Basic Theory of Coagulation

More than 50 substances that cause or affect clotting of blood have been found in blood and in the tissues. Those that promote clotting are called ***procoagulants,*** and those that inhibit clotting are called ***anticoagulants.*** The balance between these two groups of substances decides whether blood will clot or not. Normally, the anticoagulants in the blood prevent blood from clotting as long as it is circulating in the undamaged blood vessels. However, when a blood vessel is ruptured, procoagulants in the damaged area become "activated" and clotting occurs—which is a homeostatic process to prevent further loss of blood.

ESSENTIAL STAGES OF BLOOD CLOTTING

The 3 essential stages of the process of blood clotting are:

Stage 1 Generation of Prothrombin Activator (PTA; prothrombinase). The PTA is a complex of Xa + Va + phospholipids + calcium ions. Its formation can begin in either or both of the following 2 pathways:

i. **The extrinsic pathway:** Injury to cells/tissues outside (extrinsic to) the blood vessels (e.g., skin, subcutaneous tissue, etc.).
ii. **The intrinsic pathway:** Injury to the blood, cells within the blood (e.g., platelets), or injury to cells in direct contact with blood (e.g., endothelial cells and underlying collagen fibers). Outside damage is not needed.

Stage (2) Formation of Thrombin from Prothrombin: The PTA, which is a proteolytic enzyme, splits prothrombin (an alpha-2 globulin present in the plasma) into the active enzyme thrombin.

Stage (3) Formation of Fibrin Threads from Fibrinogen: Thrombin acts as a proteolytic enzyme and splits off insoluble fibrin monomers from the soluble fibrinogen. The monomers polymerize to form fibrin thread, which are stabilized (cross-linked) by factor XIII (Loki-Lorand factor) and calcium ions.

Stage (1) Generation of PTA (Figure 1-19)

The Extrinsic Pathway. The extrinsic pathway (system) has fewer steps and is explosive, i.e., it occurs within 10–15 seconds of injury. It is so named because a tissue protein called **tissue factor (TF; tissue thromboplastin TPL; or factor III)** is released from the cells outside (extrinsic to) the blood vessels (e.g. cells of skin, subcutaneous tissue, etc). It is a specific phospholipid-lipoprotein complex present on the surfaces of all cells, including platelets. The TF, which is released when cell membranes are damaged or perturbed (as by a cut, prick, or crush injury) activates factor VII to VIIa (Figure 1-19). The complex of tissue factor + VIIa + calcium ions activate factor X to Xa. Factor Xa combines with factor V and calcium ions to form the active enzyme PTA (prothrombinase). This pathway is inhibited by a tissue factor pathway inhibitor that forms a quaternary structure with TPL, factor VIIa, and factor Xa.

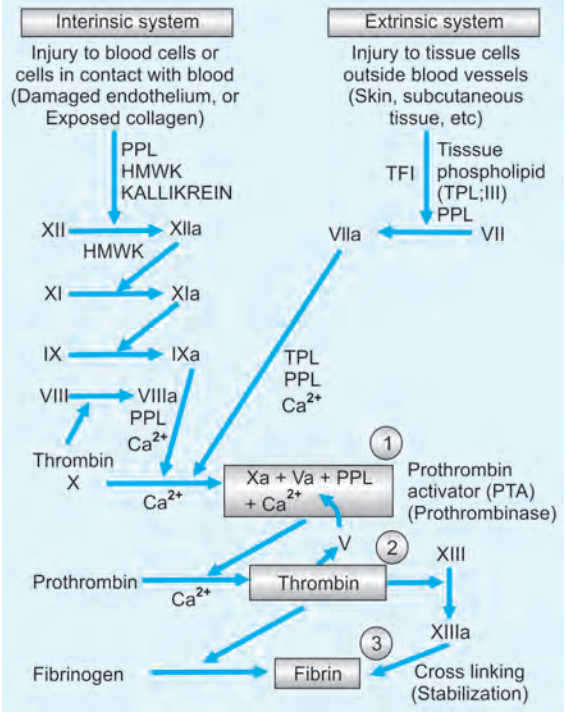

Figure 1-19: Mechanism of coagulation of blood. The intrinsic and extrinsic pathways for blood clotting are shown
Stage 1. Generation of prothrombin activator (PTA).
Stage 2. Formation of thrombin from prothrombin.
Stage 3. Formation of fibrin from fibrinogen.
HMWK: High molecular weight kininogen.
TFI: Tissue factor pathway inhibitor.
PPL: Platelet phospholipid.
(See text for details).

The Intrinsic Pathway. This pathway is more complex and occurs more slowly, usually needing several minutes. It is so named because its activators are present either within (intrinsic to) the blood (e.g., platelets), or the cells in contact with blood (endothelial cells). Injury to blood, such as by a contact with a "foreign" electronegatively charged, water-wettable surface, may occur when blood comes in contact with:

i. Roughened or damaged endothelial cells and the exposed collagen fibers under them (injury *in vivo*),
ii. The slippery glass surface of a test tube or any other water-wettable surface (injury *in vitro*).

In both cases, the damaged platelets release phospholipids (PPL), which initiates the initial reaction of conversion of factor XII to XIIa. Factor XIIa converts XI to XIa and IX to IXa (Figure 1-19). Factor IXa then forms a complex with VIIIa, PPL, and calcium ions. This complex activates factor X to Xa to form PTA.

Interaction Between Extrinsic and Intrinsic factors

It is clear from the above description of the two systems that when a blood vessel is damaged / ruptured, coagulation involves both pathways at the same time. The tissue factor starts the extrinsic system while contact of platelets and factor XII with collagen fibers in vessel walls starts the intrinsic system.

As mentioned above, the extrinsic system is explosive and with severe tissue injury, clotting can occur in 10–15 seconds. The intrinsic system requires 2–6 minutes to cause clotting.

Stages 2 and 3. The Common Pathway

Once PTA (prothrombinase) is formed, the common pathway of blood clotting follows. ***In the 2nd stage,*** PTA+ calcium converts prothrombin to thrombin. ***In the 3rd stage***, thrombin+ calcium converts fibrinogen to fibrin.

Thrombin also activates factor XIII to XIIIa. It also has 3 positive feedback effects, one on factor V, second on factor VIII, and the third on platelets, which clump together and release PPL.

Q.18 How does clotting occur in the glass capillary tube in your experiment?

The first reaction that occurs when blood comes in contact with the glass surface is injury to blood. This injury damages the platelets which release phospholipids (PPL). The PPL, along with high m.w. kininogens and kallikrein, converts factor XII to XIIa which starts the intrinsic mechanism of clotting. This pathway is supported by the extrinsic pathway in which tissue factor is released from damaged tissues at the site of skin wound.

Q.19 Name the conditions where the clotting time is increased and those where it is decreased.

The coagulation time is increased in the following conditions:

A. Hereditary Coagulation Disorders

1. **Hemophilias**—A, B, C, D (see below).
2. **von Willebrand disease.** Though usually a bleeding disease, variant forms show reduced factor VIII activity and an increase in CT. Acquired forms are caused by antibodies which inhibit vWF. The laboratory and clinical features are similar to hemophilia A.

> **Note**
> Factor VIII has 2 active components, a larger one with m.w. in millions that acts as a carrier for factor VIII and is the vWF. Its deficiency causes the bleeding disease (See Q/A 6). The smaller component has a m.w. of 230,000; loss of this smaller component causes classic hemophilia.

3. **Afibrinogenemia and dysfibrinogenemia.** The concentration of fibrinogen may be greatly reduced (normal = 250 to 300 mg%) or absent or it may be chemically abnormal, though both may be present at the same time.
4. **Deficiency of factor XIII and defective cross-linking** is a rare disorder.

B. Acquired Coagulation Disorders. These may develop in a variety of diseases as mentioned below:

1. **Vitamin K deficiency.** Deficiency of vitamin K (major sources: green vegetables, also gut bacteria) may be due to inadequate intake, intestinal malabsorption (obstructive jaundice), or loss of storage sites in liver. Since it acts a cofactor in the synthesis of prothrombin, and factors VII, IX and X, its deficiency leads to fall in their levels.
2. **Liver diseases.** There is a decrease of all clotting factors except VIII. There is

also a reduced uptake of vitamin K, and abnormalities of platelet function.

3. **Intravascular clotting.** Clotting factors are used up and bleeding may occur.
4. **Anticoagulant therapy.** Patients receiving heparin or warfarin show an increased CT.

C. Newborns. Newborns, especially premature babies sometimes have a tendency to bleed because the plasma levels of certain factors are low, especially prothrombin. Usually, these levels reach normal by the 2nd or 3rd week after birth. Vitamin K is given if bleeding persists.

The Clotting Time is decreased in:

Physiological conditions: malnutrition, parturition.

Pathological conditions: There is no pathological condition in which the CT is decreased.

Q.20 What is Hemophilia?

Hemophilia (-philia = loving) is a group of bleeding disorders that result from deficiency of factors VIII, IX, X, or XII. The 4 types have been called hemophilia A, B, C, and D. All are inherited— A and B are sex-linked, being transmitted by females (they act as carriers of the disease) to males who suffer from the disease. (Females are protected by the second X chromosome, which is usually normal.

Hemophilia A, which is also called *classical hemophilia,* is the most common hereditary coagulation disorder. The clinical features of repeated bleedings from nose, into subcutaneous tissues, joints. muscles, etc. either spontaneously or on minor injuries, start early in life, or after surgery or injuries later in life. The severity of clinical features, however, depends on the severity of factor VIII deficiency.

Hemophilia B, called Christmas disease, was discovered in 1952 in a family with the surname Christmas. Hemophilias C and D are rare.

Hemophilia has been called a royal disease because some of the children of queen Victoria of England and Czar Nicholas II of Russia had this disease. (All hemophilias are treated with fresh blood transfusions, or concentrated clotting factors).

Q.21 Why does calcium deficiency not cause a bleeding disorder though it is essential for many steps of blood coagulation?

The reason why deficiency of calcium does not cause bleeding is that only minute amounts of ionic calcium are required for clotting. A condition of bleeding due to this deficiency is not compatible with life. However, tetany may result due to lack of this mineral.

> **Note**
> Except for the first 2 stages in the intrinsic system of coagulation, calcium ions are required for the promotion of all blood clotting reactions.

Q.22 How is blood maintained in a fluid state within the body?

A balance between clotting and anticlotting mechanisms is required to prevent hemorrhage, and at the same time, to prevent intravascular clotting.

Hemofluidity within the body is maintained by the following factors:

1. **Continuous motion (circulation) of blood** does not allow clotting factors to accumulate at one point.
2. **Endothelial surface factors:**
   a. Smoothness of endothelial surface prevents contact activation of factor XII of the intrinsic pathway (damage to endothelium causes this activation).
   b. The glycocalyx layer on the endothelium repels clotting factors and platelets.
   c. Thrombomodulin secreted by these cells removes thrombin as soon as it is formed. The complex of these two activates protein C which, along with a cofactor, inactivates factors V and VIII.
   d. Prostacyclin secreted by endothelium counteracts platelet aggregation.
3. **Antithrombin action of antithrombin III-Heparin complex and fibrin.** Antithrombin III, an alpha globulin synthesized in the liver and normally present in plasma in a concentration of 15–30 mg/dl, is called the *antithrombin-heparin cofactor.* A protease inhibitor of intrinsic clotting system, it is one of the most important anticoagulants in the blood.

   Heparin, due to its low concentration in the blood, has little or no anticoagulant activity. But when it combines with antithrombin III, it increases the effectiveness of antithrombin III in removing thrombin hundreds of times. This complex also removes activated factors IX, X, XI, and XII.

The fibrin fibers that are formed when a clot is forming adsorb 85–90% of thrombin that is formed. This reduces the local concentration of thrombin, thus preventing its spread into the remaining blood and spreading of the clot.

4. **Fibrinolytic system (The Plasmin System).** Plasminogen (profibrinolysin), a plasma protein, when activated by tissue plasminogen activator (tPA) that is slowly released from injured tissues and endothelium, is changed into plasmin. Plasmin, a strong proteolytic enzyme resembling trypsin, digests and dissolves fibrin fibers, fibrinogen, prothrombin, and factors V and VIII.

   The activated protein C (APC), described above, in addition to inactivating the 2 major clotting factors (V and VIII) not blocked by antithrombin III, also enhances the activity of tPA by inhibiting the activity of a tPA inhibitor.

   Thus, once the clot has formed and succeeded in stopping blood loss, the blocked vessel is reopened by the process of fibrinolysis over the next few days, and the blood flow is restored.

Note

Human plasminogen consists of a heavy chain of 500 amino acids and a light chain of 241 amino acids. Receptors of plasminogen are present on many cells, but especially on endothelial cells. When plasminogen binds to its receptors, it gets activated into plasmin that provides a mechanism that prevents clot formation in intact vessels.

Human tPA, produced by recombinant DNA methods, is commercially available and employed for dissolving clots in coronary arteries in early treatment of myocardial infarction. Streptokinase, a bacterial enzyme and a fibrinolytic agent, is also used for the same purpose.

Q.23 What is the physiological importance of clotting of blood?

Coagulation of the blood is a homeostatic process, i.e., it maintains the integrity of the body, including its internal environment.

1. Clotting prevents further loss of blood by sealing the injured vessels.
2. The wound edges are drawn together by the fibrin threads as the clot shrinks and retracts.
3. It provides a framework for repair of the wound. Scab formation protects against loss of body fluids and drying of tissues. Finally, it forms a scar.

Q.24 What is meant by the terms thrombosis and embolism? What are the dangers associated with these conditions?

Thrombosis. Though the anticoagulating and fibrinolytic systems keep the blood in a fluid state, clotting may occur spontaneously within an unbroken vessel—a process called thrombosis (thromb- = clot; osis = a condition of). The clot thus formed is called a thrombus, (pl = thrombi). Thrombosis must be distinguished from extravascular clotting that occurs in a test tube, in wounds, or in blood vessels after death. Generally, thrombosis can begin in either of the following 2 ways:

i. **Local damage or roughness of endothelial surfaces,** e.g. atheromatous patches in arteries (e.g. coronaries, carotid, cerebral), on damaged cardiac valves, or in veins of the lower limbs. Platelets are activated and start the intrinsic system of clotting.
ii. **Slowing of blood flow (stasis)** in the pelvic and leg veins causes accumulation of clotting factors. This may happen as a complication of pregnancy, prolonged confinement to bed (fractures, surgery, severe burns), or during long flights in aeroplanes.

Embolism. The thrombus may dissolve spontaneously, or it or its fragments may get loosened and be carried away in the downstream blood. A blood clot, an air bubble, fat from broken bones, or a piece of tissue debris, transported by blood is called an embolus (em- = in; bolus = a mass). Emboli in arteries may get lodged in smaller arteries of any vital organ (e.g. brain), while emboli from the veins reach lungs and cause pulmonary embolism. Cases of thrombosis/embolism are treated with fibrinolytics and anticoagulant agents. (See Q/A 22 above).