71.

HEMOSTASIS MANUAL

1967.

INDEX

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 and 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). Arterial hemostasis is primarily accomplished by vascular contraction although fibrin formation and platelet aggregation are also required. Arterial bleeding is the most severe test of the hemostatic mechanism. 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 flow through capillaries is adjusted to local needs by precapillary sphincters. 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.

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. Perhaps 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 of unknown etiology, assumed to be immunologic, is a serious clinical condition because of the extent of involvement of the microvasculature. Small venules of the lower extremity may be affected and may be accompanied by arthritis (Schonlein's purpura); damage to the submucosal vessels of the intestinal tract (Henoch's purpura) may lead to extensive gastrointestinal bleeding; extensive pulmonary bleeding (Goodpasture's syndrome) and glomerulonephritis are also seen as manifestations of anaphylactoid purpura.

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, a congenital defect in elastic tissue of vessels, may produce more general bleeding. The purpura of senility or Cushing's disease, although involving larger vessels, 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 the hyaline deposits seen 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 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 μ^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, or partial thromboplastin). 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).

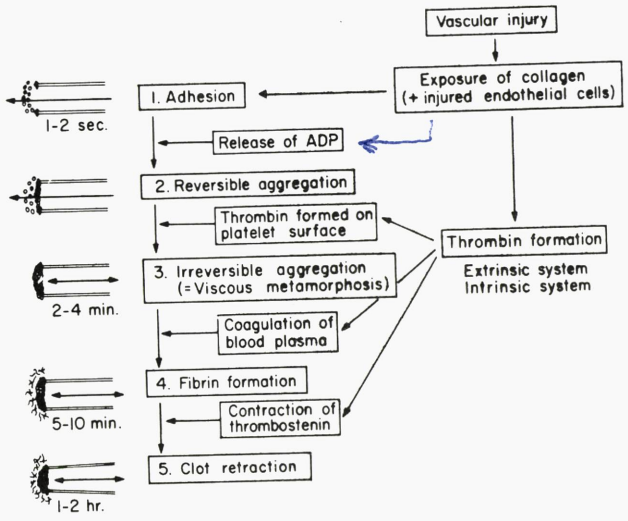


Fig. 1. Stages in the formation of a platelet plug (white thrombus).

The in vivo function of platelets may be described in a sequence of steps which follow vascular injury. Platelets first adhere to the injured vessel wall, especially to exposed collagen fibers. This adherence is accompanied by a liberation of adenosine diphosphate, which brings about aggregation of other platelets. This mass of platelets (platelet plug) abruptly undergoes irreversible change into a solid mass impervious to blood flow. In this process, which involves fibrin formation and is triggered by thrombin, platelets degranulate and liberate their phospholipid which further activates the clotting mechanism. The final solidified mass of platelets is bound to the vascular wall by fibrin and the seal is perhaps further tightened by clot retraction. – 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 taken as evidence of 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 platelet.

b. Platelet kinetics

Platelets are produced by mature megakaryocytes in the bone marrow through release of fragments of their cytoplasm into the circulation. The megakaryocyte which has a lifespan of about 10 days arises from an undifferentiated stem cell and undergoes repeated divisions of its nucleus in a common cytoplasm (endomitosis). The initial phase of nuclear multiplication takes about three days. Thereafter, the cytoplasm develops with granulation and eventual fragmentation into platelets. At any given time about 1/4 of the megakaryocytes in the marrow are non-granulated, 1/4 are partially granulated, and 1/2 are fully granulated. There is approximately one megakaryocyte for each 500 nucleated red cells.

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 curtailed; whereas platelet production is augmented by thrombocytopenia. There appear to be two mechanisms by which production is altered. The first is by change in the mitotic pattern within the megakaryocyte. In stimulated states the number of nuclei increases with a parallel increase in cytoplasm and in platelet production per megakaryocyte. The second mechanism involves an increase in total megakaryocytes. 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 or depression is 4 - 5 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.

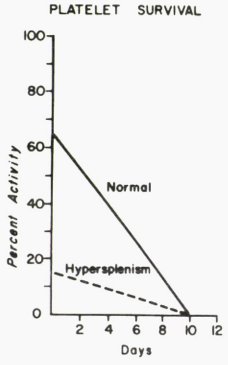


Fig. 2. Platelet survival. The normal survival curve obtained by Cr^{51} tagging is shown. Most of the initial "loss" is due to platelet sequestration in the spleen since 90% of activity is found in circulation in splenectomized subjects. Splenic localization is much greater in patients with splenomegaly.

There is no significant rapidly mobilizable reserve of platelets, although some concentration of platelets does occur within the splenic pulp amounting to about 25% of the total platelets within the vascular system.

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 preoperative degree of splenic sequestration. Platelet counts about twice normal with a decreased size of individual platelets may be seen in iron deficiency anemia. Thrombocytosis, often 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.

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

d. Disorders of production

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

2. Maturation disorders in which the number of megakaryocytes is increased but production of viable platelets is reduced (ineffective thrombopoiesis). Platelets are often variable in size and are of bizarre shapes. This condition is seen in vitamin B_{12} deficiency, folate deficiency and also in some myeloproliferative states.

e. 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 which may be detected by platelet transfusion (Fig. 2).

f. Disorders of destruction and loss

Increased destruction of platelets may be considered under two headings:

1. 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.

(a) 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 mechanisms may be obtained by measuring either the lifespan of transfused platelets or the remedial effect of large doses of steroids which are assumed to inhibit antibody production.

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

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

2. Intravascular coagulation produces profound 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. 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. The understanding of coagulation is simplified by examining first the conversion of fibrinogen to fibrin and then discussing the sequence of reactions which lead to the generation of thrombin.

a. Fibrin formation

This phase of coagulation involves the enzymatic conversion of fibrinogen to the visible end-product, fibrin. The substrate fibrinogen (factor I) is 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 16 amino acids and is released early in the reaction; polypeptide B from the center contains 14 amino acids and is released late in the reaction. Both peptides tend to inhibit further activation of fibrinogen, since they compete with fibrinogen for thrombin. In addition, peptide B stimulates vascular contraction and thus diminishes blood flow.

The next step is the spontaneous polymerization of fibrinogen molecules into the fibrin net. 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 splits off carbohydrates from the fibrin molecule. This loss of carbohydrate leads to firmer bonding between fibrin molecules and fibrin formed in the presence of factor XIII is insoluble in 5 M urea as opposed to fibrin formed in the absence of factor XIII. The ultimate orientation and tensile strength of fibrin depends also on interaction with platelets. The reactions involved in fibrin formation are shown in the following figure.

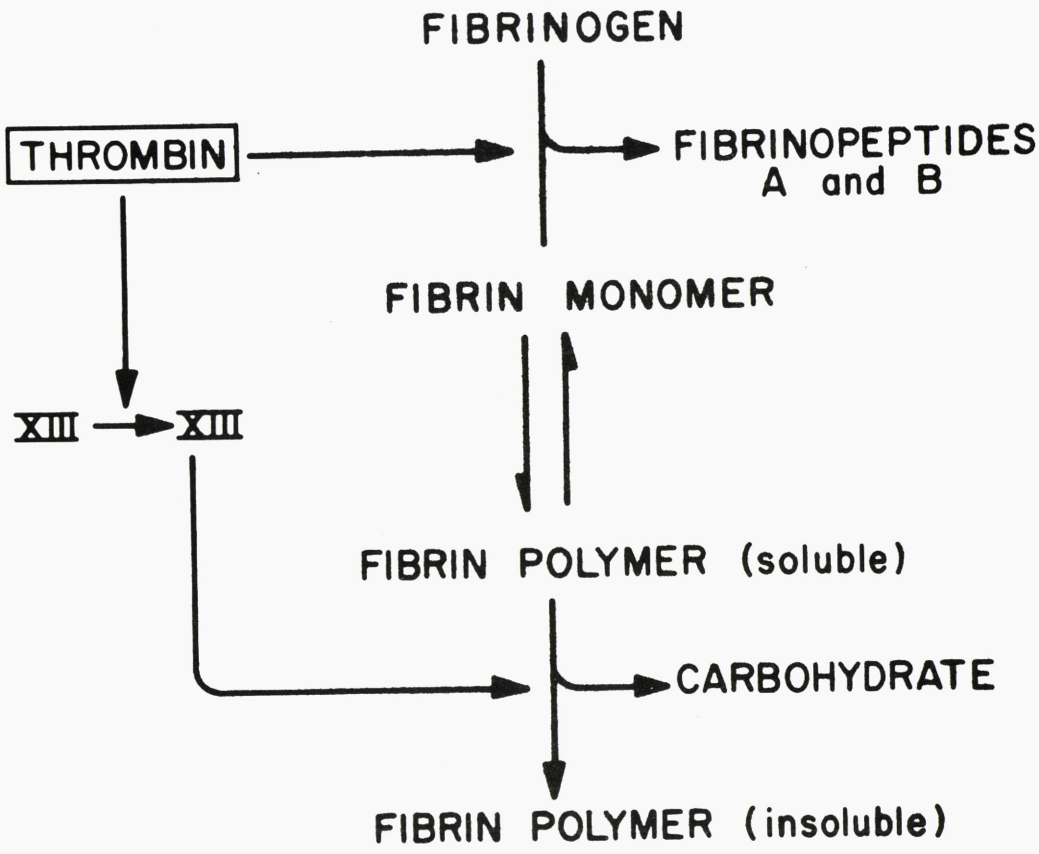


Fig. 3. Fibrin formation. The last step in coagulation.

b. 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 identity 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 1

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, Autoprothrombin I	+
VIII	Antihemophilic A factor or AHF, Antihemophilic globulin or AHG	+
IX	Antihemophilic B factor or AHB, Plasma thromboplastin component or PTC, Christmas factor, Autoprothrombin, Autoprothrombin II	+
X	Stuart or Stuart-Prower factor, Autoprothrombin III	+
XI	Plasma thromboplastin antecedent or PTA	+
XII	Hageman factor	0
XIII	Fibrin stabilizing 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.

c. 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.

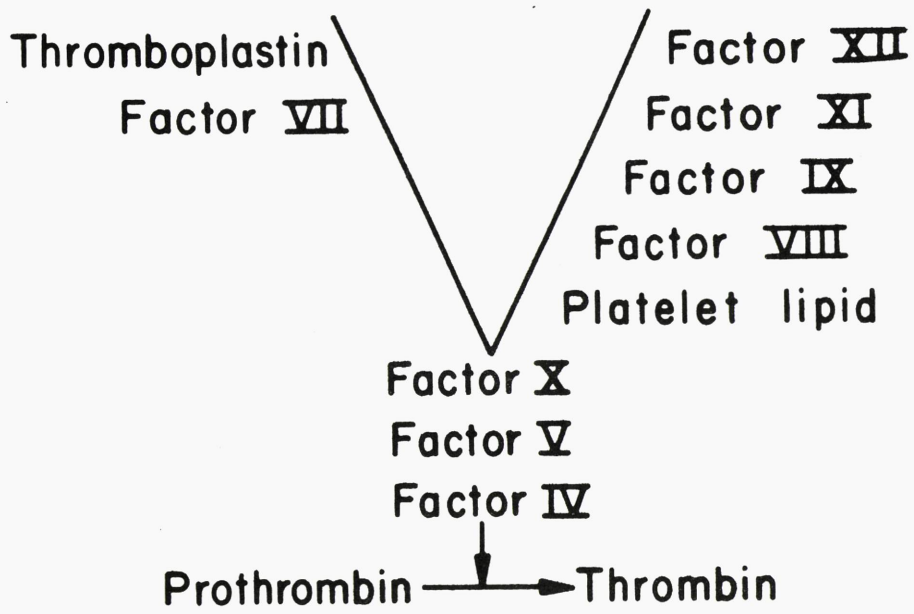


Fig. 4. Prothrombin activation.

Its basis is that all factors required for an 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. The extrinsic clotting system may well operate intravascularly also, since the vascular endothelium contains tissue thromboplastin.

The intrinsic system 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 first activated by surface contact, possibly through absorption of a specific inhibitor. Subsequent factors are successively activated, presumably by the proteolytic action of the preceding one. It is clear from isolated deficiency states that each of these factors is necessary for normal in vitro clotting and that 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.

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.

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 is 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.

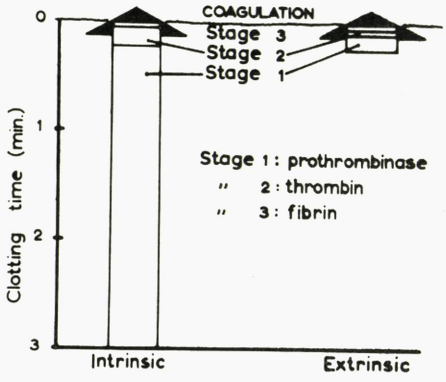


Fig. 5. Extrinsic and intrinsic clotting systems. An illustration of the time relations of the three successive stages of prothrombinase, thrombin, and fibrin formation.

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

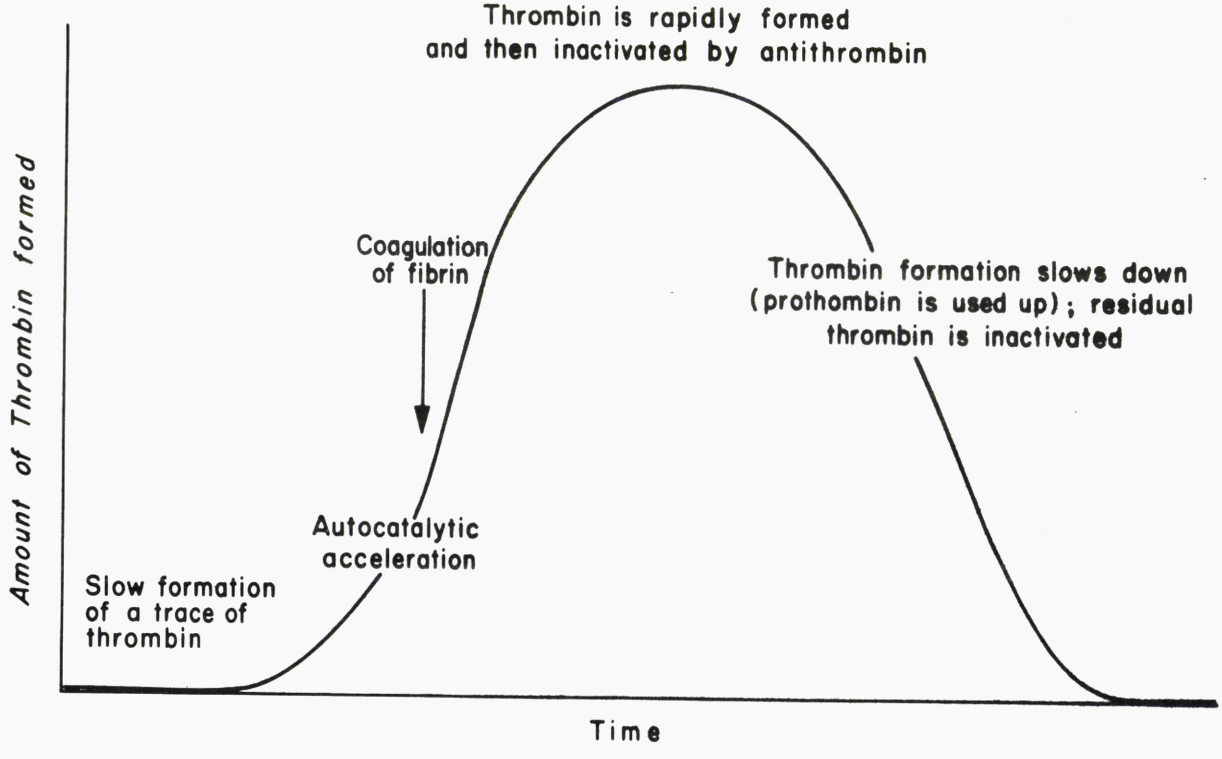


Fig. 6. Formation and inactivation of thrombin in blood.

The separation of these two 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.

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.

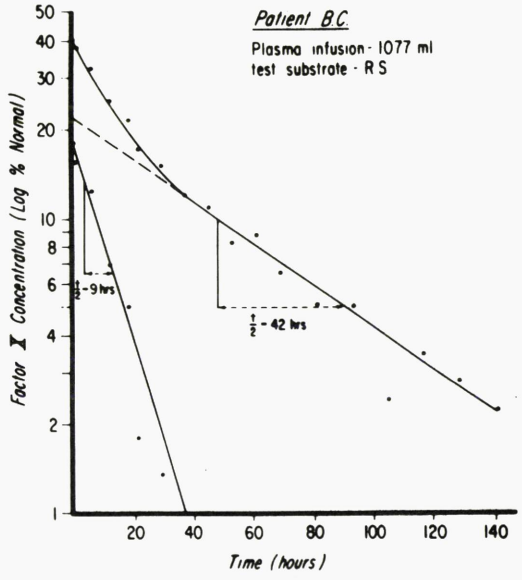


Fig. 7. Disappearance of transfused factor X in a patient congenitally deficient in this factor. The observed curve has been graphically separated into its two components, reflecting equilibration (half-time disappearance = 9 hours) and metabolic turnover (half-time disappearance = 42 hours). From H.R. Roberts, E. Lechler, W.P. Webster and G.D. Penick: Survival of transfused factor X in patients with Stuart disease. Thromb. Diathes. hemorrh. (Stuttgart) 13: 305, 1965.

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 8.

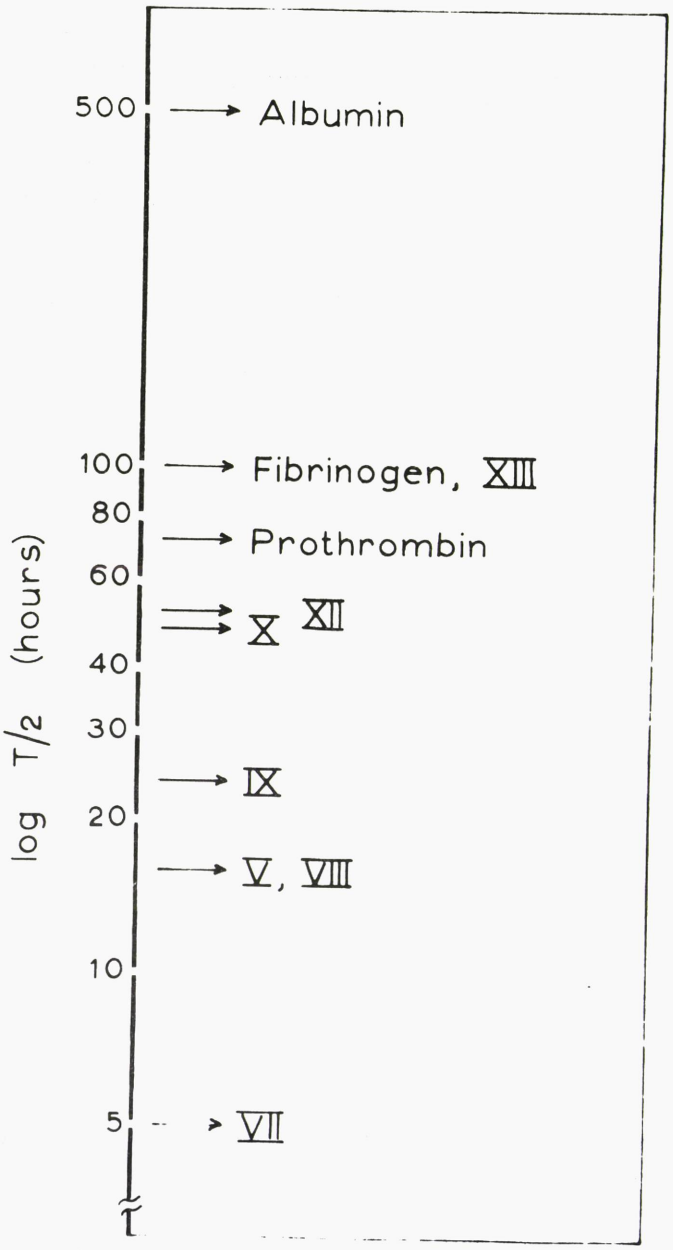


Fig. 8. Turnover times of the clotting factors.

d. 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, VIII and XIII), so-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, such as Dicumarol. 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 Dicumarol 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. They are not associated with serious hemorrhage.

e. Factors limiting clotting

It is essential with local activation of coagulation that clotting remain localized and not extend throughout 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 to 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 2

The mode of inheritance of the clotting factors

Factor	Mode of Inheritance	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 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 relate 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 either in the newborn or with broad spectrum antibiotics. A deficiency in the prothrombin group of factors may also be produced by oral anticoagulants, such as Dicumarol. 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 and factors I, II, 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 formed 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. Heparin has been suggested as a cause of prolonged clotting time with mast cell tumors.

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 affector 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 have not yet been biochemically characterized, but they may be demonstrated by immunologic technics. 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 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.

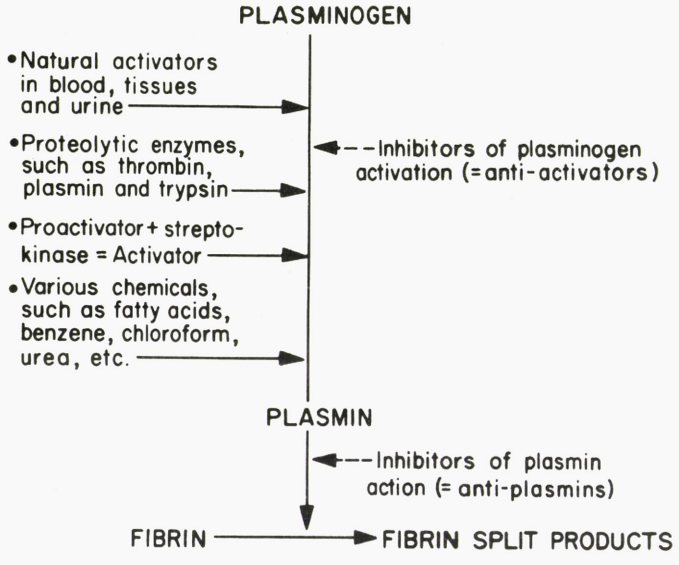


Fig. 9. A present concept of fibrinolysis. Note that plasminogen can be activated through several different mechanisms.

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 vivo 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. 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. 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

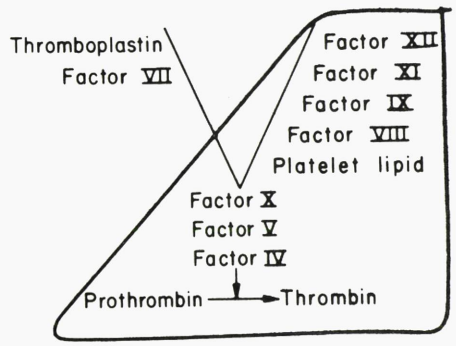
In patients with a history and physical findings suggestive of a bleeding disorder the following laboratory tests are sufficient for a qualitative diagnosis: the platelet count or examination of the blood smear, the thrombin time, the prothrombin time, and the partial thromboplastin time. These usually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

Platelets may be roughly evaluated by 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, tourniquet test, 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 20% of normal 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 remain prolonged. Since thrombin time is relatively insensitive to fibrinogen deficiency, it is desirable to have a rapid semiquantitative test for fibrinogen. One of the most convenient is the FI-test which employs antifibrinogen antibodies. This cannot only measure fibrinogen in plasma, but will also detect fibrinogen split products in 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.

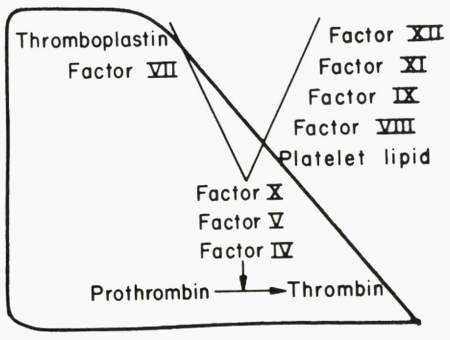
The prothrombin time reveals defects in the extrinsic system (factor VII) and in the factors common to the extrinsic and intrinsic systems (factors V and X). It also utilizes the conversion of fibrinogen to fibrin and is, therefore, influenced by factors which prolong the thrombin time.



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 absorbed by barium sulfate (containing factor V).

Fig. 10. The prothrombin test.

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 can 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.

Fig. 11. The partial thromboplastin test.

Three other tests have been employed to evaluate the intrinsic system (thromboplastin generation test, prothrombin consumption test, and whole blood clotting time). With the exception of the use of the whole blood clotting time in the control of heparin therapy, these tests have little use in the practical approach to diagnosis.

Any of the clotting tests described may be prolonged by the 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 5 M urea. 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 and factors V and VIII may be low in both situations, but a decrease in platelets provides evidence of intravascular clotting, not of fibrinolysis. On the other hand, a demonstration of fibrin 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 frequently 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 due to the ingestion of 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 transfusions 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 are obtained with all parenteral preparations and large doses may be given safely. 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. If there is evidence of both intravascular clotting and fibrinolysis, both heparin and EACA should be given since intravascular clotting is probably the primary process and EACA 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 factors I and 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 recently. 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 VIII, their turnover is so rapid that the amounts required for hemostasis are too great to be supplied effectively by any preparation but a specific concentrate.

If bleeding is confined to a local area, principles of local hemostasis are importatnt. 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 for, although it may improve the situation at the time, it usually results in later bleeding. Local vasconstriction 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; factors I, VII and VIII are usually increased in pregnancy. However, there is no good evidence that an increase in a single factor 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 the following table.

TABLE 3

	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

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 possibly 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 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 phenomenon.

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 (coumadin 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 coumadin 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 effects. 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 coumadin 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 hemophilic 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*. Streptokinase has the disadvantage of being highly antigenic but when antibodies have developed, their activity can be overcome by increasing the dose. 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

1. Tourniquet test

Technic: Inflate the blood pressure cuff to 80 mm Hg and maintain pressure for 5 minutes. Three minutes later after normal circulation has been restored, count the number of petechiae in an area 2.5 cm in diameter about 4 cm below the antecubital fossa.

Normal: not more than 5 - 10 petechiae

Comments: The test is positive in vascular and platelet disorders, but not in clotting disorders. Occasionally it is positive in normals. Many authors recommend higher cuff pressures or longer duration of the test, but this increases the number of "false" positive results.

2. Bleeding time

a. Ivy Method

Technic: Apply a blood pressure cuff in the usual manner. Maintain the pressure at 40 mm Hg throughout the test. Clean the skin of the volar surface of the forearm with alcohol, but do not rub; allow the skin to dry. With a sharp, new blade make 2 cuts about 2 cm apart and 5 mm long and deep enough to show a narrow red line in the corium. Collect the blood which oozes from the wound with the edge of a filter paper at 30-second intervals, but do not touch the wound. Measure the time until the paper is no longer stained.

Normal: 4 - 8 minutes. More than 12 minutes is definitely abnormal.

Comments: The depth of the cut is the most important point, and it must be varied according to the thickness of the skin. A mechanical device should not be used. The test is reliable if it is done with skill and consistency. It is prolonged in all platelet disorders and in von Willebrand's disease; it is often prolonged in complete afibrinogenemia, and sometimes in hemophiliacs who have recently been heavily transfused. It is normal in clotting deficiencies and usually normal in vascular disorders.

b. Duke Bleeding Time

Technic: A puncture is made in the lobe of an ear with a hemostat or Hagedorn needle to a depth of about 3 mm. The lobe should be warm but should not be cleansed with alcohol or rubbed vigorously. The blood should flow freely and the lobe must not be squeezed. As soon as the puncture is made a stopwatch is started, and at 15-second intervals the blood which has exuded is blotted by a piece of filter paper without touching the surface of the ear.

Normal: usually 1-1/2 minutes, does not exceed 3-1/2 minutes.

Comments: Within wide limits the size of the puncture is not too important. If a scalpel or spring lancet is used it is important that the cut made with these instruments should not approach the category of a wound.

3. Platelet count

Technic: The platelets should be counted in a counting chamber using a phase microscope (see method of Brecher & Cronkite, J. Appl. Physiol. 3:365, 1950), or by an electronic particle counter.

Normal: 250,000/mm^3; 95% of healthy males have counts between 140,000 and 340,000/mm^3

Comments: The indirect method of counting is misleading and should not be used.

4. Clot retraction

a. Qualitative Method

Technic: Draw 2 ml of blood and allow to clot in a test tube at 37° C. Keep the tube in the water bath for 1 hour and judge the degree of retraction: normal or abnormal.

Normal: a well-retracted clot

Comments: Always use the same kind of clean test tube, preferably silicone-coated. The test is abnormal in thrombocytopenia, but here a platelet count gives direct evidence where this test is indirect. Retraction is lacking in thrombasthenia, and here it is the decisive test. Retraction decreases with increasing concentrations of fibrinogen and red cells, and fibrinolysis may interfere with the test. The qualitative test is satisfactory for ordinary clinical work.

b. Quantitative Method

Technic: Centrifuge blood (see Technic of next method) in a siliconized tube for 10 minutes at slow speeds. In a new siliconized tube, preferably graduated to 10 ml, mix 1 ml of platelet-rich plasma, 8.8 ml saline, and 0.2 ml thrombin (20 N.I.H. units per ml). The contents clot in about 30 seconds; free the clot by swirling the tube rapidly between your hands and incubate the tube at 37° C.

Normal: After 3 hours the length of the clot should be less than 3.5 cm.

Comments: There are many modifications of this test but they all give the same type of information.

5. Collection of blood for clotting tests

Technic: Use a sharp needle, not smaller than No. 20. The puncture should be rapid and perfect. The first 2 ml of blood should be discarded ("2-syringe technic"). The blood should be collected by gravity into a tube or aspirated in a syringe ensuring rapid flow without foaming. If an anticoagulant is used, it should be mixed immediately with the blood in the collecting tube or in the syringe. Citrate anticoagulant is made by mixing 3 parts of 0.1 M sodium citrate and 2 parts 0.1 M citric acid. Mix 1 part of this anticoagulant with 9 parts blood. The blood should be centrifuged at once (2,000 - 2,500 rpm for 15 minutes in an ordinary centrifuge).

Comments: Details of blood collection for clotting tests are important when a patient with hemorrhagic disease is studied. A less rigid scheme can be used in the control of anticoagulant therapy. For very accurate work the blood should be chilled and centrifuged in the cold. For diagnostic work it is not necessary to use siliconized tubes and the speed of centrifugation is not critical. However, it is important that the studies be done at once and the plasma should be kept on ice until the tests are finished. It is often wise to freeze a portion of the plasma at -20° C. Further studies can then be carried out at a more convenient time. For all clotting studies the glassware must be scrupulously clean.

6. Whole blood clotting time (modified Lee-White method)

Technic: Using the "2-syringe technic", draw 4 ml of blood into a clean 5 ml syringe rinsed with saline. Transfer 1 ml to each of three 8 mm tubes. These 8 mm tubes should be perfectly clean and dry, and preheated in a waterbath at 37° C. Tilt all tubes at 30-second intervals until they can be turned upside down. Do not shake the tubes. The clock is started when blood enters the syringe.

Normal: about 3 - 7 minutes. Record the results for all three tubes.

Comments: Normal must be determined for each laboratory, preferably for each worker. There are endless modifications of this test and normal results vary between 2 and 60 minutes. The time is prolonged by the use of lower temperatures, siliconized surfaces, greater volume of blood, and less disturbance of the blood. This may or may not be an advantage, depending on the purpose of the test (control of heparin treatment, detection of bleeders, etc.). The main source of error is improper collection of blood which may give a shortened time. The time is prolonged only in severe clotting disturbances and thrombocytopenias. This test is an overall test of the intrinsic clotting system. It has little importance since it does not disclose the milder clotting defects, and it has been replaced by the more sensitive partial thromboplastin time.

7. Thrombin time

Reagents: Commercial thrombin (i.e., topical thrombin of Parke-Davis) prepared from prothrombin of bovine origin is used. The stock solution is diluted in a mixture of equal parts saline and glycerol. The dilute solution is kept in a plastic tube since thrombin is adsorbed to a glass surface; it is stable for several weeks when refrigerated.

Technic: A clotting tube with 0.2 ml test plasma and 0.1 ml saline is preheated for 3 minutes in waterbath at 37° C. Add 0.2 ml of the dilute thrombin solution which has also been preheated at 37° C. The clotting time is then measured.

Normal: about 20 seconds

Comments: A normal control is always run with the test plasma and the thrombin solution is adjusted to give a normal thrombin time of about 20 seconds (usually 8 - 12 N.I.H. units per ml). The thrombin time is prolonged when the concentration of fibrinogen is low, when polymerization of fibrin is blocked by fibrin split-products, or when

the blood contains heparin or a heparin-like anticoagulant. If normal plasma is substituted for the saline, the prolonged thrombin time of fibrinogenopenia becomes normal. The test can be modified to measure low concentrations of heparin. It can also be used as a bedside test for fibrinogenopenia by clotting 2 - 3 ml whole blood with 0.2 ml thrombin (150 N.IH. units per ml). A commercially prepared reagent is available for this "clot observation test." In severe fibrinogenopenia no clot or only a poor clot is formed.

8. FI-test

Technic: The FI-test is performed by a rapid slide method using 1 drop of patient's whole blood obtained by finger prick. Transfer drop to one of the bottles of glycine-saline buffer diluent and mix. Place 1 drop of diluted blood specimen in a circle on a printed card slide. Transfer 1 drop of normal control to the other circle. Add 2 drops of Latex-Anti-Human fibrinogen reagent to each circle. Mix each of the reaction mixtures on card and spread over an area approximately 20 x 25 mm, or most of the area within the circle. Tilt the card slowly from side to side for 15 - 20 seconds and observe for clumping.

Normal: 250 - 400 mg/%

Comments: Blood specimens with plasma fibrinogen levels of 100 mg/% or less will fail to show agglutination in the antigen-antibody system of the FI-test. Clumping in a degree comparable to that shown by the normal control indicates a level in the normal range of 250 - 400 mg/%. The FI-test is available as a kit of reagents for a simplified bedside or operating room procedure to screen for hypofibrinogenemia.

9. Fibrinogen titration (Schneider test)

Reagents: Thrombin (described in #7)

Technic: The test plasma is diluted in normal saline in the following manner:

	Tube Number						
	1	2	3	4	5	6	7
Plasma conc.	1/10	1/25	1/50	1/100	1/200	1/400	1/800
ml of plasma	0.2	0.2	1	1	1	1	1
ml of saline	1.8	4.8	1	1	1	1	1

From Tube 2 the dilution is geometric. The final volume is 1 ml in each tube, and the excess volume is discarded (this operation is easier if Tubes 2 and 3 are graduated). Add 0.2 ml of thrombin (300 N.I.H. units per ml) to each tube, shake well, leave tubes on the bench for 10 minutes and determine the highest dilution which still has a definite clot. This is the fibrinogen titer. The reading is easier if the tubes are shaken for a few seconds first.

Normal: The normal titer is 1/100 or 1/200. The correlation between the titer and concentration of fibrinogen is roughly:

Fibrinogen-titer >	1/400	corresponds to	> 400	mg/100 ml
-	1/400	"	300-400	-
-	1/200	"	200-300	-
-	1/100	"	75-175	-
-	1/50	"	35- 80	-
-	1/25	"	25- 40	-
-	1/10	"	15- 25	-

Comment: This is a useful modification of the old dilution principle. The test is completed in 15 - 20 minutes and it is accurate enough to guide transfusion therapy. There are several modifications of this test and the clinical laboratory should be familiar with at least one such method.

10. Partial thromboplastin time (cephalin time)

Reagents: (1) Cephalin-Kaolin: Cephalin is prepared from human brain or from commercial brain thromboplastin. Commercial preparations are also available. Stock kaolin suspension is made by suspending 2 g of kaolin powder (China Clay, Braun Chemical Co.) in 50 ml of normal saline. It is stored at room temperature; the bottle must be mixed thoroughly immediately before use. The final reagent is made by mixing equal parts of dilute cephalin and kaolin. (See Proctor & Rapaport, Amer. J. Clin. Path. 36: 212, 1961).

(2) Absorbed plasma: Draw 90 ml of human or bovine blood into 10 ml of 2.5% potassium oxalate monohydrate. After mixing, the blood is centrifuged about 2,000 rpm for 15 minutes. The plasma is pipetted off, and 100 mg of barium sulphate (C.P. "Baker") is added per ml of plasma. The mixture is stirred at room temperature for 30 minutes and then centrifuged at 2,500 rpm or faster for 30 minutes. The clear plasma (which is devoid of factors II, VII, IX and X) is pipetted off and stored in suitable aliquots at -20° C. Citrated plasma can be absorbed with aluminum hydroxide gel.

(3) Stored serum: Collect 100 ml of human blood in a dry, clean bottle. Immediately add 4 ml of human thromboplastin (as described in Prothrombin test) and stir vigorously with a heavy glass rod for 30 minutes at 37° C. The glass rod with the fibrinogen clot is removed and the blood is further incubated at 37° C for 6 hours. It is then centrifuged at 2,500 rpm for 30 minutes, the serum is pipetted off and stored in suitable aliquots at -20° C.

(4) Calcium chloride: 30 mM aqueous solution.

(5) Saline.

Technic: Mix 0.2 ml each of test plasma, saline, and cephalin-kaolin in a clean alkali-washed tube, and after 3 minutes at 37° C add 0.2 ml calcium (prewarmed at 37° C). Immediately mix by shaking the tube once; after 30 seconds, tilt the tube every 5 seconds. The end point (first clumping of the kaolin) is easier to read against a glass mirror with a strong overhead light.

Normal: 39 - 53 seconds

Comments: If the results are abnormal, repeat the test with substitution of the saline with absorbed plasma and serum. Minor technical differences may change the normal range. The test requires some experience, but it is then reproducible. Several important points are indicated: (a) When the results are normal, the patient has a normal intrinsic clotting system; i.e., he has no marked deficiency of factors I, II, V, VIII, IX, X, XI and XII. The partial thromboplastin time usually becomes prolonged when one of these factors falls below 30% of normal. (b) When the results are abnormal, the patient has a deficiency of one of these factors (he may also have a circulating anticoagulant). The longer the partial thromboplastin time, the more serious the defect. A severe hemophiliac has a cephalin time of about 100 seconds. The substitution experiments determine which factor is deficient: factors V and VIII, if the partial thromboplastin time is normalized only by the absorbed plasma; factors IX or X, if it is normalized only by serum; factors XI or XII, if it is normalized by both. (The latter two deficiencies can only be separated by mixing experiments with plasmas from patients with known deficiencies. The other deficiencies can be separated by means of this and by the following test.) The following table illustrates in simplified form the information obtained from this test:

Test plasma	Partial thromboplastin time (secs.) using:		
	Saline	Absorbed plasma	Stored serum
Normal plasma	45	45	45
Factor VIII deficiency	100	50	95
" IX "	100	100	50
" X "	100	100	50
" XI "	100	60	60
" XII "	100	60	60

11. Prothrombin time

Reagents: (1) Thromboplastin, prepared from human brain or a commercial rabbit brain preparation, is used. The reagent should contain very little blood, and therefore, lung thromboplastin should not be used.

(2) Absorbed plasma, stored serum, calcium chloride and saline as used in the partial thromboplastin test.

Technic: Mix 0.2 ml each of test plasma, saline and thromboplastin in a clean tube, and after 3 minutes at 37° C add 0.2 ml calcium chloride (prewarmed at 37° C). Immediately mix the contents, after 10 seconds tilt the tube at frequent intervals. The end point is easy to read: a solid clot.

Normal: 12 - 14 seconds

Comments: If the result is abnormal, repeat the test with substitution of absorbed plasma and serum for the saline. This test is easier than the partial thromboplastin time and requires little experience. It gives the following information: (a) When the result is normal, the patient has a normal extrinsic clotting system, i.e., he has no marked

deficiency of factors I, II, V, VII and X. The thromboplastin time usually becomes prolonged when one of these factors falls below 30% of normal. (b) When the result is abnormal, the patient has a deficiency of one of these factors (he may also have an anticoagulant). The longer the thromboplastin time, the more serious the defect (it is 70 seconds or more in a severe defect). The substitution experiments determine which factor is deficient: factor V, if the prothrombin time is normalized only by absorbed plasma; factors VII or X, if it is normalized by serum; factor II, if it is normalized by neither. The following table illustrates the information obtained from this test:

Test plasma	Prothrombin time (secs.) using:		
	Saline	Absorbed plasma	Stored plasma
Normal plasma	12	12	10-12
Factor II deficiency	70	70	60-70
" V "	70	14	70
" VII "	70	70	14
" X "	70	70	14

Observe that a deficiency of factor X gives a prolonged prothrombin time and also a prolonged partial thromboplastin time; a deficiency of factor VII gives only a prolonged prothrombin time.

12. Test for fibrinolysis

Technic: Incubate clotted blood or plasma at 37° C for 24 hours. It is convenient to use the tubes from the whole blood clotting time, for clot retraction, or for the fibrinogen titration (dilution makes the fibrinolytic system more active). Inspect the tubes at intervals and check whether the clot has disappeared by pouring the blood or plasma onto filter paper.

Normal: The clots should not dissolve; however, the smallest clot in the series of tubes for fibrinogen titration may disappear.

Comments: There may be a source of error in this test since some preparations of thrombin are weakly fibrinolytic. If the fibrinogen concentration is low, it takes only a weak fibrinolysin to dissolve the clot. Weak fibrinolysis may be induced by exercise and any form of stress. If fibrinolysis is of clinical importance, therefore, solid clots should dissolve in a few hours.

13. Test for anticoagulant

Technic: The test can be based on the thrombin time, the partial thromboplastin time, or the prothrombin time. The following table illustrates the technic.

	Thromboplastin time (secs.)	
	Patient's plasma is deficient:	Patient's plasma contains inhibitor:
Normal plasma alone	14	
Patient's plasma alone	70	
Patient's plasma + 1/10 normal pl.	16	70
Normal plasma + 1/10 patient's pl.	14	30

Comment: When slow clotting is caused by lack of some factor it takes only a small amount of normal plasma to speed up the clotting of the deficient plasma. However, when slow clotting is caused by an inhibitor, normal plasma has no effect; in fact, pathological plasma slows clotting of normal plasma. The patient may have a deficiency and an inhibitor, but this problem belongs in the specialized coagulation laboratory.

COMPILED BY

Peter Hjort, M.D.

Department of Medicine
Rikshospitalet
Oslo, Norway

and

L. A. Harker, M.D. and C. A. Finch, M.D.

Department of Medicine
University of Washington
Seattle, Washington

1967