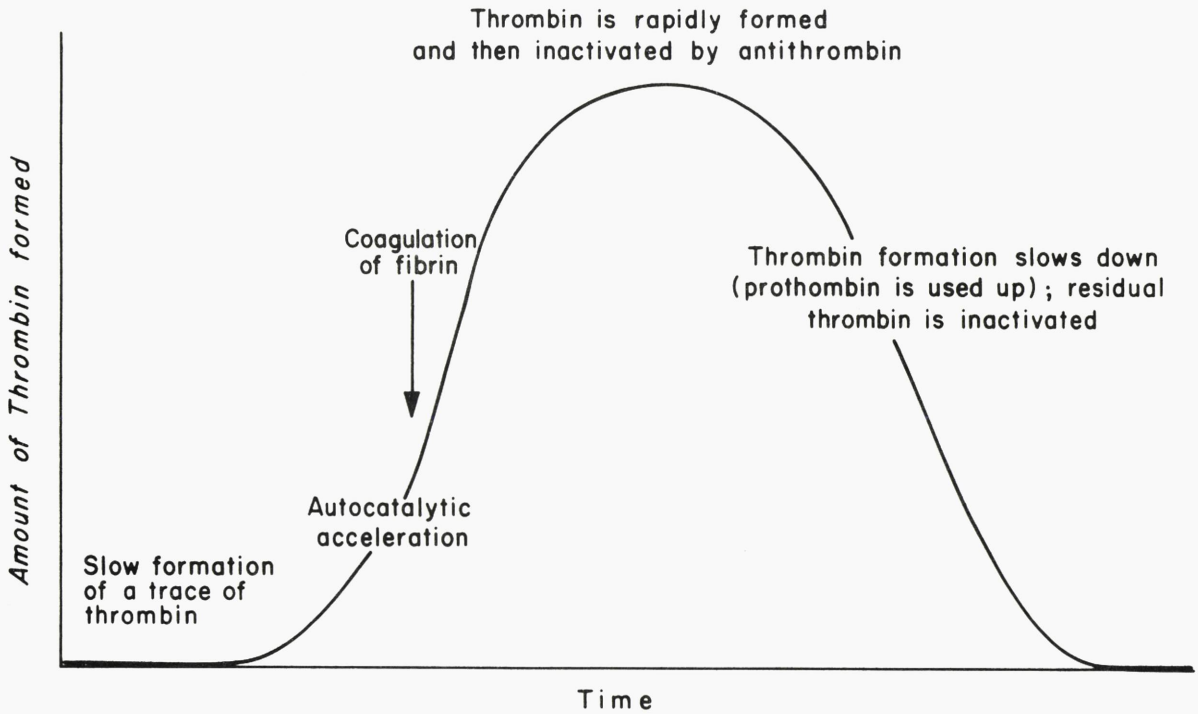


Fig. 8. Formation and inactivation of thrombin in blood.

e. Deficiency states and hemostasis

It is clear from isolated deficiency states that each of the above factors is necessary for normal *in vitro* clotting and that fibrinogen, prothrombin, factors V, VIII, IX, and X are essential for *in vivo* hemostasis. Patients lacking factor XII have little or no bleeding tendency, and patients lacking factor XI have only a mild bleeding tendency. Factors VII and XIII are also required for *in vivo* hemostasis since individuals lacking these factors also have bleeding difficulties.

The separation of the intrinsic and extrinsic clotting systems is more for the purpose of clinical evaluation of individual factors than it is a functional reality. It seems likely that tissue thromboplastin may trigger both extrinsic and intrinsic systems and that the relative participation of each may depend on the amount of tissue thromboplastin. Thus, the smaller the amount of tissue thromboplastin, the more important becomes the intrinsic system. The relative physiologic importance of one or the other system *in vivo* cannot be evaluated, but both are clearly necessary since patients with isolated deficiencies in either system bleed excessively.

f. Turnover of clotting factors

The turnover of clotting factors has been evaluated either by the injection of tagged protein (I^{131} fibrinogen) or by plasma transfusion in patients with isolated deficiencies. The disappearance rates of the members of the prothrombin group have been measured also after the blockage of synthesis by vitamin K antagonists. Turnover studies usually show biphasic curves.

The first slope represents equilibration with the extravascular pool, and the second represents the metabolic turnover of the fraction. The turnover rates of all factors are summarized in Figure 9.

g. Functional groups of clotting factors

In order to highlight certain properties of clotting factors, it is helpful to arrange them into three groups. In each group, individual members have similar properties.

1. The fibrinogen group (factors I, V, and VIII) are so-

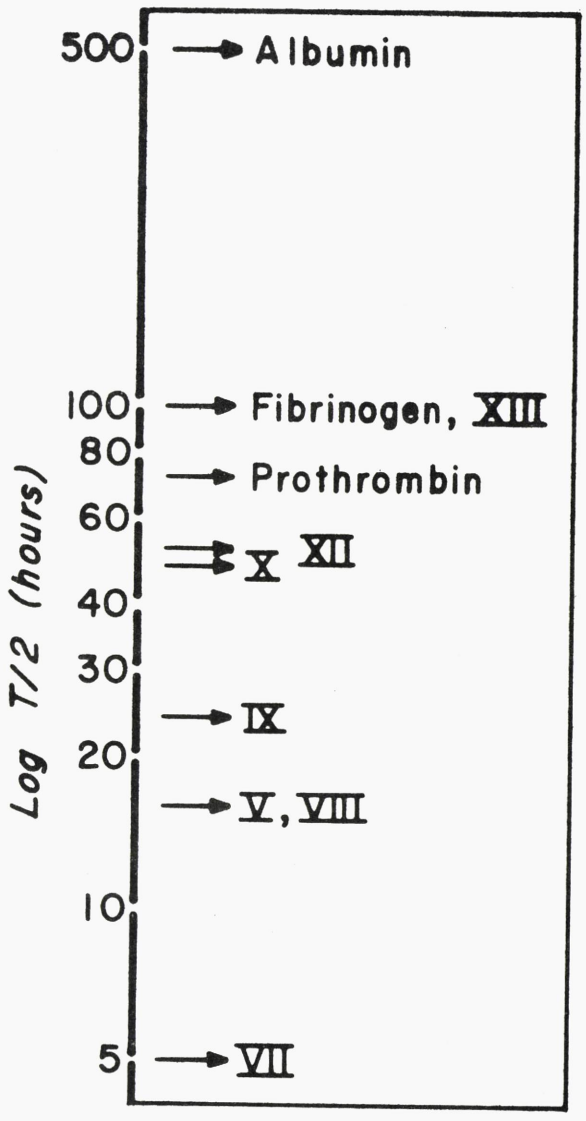


Fig. 9. Turnover times of the clotting factors.

called because all of these are in human plasma and are consumed during the coagulation process. Therefore, while present in plasma, they are absent from serum. They are not absorbed by barium sulfate or similar salts. They tend to collect in the fibrinogen fraction during various precipitation procedures. These factors are fairly susceptible to denaturation, especially factors V and VIII; thus, their activities often are reduced in stored plasma. Vitamin K is not necessary for their synthesis so that they are present in plasma of patients treated with vitamin K antagonists of the coumarin type.

Finally, these fractions tend to increase in certain "hypercoagulable" states.

2. The prothrombin group (factors II, VII, IX and X) are all produced in the liver and are quite sensitive to liver damage. They are all dependent on vitamin K for their synthesis and are absent from coumarin plasma. It has been suggested that prothrombin is the parent molecule of the other factors in this group. These factors are not activated by thrombin and, except for prothrombin, are not consumed during coagulation. They are stable and well-preserved in stored plasma.

3. The contact group is involved in the initial phase of *in vitro* activation of clotting (factors XI and XII). They are fairly stable, are not consumed during coagulation, and are not absorbed. They do not depend on vitamin K for their synthesis.

h. Factors limiting clotting

It is essential with local activation of coagulation that clotting remain localized and not extend througnout the vascular system. The need for limiting clotting becomes clear when it is appreciated that sufficient thrombin may be generated from 10 ml of plasma to clot the entire blood volume in about 15 seconds. The containment of clotting is accomplished in several ways. In the first place, normal endothelium does not activate coagulation, and normal blood does not contain any activators of clotting. When clotting has started, any activators which spill over into the circulating blood are disposed of by two mechanisms. The first involves the binding of thrombin by plasma antithrombin which renders it inactive (normal plasma is capable of binding about twice as much thrombin as it can generate from its own prothrombin). The second mechanism involves the removal of intermediate products by reticuloendothelial cells. It is possible that some inhibition of clotting may be produced by heparin liberated from mast cells, although the physiologic role of heparin is not clear, and measurable concentrations are not demonstrable in normal blood.

IV. DISORDERS OF COAGULATION

Bleeding associated with impaired coagulation is usually initiated by trauma, even though the trauma may be so slight that bleeding appears to be spontaneous. There is often a delay of several hours to a few days between the trauma and the onset of serious bleeding since platelet aggregates may stop bleeding initially, but do not seal off properly because coagulation has been inadequate to anchor the plugs. It is useful to have a general idea of the critical hemostatic levels for clotting although these also relate to the degree of associated vascular damage. Clotting defects can result from impaired or abnormal production or increased utilization of clotting factors or from anticoagulants.

a. Production abnormalities

Hereditary clotting defects usually involve one factor only, and the defect persists at a constant level throughout life. This fixed level may be different in different individuals but is usually constant in any one family. Inheritance patterns are fairly well established for the various coagulation factors.

TABLE III

The mode of inheritance of the clotting factors

Factor	Mode of Inheritance	Occurrence of Deficiency (Frequency per Million)
I	Autosomal recessive	.1
II	" "	.1
V	" "	.1
VII	" "	.1
VIII	Sexlinked recessive	30-40
IX	" "	3-4
X	Autosomal recessive	.1
XI	" "	1.0
XII	" "	.1
XIII	" "	.1
von Willebrand's disease	Autosomal dominant	100.0 (?)

(In patients, the deficient factor ranges from traces to 25% of normal, and bleeding symptoms are proportional to the degree of deficiency. In carriers, the deficient factor usually is in the range of 30 - 70%, and there is little or no bleeding tendency).

Usually defects are due to decreased production of a normal factor but may also be due to the normal production of a non-functional clotting factor. The most important of the hereditary coagulopathies is hemophilia (deficiencies of factors VIII or IX). The most frequent of the hereditary disorders is von Willebrand's disease. While this disease is not well understood, it appears to be due to two defects, a defect in factor VIII activity and prolonged bleeding time due to lack of a plasma factor. When patients with von Willebrand's are transfused with hemophilic plasma, their factor VIII level progressively increases, suggesting that there are at least two genes which may control factor VIII synthesis. Other hereditary diseases are rare.

Acquired clotting defects due to decreased synthesis are usually secondary to some underlying disease state and involve two or more clotting factors. The most important of these relates to the prothrombin group (factors II, VII, IX, and X). These factors depend on vitamin K for their production. Vitamin K deficiency may be brought about in several ways, i.e., with biliary obstruction, in sprue, with reduction in bacterial flora of the intestine. A deficiency in the prothrombin group of factors may also be produced by oral anticoagulants, such as coumarin. When synthesis is blocked, the concentration of each factor decreases according to its normal turnover. Factor VII is the first to be depressed; after a few weeks, factor X is somewhat lower than other factors. Severe liver disease affects all clotting factors except VIII and the synthesis of the prothrombin group is especially impaired.

b. Increased utilization

Increased utilization of some factors, particularly the fibrinogen group, and platelets occurs during intravascular clotting. In severe forms, a rapid defibrination occurs with depletion of platelets, fibrinogen, prothrobin, and factors V, and VIII. The defibrination mechanism is probably triggered by tissue thromboplastin entering the blood stream in large amounts. Sometimes antigen-antibody reactions appear to be the initiating factor. Characteristically, there is widespread small vessel thrombosis with necrosis, especially in the renal and adrenal cortex. The syndrome may be due in part to the inability of reticuloendothelial systems to clear fibrin and other hemostatic products from the blood. The depletion of clotting factors, the rupture of thrombosed vessels and the associated fibrinolysis may all contribute to the severe hemorrhagic state.

c. Anticoagulants

A group of rare bleeding disorders due to inhibitors (anticoagulants) has a characteristic finding in common, that small amounts of patient plasma prolong the clotting time of normal plasma. Usually this is the result of a specific anticoagulant in the form of an antibody against one of the clotting factors or intermediate products of coagulation. Antibodies against factor VIII are observed most frequently. Inhibitors against intermediates occur in disseminated lupus. Abnormal proteins (cryo- and paraproteins) may interact with clotting factors. The most common inhibitors are the breakdown products of fibrinogen which occur during or after the onset of fibrinolysis.

In certain diseases, bleeding is due to combined defects of coagulation associated with thrombocytopenia, vascular defects, and abnormal fibrinolysis. All combinations and degrees are possible and such patients must be studied in detail. Bleeding in acute leukemia, severe liver disease, and uremia illustrate this type of complex hemostatic breakdown.

V. FIBRINOLYSIS

Blood contains a powerful proteolytic system capable of digesting fibrin and thus re-establishing circulation where it has been obstructed by the hemostatic response to injury. The proteolytic system is designed much like the clotting system, with inactive profactors, activators, and inhibitors. The effector substance is plasmin, a proteolytic enzyme similar to trypsin in its ability to digest many proteins, such as fibrin, fibrinogen, factors V and VIII, etc. The site of action is the arginine-lysine bond of fibrin. During fibrinolysis, fibrin breaks down into successively smaller and finally plasmin-resistant fragments, the so-called "fibrin split products." These products may be demonstrated by immunologic techniques. The early hydrolysis products, formed within the first 30 minutes, inhibit the aggregation of platelets. Late hydrolysis products interfere with the polymerization of fibrin. Thus, the presence of split products in sufficient amounts inhibit several important steps of hemostasis and may cause abnormal bleeding. They circulate for one or two days after release from the clot. While plasmin may attack fibrinogen as well as fibrin, it usually appears to function in vivo selectively on fibrin. Two explanations have been given for this specificity of action. One suggests that plasminogen penetrates and adheres to the clot whereas anti-activators are not so concentrated; consequently, there is selective activation of plasminogen to plasmin within the clot. The other suggests that plasmin is held in an inactive form in general circulation and that it dissociates when it reaches fibrin due to its greater affinity for fibrin.

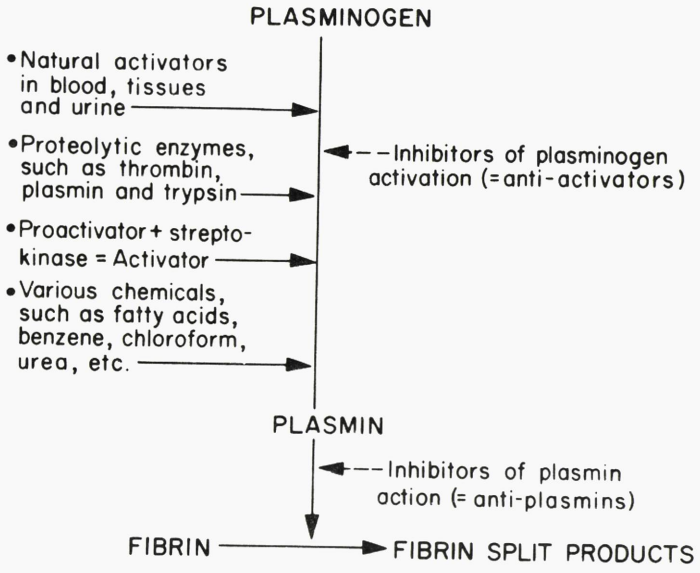


Fig. 10. A present concept of fibrinolysis. Note that plasminogen can be activated through several different mechanisms.

Plasminogen (profibrinolysin) is the inactive precursor of plasmin present in concentrations of 10 - 20 mg percent as a serum globulin with a molecular weight of about 90,000. Activators for plasminogen are present in tissues, plasma and urine. All are relatively heat-stable proteins which act directly on plasminogen by splitting peptide bonds. The tissue activator, present in most tissues, is bound to particulate cellular matter; it is also found in vessel walls, both in adventitia

and endothelium. Plasma activator is an euglobulin which may have its origin in the vascular wall and represents a circulating form of tissue activator. The urinary activator (urokinase) is a trace protein in normal urine. Similar activators are also found in tears and in breast milk.

Physiologically, the fibrinolytic system is activated by vigorous exercise, by anoxia, by stress and by adrenalin injection. In disease, plasminogen is activated by tissue damage, by necrotizing tumors and by severe infection. There are, in addition, intimate relationships between activation of clotting and fibrinolysis. Thrombin activates plasminogen directly, tissue damage appears to initiate both systems, and activation of factor XII likewise triggers both clotting and fibrinolysis. Thus, it would appear that fibrinolysis automatically is associated with clotting.

VI. APPROACH TO BLEEDING DISORDERS

a. History and physical examination

The history should record the many in vivo hemostatic tests inflicted on the patient by his environment. Such episodes are more significant for detecting the presence of a hemostatic disorder than any in vitro reactions of his blood to artificial reagents. The essential decision is whether the bleeding was excessive for the amount of trauma induced. The following five points should be covered by an adequate history:

1. The type of bleeding present (petechial, ecchymotic, single versus multiple bleeding points).

2. The onset and duration of the abnormal bleeding tendency (bleeding at birth, circumcision, trauma, operations, menstruation, dental extraction).

3. Family incidence (and, if present, the mode of inheritance as derived from the family tree).

4. Previous or current drugs or transfusions.

5. Local or general underlying disease.

In the physical examination one should look for the type of bleeding, for vascular abnormalities, and for swelling and deformities of joints due to bleeding. In patients with acquired bleeding tendency, one should search for evidence of generalized disease such as leukemia, uremia, and liver disease.

The clinical appearance of bleeding often suggests its cause. Petechial bleeding occurs with platelet and vascular abnormalities. When due to vascular disease the petechiae are often elevated, and thus

palpable due to an associated increase in capillary permeability. Petechial lesions of thrombocytopenic purpura are not elevated and, while they may be widespread, they are especially located where capillaries are under pressure or are traumatized. Minor cuts bleed for a long time in patients with platelet disorders because platelet plugs do not form. Mucosal, renal, and gastrointestinal bleeding are common in thrombocytopenia and are also seen in the more generalized vascular purpuras. Widespread ecchymotic bleeding associated with bleeding from the gastrointestinal and urinary tracts is most often associated with acquired clotting defects. Since these multiple defects result in a more generalized breakdown of hemostasis, bleeding often occurs without evident trauma. Single large bleeding episodes, especially into the joints, are characteristic of the congenital clotting defects of factors VIII and IX. Since arterial bleeding provides the single most severe test of hemostasis, episodes of arterial or "blow-out" hemorrhage in patients with congenital defects may be life-threatening. Each episode is provoked by trauma which, however, may be so trivial that it is overlooked. There may be a lag phase of several hours to a few days between trauma and serious bleeding because platelet plugs may form but do not last. Rebleeding is also common several days after the initial episode.

b. Laboratory diagnosis

In patients with a history and physical findings suggestive of a bleeding disorder, the following laboratory tests are sufficient for a qualitative diagnosis: the platelet count or examination of the blood smear, the thrombin time, the prothrombin time, and the partial thromboplastin time. These usually permit the classification of bleeding as a platelet disorder, coagulation abnormality or, by exclusion, a vascular defect.

Vascular abnormalities resulting in bleeding often cannot be identified by specific tests. The evidence of increased vascular permeability shown by the elevation of the lesion is often helpful. An increased rupture of vessels when venous obstruction is produced (tourniquet test) is usually not helpful. Skin biopsy may show specific lesions.

Platelets may be evaluated from a blood film, but more accurately by a count. If the number of platelets is below $50,000/mm^3$, the thrombocytopenia may contribute to bleeding, but the platelet count is usually much lower before the patient bleeds from a platelet deficiency alone. Bleeding time and clot retraction, which have been used extensively to evaluate petechial bleeding and platelet function, are abnormal to variable degrees when the platelet count is below $80,000/mm^3$. These tests do not give further information when the platelet count is obviously reduced, but they should be done when the platelet count is normal and qualitative platelet defects are suspected. They are particularly helpful in functional disorders of platelets, such as von Willebrand's disease, thrombasthenia, and the thrombopathies. With vascular purpura, bleeding time and tourniquet test may occasionally be abnormal.

Assessment of the clotting mechanism is best approached by three tests. The thrombin time, the time required for a standard amount of thrombin to clot plasma, is prolonged when fibrinogen is reduced to less than 100 mg/ 100 ml and when anticoagulants, such as early fibrin breakdown products, abnormal plasma proteins, or administered heparin interfere with the action of thrombin. Fibrinogen deficiency may be differentiated from anticoagulants by the thrombin time of a 1:1 mixture of normal and patient plasma. With deficiency the time will be corrected; whereas with anticoagulant, it will reamin prolonged. This test is most valuable for detection of intravascular coagulation and fibrinogen breakdown products. A convenient and rapid semiquantitative test for fibrinogen is the FI-test which employs antifibrinogen antibodies. This will measure fibrinogen in plasma and will also detect fibrinogen split products in plasma or serum. In the fibrinogen titration (Schneider test), dilutions of whole blood or plasma are clotted by standard solutions of thrombin, and fibrinogen level is estimated from the highest dilution which still gives a visible clot. Since the dilution of plasma has an enhancing effect on fibrinolysis, a decrease in fibrinogen titer after incubation is also a sensitive indicator of fibrinolytic activity.

The prothrombin time reveals defects in the extrinsic system (factor VII) and in the factors common to the extrinsic and intrinsic systems (factors V and X). It also utilizes the conversion of fibrinogen to fibrin and is, therefore, influenced by factors which prolong the thrombin time.

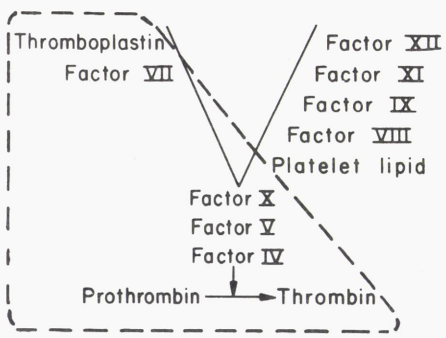


Fig. 11. The prothrombin test.

If the prothrombin time is prolonged and the thrombin time is normal, the defect involves the formation of thrombin. The cause of the defect can be qualitatively evaluated by repeating the test with addition of either normal serum (containing factors VII and X) or normal plasma adsorbed by barium sulfate (containing factor V). This test is most valuable in measuring defects in the prothrombin group (II, VII, IX, X) due to vitamin K deficiency, liver disease, or anticoagulant therapy.

The partial thromboplastin time reveals defects in the intrinsic system (factor VIII, IX, XI and XII) and in the factors common to both systems (factors V and X) and in the conversion of fibrinogen to fibrin. If it is prolonged and the thrombin time is normal, the cause may be found by repeating the test with supplements of normal serum (containing factors IX, X, XI, and XII) or absorbed plasma (containing factors V, VIII, XI and XII). If the prothrombin time is normal, deficiencies of factors V and X are excluded. This test is most valuable in detecting and following the treatment of hemophilia (VIII and IX deficiencies).

Any of the clotting tests described may be prolonged by the

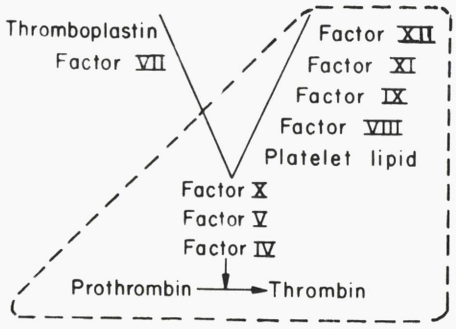


Fig. 12. The partial thromboplastin test.

presence of anticoagulants. Tests for circulating anticoagulants are based on the observation that normal plasma will have little effect on prolonged clotting due to an anticoagulant whereas it will correct clotting due to a deficiency. This principle may be applied to the thrombin, prothrombin, and partial thromboplastin time, in order to localize the action of an anticoagulant.

A deficiency of factor XIII does not reveal itself in any of these clotting tests and can only be detected by measuring the solubility of a clot in one molar monochloracetic acid. Performance of this test is indicated if the basis for hemorrhagic defect is not elucidated by the other tests.

The presence of active fibrinolysis is detected by the incubation of clots, either of whole blood, plasma, or better, of diluted plasma. If clots fail to dissolve within 3 hours, important fibrinolysis is unlikely. However, fibrinolysis may also have occurred in significant amounts prior to the time of testing, and this may be demonstrated by the presence of split products of fibrinogen or fibrin. These are demonstrated either by a prolonged thrombin time which is not corrected by a 1:1 mixture with normal plasma or by a positive FI-test on serum. In bleeding disorders involving destruction of clotting factors, it is often a problem to distinguish between acute intravascular clotting and fibrinolysis. Fibrinogen may be low in both situations. A decrease in platelets provides evidence of intravascular clotting while a demonstration of fibrinogen split products indicates fibrinolysis. It should be emphasized that the presence of fibrinolysis or split products does not preclude intravascular clotting since the former is usually the consequence of the latter.

c. General therapeutic consideration in bleeding disorders

1. Vascular disorders. The agents known to produce vascular damage should be sought. These include bacterial toxins and sepsis, drug allergy, congenital anomalies such as familial telangiectasia, acquired disorders such as Cushing's disease, and vitamin C deficiency. Familiarity with some bleeding lesions such as senile purpura will dispell concern about a generalized disorder of hemostasis. Steroids are probably of little effect with vascular lesions. Unless a large portion of the vascular system is involved, vascular purpura is usually benign.

2. Platelet disorders must always be regarded seriously because of the critical role of the platelet in hemostasis. The major categories of thrombocytopenia have been previously discussed. Great diligence is required in detection of drugs which may impair production or cause increased destruction (i.e., thrombocytopenia from quinine present in "gin and tonic"). Steroids may reduce vascular manifestations despite a

continued thrombocytopenia. In immunologic thrombocytopenic purpura steroids may be also of value by depressing antibody formation. Splenectomy may modify the rate of destruction of platelets in this condition since the spleen plays an important role in destroying sensitized platelets. Platelet transfusion can be effective in therapy, but much more so when thrombocytopenia is due to impaired production rather than to increased destruction, since the life of the transfused platelets will be much longer in the former instance. Platelet transfusion requires the use of either fresh blood or platelet concentrates prepared within 4 hours of phlebotomy, since platelets rapidly lose viability in vitro. The development of iso-antibodies after repeated transfusion limits the usefulness of platelet therapy in some patients.

3. Clotting disorders. Acquired clotting abnormalities are sometimes amenable to specific therapy. A patient with vitamin K deficiency, for example, when treated with vitamin K or K_1 will show increases in factors II, VII, IX, and X within hours, and normal values may be expected within 1 - 2 days. When the deficiency is due to biliary obstruction or intestinal disease, good results of all parenteral preparations are effective. When given to counteract the action of coumarin anticoagulants, K_1 should always be used since K cannot compete with the anticoagulant. When the prothrombin complex is deficient because of liver disease, there is a variable response to therapy with either K or K_1 depending on the relative importance of a decreased supply of K to the liver versus the decreased synthesizing capacity of the liver.

Highly effective therapeutic agents exist for the treatment of intravascular clotting and fibrinolysis. When hemorrhage is due to intravascular clotting with depletion of clotting factors and platelets, the coagulation process may be arrested by heparin. When hemorrhage follows the reduction in clotting factors accompanying fibrinolysis, epsilon-aminocaproic acid (EACA) may be injected. This chemical is a powerful inhibitor of plasminogen activation and a relatively weak inhibitor of plasmin. The bovine kallikrein inhibitor ("Trasylol") not only inhibits plasminogen activation but also plasmin itself, and appears to have some antithromboplastin function. If there is evidence of both intravascular clotting and fibrinolysis, both heparin and a fibrinolytic inhibitor should be given since intravascular clotting is probably the primary process and inhibition of fibrinolysis alone would increase coagulation. It may be necessary, in addition, to replace depleted clotting factors.

In the congenital abnormalities, replacement therapy is limited by the availability of clotting factor concentrates and by the rate at which these fractions disappear from the blood. Only with fibrinogen and factor VIII (and platelets to a lesser degree) are adequate concentrates available. The importance of truly adequate therapy in hemophilia (factor VIII deficiency) has been recognized only in the last decade. Initially, amounts equivalent to 50% of the AHG in circulation of the normal individual may be required every 8 - 12 hours. It is essential to have accurate quantitative assays of clotting factors available to gauge such replacement therapy. While whole blood

and plasma may supply factors other than fibrinogen and VIII, turnover is so rapid as to greatly limit the effectiveness of such therapy.

If bleeding is confined to a local area, principles of local hemostasis are important. They include complete immobilization until the bleeding is stopped, cleaning of old clots, and the use of fibrin foam and thrombin on the wound. Cauterization is usually not useful because, although it may improve the situation at the time, it usually results in later bleeding. Local vasoconstriction by cooling or vasoconstricting drugs may be of some use.

VII. THROMBOSIS

While in small vessels the major expression of abnormal hemostasis is bleeding, in large vessels it is thrombosis. Thrombosis is defined as a vascular occulsion formed by coagulant materials from the blood.

a. Pathogenesis

The word "hypercoagulability" has been used to indicate an increased tendency to thrombosis on the basis of an elevation of one or more clotting factors. Such elevated levels are quite common. For example, factors I and VIII are markedly increased after operation and in many inflammatory states; fibrinogen, factors VII, VIII, IX and X are usually increased in pregnancy. However, there is no good evidence that an increase in clotting factors per se can produce thrombosis. Causative factors are still poorly defined, but it would appear that thrombosis is the end result of interaction of several factors. Prominent among these are abnormalities of the vessel wall, changes in blood flow and changes in the blood itself.

Damaged endothelium and exposed collagen of the vessel wall provide a focus for adhesion and aggregation of platelets. This represents the first and essential step in arterial thrombosis, whereas in venous thrombosis, it is probably less important. The effects of blood flow are illustrated by the different nature of thromboses within different parts of the vascular system. In active circulation, blood is usually defibrinated without thrombus formation. If thrombi are formed, they are composed largely of platelets. In sluggish areas of circulation solid red clots are formed. The general characteristics of each are shown in Table IV.

Since the venous system has the slowest blood flow, it is most susceptible to thrombosis. Obstruction to flow and possible vascular damage through compression may lead to venous thrombosis as, for example, cramped positions. High levels of platelets may contribute to arterial thrombosis and possible also to venous thrombosis, but the relation is not a prominent one. Decreased fibrinolysis may also contribute to thrombosis. For example, repeated venous thromboses have been observed in rare patients with high levels of fibrinolytic inhibitors. However, there is no convincing evidence that decreased

TABLE IV

	Red Thrombus	White Thrombus
Blood flow	Slow or stagnant	Rapid
Main mechanism	Coagulation	Platelet plug formation
Structure	Clot	Platelet plug
Role of vascular injury	Little or none	Essential
Vessel involved	Veins	Arteries
Tendency to propagation	Large	Small
Preventive effect of anticoagulants	Good	Poor

fibrinolysis is an important contributing mechanism in the usual thrombus formation. Increased viscosity which may occur with increased concentration of red cells or abnormal plasma protein may contribute to thrombus formation; however, many patients with increased viscosity live for years without thrombotic phenomena.

One of the most important factors leading to intravascular coagulation is the presence in circulation of active products of clotting. These substances are associated with a shortened whole blood clotting time, and their effects may be demonstrated after the intravenous injection of fresh serum by the production of thrombosis in distant, partially occluded veins. This mechanism may be important after trauma or after operation since tissue products may enter circulation, activating clotting and producing thrombosis in veins with slow blood flow.

It is, therefore, useful to think of thrombosis as the end result of several cumulative or synergistic mechanisms which produce thrombosis when the collective "thrombosis score" has reached a certain critical level. A contribution of individual factors may vary greatly. Thus, postoperative venous thrombosis is probably due to slow venous flow during bedrest and to some vein wall damage during tight strapping on the operative table and to some activation of clotting factors during the postoperative period. Arterial thrombosis, on the other hand, is probably mainly due to changes in the vascular wall, possibly aided by minor changes in clotting factors, platelets, viscosity, and fibrinolysis.

b. Clinical approach

The clinical manifestations of thrombotic disease relate to local vascular obstruction and to embolization of the thrombus. With arterial lesions, the major manifestation is impairment of the blood supply to a tissue with ischemia or infarction following. Usually there has been vessel narrowing due to extensive disease of the arterial

wall, and the clot represents the final episode in the process. Venous thrombosis, on the other hand, can occur in widely patent vessels. The initial thrombotic process most often occurs in the leg or pelvic veins and in most instances is asymptomatic. The fact that these thrombi are often bilateral is taken as an indication of the capacity of the hypercoagulable state to produce lesions distant to the primary thrombus. Local venous obstruction with prominence of the remaining patent superficial veins, edema of the obstructed area, and deep tenderness over the involved vein may be observed. A distinct hazard is the embolization of the clot to the lungs. Obstruction of blood flow in the lungs can result in acute right heart strain, cardiac arrhythmia, pulmonary infiltration with hemoptysis and pleurisy. While it is difficult to locate the thrombi in peripheral veins directly because of the multiplicity of venous channels, angiography and lung scanning permit visualization of the extent of embolization in the lung and its effect on pulmonary perfusion. Thromboembolic disease is often unrecognized, either because it does not reach the symptomatic level, or because its manifestations are not sufficiently specific to permit diagnosis.

c. General approach to therapy

The role of the coagulation apparatus in the propagation of the thrombus and embolization of the clot is an important one, especially on the venous side. Therefore, anticoagulant therapy might be expected to be effective in situations where thrombosis is likely. When thrombosis is already present, the anticoagulant would be expected to reverse the hypercoagulable state and to permit the fibrinolytic mechanism to resolve the clot.

Congestive heart failure is an example of a high risk state where approximately 50% of mortality is due to the complications of thromboembolic disease. Here a number of factors predispose the individual to thrombosis (decreased blood flow, anoxia, immobilization, and venous obstruction). In such patients, the use of drugs which block the synthesis of prothrombin complex (coumarin group) can result in virtual elimination of the hazard of thrombosis. Similar evidence obtained from patients during postoperative or post-traumatic periods provides further documentation of the role of the hypercoagulable state in thrombosis and of anticoagulants in its prevention. With acute thromboembolic phenomena, heparin is often used in preference to coumarin with the belief that it is a more potent anticoagulant and that it is safer to use at the high levels which provide maximum anticoagulant effect. Familiarity with these two anticoagulants is important because of their frequent use and because they can, by themselves, induce potentially serious coagulation defects. They may occasionally provoke local hemorrhage due to an underlying disease process effecting vascular integrity (latent ulcerating lesions of the bowel). The coumarin or indanedione drugs completely block the synthesis within the liver of factors II, VII, IX, and X. Depending on the drug employed, there is usually a latent period of 2 - 4 days before an effective depression of clotting factors is achieved (15 - 20% by the prothrombin time). Levels of less than 5% may be associated with ecchymoses, gastrointestinal and renal bleeding. The effect of

these anticoagulants may be counteracted by vitamin K_1 (not by vitamin K); however, large doses of K_1, while removing the danger of hemorrhage, may bring about a return of the hypercoagulable state. Heparin is most dependable when given intravenously. Its effect is measured by the clotting time which should be allowed to come to normal before the next dose is given unless it is being used to treat generalized intravascular clotting. Clinically, the heparinized patient behaves like the hemophiliac in that the greatest hazard is hemorrhage from a single artery which may be traumatized by a needle or surgical procedure. Protamine sulfate is an effective antidote for heparin and will immediately bring the clotting time to normal.

The use of fibrinolytic therapy in thromboembolic states is still in the experimental stage. At present, there are two potentially useful preparations, both of which depend on the activation of plasminogen in vivo. Because streptokinase has the disadvantage of being highly antigenic and to give safe and effective levels after the antibody has been overcome, titration is required. Consequently, streptokinase is not a practical form of fibrinolytic therapy. Urokinase does not have this disadvantage, but it is expensive since it must be laboriously isolated from human urine. These fibrinolytic substances are capable of dissolving recent thrombi, but uncontrolled activation produces fibrinolytic bleeding.

Another therapeutic approach designed to prevent further embolization is the surgical ligation of the venous bed from which the thrombi originate. Ligation or plication of the inferior vena cava when emboli originate below this point has undoubtedly been lifesaving in certain individuals in whom anticoagulant therapy has proved ineffective. However, this approach is not without complication, including thrombosis in the peripheral venous system and even emboli from above or from collateral circulation around the area of obstruction.

VIII. METHODS

In this section those tests are described which most efficiently establish the presence or absence of a hemostatic disorder, i.e., screening tests. The platelet count, bleeding time, and clot retraction screen for platelet-vascular defects. Coagulation is evaluated by the thrombin time, prothrombin time, and partial thromboplastin time. Fibrinogen is estimated by titration or immunologic tests. Abnormal fibrinolysis is screened for by tests of clot lysis. Specific factor assay methods are referred to only.

Blood for use in performing coagulation tests should be obtained by clean venipuncture using a 19 gauge, short-beveled, disposable needle and minimal stasis. The initial blood is suitable for measurements of clot retraction and clot lysis. The coagulation screening tests should be performed on blood obtained in a second plastic syringe and anticoagulated with citrate (one part per nine parts of blood). To prevent activation of coagulation factors, plastic tubes and refrigerated centrifugation are recommended in the preparation of platelet-poor

plasma. The platelet count is performed on blood anticoagulated with EDTA.

An abnormality in the clotting screens indicates either a factor deficiency or a circulating anticoagulant effect. Differentiation between these two is achieved by repeating any abnormal test on a plasma mixture composed of equal volumes of normal and patient's plasma, i.e., 1:1 mix. A clotting deficiency will be corrected to normal values with such a mixture, whereas a circulating anti-coagulant effect will show persistent prolongation of the clotting time. Interpretation of abnormal clotting tests requires such testing.

1. Bleeding time (Modified Ivy Method)

A blood pressure cuff is applied and the pressure maintained at 40 mm of mercury throughout the test (for children, half the diastolic reading is used). The volar surface of the forearm is cleaned with alcohol and allowed to dry. With a new No. 11 Bard-Parker blade, two cuts about 2 cm apart and 1/2 cm long are made deep enough to show a narrow red line in the corium. The top of the blood which oozes from the cuts is touched every 30 seconds with filter paper. The wound itself should not be touched. The end point is the cessation of staining the paper.

Interpretation: Normal subjects have a bleeding time of less than 12 minutes. Prolongation is seen in platelet disorders, von Willebrand's disease, and occasionally as an isolated finding. The bleeding time is normal in factor deficiencies.

Reference: Borchgrevink, C.F. and Waaler, B.A. The secondary bleeding time. A new method for the differentiation of hemorrhagic diseases. Acta Med. Scan. 162: 362, 1958.

2. Platelet count

EDTA anticoagulated blood is diluted with appropriate amounts of ammonium oxalate which lyses red cells and white cells. At least two counting chambers are charged with the resultant platelet suspension. Platelets are then enumerated by phase microscopy. In many centers, the electronic particle counter has been adapted for the enumeration of platelets.

Interpretation: The normal platelet count is 250,000 per mm^3 $\pm$ 75,000. In the absence of a qualitative defect of platelet function, bleeding due to thrombocytopenia usually does not occur until the peripheral count is less than 25,000 - 30,000/mm^3. Spontaneous bleeding due to thrombocytopenia seldom presents until the peripheral platelet count falls below about 5,000 - 10,000/mm^3.

Reference: Brecher, G. and Cronkite, E.P. Morphology and enumeration of human platelets. J. Appl. Physiol. 3: 365, 1950.
Bull, B.S., Schneiderman, M.D. and Brecher, G. Platelet counts with the Coulter Counter. Amer. J. Clin. Path. 44: 678, 1965.

3. Thrombin time

This test evaluates fibrin formation from the patient's plasma by exogenous thrombin. 0.2 ml of plasma is placed in a glass tube and incubated for 60 seconds at 37°C. 0.1 ml of thrombin (37°C) is blown in as a stop watch is started, and the clotting time is observed. Commercial bovine thrombin is used which is diluted with buffered saline to give a 15 second clotting time with normal plasma.

Interpretation: The thrombin time is prolonged if the plasma fibrinogen content is less than 100 mg / 100 ml, if heparin is present, or if antithrombin V or VI (fibrinogen breakdown products) are present. Of the coagulation screening tests, the thrombin time is by far the most sensitive in detecting equivalent amounts of heparin.

Reference: Biggs, R. and MacFarlane, R.G. Human blood coagulation and its disorders. 3rd ed., Philadelphia, F.A. Davis Co., 1962.

4. Prothrombin time

This test measures the extrinsic clotting system and consists of adding brain thromboplastin and calcium to plasma and measuring the clotting time. 0.1 ml of plasma and 0.1 ml of brain thromboplastin are combined in a glass test tube and incubated for 60 seconds at 37° C. 0.1 ml of M/40 $CaCl_2$ is blown in, and the stop watch is started. The clotting time may be observed by repetitive tilting or by the use of hooks.

Interpretation: The normal prothrombin time is 14 - 16 seconds, although additional shortening may occur with undue exposure to glass or room temperature. A normal prothrombin time implies an intact extrinsic system. Prolongation of the prothrombin time is seen when the fibrinogen concentration is below 100 mg / 100 ml or with deficiencies of the other extrinsic clotting factors, i.e., II, VII (and V, X), although, paradoxically, clinically significant prothrombin deficiency may show only minimal prolongation of the prothrombin time. Prolongation also occurs in the presence of the circulating anticoagulants such as that seen with lupus erythematosus or in heparin therapy. The differential between factor deficiencies and circulating anticoagulant is established by repeating the test using a 1:1 plasma mixture of patient's and normal plasma.

Reference: Biggs, R. and MacFarlane, R.G. Human blood coagulation and its disorders. 3rd ed., Philadelphia, F.A. Davis Co., 1962.

5. Partial thromboplastin time

The PTT is the screening test for the intrinsic clotting system and involves the determination of the clotting time in a mixture composed of cephalin as a platelet substitute, kaolin for maximal activation of factor XII, and calcium together with citrated

plasma. Kaolin-cephalin reagent is prepared by adding 1 ml of a 4 g/100 ml saline suspension to 1 ml of cephalin-stock diluted 1 to 50 in veronal buffer. 0.2 ml of plasma is placed in a glass tube in a 37° water bath. The kaolin-cephalin reagent is thoroughly suspended, and 0.2 ml is added to the plasma and mixed. After a 5 minute incubation at 37° C, 0.2 ml of M/30 $CaCl_2$ is blown into the mixture and the stop watch is started. The tube is shaken, returned to the water bath for 30 seconds, and time of clotting is observed by slow rotation of the tube, tilted at 45° angle.

Interpretation: The normal range is 35 - 55 seconds. This test reflects the intrinsic clotting system and is therefore normal in factor VII deficiency. The test is prolonged if the fibrinogen level is below 100 mg / 100 ml, but it may be normal in clinically significant prothrombin deficiency. Prolongation is also seen when the other intrinsic clotting factors are less than about 30% of normal, although exceptions do occur. Inasmuch as the prothrombin time also detects abnormalities of factors V and X, this test is primarily useful in detecting a deficiency of factors VIII, IX, XI, or XII. Long PTT values are also seen in the presence of circulating anticoagulants such as heparin, fibrinogen breakdown products, circulating anticoagulants directed against factor VIII or factor IX, or the variety found in 10% of patients with lupus erythematosus. This differential is established by the 1:1 mixture. In the presence of a prolonged PTT due to factor deficiency, the test can be expanded to a four component-PTT to distinguish between deficiencies of factor VIII, IX or XI. If the test is corrected by the $BaSO_4$ adsorbed plasma but not by serum, factor VIII deficiency is suggested. If serum, but not the $BaSO_4$ adsorbed plasma, corrects the PTT, factor IX deficiency is suggested. If both adsorbed plasma and serum correct the prolonged PTT, then deficiency of factor XI or XII is suggested.

References: Proctor, R.R. and Rapaport, S.I. The partial thromboplastin time with kaolin. Amer. J. Clin. Path. 36: 212, 1961

6. Fibrinogen determination

a. FI-test

This test is a rapid slide method which requires only one drop of patient's whole blood and depends upon Latex-Anti-Human fibrinogen reagent to produce visible flocculation in the presence of fibrinogen in amounts over 100 mg / 100 ml.

Interpretation: Agglutination will be visible with fibrinogen levels exceeding 100 mg / 100 ml, and the degree of clumping is roughly proportional to the fibrinogen content. The test is available as a kit of reagents with precise instructions included and is useful as a simple, rapid bedside or operating room procedure to screen for hypofibrinogenemia. The test can be positive in the presence of fibrinogen breakdown products despite

low levels of functional fibrinogen below 100 mg / 100 ml. Some workers have suggested the use of this latter observation in the differentiation of consumptive coagulopathy and pathological fibrinolysis.

b. Fibrinogen titration

This test is a rapid, semiquantitative measure of clottable fibrinogen as estimated by the highest saline dilution of plasma which can be clotted by thrombin. Citrated plasma is diluted with saline 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128. 0.5 ml of each plasma dilution is added to a glass test tube in a 37° C bath. After 3 minutes, 0.1 ml of a thrombin dilution is added. The presence or absence of clots is noted after 15 minutes. The thrombin dilution is prepared by diluting the thrombin with buffered saline which is M/40 with $CaCl_2$.

Interpretation: Normally, clots will be present up through the 1/64 dilution. When fibrinogen is decreased to levels below 100 mg/100 ml, clots will be seen in one or more dilutions below 1/64. The appearance of the clot is also inhibited by heparin or fibrinogen breakdown products.

Reference: Biggs, R. and MacFarlane, R.G. Human blood coagulation and its disorders. 3rd ed., Philadelphia, F.A. Davis Co., 1962.

7. Fibrinolysis

The simplest documentation of increased fibrinolysis is the shortened lysis time of incubated clotted blood or plasma at 37°C. If the clot is unusually friable because of fibrinogen deficiency, abnormally rapid lysis can be produced by a weak fibrinolysin. Accordingly, a modification has been suggested which employs placing patient's plasma on the top of a normal clot and observing the period for lysis.

A preferable screening test for fibrinolysis is an adaptation of the fibrinogen titer. In this test, duplicate samples of blood are drawn, one in citrate and the second with 3.13% citrate being 0.02 M with respect to episilon-aminocaproic acid (EACA). Using these plasma samples, duplicate sets of dilutions are set up as in the fibrinogen titer methods. The formation of clots is observed after 15 minutes following the addition of the thrombin-calcium reagent. Observation of lysis is repeated over the next 6 hours and again at 24 hours.

Interpretation: Clots do not normally dissolve within 24 hours, although the smallest clot in a dilution series may disappear. Artifactual shortening of fibrinolysis is not unusual. Therefore, the time for clot lysis must be within at least six hours to be of clinical importance. In the modification of the fibrinogen titration, significant fibrinolysis is present when clot lysis is observed in the plain plasma two dilutions or more below that seen with the EACA plasma within the 6 hour period.

Reference: Hardisty, R.M., Ingram, G.I.C., and Sharp, A.A. The laboratory investigation control of defibrination syndrome. The Assoc. Clin. Path. No. 48, June, 1964.

8. Clotting time

Blood is obtained by the "two-syringe" technique, and 1 ml is added to each of three glass test tubes. Each tube is tipped horizontally every 30 seconds, but maintained upright inbetween tips. The endpoint is failure of the blood to flow when the tube is held horizontally. Usually the test is run at room temperature, although better controls are claimed for 37° C incubation.

Interpretation: Normal clotting times range from five to ten minutes (performed at room temperature). This test reflects the intrinsic clotting system, but should never be used diagnostically because of the great incidence of false-negatives. It is used primarily to follow heparin therapy.

Reference: Biggs, R. and MacFarlane, R.G. Human blood coagulation and its disorders. 3rd ed., Philadelphia, F.A. Davis Co., 1962.

9. Clot retraction

5 ml of blood is added to each of two calibrated conical glass centrifuge tubes, each of which contains a coil of wire formed by wrapping the wire in a spiral fashion around a lead pencil. The tubes of blood are allowed to clot at room temperature and then are incubated at 37°C for exactly one hour. At that time the wire coils with attached clots are lifted to the top of the tube, drained and removed. The fluid volume remaining, divided by the initial volume of blood, multiplied by 100, gives clot retraction in percent.

Interpretation: Normal values range from 35 - 65 %. The decreased clot retraction is seen with platelet counts below 100,000/mm^3 or in the presence of thrombasthenia. Increased percent of retraction is seen with anemia or hypofibrinogenemia.

Specific Assay

Specific assays for all of the known established clotting factors are well described in current texts of blood coagulation (e.g., Human Blood Coagulation and Its Disorders by Biggs and MacFarlane, 1962), and all of these assays are available in established clotting laboratories. Fibrinogen is most accurately determined by the method of Ratnoff and Menzies, although simpler, less reliable turbidometric methods have appeared. Assays for factors II, V, VII, VIII, IX, X, XI, and XII have in common the use of a substrate plasma which lacks only the factor being measured. Such substrate plasma has a prolonged clotting time, whether this be measured in an extrinsic or intrinsic thromboplastin system. Accurate dilutions of normal plasma induces

shortening of this prolonged clotting time which is proportional to the amount of normal plasma added. The clotting times obtained with a series of such dilutions of normal plasma can be used to construct a calibration curve, which in turn may be used to estimate the factor content of unknown plasma. The substrates for assaying factors VII, VIII, IX, XI and XII are obtained from patients with the respective specific congenital deficiency. Substrate plasma deficient in factors II, V, or VII/X complex can be prepared by manipulation of normal plasma. Performing such factor assays is consequently dependent upon an adequate supply of substrate plasma. The diagnostic clotting laboratory must, therefore, have such a supply on hand which is maintained frozen at -70°C. While these assays are most valuable in the diagnosis and management of clotting disorders, detailed knowledge of the techniques employed is unnecessary for the student.

IX. REFERENCES

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