Hemostasis and blood coagulation

The term hemostasis means prevention of blood loss. Whenever a vessel is severed or ruptured, hemostasis is achieved by several mechanisms:

(1) vascular constriction,

(2) formation of a platelet plug,

(3) formation of a blood clot as a result of blood coagulation, and

(4) eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently.

Vascular spasm:

Vasoconstriction is produced by vascular smooth muscle cells, and is the blood vessel's first response to injury. When a blood vessel is damaged, there is an immediate reflex, initiated by local sympathetic pain receptors, which helps promote vasoconstriction. The damaged vessels will constrict (vasoconstrict) which reduces the amount of blood flow through the area and limits the amount of blood loss. Collagen is exposed at the site of injury; the collagen promotes platelets to adhere to the injury site. Platelets release cytoplasmic granules which contain serotonin, ADP and thromboxane A2, all of which increase the effect of vasoconstriction. The spasm response becomes more effective as the amount of damage is increased. Vascular spasm is much more effective in smaller blood vessels.

Formation of the Platelet Plug

Physical and Chemical Characteristics of Platelets

Platelets (also called thrombocytes) are minute discs 1 to 4 micrometers in diameter. They are formed in the bone marrow from megakaryocytes, which are extremely large cells of the hematopoietic series in the marrow; the megakaryocytes fragment into the minute platelets either in the bone

marrow or soon after entering the blood, especially as they squeeze through capillaries.

The normal concentration of platelets in the blood is between 150,000 and 300,000 per microliter.

Platelets have many functional characteristics of whole cells, even though they do not have nuclei and cannot reproduce. In their cytoplasm are such active factors as

(1) actin and myosin molecules, which are contractile proteins similar to those found in muscle cells, and still another contractile protein, thrombasthenia, that can cause the platelets to contract;

(2) residuals of both the endoplasmic reticulum and the Golgi apparatus that synthesize various enzymes and especially store large quantities of calcium ions;

(3) mitochondria and enzyme systems that are capable of forming adenosine triphosphate (ATP) and adenosine diphosphate (ADP);

(4) enzyme systems that synthesize prostaglandins, which are local hormones that cause many vascular and other local tissue reactions;

(5) an important protein called fibrin-stabilizing factor, which we discuss later in relation to blood coagulation; and

(6) a growth factor that causes vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus causing cellular growth that eventually helps repair damaged vascular walls

The cell membrane of the platelets is also important. On its surface is a coat of glycoproteins that repulses adherence to normal endothelium and yet causes adherence to injured areas of the vessel wall, especially to injured endothelial cells and even more so to any exposed collagen from deep within the vessel wall.

Mechanism of the Platelet Plug

Platelet repair of vascular openings is based on several important functions of the platelet itself. When platelets come in contact with a damaged

vascular surface, especially with collagen fibers in the vascular wall, the platelets themselves immediately change their own characteristics drastically. They begin to swell; they assume irregular forms with numerous irradiating pseudopods protruding from their surfaces;

- Their contractile proteins contract forcefully and cause the release of granules that contain multiple active factors; they become sticky so that they **adhere to collagen in the tissues and to a protein called von Willebrand factor** that leaks into the traumatized tissue from the plasma; -they secrete large quantities of **ADP**; and their enzymes form **thromboxane A2**.

-The ADP and thromboxane in turn act on nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the original activated platelets. Therefore, at the site of any opening in a blood vessel wall, the damaged vascular wall activates successively increasing numbers of platelets that themselves attract more and more additional platelets, thus forming a platelet plug. This is at first a loose plug, but it is usually successful in blocking blood loss if the vascular opening is small. Then, during the subsequent process of blood coagulation, fibrin threads form.

These attach tightly to the platelets, thus constructing unyielding plug.

Mechanism of Blood Coagulation

Basic Theory. More than 50 important substances that cause or affect blood coagulation have been found in the blood and in the tissues—some that promote coagulation, called procoagulants, and others that inhibit coagulation, called anticoagulants. Whether blood will coagulate depends on the balance between these two groups of substances. In the blood stream, the anticoagulants normally predominate, so that the blood does not coagulate while it is circulating in the blood vessels. But when a vessel is ruptured, procoagulants from the area of tissue damage become "activated" and override the anticoagulants, and then a clot does develop.

Conversion of Prothrombin to Thrombin

First, prothrombin activator is formed as a result of rupture of a blood vessel or as a result of damage to special substances in the blood

Second, the prothrombin activator, of ionic Ca++, causes conversion of prothrombin to thrombin. **Third**, the thrombin causes polymerization of fibrinogen molecules into fibrin fibers within another 10 to 15 seconds. Thus, the rate-limiting factor in causing blood coagulation is usually the formation of prothrombin activator and not the subsequent reactions beyond that point, because these terminal steps normally occur rapidly to form the clot itself. Platelets also play an important role in the conversion of prothrombin to thrombin because much of the prothrombin first attaches to prothrombin receptors on the platelets already bound to the damaged tissue.

Conversion of Fibrinogen to Fibrin— Formation of the Clot Fibrinogen. Fibrinogen is a high-molecular-weight protein (MW = 340,000) that occurs in the plasma in quantities of 100 to 700 mg/dl. Fibrinogen is formed in the liver, and liver disease can decrease the concentration of circulating fibrinogen, as it does the concentration of prothrombin, pointed out above. Because of its large molecular size, little fibrinogen normally leaks from the blood vessels into the interstitial fluids, and because fibrinogen is one of the essential factors in the coagulation process, interstitial fluids ordinarily do not coagulate. Yet, when the permeability of the capillaries becomes pathologically increased, fibrinogen does then leak into the tissue fluids in sufficient quantities to allow clotting of these fluids in much the same way that plasma and whole blood can clot.

Extrinsic Pathway for Initiating Clotting

The extrinsic pathway for initiating the formation of prothrombin activator begins with a traumatized vascular wall or traumatized extravascular tissues that come in contact with the blood. This leads to the following steps,

1- Release of tissue factor. Traumatized tissue releases a complex of several factors called tissue factor or tissue thromboplastin. This factor is composed especially of phospholipids from the membranes of the tissue plus a lipoprotein complex that functions mainly as a proteolytic enzyme.

2. Activation of Factor X—role of Factor VII and tissue factor. The lipoprotein complex of tissue factor further complexes with blood

coagulation Factor VII and, in the presence of calcium ions, acts enzymatically on Factor X to form activated Factor X (Xa).

3. Effect of activated Factor X (Xa) to form prothrombin activator—role of Factor V. The activated Factor X combines immediately with tissue phospholipids that are part of tissue factor or with additional phospholipids released from platelets as well as with Factor V to form the complex called prothrombin activator. Within a few seconds, in the presence of calcium ions (Ca^{++}), this splits prothrombin to form thrombin, and the clotting process proceeds as already explained. At first, the Factor V in the prothrombin activator complex is inactive, but once clotting begins and thrombin begins to form, the proteolytic action of thrombin activates Factor V. This then becomes an additional strong accelerator of prothrombin activation. Thus, in the final prothrombin activator complex, activated Factor X is the actual protease that causes splitting of prothrombin to form thrombin; activated Factor V greatly accelerates this protease activity, and platelet phospholipids act as a vehicle that further accelerates the process. Note especially the positive feedback effect of thrombin, acting through Factor V, to accelerate the entire process once it begins

Intrinsic Pathway for Initiating Clotting

The second mechanism for initiating formation of prothrombin activator, and therefore for initiating clotting, begins with trauma to the blood itself or exposure of the blood to collagen from a traumatized blood vessel wall. Then the process continues through the series of cascading reactions.

1.Blood trauma causes
(1) activation of Factor XII and

(2) release of platelet phospholipids. Trauma to the blood or exposure of the blood to vascular wall collagen alters two important clotting factors in the blood: Factor XII and the platelets. When Factor XII is disturbed, such as by coming into contact with collagen or with a wettable surface such as glass, it takes on a new molecular configuration that converts it into a proteolytic enzyme called “activated Factor XII.” Simultaneously, the

blood trauma also damages the platelets because of adherence to either collagen or a wettable surface (or by damage in other ways), and this

releases platelet phospholipids that contain the lipoprotein called platelet factor 3, which also plays a role in subsequent clotting reactions.

2. Activation of Factor XI. The activated Factor XII acts enzymatically on Factor XI to activate this factor as well, which is the second step in the intrinsic pathway. This reaction also requires HMW (high-molecular-weight) kininogen and is accelerated by prekallikrein.

3. Activation of Factor IX by activated Factor XI. The activated Factor XI then acts enzymatically on Factor IX to activate this factor also.

4. Activation of Factor X—role of Factor VIII. The activated Factor IX, acting in concert with activated Factor VIII and with the platelet phospholipids and factor 3 from the traumatized platelets, activates Factor X. It is clear that when either Factor VIII or platelets are in short supply, this step is deficient. Factor VIII is the factor that is missing in a person who has classic hemophilia, for which reason it is called antihemophilic factor. Platelets are the clotting factor that is lacking in the bleeding disease called thrombocytopenia.

5. Action of activated Factor X to form prothrombin activator—role of Factor V. This step in the intrinsic pathway is the same as the last step in the extrinsic pathway. That is, activated Factor X combines with Factor V and platelet or tissue phospholipids to form the complex called prothrombin activator. The prothrombin activator in turn initiates within seconds the cleavage of prothrombin to form thrombin, thereby setting into motion the final clotting process, as described earlier.

Prevention of Blood Clotting in the Normal Vascular System—Intravascular Anticoagulants Endothelial Surface Factors.

Probably the most important factors for preventing clotting in the normal vascular system are

(1) the smoothness of the endothelial cell surface, which prevents contact activation of the intrinsic clotting system;

(2) a layer of glycocalyx on the endothelium (glycocalyx is a mucopolysaccharide adsorbed to the surfaces of the endothelial cells),

which repels clotting factors and platelets, thereby preventing activation of clotting; and

(3) a protein bound with the endothelial membrane, thrombomodulin, which binds thrombin. Not only does the binding of thrombin with thrombomodulin slow the clotting process by removing thrombin, but the thrombomodulin-thrombin complex also activates a plasma protein, protein C, that acts as an anticoagulant by inactivating activated Factors V and VIII. When the endothelial wall is damaged, its smoothness and its glycocalyx-thrombomodulin layer are lost, which activates both Factor XII and the platelets, thus setting off the intrinsic pathway of clotting. If Factor XII and platelets come in contact with the subendothelial collagen, the activation is even more powerful.

Antithrombin Action of Fibrin and Antithrombin III.

Among the most important anticoagulants in the blood itself are those that remove thrombin from the blood. The most powerful of these are

(1) the fibrin fibers that themselves are formed during the process of clotting and (2) an alpha-globulin called antithrombin III or antithrombin-heparin cofactor. While a clot is forming, about 85 to 90 per cent of the thrombin formed from the prothrombin becomes adsorbed to the fibrin fibers as they develop. This helps prevent the spread of thrombin into the remaining blood and, therefore, prevents excessive spread of the clot.

The thrombin that does not adsorb to the fibrin fibers soon combines with antithrombin III, which further blocks the effect of the thrombin on the fibrinogen and then also inactivates the thrombin itself during the next 12 to 20 minutes.

Heparin. Heparin is another powerful anticoagulant, but its concentration in the blood is normally low, so that only under special physiologic conditions does it have significant anticoagulant effects. However, heparin is used widely as a pharmacological agent in medical practice in much higher concentrations to prevent intravascular clotting.

Prevention of Blood Coagulation Outside the Body

Although blood removed from the body and held in a glass test tube normally clots in about 6 minutes, blood collected in siliconized containers often does not clot for 1 hour or more. The reason for this delay is that

preparing the surfaces of the containers with silicone prevents contact activation of platelets and Factor XII, the two principal factors that initiate the intrinsic clotting mechanism. Conversely, untreated glass containers allow contact activation of the platelets and Factor XII, with rapid development of clots. Heparin can be used for preventing coagulation of blood outside the body as well as in the body. Heparin is especially used in surgical procedures in which the blood must be passed through a heart-lung machine or artificial kidney machine and then back into the person. Various substances that decrease the concentration of calcium ions in the blood can also be used for preventing blood coagulation outside the body. For instance, a soluble oxalate compound mixed in a very small quantity with a sample of blood causes precipitation of calcium oxalate from the plasma and thereby decreases the ionic calcium level so much that blood coagulation is blocked. Any substance that deionizes the blood calcium will prevent coagulation. The negatively charged citrate ion is especially valuable for this purpose, mixed with blood usually in the form of sodium, ammonium, or potassium citrate. The citrate ion combines with calcium in the blood to cause an un-ionized calcium compound, and the lack of ionic calcium prevents coagulation. Citrate anticoagulants have an important advantage over the oxalate anticoagulants because oxalate is toxic to the body, whereas moderate quantities of citrate can be injected intravenously. After injection, the citrate ion is removed from the blood within a few minutes by the liver and is polymerized into glucose or metabolized directly for energy. Consequently, 500 milliliters of blood that has been rendered incoagulable by citrate can ordinarily be transfused into a recipient within a few minutes without dire consequences. But if the liver is damaged or if large quantities of citrated blood or plasma are given too rapidly (within fractions of a minute), the citrate ion may not be removed quickly enough, and the citrate can, under these conditions, greatly depress the level of calcium ion in the blood, which can result in tetany and convulsive death.

Lysis of Blood Clots—Plasmin The plasma proteins contain a euglobulin called plasminogen (or profibrinolysin) that, when activated, becomes a substance called plasmin (or fibrinolysin). Plasmin is a proteolytic enzyme that resembles trypsin, the most important proteolytic digestive enzyme of pancreatic secretion. Plasmin digests fibrin fibers and some other protein coagulants such as fibrinogen, Factor V, Factor VIII, prothrombin, and

Factor XII. Therefore, whenever plasmin is formed, it can cause lysis of a clot by destroying many of the clotting factors, thereby sometimes even causing hypocoagulability of the blood.

Activation of Plasminogen to Form Plasmin: Then Lysis of Clots. When a clot is formed, a large amount of plasminogen is trapped in the clot along with other plasma proteins. This will not become plasmin or cause lysis of the clot until it is activated. The injured tissues and vascular endothelium very slowly release a powerful activator called tissue plasminogen activator (t-PA) that a few days later, after the clot has stopped the bleeding, eventually converts plasminogen to plasmin, which in turn removes the remaining unnecessary blood clot. In fact, many small blood vessels in which blood flow has been blocked by clots are reopened by this mechanism. Thus, an especially important function of the plasmin system is to remove minute clots from millions of tiny peripheral vessels that eventually would become occluded were there no way to clear them,

Blood disease

1.Decreased Prothrombin, Factor VII, Factor IX, and Factor X Caused by Vitamin K Deficiency.

With few exceptions, almost all the blood-clotting factors are formed by the liver. Therefore, diseases of the liver such as hepatitis, cirrhosis, and acute yellow atrophy can sometimes depress the clotting system so greatly that the patient develops a severe tendency to bleed. Another cause of depressed formation of clotting factors by the liver is vitamin K deficiency. Vitamin K is necessary for liver formation of five of the important clotting factors: prothrombin, Factor VII, Factor IX, Factor X, and protein C. In the absence of vitamin K, subsequent insufficiency of these coagulation factors in the blood can lead to serious bleeding tendencies.

2. Hemophilia

Hemophilia is a group of sex-linked inherited blood disorders characterized by prolonged clotting time. In this disorder, males are

affected and the females are the carriers. Because of prolonged clotting time, even a mild

Types of hemophilia
Depending upon the deficiency of the factor involved, hemophilia is classified into three types:
i. Hemophilia A or classic hemophilia that is due to the deficiency of factor VIII. 85 percent of people with hemophilia are affected by hemophilia A.
ii. Hemophilia B or Christmas disease which is due to the deficiency of factor IX. 15 percent of people with hemophilia are affected by hemophilia B.
iii. Hemophilia C which is due to the deficiency of factor XI. It is a very rare blood disorder.

3. Purpura

It is a disorder characterized by prolonged bleeding time. However, the clotting time is normal. The characteristic feature of this disease is spontaneous bleeding under the skin from ruptured capillaries. It causes small tiny hemorrhagic spots under the skin which are called purpuric spots (purple colored patch like appearance). That is why this disease is called purpura.

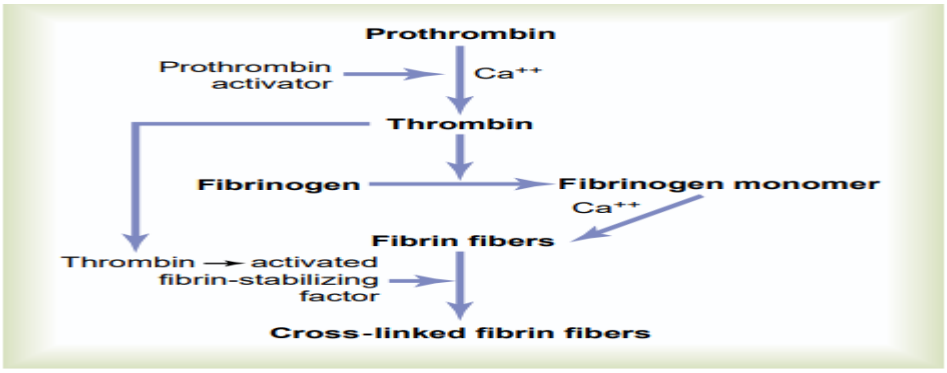
Types and causes of purpura
The purpura is classified into different types depending upon the causes.
Thrombocytopenic purpura
Thrombocytopenic purpura is due to the deficiency of platelets (thrombocytopenia). In bone marrow disease, platelet production is affected leading to deficiency of platelets.
Idiopathic thrombocytopenic purpura Purpura due to some unknown cause is called idiopathic thrombocytopenic purpura. It is believed that platelet count decreases due to the development of antibodies against platelets, which occurs after blood transfusion.

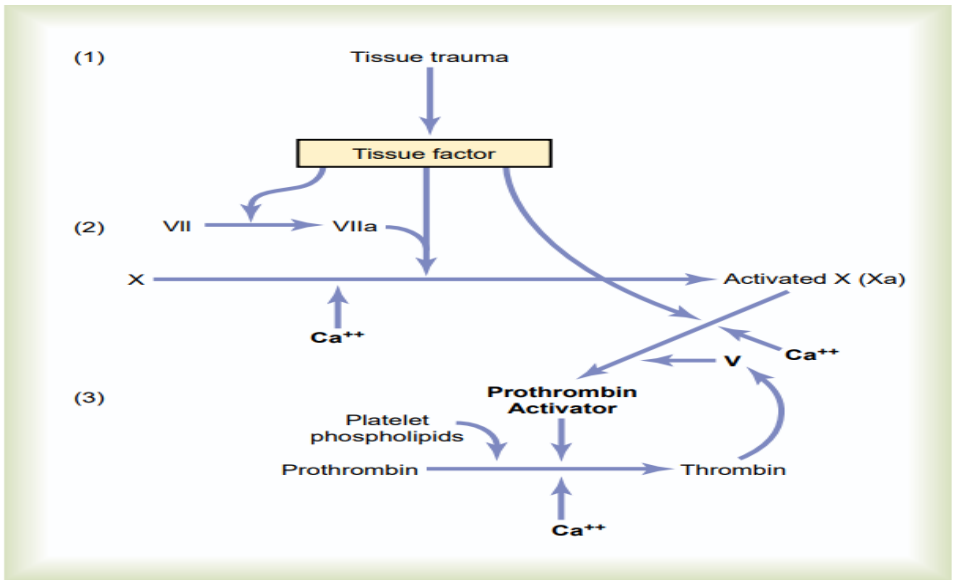
Thrombasthenia purpura
It is due to structural or functional abnormality of platelets. However, the platelet count is normal. It is characterized by normal clotting time, normal or prolonged bleeding time but defective clot retraction.

4. von Willebrand Disease

von Willebrand disease is a bleeding disorder characterized by excess bleeding even with a mild injury. It is due to inherited deficiency of von Willebrand factor which is a protein secreted by endothelium of damaged blood vessels and platelets. This protein is responsible for adherence of platelets to endothelium of blood vessels during hemostasis after an injury. It is also responsible for the survival and maintenance of factor VIII in plasma.

The deficiency of von Willebrand factor suppresses platelet adhesion. It also causes deficiency of factor VIII. This results in excess bleeding which resembles the bleeding that occurs during platelet dysfunction or hemophilia.





Clotting Factors in Blood and Their Synonyms

Clotting Factor	Synonyms
Fibrinogen	Factor I
Prothrombin	Factor II
Tissue factor	Factor III; tissue thromboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thromboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor; HMWK (high-molecular-weight) kininogen
Platelets