

Blood Plasma Proteins, Coagulation, and Fibrinolysis



thePoint For additional ancillary materials related to this chapter, please visit [thePoint](#).

The blood is the body's main transport system. Although the transport and delivery of oxygen to the cells of the tissues is carried out by specialized cells, other vital components such as nutrients, metabolites, electrolytes, and hormones are all carried in the noncellular fraction of the blood, the **plasma**. Some components, such as glucose, are dissolved in the plasma; others—for example, lipids and steroid hormones—are bound to carrier proteins for transport. The **osmotic pressure** exerted by the plasma proteins regulates the distribution of water between the blood and the tissues. Plasma proteins in conjunction with platelets maintain the integrity of the circulatory system through the process of clotting.

Blood normally circulates through endothelium-lined blood vessels. When a blood vessel is severed, a **blood clot** (called a **thrombus**, which is formed by the process of **thrombosis**) forms as part of **hemostasis**, the physiologic response that stops bleeding. Blood clots also form to repair damage to the endothelial lining, in which components of the **subendothelial layer** become accessible to plasma proteins.

In hemostasis and thrombosis, a primary hemostatic plug, consisting of

aggregated platelets and a **fibrin clot**, forms at the site of injury. Platelets are attached to the subendothelial layer of the vessel principally through the protein **von Willebrand factor** (vWF) and are activated to bind fibrinogen. **Fibrinogen** binds the platelets to each other to form a platelet aggregate. The platelet aggregate is rapidly covered with layers of **fibrin**, which are formed from fibrinogen by the proteolytic enzyme **thrombin**.

Injury to the endothelium and exposure of **tissue factor** on the **subendothelial layer** to plasma proteins also activate the blood coagulation cascade, which ultimately activates thrombin and **factor XIII**. Factor XIII **cross-links** strands of polymerized fibrin monomers to form a stable clot (the **secondary hemostatic plug**). The blood coagulation cascade consists of a series of enzymes (such as **factor X**), which are inactive until they are proteolytically cleaved by the preceding enzyme in the cascade. Other proteins (**factor V** and **factor VIII**) serve as binding proteins, which assemble factor complexes at the site of injury. Ca^{2+} and **γ -carboxyglutamate** residues in the proteins (formed by a vitamin K–dependent process in the liver) attach the factor complexes to **phospholipids** exposed on platelet membranes. Consequently, thrombus formation is rapidly accelerated and localized to the site of injury.

Regulatory mechanisms within the blood coagulation cascade and **antifibrinolytic mechanisms** prevent random coagulation within blood vessels that might obstruct blood flow. Impairments in these mechanisms lead to thrombosis.

THE WAITING ROOM



Peter K., a 6-month-old male infant, was brought to his pediatrician's office with a painful, expanding mass in his right upper thigh that was first noted just hours after he fell down three uncarpeted steps in his home. The child appeared to be in severe distress.

A radiograph showed no fractures, but a soft tissue swelling, consistent with a hematoma (bleeding into the tissues), was noted. Peter's mother related that soon after he began to crawl, his knees occasionally became swollen and seemed painful.

The pediatrician suspected a disorder of coagulation. A screening coagulation profile suggested a possible deficiency of factor VIII, a protein involved in the formation of blood clots. Peter's plasma factor VIII level was found to be only 3% of the average level found in normal subjects. A diagnosis of hemophilia A was made.


I. Plasma Proteins Maintain Proper Distribution of Water between Blood and Tissues

When cells are removed from the blood, the remaining plasma is composed of water, nutrients, metabolites, hormones, electrolytes, and proteins. Plasma has essentially the same electrolyte composition as other extracellular fluids and constitutes approximately 25% of the body's total extracellular fluid. The plasma proteins serve several functions, including maintaining the proper distribution of water between the blood and the tissues; transporting nutrients, metabolites, and hormones throughout the body; defending against infection; and maintaining the integrity of the circulation through clotting. Many diseases alter the amounts of plasma proteins produced and hence their concentration in the blood. These changes can be determined by electrophoresis of plasma proteins over the course of a disease.

A. Body Fluid Maintenance between Tissues and Blood

As the arterial blood enters the capillaries, fluid moves from the intravascular space into the interstitial space (that surrounding the capillaries) owing to Starling's forces. The hydrostatic pressure in the arteriolar end of the capillaries (~37 mm Hg) exceeds the sum of the tissue pressure (~1 mm Hg) and the osmotic pressure of the plasma proteins (~25 mm Hg). Thus, water tends to leave the capillaries and enter extravascular spaces. At the venous end of the capillaries, the hydrostatic pressure falls to approximately 17 mm Hg, whereas the osmotic pressure and the tissue pressure remain constant, resulting in movement of fluid back from the extravascular (interstitial) spaces and into the blood. Thus, most of the force bringing water back from the tissues into the plasma is the osmotic pressure mediated by the presence of proteins in the

plasma.

 There are two basic assays for measuring the activity of factor VIII in a blood sample. The first is a functional (clotting) assay; the second is a coupled (to factor X activation) enzyme system leading to the cleavage of a chromogenic substrate, generating a colored product.

For the functional assay, a sample of the patient's plasma is added to a factor VIII-deficient sample of plasma (which can be obtained commercially), and the time to generate clot formation is determined. Although clot formation is the end-product of this assay, one is measuring the eventual activation of factor II to IIa, which allows clot formation to initiate.

In the automated chromogenic assay, a sample of the unknown is mixed with purified factor IXa, Ca^{2+} , phospholipid vesicles, the chromogenic substrate, and purified factor X. If the unknown sample contains factor VIII, factors VIII and IXa will synergize and activate factor X to Xa. Factor Xa will cleave the chromogenic substrate, forming a colored product whose concentration can be determined spectrophotometrically. Comparison to a standard curve of factor VIII addition enables an accurate estimate of the level of active factor VIII in the sample.

B. The Major Serum Protein, Albumin

As indicated in [Table 43.1](#), the liver produces several transport or binding proteins and secretes them into the blood. The major protein synthesized is albumin, which constitutes approximately 60% of the total plasma protein, but because of its relatively small size (69 kDa), it is thought to contribute 70% to 80% of the total osmotic pressure of the plasma. Albumin, like most plasma proteins, is a glycoprotein (GP) and is a carrier of free fatty acids, calcium, zinc, steroid hormones, copper, and bilirubin. Many drugs also bind to albumin, which may have important pharmacologic implications. For example, when a drug binds to albumin, such binding will likely lower the effective concentration of that drug and may lengthen its lifetime in the circulation. Drug dosimetry may

need to be recalculated if a patient's plasma protein concentration is abnormal.

TABLE 43.1 Specific Plasma-Binding Proteins Synthesized in the Liver	
Albumin	Binds free fatty acids, calcium, zinc, steroid hormones, copper, bilirubin
Ceruloplasmin	Binds copper; appears to be more important as a copper storage pool than as a transport protein; integrates iron and copper homeostasis
Corticosteroid-binding globulin	Binds cortisol
Haptoglobin	Binds extracorpuscular heme
Lipoproteins	Transport cholesterol and fatty acids
Retinol-binding protein	Binds vitamin A
Sex hormone-binding globulin	Binds estradiol and testosterone
Transferrin	Transports iron
Transthyretin	Binds tetraiodothyronine (thyroxine; T ₄); also forms a complex with retinol-binding protein



In spite of the importance of albumin in the maintenance of osmotic pressure in the blood, individuals who lack albumin (analbuminemia) have only moderate edema. Apparently, the concentration of other plasma proteins is increased to compensate for the lack of albumin. The frequency of analbuminemia is <1 per million individuals.

II. The Plasma Contains Proteins that Aid in Immune Defense

Two different sets of proteins aid in the immune response, the immunoglobulins and the complement proteins. The immunoglobulins are secreted by a subset of differentiated B-lymphocytes termed *plasma cells* and bind antigens at binding sites formed by the hypervariable regions of the proteins (see [Chapter 7](#)). Once the antibody–antigen complex is formed, it must be cleared from the circulation. The complement system participates in this function. The complement system, consisting of approximately 20 proteins, becomes activated in either of two ways. The first is interaction with antigen–antibody complexes, and the second, specific for bacterial infections, is through interaction of bacterial cell polysaccharides with complement protein C3b. Activation of the complement system by either trigger results in a proteolytic activation cascade of the proteins

of the complement system, resulting in the release of biologically active peptides or polypeptide fragments. These peptides mediate the inflammatory response, attract phagocytic cells to the area, initiate degranulation of granulocytes, and promote clearance of antigen–antibody complexes.

Protease inhibitors in plasma serve to carefully control the inflammatory response. Activated neutrophils release lysosomal proteases from their granules that can attack elastin, the various types of collagen, and other extracellular matrix proteins. The plasma proteins α_1 -antiproteinase (α_1 -antitrypsin) and α_2 -macroglobulin limit proteolytic damage by forming noncovalent complexes with the proteases, thereby inactivating them. However, the product of neutrophil myeloperoxidase, HOCl, inactivates the protease inhibitors, thereby ensuring that the proteases are active at the site of infection.



α_1 -Antiproteinase (AAP), also known as α_1 -antitrypsin, is the main serine protease inhibitor of human plasma. Individuals with a point mutation in exon 5 of the *AAP* gene, which results in a single amino acid substitution in the protein, have diminished secretion of AAP from the liver. These individuals are at increased risk for developing emphysema. When neutrophils degranulate in the lungs as part of the body's defense against microorganisms, insufficient levels of AAP are present to neutralize the elastase and other proteases released. The excess proteolytic activity damages lung tissue, leading to loss of alveolar elasticity and the development of emphysema.

Methionine 358 of AAP is necessary for AAP binding to the proteases. Oxidation of this methionine destroys the protease-binding capacity of AAP. Chemicals within cigarette smoke lead to the oxidation of Met-358, thereby increasing the risk of emphysema through the inactivation of AAP.

III. Plasma Proteins Maintain the Integrity of the Circulatory System

Blood is lost from the circulation when the endothelial lining of the blood

vessels is damaged and the vessel wall is partly or wholly severed. When this occurs, the subendothelial cell layer is exposed, consisting of the basement membrane of the endothelial cells and smooth muscle cells. In response to the damage, a barrier (the hemostatic plug, a fibrin clot), initiated by platelet binding to the damaged area, is formed at the site of injury. Regulatory mechanisms limit clot formation to the site of injury and control its size and stability. As the vessel heals, the clot is degraded (fibrinolysis). Plasma proteins are required for these processes to occur.

A. Formation of the Hemostatic Plug

1. The Platelet

Platelets are nonnucleated cells in the blood whose major role is to form mechanical plugs at the site of vessel injury and to secrete regulators of the clotting process and vascular repair. In the absence of platelets, leakage of blood from rents in small vessels is common. Platelets are derived from megakaryocytes in the bone marrow. Megakaryocytes differentiate from the hematopoietic stem cell. As the megakaryocyte matures, it undergoes synchronous nuclear replication without cellular division, to form a cell with a multilobed nucleus and enlarged cytoplasm. At approximately the eight-nucleus stage, the cytoplasm becomes granular, and the platelets are budded off the cytoplasm. A single megakaryocyte gives rise to approximately 4,000 platelets.



Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease in which antibodies to platelet GPs are produced. Antibody binding to platelets results in their clearance by the spleen. An early symptom of the disorder is the appearance of small red spots on the skin (petechial hemorrhages) caused by blood leakage from capillaries. Minor damage to vascular endothelial cells is constantly being caused by mechanical forces related to blood flow. In patients with ITP, few platelets are available to repair the damage.

In the nonactivated platelet (a platelet that is not involved in forming a clot),

the plasma membrane invaginates extensively into the interior of the cell, forming an open membrane (canalicular) system. Because the plasma membrane contains receptors and phospholipids that accelerate the clotting process, the canalicular structure substantially increases the membrane surface area that is potentially available for clotting reactions. The platelet interior contains microfilaments and an extensive actin/myosin system. Platelet activation in response to endothelial injury causes Ca^{2+} -dependent changes in the contractile elements, which, in turn, substantially change the architecture of the plasma membrane. Long pseudopodia are generated, increasing the surface area of the membrane as clot formation is initiated.

Platelets contain three types of granules. The first are electron-dense granules, which contain calcium, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and serotonin. The second type of granule is the α -granule, which contains a heparin antagonist (heparin interferes with blood clotting; see “[Biochemical Comments](#)”), platelet-derived growth factor, β -thromboglobulin, fibrinogen, vWF, and other clotting factors. The third type of granule is the lysosomal granule, which contains hydrolytic enzymes. During activation, the contents of these granules, which modulate platelet aggregation and clotting, are secreted.



vWF is a large multimeric GP with a subunit molecular weight of 220,000 Da. Its size in the circulation ranges between 500 and 20,000 kDa, and its role in circulation is to stabilize factor VIII and protect it from degradation. The high-molecular-weight forms are concentrated in the endothelium of blood vessels and are released in response to stress hormones and endothelial damage. High-molecular-weight vWF released by the endothelium is cleaved by a specific metalloprotease in the serum, reducing the size of the circulating vWF. Large vWF multimers are more effective at forming complexes with platelets than are small vWF multimers.

vWF deficiency is the most common cause of inherited bleeding disorders. Both platelet adherence and the clotting cascade are affected because levels of factor VIII are low. In the absence of vWF, factor VIII is rapidly cleared from the system. The vWF gene is large, covering

approximately 180 kb, and contains 52 exons. Multiple mutations are known, with varying clinical presentations.

2. Platelet Activation

Three fundamental mechanisms are involved in platelet function during blood coagulation: adhesion, aggregation, and secretion. Adhesion sets off a series of reactions termed platelet activation, which leads to platelet aggregation and secretion of platelet granule contents.

The *adhesion step* refers primarily to the platelet–subendothelial interaction that occurs when platelets initially adhere to the sites of blood vessel injury (Fig. 43.1). Blood vessel injury exposes collagen, subendothelial matrix-bound vWF, and other matrix components. vWF is a protein synthesized in endothelial cells and megakaryocytes and is located in the subendothelial matrix, in specific platelet granules, and in the circulation bound to factor VIII. The platelet cell membrane contains GPs that bind to collagen and to vWF, causing the platelet to adhere to the subendothelium. Binding to collagen by GPIa ($\alpha_2\beta_1$ integrin) causes the platelet to change its shape from a flat disc to a spherical cell. The latter extrudes long pseudopods, which promote platelet–platelet interactions. Binding of subendothelial vWF by GPIb causes changes in the platelet membrane that expose GPIIb-IIIa ($\alpha_{IIb}\beta_3$ integrin)–binding sites to fibrinogen and vWF.

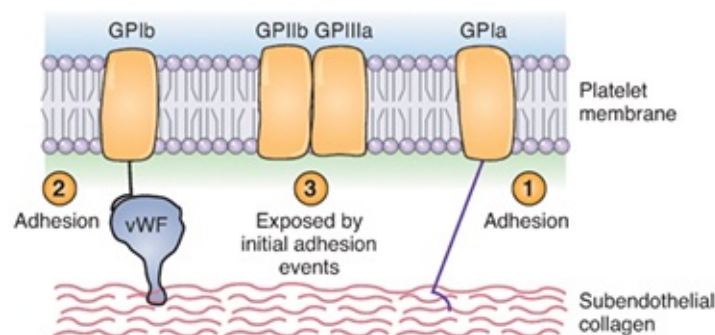


FIGURE 43.1 Adhesion of platelets to the subendothelial cell layer. (1) glycoprotein Ia (GPIa) initially binds to the exposed collagen, which results in changes in the three-dimensional configuration of the complex, allowing GPIb to bind to von Willebrand factor (vWF) (2). (3) This second binding event exposes the GPIIb–GPIIIa complex, which also can bind to vWF and fibrinogen.

The initial adherence of platelets sets off a series of reactions (platelet activation) that results in more platelets being recruited and aggregated at the site

of injury. After initial adherence, some of the platelets release the contents of their dense granules and their α -granules, with ADP release being of particular importance because ADP is a potent platelet activator. ADP released from the platelets and from damaged red blood cells binds to a receptor on the platelet membrane, which leads to the further unmasking of GPIIb-IIIa-binding sites. Aggregation of platelets cannot take place without ADP stimulation because ADP induces swelling of the activated platelets, promoting platelet-platelet contact and adherence.



Mutations in GPIb cause a bleeding disorder known as *Bernard-Soulier syndrome*. Platelet aggregation is affected because of the inability of GPIb to adhere to subendothelial vWF.

Fibrinogen is a protein that circulates in the blood and is also found in platelet granules. It consists of two triple helices held together with disulfide bonds (Fig. 43.2). Binding of fibrinogen to activated platelets is necessary for aggregation, providing, in part, the mechanism by which platelets adhere to one another. Cleavage of fibrinogen by thrombin (a protease that is activated by the coagulation cascade) produces fibrin monomers that polymerize and, together with platelets, form a “soft” clot. Thrombin itself is a potent activator of platelets, through binding to a specific receptor on the platelet surface.

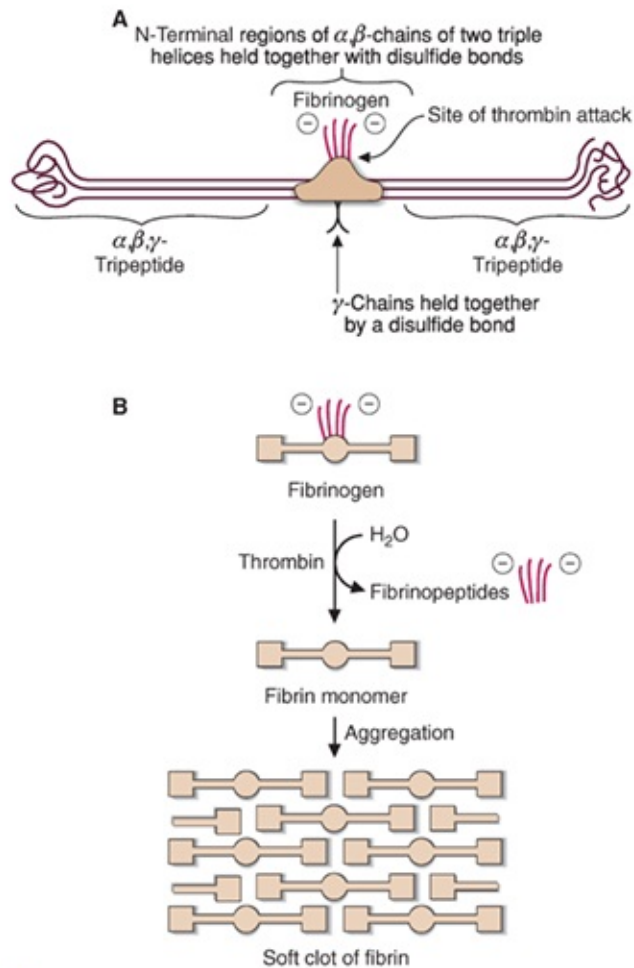


FIGURE 43.2 Cleavage of fibrinogen results in clot formation. **A.** Fibrinogen, the precursor protein of fibrin, is formed from two triple helices joined together at their N-terminal ends. The α,β -peptides are held together by disulfide bonds, and the γ -peptides are joined to each other by disulfide bonds. The terminal α,β -peptide regions, shown in red, contain negatively charged glutamate and aspartate residues that repel each other and prevent aggregation. **B.** Thrombin, a serine protease, cleaves the terminal portions of fibrinogen that contain negative charges. The fibrin monomers can then aggregate and form a "soft" clot. The soft clot is subsequently cross-linked by another enzyme.



Thrombotic thrombocytopenic purpura (TTP) is a disease characterized by the formation in the circulation of microclots (microthrombi) consisting of aggregated platelets. The microthrombi collect in the microvasculature and damage red blood cells, resulting in hemolytic anemia. They also damage vascular endothelium, exposing collagen and releasing high-molecular-weight vWF, promoting more platelet aggregation. The subsequent depletion of platelets renders the patient susceptible to internal hemorrhage. Mortality in untreated TTP can approach 90%.

Familial TTP, which is a less common cause of TTP, is associated with mutations in the vWF-specific metalloprotease, although not all individuals with defective protease develop TTP. Sporadic cases of TTP are associated with the development of an antibody to the metalloprotease.

B. The Blood Coagulation Cascade

Thrombus (clot) formation is enhanced by thrombin activation, which is mediated by the complex interaction that constitutes the blood coagulation cascade. This cascade ([Fig. 43.3](#)) consists primarily of proteins that serve as enzymes or cofactors, which function to accelerate thrombin formation and localize it at the site of injury. These proteins are listed in [Table 43.2](#). All of these proteins are present in the plasma as proproteins (zymogens). These precursor proteins are activated by cleavage of the polypeptide chain at one or more sites. The key to successful and appropriate thrombus formation is the regulation of the proteases that activate these zymogens.

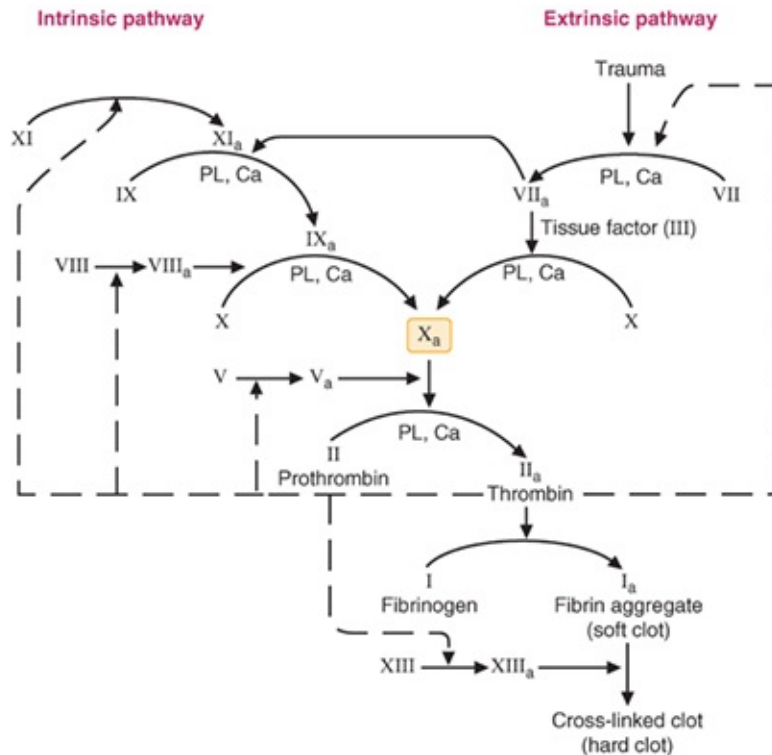


FIGURE 43.3 The blood coagulation cascade. Activation of clot formation occurs through interlocking pathways, termed the *intrinsic* and *extrinsic* pathways. The intrinsic pathway is activated when factor XI is converted to factor XI_a by thrombin. The extrinsic pathway (external damage, such as a cut) is activated by tissue factor. The reactions designated by PL, Ca are occurring through cofactors bound to phospholipids (PL) on the platelet and blood vessel endothelial cell surface in a Ca²⁺-coordination complex. Factors XI_a, IX_a, VII_a, X_a, and thrombin are serine proteases. Note the positive feedback regulation of thrombin on the activation of proteases earlier in the cascade sequence (indicated by the *dashed lines*).

TABLE 43.2 Proteins of Blood Coagulation

FACTOR	DESCRIPTIVE NAME	FUNCTION/ACTIVE FORM
Coagulation Factors		
I	Fibrinogen	Fibrin
II	Prothrombin	Serine protease
III	Tissue factor	Receptor and cofactor
IV	Ca ²⁺	Cofactor
V	Proaccelerin, labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihemophilia factor A	Cofactor
IX	Antihemophilia factor B, Christmas factor	Serine protease
X	Stuart-Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XIII	Fibrin-stabilizing factor	Ca ²⁺ -dependent transglutaminase
Regulatory Proteins		
Thrombomodulin	Endothelial cell receptor, binds thrombin	
Proteins C and S	Activated by thrombomodulin-bound thrombin; is a serine protease cofactor; binds activated protein C	

The proenzymes (factors VII, XI, IX, X, and prothrombin) are serine

proteases that, when activated by cleavage, cleave the next proenzyme in the cascade. Because of the sequential activation, great acceleration and amplification of the response is achieved. That cleavage and activation have occurred is indicated by the addition of an “a” to the name of the proenzyme (e.g., factor IX is cleaved to form the active factor IXa).

The cofactor proteins (tissue factor, factors V and VIII) serve as binding sites for other factors. Tissue factor is not related structurally to the other blood coagulation cofactors and is an integral membrane protein that does not require cleavage for active function. Factors V and VIII serve as procofactors, which, when activated by cleavage, function as binding sites for other factors.

Two additional proteins that are considered part of the blood coagulation cascade, protein S and protein C, are regulatory proteins. Only protein C is regulated by proteolytic cleavage, and when it is activated, it is itself a serine protease.

In addition, in response to collagen and thrombin, platelets release vasoconstrictors. Serotonin is released from the dense granules of the platelets, and the synthesis of thromboxane A₂ is stimulated. This reduces blood flow to the damaged area. Platelet-derived growth factor, which stimulates proliferation of vascular cells, is also released into the environment surrounding the damage.

C. The Process of Blood Coagulation

Activation of the blood coagulation cascade is triggered by the reaction of plasma proteins with the subendothelium at the same time that platelets are adhering to the subendothelial layer. Historically, two different pathways were discovered, one dependent on external stimuli (such as blunt trauma, which initiates the extrinsic pathway) and one using internal stimuli (the intrinsic pathway). As our understanding of blood clotting has expanded, it has become obvious that these distinctions are no longer correct because there is overlap between the pathways, but the terms have persisted in the description of the pathways.

The use of an active-site serine to cleave a peptide bond is



common to a variety of enzymes referred to as *serine proteases*. Serine proteases are essential for activating the formation of a blood clot from fibrin. Fibrin and many of the other proteins involved in blood coagulation are present in the blood as inactive precursors or zymogens, which must be activated by proteolytic cleavage. Thrombin, the serine protease that converts fibrinogen to fibrin, has the same aspartate–histidine–serine catalytic triad found in chymotrypsin and trypsin.

Thrombin is activated by proteolytic cleavage of its precursor protein, prothrombin. The sequence of proteolytic cleavages leading to thrombin activation requires factor VIII, the blood-clotting protein that is deficient in **Peter K**.

In the case of external trauma, damaged tissues present tissue factor to the blood at the injured site, thereby triggering the extrinsic phase of blood coagulation. Circulating factor VII binds to tissue factor, which autocatalyzes its own activation to factor VIIa. Factor VIIa then activates factor X (to Xa) in the extrinsic pathway and factor IX (to IXa) in the intrinsic pathway. Factor IXa, as part of the intrinsic pathway, also activates factor X. Therefore, activation of both the extrinsic and intrinsic pathways results in the conversion of factor X to factor Xa. All of these conversions require access to membranes and calcium; the platelet membrane, which had adhered to the damaged site, is used as a scaffold for the activation reactions to occur. The γ -carboxylated clotting proteins are chelated to membrane surfaces via electrostatic interactions with calcium and negatively charged phospholipids of the platelet membrane. The protein cofactors VIIIa and Va serve as sites for assembling enzyme–cofactor complexes on the platelet surface, thereby accelerating and localizing the reaction. The result is thrombin formation, which augments its own formation by converting factors V, VIII, and XI into activated cofactors and stimulating platelet degranulation. The initial activation of prothrombin is slow because the activator cofactors, VIIIa and Va, are present only in small amounts. However, once a small amount of thrombin is activated, it accelerates its own production by cleaving factors V and VIII to their active forms. Note that these factors are in the intrinsic pathway. The intrinsic pathway is thought to sustain the coagulation response initiated by the extrinsic pathway. The major substrate of

thrombin is fibrinogen, which is hydrolyzed to form fibrin monomers that undergo spontaneous polymerization to form the fibrin clot. This is considered a “soft” clot because the fibrin monomers are not cross-linked. Cross-linking requires factor XIIIa, which is activated by thrombin cleavage of factor XIII.

1. Cross-Linking of Fibrin

Factor XIIIa catalyzes a transamidation reaction between glutamine and lysine side chains on adjacent fibrin monomers. The covalent cross-linking takes place in three dimensions, creating a strong network of fibers that is resistant to mechanical and proteolytic damage. This network of fibrin fibers traps the aggregated platelets and other cells, forming the clot that plugs the vent in the vascular wall (Fig. 43.4). Factor XIIIa is the only enzyme in the blood coagulation cascade that is not a serine protease.

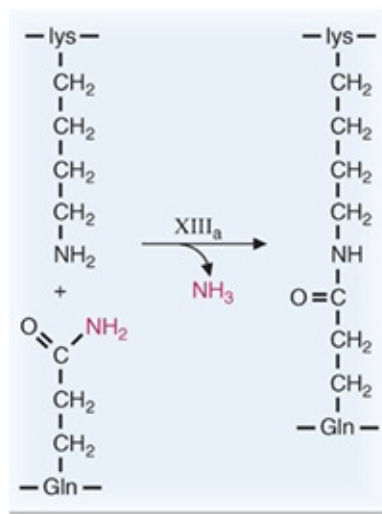


FIGURE 43.4 The transamidation reaction catalyzed by factor XIIIa, transglutaminase. This reaction cross-links fibrin monomers, allowing “hard” clots to form.

2. Factor Complexes

In several of the essential steps in the blood coagulation cascade, the activated protease is bound in a complex attached to the surfaces of the platelets that have aggregated at the site of injury. Factors VII, IX, X, and prothrombin contain a domain in which one or more glutamate residues are carboxylated to γ -carboxyglutamate in a reaction that requires vitamin K (Fig. 43.5). Prothrombin

and factor X both contain 10 or more γ -carboxyglutamate residues that bind Ca^{2+} . The Ca^{2+} forms a coordination complex with the negatively charged platelet membrane phospholipids and the γ -carboxyglutamates, thereby localizing the complex assembly and thrombin formation to the platelet surface.

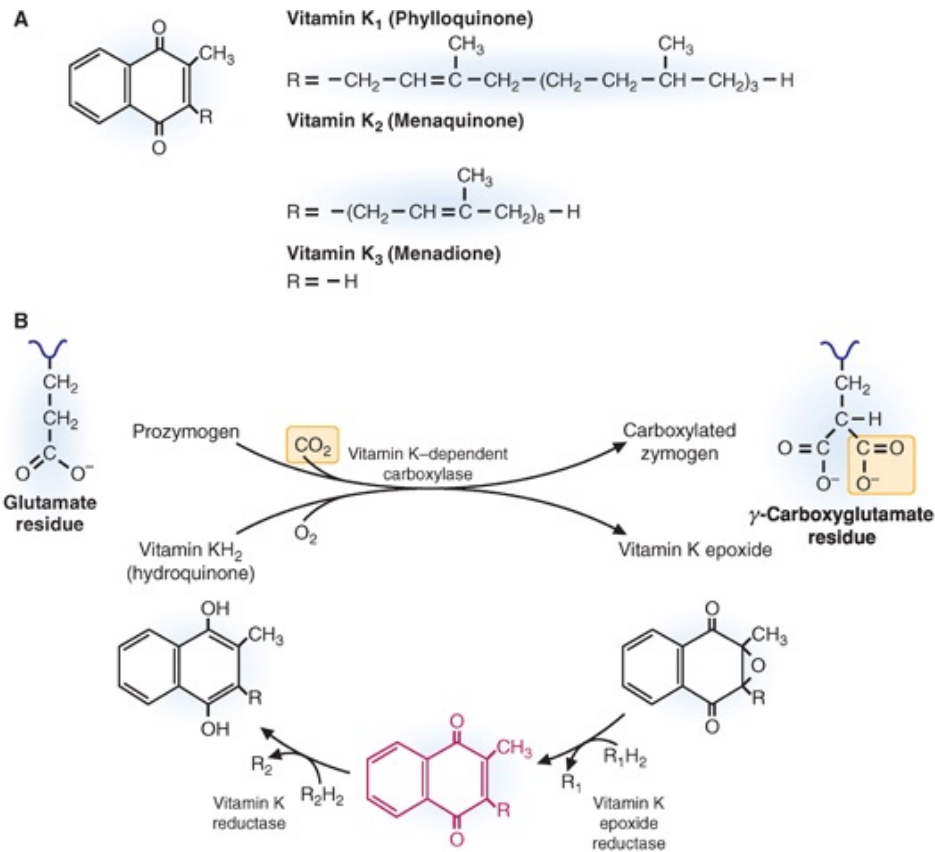
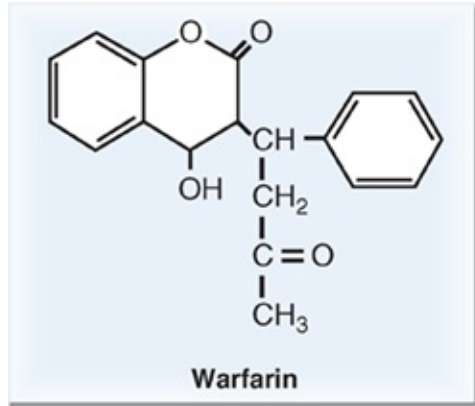


FIGURE 43.5 **A.** Structures of vitamin K derivatives. Phylloquinone is found in green leaves, and intestinal bacteria synthesize menaquinone. Humans convert menadione to a vitamin K active form. **B.** Vitamin K–dependent formation of γ -carboxyglutamate residues. Thrombin and factors VII, IX, and X are bound to their phospholipid activation sites on cell membranes by Ca^{2+} . The vitamin K–dependent carboxylase, which adds the extra carboxyl group, uses a reduced form of vitamin K (KH_2) as the electron donor and converts vitamin K to an epoxide. Vitamin K epoxide is reduced, in two steps, back to its active form by the enzymes vitamin K epoxide reductase and vitamin K reductase.



Warfarin (Coumadin) is a slow- and long-acting blood anticoagulant with a structure resembling that of vitamin K. The structural similarity allows the compound to compete with vitamin K and prevent γ -carboxylation of glutamate residues in factors II, VII, IX, and X and proteins C and S. The noncarboxylated blood clotting protein precursors increase in both the blood and plasma, but they are unable to promote blood coagulation because they cannot bind calcium and thus cannot bind to their phospholipid membrane sites of activation.



Cofactor Va contains a binding site for both factor Xa and prothrombin, the zymogen substrate of factor Xa. Upon binding to the factor Va–platelet complex, prothrombin undergoes a conformational change, rendering it more susceptible to enzymatic cleavage. Binding of factor Xa to the factor Va–prothrombin–platelet complex allows the prothrombin-to-thrombin conversion. Complex assembly accelerates the rate of this conversion 10,000- to 15,000-fold as compared with non-complex formation.

Factor VIIIa forms a similar type of complex on the surface of activated platelets but binds factor IXa and its zymogen substrate, factor X. Tissue factor works slightly differently because it is an integral membrane protein. However, once it is exposed by injury, it also binds factor VIIa and initiates complex formation.

Complex assembly has two physiologically important consequences. First, it enhances the rate of thrombin formation by as much as several hundred thousand-fold, enabling the clot to form rapidly enough to preserve hemostasis. Second, such explosive thrombin formation is localized to the site of vascular injury at which the negatively charged phospholipids are exposed. From our knowledge of the location of such phospholipids in cellular and subcellular organelle membranes, these surface binding sites are only exposed at an injury site in which cell rupture exposes the internal membrane surface (recall that certain phospholipids face only the cytoplasm; if these lipids are now exposed to the environment, substantial cell damage must have occurred).

3. Vitamin K Requirement for Blood Coagulation

The formation of the γ -carboxyglutamate residues on blood coagulation factors takes place in the hepatocyte before release of the protein. Within the hepatocyte,

vitamin K (which is present in the quinone form) is reduced to form vitamin KH_2 by a microsomal quinone reductase (see Fig. 43.5). Vitamin KH_2 is a cofactor for carboxylases that add a carboxyl group to the appropriate glutamate residues in the proenzyme to form the carboxylated proenzyme (e.g., prothrombin). In the same reaction, vitamin K is converted to vitamin K epoxide. To recover active vitamin KH_2 , vitamin K is first reduced to the quinone form by vitamin K epoxide reductase, and then to the active hydroquinone form.

D. Regulation through Feedback Amplification and Inhibition

Once the formation of the clot (thrombus) begins, clot formation is accelerated in an almost explosive manner by several processes that are collectively termed feedback amplification.



Warfarin is a commonly used rat poison and thus is occasionally encountered in emergency departments in cases of accidental poisoning. It is effective as a rat poison because it takes many hours to develop pathologic symptoms, which allows one poisoned trap to kill more than one rat.

1. The Role of Thrombin in Regulation

Thrombin has both a prothrombotic regulatory role (feedback amplification) and an antithrombotic regulatory role (feedback inhibition). The prothrombotic action is initiated when thrombin stimulates its own formation by activating factors V, VIII, and XI, thereby accelerating the rate of clot formation (see Fig. 43.3). Thrombin also promotes clot formation by activating platelet aggregation, stimulating the release of factor VIII from vWF, and cleaving factor XIII to factor XIIIa.

Antithrombotic effects of thrombin result from its binding to an endothelial cell receptor called *thrombomodulin* (Fig. 43.6). Thrombomodulin abolishes the clotting function of thrombin and allows thrombin to activate protein C, which has anticoagulant effects.

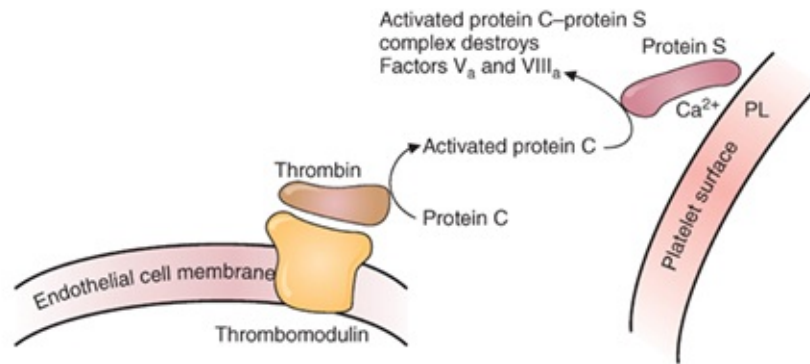


FIGURE 43.6 Antithrombotic effects of thrombin. Thrombin, bound to thrombomodulin on the endothelial cell surface, activates protein C. Activated protein C, in complex with protein S, binds to the platelet membrane, and the activated complex destroys factors V_a and VIII_a, thereby inhibiting the coagulation cascade. PL, phospholipids.

2. Proteins S and C

Protein C and its cofactor protein S serve to suppress the activity of the coagulation cascade. After activation, protein C forms a complex with protein S. Protein S anchors the activated protein C complex (APC) to the clot through Ca^{2+} / γ -carboxyglutamate binding to platelet phospholipids. The APC destroys the active blood coagulation cofactors factor VIII_a and V_a by proteolytic cleavage, decreasing the production of thrombin. The APC also stimulates endothelial cells to increase secretion of the prostaglandin I₂ (PGI₂), which reduces platelet aggregation.



Deficiency in the amount or functionality of protein C or protein S increases the risk of venous thromboembolism. Individuals who are homozygous for these mutations do not survive the neonatal period unless they are given replacement therapy.

3. Serpins

Many proteases of the blood coagulation enzyme system are serine proteases. Because uncontrolled proteolytic activity would be destructive, modulating mechanisms control and limit intravascular proteolysis. The serpins (*serine protease inhibitors*) are a group of naturally occurring inhibitory proteins that are present in the plasma at high concentration (~10% of the plasma proteins are serpins). At least eight major inhibitors have been found that share a common

mechanism of action and inhibit proteases involved in coagulation and clot dissolution (fibrinolysis). Each inhibitor possesses a reactive site that appears to be an ideal substrate for a specific serine protease and thus acts as a trap for that protease. The bound serine protease attacks a peptide bond located at a critical amino acid residue within the serpin and forms a tight enzyme–inhibitor complex.



In European populations, a point mutation in the factor V gene (factor V Leiden) causes the replacement of an Arg with a Gln in the preferred site for cleavage by activated protein C, rendering factor Va Leiden resistant to APC. Heterozygous individuals have a sixfold to eightfold increased risk of deep vein thromboses, and homozygous individuals have a 30- to 140-fold increased risk. The factor V Leiden mutation does not appear to be associated with increased risk of arterial thrombosis, such as myocardial infarction, except in young women who smoke.

Genetic studies suggest that the factor V Leiden mutation arose after the separation of the European, Asian, and African populations. The frequency of this variant indicates that it conferred some selective advantage at one time. In the developed world, inherited APC resistance is the most prevalent risk factor for familial thrombotic disease.

The activity of thrombin is controlled by the serpin antithrombin III (ATIII). Regulation of blood coagulation at the level of thrombin is critical because this enzyme affects the pathways of both coagulation and fibrinolysis (see [Section III.F](#)). One molecule of ATIII irreversibly inactivates one molecule of thrombin through reaction of an arginine residue in ATIII with the active-site serine residue of thrombin. ATIII–thrombin complex formation is markedly enhanced in the presence of heparin. Heparin is a glycosaminoglycan (see [Chapter 47](#)) found in the secretory granules of mast cells and in the loose connective tissue around small vascular beds. Heparin binds to lysyl residues on ATIII and dramatically accelerates its rate of binding to thrombin. This is because of an allosteric alteration in ATIII such that the position of the critical arginine residue of ATIII is more readily available for interaction with thrombin. The formation

of the ATIII–thrombin complex releases the heparin molecule so that it can be reused, and, therefore, the function of heparin is catalytic. Thrombin that is attached to a surface—for example, to thrombomodulin on the endothelial cell membrane—is no longer participating in clot formation and is not readily attacked by ATIII or the ATIII–heparin complex. The ATIII–heparin complex can also inactivate the serine protease factors XIIIa, XIa, IXa, and Xa but has no effect on factor VIIa or activated protein C.

E. Thromboresistance of Vascular Endothelium

Endothelial cells of blood vessels provide a selectively permeable barrier between the circulating blood and the tissues. The normal endothelial cell lining neither activates coagulation nor supports platelet adhesion; thus, it is called a *nonthrombogenic surface*. The thromboresistance of normal vascular endothelium is contributed to by several properties. Endothelial cells are highly negatively charged, a feature that may repel the negatively charged platelets. Endothelial cells synthesize PGI₂ and nitric oxide, vasodilators and powerful inhibitors of platelet aggregation. PGI₂ synthesis is stimulated by thrombin, epinephrine, and local vascular injury. Endothelial cells also synthesize two cofactors that each inhibit the action of thrombin: thrombomodulin and heparan sulfate. Heparan sulfate is a glycosaminoglycan similar to heparin that potentiates ATIII, but not as efficiently. The inactivation of thrombin is accelerated by heparan sulfate present on the endothelial cell surface. Thus, the intact endothelium has the capability of modifying thrombin action and inhibiting platelet aggregation.

F. Fibrinolysis

After successful formation of a hemostatic plug, further propagation of the clot must be prevented. This is accomplished in part by switching off blood coagulation and in part by turning on fibrinolysis. Fibrinolysis involves the degradation of fibrin in a clot by plasmin, a serine protease that is formed from its zymogen, plasminogen. Plasminogen is a circulating serum protein that has a high affinity for fibrin, promoting the incorporation of plasminogen in the developing clot. The activity of plasminogen is mediated by proteins known as

plasminogen activators. The conversion of plasminogen to plasmin by plasminogen activators can occur both in the liquid phase of the blood and at the clot surface; however, the latter process is by far more efficient. Activated protein C (APC), in addition to turning off the blood coagulation cascade, also stimulates the release of plasminogen activator from tissues (tissue plasminogen activator [tPA]) and simultaneously inactivates an inhibitor of plasminogen activator, PAI-1.

Plasminogen activator release can lead to plasmin formation in the circulation. However, the circulating plasmin is rapidly inactivated by binding with α_2 -antiplasmin, a circulating protease inhibitor. Clot-bound plasmin is not readily inactivated by α_2 -antiplasmin. Thus, plasminogen binding to fibrin facilitates its activation to plasmin, protects it from blood serpins, and localizes it on the fibrin substrate for subsequent efficient proteolytic attack. This mechanism allows for dissolution of fibrin in pathologic thrombi or oversized hemostatic plugs, and at the same time it prevents degradation of fibrinogen in the circulating blood.

Two endogenous plasminogen activators are most important; both are synthesized in a variety of cells. tPA is produced chiefly by the vascular endothelial cells, has a high binding affinity for fibrin, and plays a major role in fibrinolysis. Single-chain urokinase (uPA) is synthesized in most cells and tissues and has a moderate affinity for fibrin. Streptokinase, the bacterial exogenous plasminogen activator from β -hemolytic streptococci, is not an enzyme but an allosteric modifier of human plasminogen that allows an autocatalytic reaction such that plasmin is formed. In vivo, physical stress, hypoxia, and large numbers of low-molecular-weight organic compounds promote increased synthesis and release of tPA and uPA from tissues into the blood. The balance between release of plasminogen activators, the availability of fibrin, and inhibitors of the activators and plasmin determines regulation of the fibrinolytic response, as indicated in [Figure 43.7](#).

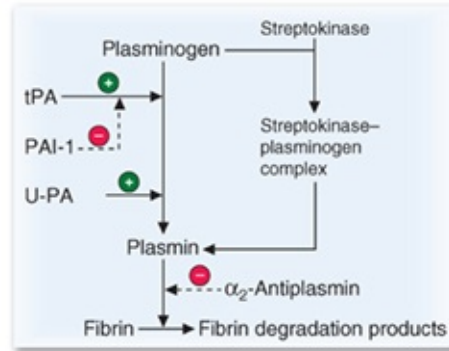


FIGURE 43.7 Regulation of plasmin activation. Plasminogen can be activated by either tissue plasminogen activator (tPA) or urokinase (uPA) (⊕). Plasminogen activator inhibitor I (PAI-I) blocks tPA action (⊖). Streptokinase binding to plasminogen allows autocatalysis to form plasmin. Circulating α_2 -antiplasmin blocks (⊖) the activity of any soluble plasmin that may be in the blood.



Both streptokinase and tPA have been approved for the treatment of myocardial infarction but have been used less frequently over the last several years. If there is cardiac catheterization available, this procedure will be used to treat a myocardial infarction. tPA can also be used to dissolve clots in an acute cerebral vascular accident (CVA; or stroke) or a massive pulmonary embolus (lung clot). Owing to the availability of newer drugs, streptokinase is rarely used at this time.

G. Regulation of Fibrinolysis

Antiactivators regulate interaction of plasminogen in blood with plasminogen activators in a dynamic equilibrium. Even if minute amounts of plasmin are generated (e.g., after stress-induced release of vascular plasminogen activator), the enzyme is probably inactivated by antiplasmin. Upon activation of the blood coagulation system, a fibrin clot is formed, which not only strongly binds tPA and plasminogen from blood but also accelerates the rate of plasmin activation. The clot-bound plasmin is protected from inhibitors while attached to fibrin. The enzyme is inactivated by α_2 -antiplasmin and α_2 -macroglobulin after proteolytic dissolution of fibrin and its liberation into the liquid phase of blood. Thus, the fibrin network catalyzes both initiation and regulation of fibrinolysis.

CLINICAL COMMENTS



Peter K. Peter K. has hemophilia A, the most frequently encountered serious disorder of blood coagulation in humans, occurring in 1 in every 10,000 males. The disease is transmitted with an X-linked pattern of inheritance.

The most common manifestations of hemophilia A are those caused by bleeding into soft tissues (hematomas) such as muscle or into body spaces like the peritoneal cavity, joint spaces, or the lumen of the gastrointestinal tract. When bleeding occurs repeatedly into joints (hemarthrosis), the joint may eventually become deformed and immobile.

In the past, bleeding episodes have been managed primarily by administration of factor VIII, sometimes referred to as *antihemophilia cofactor*. Unfortunately, the concentration of factor VIII in plasma is quite low (0.3 nM, compared with 8,800 nM for fibrinogen), requiring that it be prepared from multiple human donors. Before donor screening and virus-inactivation procedures during preparation essentially eliminated transmission with blood transfusions, >50% of patients with hemophilia who were treated with factor VIII during the 1980s in Western Europe or North America became infected with HIV and hepatitis C. Recombinant factor VIII is now available for clinical use and mitigates the threat of HIV/hepatitis C transmission in these patients.



Another X-linked bleeding disorder is hemophilia B, which is caused by mutations in the gene for factor IX. Lack of factor IX activity leads to an inability to convert prothrombin to thrombin and to impaired clotting.

BIOCHEMICAL COMMENTS



Drugs that Inhibit Blood Coagulation. Several drugs have been developed that inhibit the blood coagulation cascade. Such drugs are useful in cases in which patients develop spontaneous thrombi, which, if left untreated, would result in a fatal pulmonary embolism. There are several major classes of such drugs: the heparins, vitamin K antagonists, specific inhibitors of thrombin, and P2Y₁₂ inhibitors.

Heparin binds to and activates ATIII, which leads to thrombin inactivation.

ATIII also blocks the activity of factors VIIIa, IXa, Xa, and XIa. Heparin can be administered in either of two forms: unfractionated, or high-molecular-weight (HMW) heparin; and fractionated, or low-molecular-weight (LMW) heparin. HMW heparin is a heterogenous mixture of glycosaminoglycans that have an average chain length of 43 monosaccharides with an average molecular weight of 15 kDa (range, 3 to 30 kDa). LMW heparins are fragments of HMW heparin, containing fewer than 18 monosaccharides with an average molecular weight of 4 to 5 kDa.

HMW heparin binds to plasma proteins and cell surfaces in addition to its prime target, ATIII. Because different individuals synthesize different levels of plasma proteins, the use of this form of heparin as an anticoagulant requires constant monitoring of the patient to ensure that the correct dosage has been given so that spontaneous thrombi do not develop but not so much that spontaneous bleeding occurs. LMW heparin has fewer nonspecific interactions than HMW heparin, and its effects are easier to predict on patients, so constant monitoring is not required.

A major complication of heparin therapy is heparin-induced thrombocytopenia (HIT; excessive blood clotting with a reduction in the number of circulating platelets). This unexpected result of heparin treatment is caused by heparin binding to a platelet protein, platelet factor 4 (PF4), which induces a conformational change in PF4 such that the immune system believes the complex is foreign. Thus, antibodies are developed against the heparin–PF4 complex. When the antibodies bind to the platelets, the platelets become activated, and thrombi develop. Treatment consists of removing the heparin and using a different form of antithrombotic agent.

The classic vitamin K antagonist is warfarin. Warfarin acts by blocking the vitamin K reductase enzymes required to regenerate active vitamin K (see [Fig. 43.5](#)). This results in reduced γ -carboxylation of factors II, VII, IX, and X. In the absence of γ -carboxylation, the factors cannot bind calcium or form the complexes necessary for the coagulation cascade to be initiated. However, warfarin also blocks the activity of proteins S and C, so both blood clotting and the regulation of clotting are impaired by warfarin administration.

Both heparin and warfarin therapy suffer from their lack of specificity, so drugs specific for single steps in the blood coagulation pathway have been

sought and identified. Analysis of heparin potentiation of factor Xa binding to ATIII showed that a unique pentasaccharide sequence was required. An appropriate pentasaccharide, named *fondaparinux*, was developed that would specifically enhance ATIII interactions with factor Xa. Fondaparinux stimulates the binding of ATIII to factor Xa by 300-fold and is specific for factor Xa inhibition. Fondaparinux does not affect thrombin or platelet activity, and it is not an activating agent of platelets. Because fondaparinux does not bind to PF4, HIT is not a complication with this therapy.

Direct thrombin inhibitors are based on the hirudin molecules, which were initially discovered in leeches and other blood-sucking organisms. These organisms would not be able to feed if the blood clotted at the site of the puncture wound, so the organisms secrete thrombin inhibitors to prevent clotting from occurring. Hirudin treatment itself is dangerous in that formation of the hirudin–thrombin complex is irreversible, and use of the drug requires constant monitoring of the patient. Thus, to overcome this problem, rational drug design based on the hirudin structure was used, and a synthetic 20-amino-acid peptide known as *bivalirudin* was synthesized. This agent has a high binding affinity and specificity for thrombin, although its effects on thrombin are transient (not irreversible), making this a safer agent for long-term use. More recently, there have been multiple other direct-acting anticoagulants developed that inhibit either thrombin or factor Xa.

The P2Y₁₂ receptor inhibitors (examples of which are clopidogrel [Plavix] and ticagrelor [Brilinta]) inhibit platelet aggregation by binding tightly to the ADP receptor on the platelet surface. These drugs are used in patients to prevent strokes and heart attacks in patients who have had a previous stroke or heart attack as well as in patients with cardiovascular disease who have had stents placed to correct narrowed coronary arteries. The use of the drugs prevents platelet aggregation on the newly placed stent. The P2Y₁₂ receptor is coupled to an inhibitory G-protein. ADP is found in the platelet-dense granule, and when the platelet encounters an appropriate agent (such as thrombin), the granular contents are released and ADP binds to its receptor, which amplifies the platelet response and contributes to platelet aggregation. Blocking ADP binding to its receptor will prevent efficient platelet aggregation.

KEY CONCEPTS

- The plasma contains water, nutrients, metabolites, hormones, electrolytes, and proteins.
- Plasma proteins provide osmotic pressure to maintain fluid balance between the tissues and the blood.
- The plasma proteins provide transport for many molecules and also aid in immune defense.
- The plasma proteins, in association with platelets, maintain the integrity of the circulatory system.
 - Platelets form mechanical plugs at the site of vessel injury and secrete regulators of the blood-clotting process.
 - Platelets become activated when bound to a site of injury, which leads to more platelet binding and aggregation at the injured site.
 - Circulating fibrinogen also binds to the activated platelets at sites of injury.
- Clot formation is carefully regulated such that overclotting (thrombosis) or underclotting (bleeding) does not occur.
 - The clotting cascade consists of a series of protease activation steps leading to the activation of thrombin, which converts fibrinogen to fibrin.
 - Thrombin also activates transglutaminase, which cross-links the fibrin and leads to “hard” clot formation.
 - Proteins S and C regulate the clotting cascade and are activated by thrombin.
 - In order to form a clot, protein complexes must adhere to the activated platelets, which occurs via γ -carboxyglutamate binding to calcium and platelet membranes.
 - Serpins are serine protease inhibitors; the serpin antithrombin III (ATIII) aids in regulating blood coagulation by modulating thrombin activity.
 - Heparin enhances the interaction of thrombin with ATIII.
 - Plasmin, the active product of plasminogen, is the only protease that can dissolve fibrin clots.

- Hemophilia A is caused by a lack of factor VIII, an essential factor required for thrombin activation.
- Diseases discussed in this chapter are summarized in [Table 43.3](#).

TABLE 43.3 Diseases Discussed in Chapter 43

DISEASE OR DISORDER	ENVIRONMENTAL OR GENETIC	COMMENTS
Factor V Leiden	Genetic	Factor V is altered such that it cannot be inactivated by activated protein C, leading to hypercoagulation and deep vein thrombosis (excessive clotting).
Hemophilia B	Genetic	Lack of factor IX in the blood clotting cascade, such that clots cannot be formed
Hemophilia A	Genetic	Lack of factor VIII in the blood clotting cascade, leading to a bleeding disorder (reduced clot formation)
α_1 -Antitrypsin deficiency	Genetic	Lack of α_1 -antitrypsin leads to elastase destruction of lung tissue, progressing to emphysema and chronic obstructive pulmonary disease (COPD).
Kwashiorkor	Environmental	Protein deficiency in a calorie-sufficient diet leads to a reduction in circulating blood proteins, resulting in edema.
Thrombocytopenic purpura	Both	Formation of microscopic thrombi which lead to hemolysis and organ failure, if untreated. The idiopathic type may be caused by autoimmune destruction of platelets.
von Willebrand factor deficiency	Both	A defect in von Willebrand factor (vWF), a necessary factor for initiation of the blood clotting cascade. In its absence, clotting will not occur and bleeding disorders will result.
Bernard-Soulier syndrome	Genetic	Lack of glycoprotein Ib, the receptor for vWF. Leads to lack of function of vWF and a bleeding disorder because the coagulation cascade cannot be fully initiated.

REVIEW QUESTIONS—CHAPTER 43

1. The edema observed in patients with non-calorie-protein malnutrition is caused by which of the following?
 - A. Loss of muscle mass
 - B. Ingestion of excess carbohydrates
 - C. Increased fluid uptake
 - D. Reduced protein synthesis in the liver
 - E. Increased production of ketone bodies

2. A recent surgery patient receiving warfarin therapy was found to be bleeding internally. The clotting process is impaired in this patient primarily because of which one of the following?
 - A. Inability of the liver to synthesize clotting factors
 - B. Specific inhibition of factor XIII activation
 - C. Inability to form clotting factor complexes on membranes
 - D. Reduction of plasma calcium levels
 - E. Enhancement of protein C activity
3. An inactivating mutation in which one of the following proenzymes would be expected to lead to thrombosis (uncontrolled blood clotting)?
 - A. Factor XIII
 - B. Prothrombin
 - C. Protein C
 - D. Factor VIII
 - E. Tissue factor
4. Classic hemophilia A results in an inability to directly activate which one of the following factors?
 - A. Factor II
 - B. Factor IX
 - C. Factor X
 - D. Protein S
 - E. Protein C
5. Hemophilia B results in an inability to directly activate which one of the following factors?
 - A. Factor II
 - B. Factor IX
 - C. Factor X
 - D. Protein S
 - E. Protein C
6. An infant is born with a severe bleeding disorder. It is quickly determined via analysis of blood components that the patient has greatly decreased levels of circulating vWF. The bleeding disorder most likely results from which one of the following as a primary cause?
 - A. Inability to activate factor II

- B. Inability of platelets to bind to blood vessel walls
 - C. Inability to activate factor IX
 - D. Inability to activate protein C
 - E. Inability of platelets to bind fibrinogen
7. An individual has a family history of factor V Leiden. Factor V Leiden leads to an increased risk of deep vein thrombosis owing to which one of the following?
- A. The activated form of factor V Leiden is resistant to protein C cleavage.
 - B. Factor V Leiden does not need to be cleaved to be activated.
 - C. Factor V Leiden enhances factor XIIIa formation.
 - D. Factor V Leiden has a reduced need for phospholipids and calcium to activate other factors.
 - E. Factor V Leiden directly converts fibrinogen to fibrin.
8. An individual accidentally ingested rat poison that interfered with vitamin K action. The activity of which one of the following clotting factors is vitamin K-dependent?
- A. Factor II
 - B. Factor III
 - C. Factor V
 - D. Factor VIII
 - E. Factor XIII
9. A patient had a stroke (a cerebrovascular accident [CVA]) caused by a clot in the carotid circulation and received “clot busters” intravenously as emergency treatment. Which clot buster below is not normally found in the human body?
- A. APC
 - B. PAI-1
 - C. tPA
 - D. Streptokinase
 - E. uPA
10. A patient had a deep vein thrombosis complicated by a pulmonary embolus and was placed on an anticoagulant as prevention of future clots. He decided to adopt a healthier lifestyle and changed his diet from primarily meat and potatoes to strict vegetarian. This change in diet could affect the

efficiency of which one of his following medications?

- A. Unfractionated heparin
- B. LMW heparin
- C. Warfarin
- D. Fondaparinux
- E. Direct thrombin inhibitor

ANSWERS TO REVIEW QUESTIONS

1. **The answer is D.** Under conditions of reduced protein ingestion, essential amino acids are scarce and the liver reduces protein synthesis, including circulating plasma proteins. The reduction of protein in the plasma results in a lower osmotic pressure, so excess fluid in the extravascular spaces cannot return to the blood and remains outside of the circulation, collecting in tissues.
2. **The answer is C.** Warfarin inhibits the reduction of vitamin K epoxide, so active vitamin K levels decrease. The reduction in active vitamin K levels reduces the γ -carboxylation of clotting factors. In the absence of γ -carboxylation, the clotting factors cannot bind to calcium to form membrane-associated complexes with other clotting factors. Warfarin has no effect on the liver's ability to synthesize the clotting factor (the synthesized factor is not modified), nor does warfarin specifically inhibit the activation of factor XIII. The inhibition is more global than just attacking one step in the coagulation cascade. Plasma calcium levels are not altered by warfarin, and protein C activity is actually decreased in the presence of warfarin because protein C is one of the proteins that is γ -carboxylated in a vitamin K–dependent reaction.
3. **The answer is C.** APC turns off the clotting cascade; in the absence of protein C, regulation of clotting is impaired and clots can develop when not required. Mutations in any of the other answers listed would lead to excessive bleeding, because an essential component of the clotting cascade would be inactivated.
4. **The answer is C.** Classic hemophilia is absence of factor VIII, which is a necessary cofactor for the activation of factor X by factor IXa. Factor II

- is directly activated by factor Xa, and factor IX is directly activated by factor XIa. Proteins and S are directly activated by thrombin, factor IIa.
5. **The answer is B.** Hemophilia B is an inactivating mutation in factor IX, such that factor IXa cannot be formed. Factor XIa is formed but its substrate, factor IX, is defective, and a nonactive protein results.
 6. **The answer is B.** GPIb on the platelet membrane binds to vWF exposed on the subendothelial cell layer. In this patient, vWF levels are reduced and the initial platelet adhesion event is disturbed. Inability of platelets to adhere to the sites of injury leads to blockage of platelet aggregation, fibrinogen deposition, and clot formation. Owing to this problem, most clotting factors will not be activated, but the inability to activate these factors is not the primary cause of the disorder.
 7. **The answer is A.** Factor V Leiden still needs to be converted to factor Va to be active, but once active, it is not inactivated by APC. Continued Va activity leads to dysregulation of the clotting cascade and the potential for thrombi formation when none is required. Factor Va aids in factor II activation, not factor XIII activation, and factor V Leiden does not have an altered phospholipid or calcium requirement for complex formation. Factor V Leiden also cannot convert fibrinogen to fibrin, which is the function of thrombin (factor IIa).
 8. **The answer is A.** Factors VII, IX, X, and prothrombin (factor II) contain a domain in which glutamate residues are carboxylated to γ -carboxyglutamate in a reaction that requires vitamin K (vitamin K–dependent). The carboxylation is required to allow the factors to bind to positive charges on the forming clot surface, many times aided by the presence of calcium. Factors III, V, VIII, and XIII are not carboxylated in a vitamin K–dependent reaction.
 9. **The answer is D.** Streptokinase is produced by β -hemolytic streptococci but has the ability to activate plasminogen to plasmin. All the other proteins listed are produced in the human body. PAI-1 is an inhibitor of plasminogen activator promoting clot retention instead of “clot busting.” uPA activates plasminogen to plasmin, as does tPA. Protein C is a normal component of the body and when activated works with protein S to inhibit the clotting cascade.

10. **The answer is C.** Warfarin acts as a vitamin K antagonist. Green leafy vegetables are high in vitamin K and are a staple in vegetarian diets. The increased vitamin K in the patient's diet, and subsequently in his circulation, would greatly reduce warfarin's action and could put the patient at risk for further clots. Both types of heparin bind to and activate ATIII, which, when activated, directly inhibits thrombin. Direct thrombin inhibitors bind to thrombin directly to inhibit clotting. Fondaparinux enhances ATIII interaction with factor Xa, inhibiting Xa and blocking the clotting cascade. The mechanism of action of the heparins, fondaparinux, and direct thrombin inhibitors would not be influenced by a vegetarian diet and an increase in vitamin K levels.