Coagulation or clotting

The process in which blood loses its fluidity and becomes a jelly-like mass few minutes after it is shed out or collected in a container.

FACTORS INVOLVED IN BLOOD CLOTTING

Thirteen clotting factors are identified:

Factor I **Fibrinogen**
Factor II **Prothrombin**
Factor III **Thromboplastin (Tissue factor)**
Factor IV **Calcium**
Factor V **Labile factor (Proaccelerin or accelerator globulin)**
Factor VI **Presence has not been proved**
Factor VII **Stable factor**
Factor VIII (Antihemophilic globulin) (Antihemophilic globulin)
Factor IX **Christmas factor**
Factor X **Stuart-Prower factor**
Factor XI **Plasma thromboplastin antecedent**
Factor XII **Hageman factor (Contact factor)**
Factor XIII **Fibrin-stabilizing factor (Fibrinase).**

Clotting factors were named after the scientists who discovered them or as per the activity, except factor IX. Factor IX or Christmas factor was named after the patient in whom it was discovered.

SEQUENCE OF CLOTTING MECHANISM

ENZYME CASCADE THEORY

Most of the clotting factors are proteins in the form of enzymes. Normally, all the factors are present in the form of inactive proenzyme. These proenzymes must be activated into enzymes to enforce clot formation. It is carried out by a series of proenzyme-enzyme conversion reactions. First one of the series is converted into an active enzyme that activates the second one, which activates the third one; this continues till the final active enzyme thrombin is formed. Enzyme cascade theory explains how various reactions, involved in the conversion of proenzymes to active enzymes take place in the form of a cascade. Cascade refers to a process that occurs through a series of steps, each step initiating the next, until the final step is reached.

Stages of Blood Clotting :

1. Formation of prothrombin activator
2. Conversion of prothrombin into thrombin
3. Conversion of fibrinogen into fibrin.

STAGE 1: FORMATION OF PROTHROMBIN ACTIVATOR

Blood clotting commences with the formation of a substance called prothrombin activator, which converts prothrombin into thrombin. Its formation is initiated by substances produced either within the blood or outside the blood. Thus, formation of prothrombin activator occurs through two pathways:

i. **Intrinsic pathway**
ii. **ii. Extrinsic pathway**.

i. **Intrinsic Pathway for the Formation of Prothrombin Activator**

In this pathway, the formation of prothrombin activator is initiated by platelets, which are within the blood itself (Fig. 20.1).

Sequence of Events in Intrinsic pathway

i. During the injury, the blood vessel is ruptured. Endothelium is damaged and collagen beneath the endothelium is exposed.

ii. When factor XII (Hageman factor) comes in contact with collagen, it is converted into activated factor XII in the presence of kallikrein and high molecular weight (HMW) kinogen.

iii. The activated factor XII converts factor XI into activated factor XI in the presence of HMW kinogen.

iv. The activated factor XI activates factor IX in the presence of factor IV (calcium).

v. Activated factor IX activates factor X in the presence of factor VIII and calcium.

vi. When platelet comes in contact with collagen of damaged blood vessel, it gets activated and releases phospholipids.

vii. Now the activated factor X reacts with platelet phospholipid and factor V to form prothrombin activator. This needs the presence of calcium ions.

viii. Factor V is also activated by positive feedback effect of thrombin.

ii. Extrinsic Pathway for the Formation of Prothrombin Activator

In this pathway, the formation of prothrombin activator is initiated by the tissue thromboplastin, which is formed from the injured tissues.

Sequence of Events in Extrinsic Pathway

i. Tissues that are damaged during injury release tissue thromboplastin (factor III). Thromboplastin contains proteins, phospholipid and glycoprotein, which act as proteolytic enzymes.

ii. Glycoprotein and phospholipid components of thromboplastin convert factor X into activated factor X, in the presence of factor VII.

iii. Activated factor X reacts with factor V and phospholipid component of tissue thromboplastin to form prothrombin activator. This reaction requires the presence of calcium ions.

STAGE 2: CONVERSION OF PROTHROMBIN INTO THROMBIN

Blood clotting is all about thrombin formation. Once thrombin is formed, it definitely leads to clot formation.

Sequence of Events in Stage 2

i. Prothrombin activator that is formed in intrinsic and extrinsic pathways converts prothrombin into thrombin in the presence of calcium (factor IV).

ii. Once formed thrombin initiates the formation of more thrombin molecules.

The initially formed thrombin activates Factor V. Factor V in turn accelerates formation of both extrinsic and intrinsic prothrombin activator, which converts prothrombin into thrombin.

This effect of thrombin is called positive feedback effect .

STAGE 3: CONVERSION OF FIBRINOGEN INTO FIBRIN

The final stage of blood clotting involves the conversion of fibrinogen into fibrin by thrombin.

Sequence of Events in Stage 3

i. Thrombin converts inactive fibrinogen into activated fibrinogen due to loss of 2 pairs of polypeptides from each fibrinogen molecule. The activated fibrinogen is called fibrin monomer.

ii. Fibrin monomer polymerizes with other monomer molecules and form loosely arranged strands of fibrin.

iii. Later these loose strands are modified into dense and tight fibrin threads by fibrin-stabilizing factor (factor XIII) in the presence of calcium ions.

All the tight fibrin threads are aggregated to form a meshwork of stable clot.

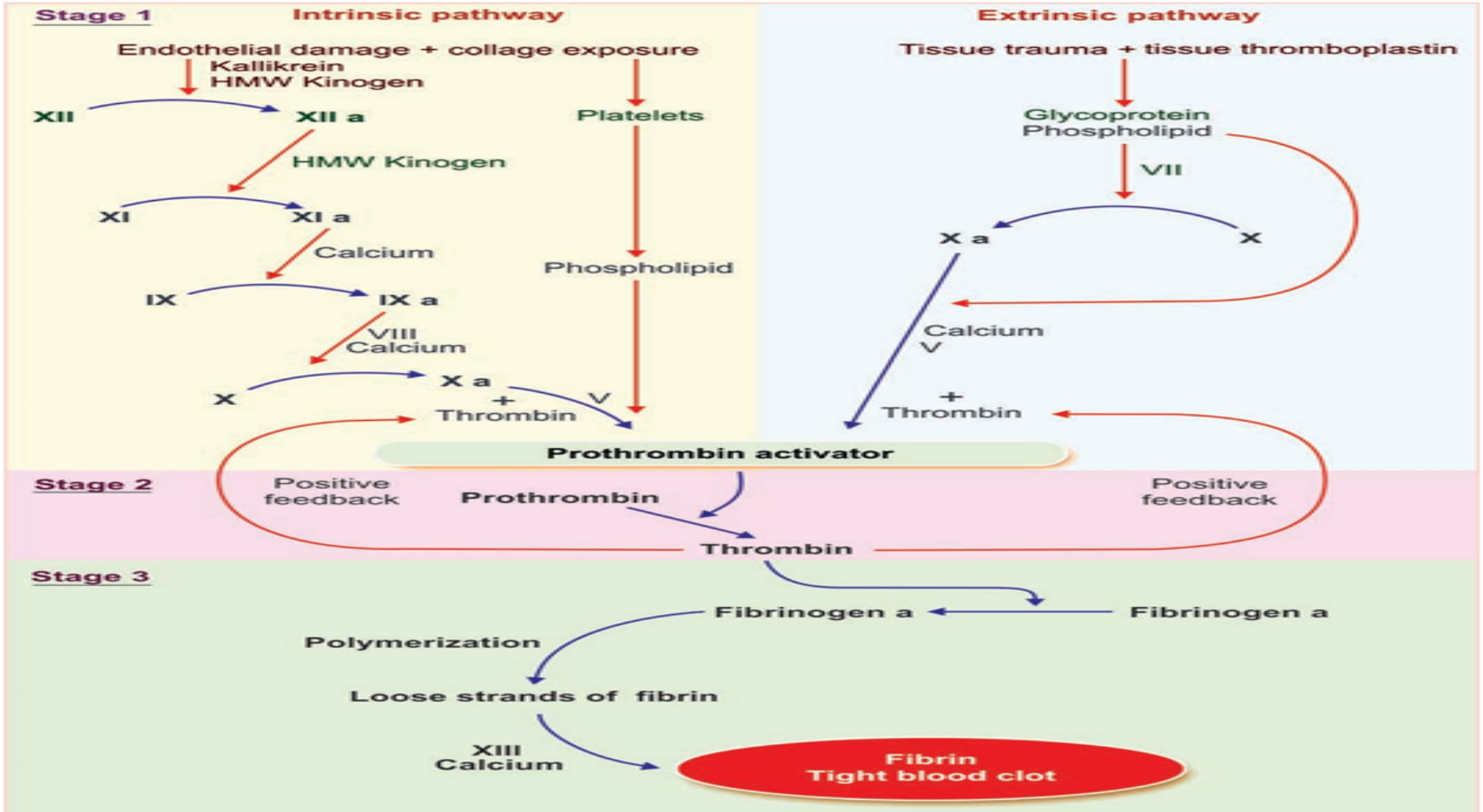

FIGURE 20.1: Stages of blood coagulation. a = Activated, + = Thrombin induces formation of more thrombin (positive feedback); HMW = High molecular weight.

BLOOD CLOT

Blood clot is defined as the mass of coagulated blood which contains RBCs, WBCs and platelets entrapped in fibrin meshwork. RBCs and WBCs are not necessary for clotting process. However, when clot is formed, these cells are trapped in it along with platelets.

The trapped RBCs are responsible for the red color of the clot. The external blood clot is also called scab. It adheres to the opening of damaged blood vessel and prevents blood loss.

CLOT RETRACTION

After the formation, the blood clot starts contracting. And after about 30 to 45 minutes, the straw-colored serum oozes out of the clot. The process involving the contraction of blood clot and oozing of serum is called clot retraction.

Contractile proteins, namely actin, myosin and thrombo-sthenin in the cytoplasm of platelets are responsible for clot retraction.

FIBRINOLYSIS

Lysis of blood clot inside the blood vessel is called fibrinolysis. It helps to remove the clot from lumen of the blood vessel. This process requires a substance called plasmin or fibrinolysin.

- Plasmin is formed from inactivated glycoprotein called plasminogen. Plasminogen is synthesized in liver and it is incorporated with other proteins in the blood clot.
- Plasminogen is converted into plasmin by **tissue plasminogen activator (t-PA), lysosomal enzymes and thrombin**.
- The **t-PA and lysosomal enzymes** are released from **damaged tissues and damaged endothelium**.
- **Thrombin** is derived from **blood**.
- The t-PA is always inhibited by a substance called t-PA inhibitor. It is also inhibited by factors V and VIII.
- Besides t-PA, there is another plasminogen activator called urokinase plasminogen activator (u-PA). It is derived from blood.

Sequence of Events Involved in the Activation of Plasminogen

1. During intravascular clotting, the endothelium of the blood vessel secretes a thrombin-binding protein, the thrombomodulin. It is secreted by the endothelium of all the blood vessels, except the minute vessels of brain.
2. Thrombomodulin combines with thrombin and forms a thrombomodulin-thrombin complex
3. Thrombomodulin-thrombin complex activates protein C
4. Activated protein C inactivates factor V and VIII in the presence of a cofactor called protein S
5. Protein C also inactivates the t-PA inhibitor
6. Now, the t-PA becomes active
7. Activated t-PA and lysosomal enzymes activate plasminogen to form plasmin.

Plasminogen is also activated by thrombin and u-PA

ANTICLOTTING MECHANISM IN THE BODY

Under physiological conditions, intravascular clotting does not occur. It is because of the presence of some physicochemical factors in the body.

1. **Physical Factors**

i. Continuous circulation of blood.

ii. Smooth endothelial lining of the blood vessels.

2. Chemical Factors – Natural Anticoagulants

i Presence of natural anticoagulant called heparin that is produced by the liver

ii. Production of thrombomodulin by endothelium of the blood vessels (except in brain capillaries).

Thrombomodulin is a thrombin-binding protein. It binds with thrombin and forms a thrombomodulin-thrombin complex. This complex activates protein C. Activated protein C along with its cofactor protein S inactivates Factor V and Factor VIII. Inactivation of these two clotting factors prevents clot formation

iii. All the clotting factors are in inactive state.

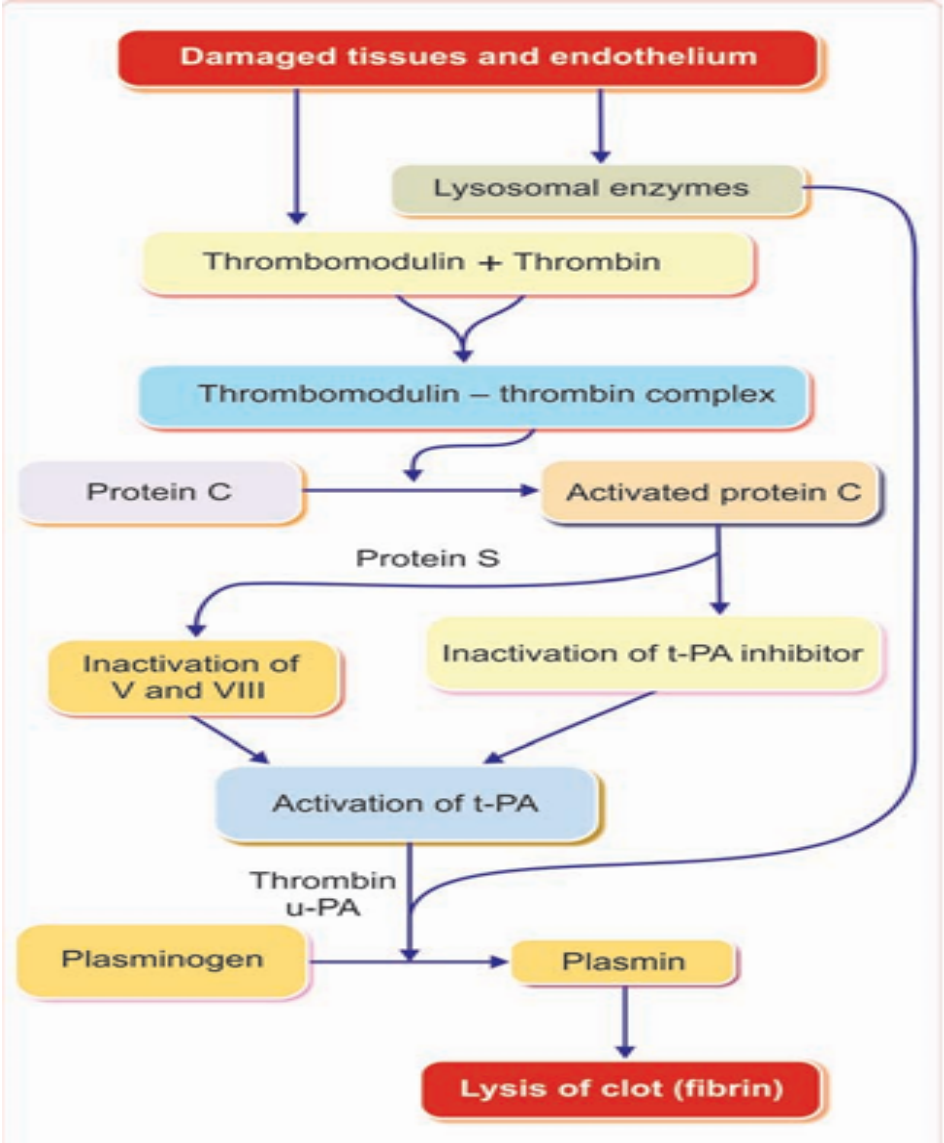

FIGURE 20.2: Fibrinolysis. t-PA = Tissue plasminogen activator, u-PA = Urokinase plasminogen activator.

ANTICOAGULANTS

Substances which prevent or postpone coagulation of blood are called anticoagulants.

Anticoagulants are of three types:

1. Anticoagulants used to prevent blood clotting inside the body, i.e. in vivo.
2. Anticoagulants used to prevent clotting of blood that is collected from the body, i.e. in vitro.
3. Anticoagulants used to prevent blood clotting both in vivo and in vitro.

1. HEPARIN

Heparin is a naturally produced anticoagulant in the body. It is produced by mast cells which are the wandering cells present immediately outside the capillaries in many tissues or organs that contain more connective tissue. These cells are abundant in liver and lungs. Basophils also secrete heparin. Heparin is a conjugated polysaccharide.

Mechanism of Action of Heparin

i Prevents blood clotting by its antithrombin activity. It directly suppresses the activity of thrombin

ii. Combines with antithrombin III (a protease inhibitor present in circulation) and removes thrombin from circulation

iii. Activates antithrombin III iv. Inactivates the active form of other clotting factors like IX, X, XI and XII (Fig. 20.3).

Heparin is used as an anticoagulant both in vivo and in vitro. Clinical use Intravenous injection of heparin (0.5 to 1 mg/kg body weight) postpones clotting for 3 to 4 hours (until it is destroyed by the enzyme heparinase). So, it is widely used as an anticoagulant in clinical practice.

In clinics, heparin is used for many purposes such as:

i. To prevent intravascular blood clotting during surgery.

ii. While passing the blood through artificial kidney for dialysis.

iii. During cardiac surgery, which involves heart lung machine.

iv. To preserve the blood before transfusion. Use in the laboratory Heparin is also used as anticoagulant in vitro while collecting blood for various investigations. About 0.1 to 0.2 mg is sufficient for 1 mL of blood. It is effective for 8 to 12 hours. After that, blood will clot because heparin only delays clotting and does not prevent it. Heparin is the most expensive anticoagulant

2. **COUMARIN DERIVATIVES**

Warfarin and dicoumoral are the derivatives of coumarin.

Mechanism of Action

Coumarin derivatives prevent blood clotting by inhibiting the action of vitamin K. Vitamin K is essential for the formation of various clotting factors, namely II, VII, IX and X Dicoumoral and warfarin are the commonly used oral anticoagulants (in vivo). Warfarin is used to prevent myocardial infarction (heart attack), strokes and thrombosis.

3. **EDTA Ethylenediaminetetraacetic acid**

(EDTA) is a strong anticoagulant. It is available in two forms: i. Disodium salt (Na2 EDTA). ii. Tripotassium salt (K3 EDTA).

Mechanism of Action

These substances prevent blood clotting by removing calcium from blood.

EDTA is used as an anticoagulant both in vivo and in vitro. It is:

i. Commonly administered intravenously, in cases of lead poisoning.

ii. Used as an anticoagulant in the laboratory (in vitro). 0.5 to 2.0 mg of EDTA per mL of blood is sufficient to preserve the blood for at least 6 hours. On refrigeration, it can preserve the blood up to 24 hours.

4**. Oxalate compounds**

Prevent coagulation by forming calcium oxalate, which is precipitated later. Thus, these compounds reduce the blood calcium level. Earlier sodium and potassium oxalates were used. Nowadays, mixture of ammonium oxalate and potassium oxalate in the ratio of 3 : 2 is used. Each salt is an anticoagulant by itself. But potassium oxalate alone causes shrinkage of RBCs. Ammonium oxalate alone causes swelling of RBCs. But together, these substances do not alter the cellular activity.

Mechanism of Action

Oxalate combines with calcium and forms insoluble calcium oxalate. Thus, oxalate removes calcium from blood and lack of calcium prevents coagulation. Uses Oxalate compounds are used only as in vitro anticoagulants. 2 mg of mixture is necessary for 1 ml of blood. Since oxalate is poisonous, it cannot be used in vivo.

5. CITRATES

Sodium, ammonium and potassium citrates are used as anticoagulants.

Mechanism of Action

Citrate combines with calcium in blood to form insoluble calcium citrate. Like oxalate, citrate also removes calcium from blood and lack of calcium prevents coagulation.

Citrate is used as in vitro anticoagulant.

i. It is used to store blood in the blood bank as:

a. Acid citrate dextrose (ACD): 1 part of ACD with 4 parts of blood

b. Citrate phosphate dextrose (CPD): 1 part of CPD with 4 parts of blood

ii. Citrate is also used in laboratory in the form of formol-citrate solution (Dacie's solution) for RBC and platelet counts.

OTHER SUBSTANCES WHICH PREVENT BLOOD CLOTTING

Peptone, C-type lectin (proteins from venom of viper snake) and hirudin (from the leach Hirudinaria manillensis) are the known anticoagulants.

PHYSICAL METHODS TO PREVENT BLOOD CLOTTING

COLD

Reducing the temperature to about 5°C postpones the coagulation of blood.

COLLECTING BLOOD IN A CONTAINER WITH SMOOTH SURFACE

Collecting the blood in a container with smooth surface like a silicon-coated container prevents clotting. The smooth surface inhibits the activation of factor XII and platelets. So, the formation of prothrombin activator is prevented.

PROCOAGULANTS

Procoagulants or hemostatic agents are the substances which accelerate the process of blood coagulation.

Procoagulants are: **THROMBIN**

Thrombin is sprayed upon the bleeding surface to arrest bleeding by hastening blood clotting.

SNAKE VENOM

Venom of some snakes (vipers, cobras and rattle snakes) contains proteolytic enzymes which enhance blood clotting by activating the clotting factors.

EXTRACTS OF LUNGS AND THYMUS

Extract obtained from the lungs and thymus has thromboplastin, which causes rapid blood coagulation.

SODIUM OR CALCIUM ALGINATE

Sodium or calcium alginate substances enhance blood clotting process by activating the Hageman factor.

OXIDIZED CELLULOSE

Oxidized cellulose causes clotting of blood by activating the Hageman factor

BLEEDING DISORDERS

Bleeding disorders are the conditions characterized by prolonged bleeding time or clotting time. Bleeding disorders are of three types: 1.Hemophilia. 2. Purpura. 3. von Willebrand disease.

1. Hemophilia

Hemophilia is a group of sex-linked inherited blood disorders, characterized by prolonged clotting time. However, the bleeding time is normal. Usually, it affects the males, with the females being the carriers. Because of prolonged clotting time, even a mild trauma causes excess bleeding which can lead to death.

Hemophilia occurs due to lack of formation of prothrombin activator. That is why the coagulation time is prolonged. The formation of prothrombin activator is affected due to the deficiency of factor VIII, IX or XI.

Types of hemophilia Depending upon the deficiency of the factor involved, hemophilia is classified into three types:

i. Hemophilia A or classic hemophilia: Due to the deficiency of factor VIII. 85% of people with hemophilia are affected by hemophilia A.

ii. Hemophilia B or Christmas disease: Due to the deficiency of factor IX. 15% of people with hemophilia are affected by hemophilia B.

iii. Hemophilia C or factor XI deficiency: Due to the deficiency of factor XI. It is a very rare bleeding disorder.

Symptoms of hemophilia

i. Spontaneous bleeding.

ii. Prolonged bleeding due to cuts, tooth extraction and surgery.

iii. Hemorrhage in gastrointestinal and urinary tracts.

iv. Bleeding in joints followed by swelling and pain

v. Appearance of blood in urine.

Treatment for hemophilia

Effective therapy for classical hemophilia involves replacement of missing clotting factor.

2. Purpura

Purpura is a disorder characterized by prolonged bleeding time. However, the clotting time is normal. Characteristic feature of this disease is spontaneous bleeding under the skin from ruptured capillaries. It causes small tiny hemorrhagic spots in many areas of the body. The hemorrhagic spots under the skin are called purpuric spots (purple colored patch like appearance). That is why this disease is called purpura. Blood also sometimes collects in large areas beneath the skin which are called ecchymoses. Purpura is classified into three types depending upon the causes:

i. **Thrombocytopenic purpura**. This is due to the deficiency of platelets (thrombocytopenia). In bone marrow disease, platelet production is affected leading to the deficiency of platelets.
ii. **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.
iii. **Thrombasthenic purpura:** This 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.

3. von Willebrand Disease

von Willebrand disease is a bleeding disorder, characterized by excess bleeding even with a mild injury. It is due to 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. 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

THROMBOSIS

Thrombosis or intravascular blood clotting refers to coagulation of blood inside the blood vessels. Normally, blood does not clot in the blood vessel because of some factors which are already explained. But some abnormal conditions cause thrombosis.

Causes of Thrombosis

1. Injury to blood vessels During infection or mechanical obstruction, the endothelial lining of the blood vessel is damaged and it initiates thrombosis.
2. Roughened endothelial lining In infection, damage or arteriosclerosis, the endothelium becomes rough and this initiates clotting.
3. Sluggishness of blood flow Decreased rate of blood flow causes aggregation of platelets and formation of thrombus. Slowness of blood flow occurs in reduced cardiac action, hypotension, low metabolic rate, prolonged confinement to bed and immobility of limbs.
4. Agglutination of RBCs Agglutination of the RBCs leads to thrombosis. Agglutination of RBCs occurs by the foreign antigens or toxic substances.
5. Toxic thrombosis is common due to the action of chemical poisons like arsenic compounds, mercury, poisonous mushrooms and snake venom.
6. Congenital absence of protein C Protein C is a circulating anticoagulant, which inactivates factors V and VIII. Thrombosis occurs in the absence of this protein. Congenital absence of protein C causes thrombosis and death in infancy.

Complications of Thrombosis

1.During thrombosis, lumen of blood vessels is occluded. The solid mass of platelets, red cells and/or clot, which obstructs the blood vessel, is called thrombus. The thrombus formed due to agglutination of RBC is called agglutinative thrombus.

2. Embolism and embolus Embolism is the process in which the thrombus or a part of it is detached and carried in bloodstream and occludes the small blood vessels, resulting in arrests of blood flow to any organ or region of the body. Embolus is the thrombus or part of it, which arrests the blood flow. The obstruction of blood flow by embolism is common in lungs (pulmonary embolism), brain (cerebral embolism) or heart (coronary embolism).

3. Ischemia Insufficient blood supply to an organ or area of the body by the obstruction of blood vessels is called ischemia. Ischemia results in tissue damage because of hypoxia (lack of oxygen). Ischemia also causes discomfort, pain and tissue death. Death of body tissue is called necrosis.

4. Necrosis and infarction Necrosis is a general term that refers to tissue death caused by loss of blood supply, injury, infection, inflammation, physical agents or chemical substances. Infarction means the tissue death due to loss of blood supply. Loss of blood supply is usually caused by occlusion of an artery by thrombus or embolus and sometimes by atherosclerosis. Area of tissue that undergoes infarction is called infarct. Infarction commonly occurs in heart, brain, lungs, kidneys and spleen

FUNCTIONS OF PLATELETS

Normally, platelets are inactive and execute their actions only when activated. Activated platelets immediately release many substances. This process is known as platelet release reaction.

Functions of platelets are:

1. **ROLE IN BLOOD CLOTTING**

Platelets are responsible for the formation of intrinsic prothrombin activator. This substance is responsible for the onset of blood clotting.

2. **ROLE IN CLOT RETRACTION**

In the blood clot, blood cells including platelets are entrapped in between the fibrin threads. Cytoplasm of platelets contains the contractile proteins, namely actin, myosin and thrombosthenin, which are responsible for clot retraction.

3. **ROLE IN PREVENTION OF BLOOD LOSS (HEMOSTASIS)**

Platelets accelerate the hemostasis by three ways: i. Platelets secrete 5-HT, which causes the cons triction of blood vessels. ii. Due to the adhesive property, the platelets seal the damage in blood vessels like capillaries. iii. By formation of temporary plug, the platelets seal the damage in blood vessels.

4. **ROLE IN REPAIR OF RUPTURED BLOOD VESSEL**

Platelet-derived growth factor (PDGF) formed in cytoplasm of platelets is useful for the repair of the endothelium and other structures of the ruptured blood vessels.

5. **ROLE IN DEFENSE MECHANISM**

By the property of agglutination, platelets encircle the foreign bodies and destroy them.