Platelets

Chapter 18

INTRODUCTION

Platelets or thrombocytes are the formed elements of blood. Platelets are small colorless, non-nucleated and moderately refractive bodies. These formed elements of blood are considered to be the fragments of cytoplasm.

Size of Platelets

Diameter : 2.5 μ (2 to 4 μ)
Volume : 7.5 cu μ (7 to 8 cu μ).

Shape of Platelets

Normally, platelets are of several shapes, viz. spherical or rod-shaped and become oval or disk-shaped when inactivated. Sometimes, the platelets have dumbbell shape, comma shape, cigar shape or any other unusual shape. Inactivated platelets are without processes or filopodia and the activated platelets develop processes or filopodia (see below).

STRUCTURE AND COMPOSITION

Platelet is constituted by:

1. Cell membrane or surface membrane
2. Microtubules
3. Cytoplasm.

CELL MEMBRANE

Cell membrane of platelet is 6 nm thick. Extensive invagination of cell membrane forms an open **canalicular system** (Fig. 18.1). This canalicular system is a delicate tunnel system through which the platelet granules extrude their contents.

Cell membrane of platelet contains lipids in the form of phospholipids, cholesterol and glycolipids, carbohydrates as glycocalyx and glycoproteins and proteins. Of these substances, glycoproteins and phospholipids are functionally important.

Glycoproteins

Glycoproteins prevent the adherence of platelets to normal endothelium, but accelerate the adherence of platelets to collagen and damaged endothelium in ruptured blood vessels. Glycoproteins also form the receptors for adenosine diphosphate (ADP) and thrombin.

Phospholipids

Phospholipids accelerate the clotting reactions. The phospholipids form the precursors of thromboxane A_2 and other prostaglandin-related substances.

MICROTUBULES

Microtubules form a ring around cytoplasm below the cell membrane. Microtubules are made up of polymerized proteins called **tubulin.** These tubules provide structural support for the inactivated platelets to maintain the disk-like shape.

CYTOPLASM

Cytoplasm of platelets contains the cellular organelles, Golgi apparatus, endoplasmic reticulum, mitochondria, microtubule, microvessels, filaments and granules.

Cytoplasm also contains some chemical substances such as proteins, enzymes, hormonal substances, etc.

Proteins

1. *Contractile proteins*
   i. Actin and myosin: Contractile proteins, which are responsible for contraction of platelets.
   ii. Thrombosthenin: Third contractile protein, which is responsible for clot retraction.
2. *von Willebrand factor:* Responsible for adherence of platelets and regulation of plasma level of factor VIII.
3. *Fibrin-stabilizing factor:* A clotting factor.
4. *Platelet-derived growth factor (PDGF):* Responsible for repair of damaged blood vessels and wound healing. It is a potent mytogen (chemical agent that promotes mitosis) for smooth muscle fibers of blood vessels.
5. *Platelet-activating factor (PAF):* Causes aggregation of platelets during the injury of blood vessels, resulting in prevention of excess loss of blood.
6. *Vitronectin (serum spreading factor):* Promotes adhesion of platelets and spreading of tissue cells in culture.
7. *Thrombospondin:* Inhibits angiogenesis (formation of new blood vessels from pre-existing vessels).

Enzymes

1. Adensosine triphosphatase (ATPase)
2. Enzymes necessary for synthesis of prostaglandins.

Hormonal Substances

1. Adrenaline
2. 5-hydroxytryptamine (5-HT; serotonin)
3. Histamine.

Other Chemical Substances

1. Glycogen
2. Substances like blood group antigens
3. Inorganic substances such as calcium, copper, magnesium and iron.

Platelet Granules

Granules present in cytoplasm of platelets are of two types:

1. Alpha granules
2. Dense granules.

Substances present in these granules are given in Table 18.1.

Alpha granules

Alpha granules contain:

1. Clotting factors – fibrinogen, V and XIII
2. Platelet-derived growth factor
3. Vascular endothelial growth factor (VEGF)
4. Basic fibroblast growth factor (FGF)
5. Endostatin
6. Thrombospondin.

Dense granules

Dense granules contain:

1. Nucleotides
2. Serotonin
3. Phospholipid
4. Calcium
5. Lysosomes.

NORMAL COUNT AND VARIATIONS

Normal platelet count is 2,50,000/cu mm of blood. It ranges between 2,00,000 and 4,00,000/cu mm of blood.

PHYSIOLOGICAL VARIATIONS

1. *Age:* Platelets are less in infants (1,50,000 to 2,00,000/cu mm) and reaches normal level at 3rd month after birth.
2. *Sex:* There is no difference in the platelet count between males and females. In females, it is reduced during menstruation.
3. *High altitude:* Platelet count increases.
4. *After meals:* After taking food, the platelet count increases.

TABLE 18.1: Substances present in platelet granules

Alpha granules	Dense granules
Clotting factors: fibrinogen, V and XIII Platelet-derived growth factor Vascular endothelial growth factor Basic fibroblast growth factor Endostatin Thrombospondin	Nucleotides Serotonin Phospholipid Calcium Lysosomes

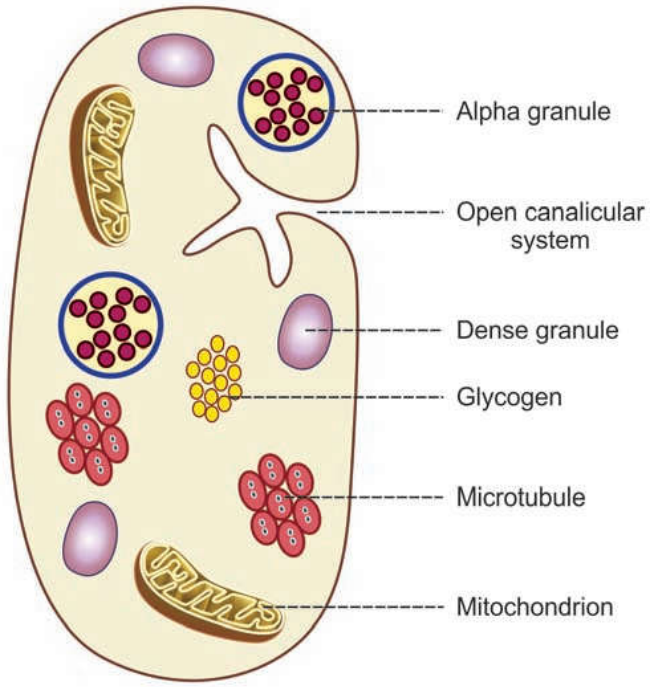

FIGURE 18.1: Platelet under electron microscope

PATHOLOGICAL VARIATIONS

Refer applied physiology of this chapter.

PROPERTIES OF PLATELETS

Platelets have three important properties (three 'A's):

1. Adhesiveness
2. Aggregation
3. Agglutination.

ADHESIVENESS

Adhesiveness is the property of sticking to a rough surface. During injury of blood vessel, endothelium is damaged and the subendothelial collagen is exposed. While coming in contact with collagen, platelets are activated and adhere to collagen. Adhesion of platelets involves interaction between **von Willebrand factor** secreted by damaged endothelium and a receptor protein called glycoprotein Ib situated on the surface of platelet membrane. Other factors which accelerate adhesiveness are collagen, thrombin, ADP, Thromboxane A_2, calcium ions, P-selectin and vitronectin.

AGGREGATION (GROUPING OF PLATELETS)

Aggregation is the grouping of platelets. Adhesion is followed by activation of more number of platelets by substances released from dense granules of platelets.

During activation, the platelets change their shape with elongation of long filamentous pseudopodia which are called processes or filopodia (Fig. 18.2).

Filopodia help the platelets aggregate together. Activation and aggregation of platelets is accelerated by ADP, thromboxane A_2 and platelet-activating factor (PTA: cytokine secreted by neutrophils and monocytes; Chapter 16).

AGGLUTINATION

Agglutination is the clumping together of platelets. Aggregated platelets are agglutinated by the actions of some platelet agglutinins and platelet-activating factor.

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 carried out by these substances.

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 (Chapter 20).

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 (Chapter 20).

3. ROLE IN PREVENTION OF BLOOD LOSS (HEMOSTASIS)

Platelets accelerate the hemostasis by three ways:

i. Platelets secrete 5-HT, which causes the constriction 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 (Chapter 19).

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.

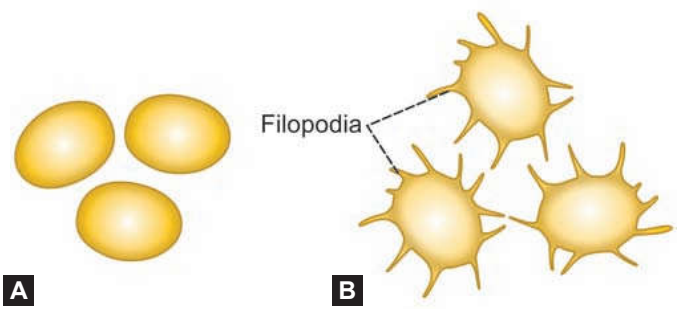

FIGURE 18.2: A. Inactive platelets. **B.** Activated platelets.

5. ROLE IN DEFENSE MECHANISM

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

ACTIVATORS AND INHIBITORS OF PLATELETS

ACTIVATORS OF PLATELETS

1. Collagen, which is exposed during damage of blood vessels
2. von Willebrand factor
3. Thromboxane A_2
4. Platelet-activating factor
5. Thrombin
6. ADP
7. Calcium ions
8. P-selectin: Cell adhesion molecule secreted from endothelial cells
9. Convulxin: Purified protein from snake venom.

INHIBITORS OF PLATELETS

1. Nitric oxide
2. Clotting factors: II, IX, X, XI and XII
3. Prostacyclin
4. Nucleotidases which breakdown the ADP.

DEVELOPMENT OF PLATELETS

Platelets are formed from bone marrow. Pluripotent stem cell gives rise to the colony forming unit-megakaryocyte (CFU-M). This develops into megakaryocyte. Cytoplasm of megakaryocyte form **pseudopodium.** A portion of pseudopodium is detached to form platelet, which enters the circulation (Fig. 10.2).

Production of platelets is influenced by colony-stimulating factors and **thrombopoietin.** Colony-stimulating factors are secreted by monocytes and T lymphocytes. Thrombopoietin is a glycoprotein like erythropoietin. It is secreted by liver and kidneys.

LIFESPAN AND FATE OF PLATELETS

Average lifespan of platelets is 10 days. It varies between 8 and 11 days. Platelets are destroyed by tissue macrophage system in spleen. So, **splenomegaly** (enlargement of spleen) decreases platelet count and **splenectomy** (removal of spleen) increases platelet count.

APPLIED PHYSIOLOGY – PLATELET DISORDERS

Platelet disorders occur because of pathological variation in platelet count and dysfunction of platelets.

Platelet disorders are:

1. Thrombocytopenia
2. Thrombocytosis
3. Thrombocythemia
4. Glanzmann's thrombasthenia.

1. *Thrombocytopenia*

Decrease in platelet count is called thrombocytopenia. It leads to thrombocytopenic purpura (Chapter 20).

Thrombocytopenia occurs in the following conditions:

i. Acute infections
ii. Acute leukemia
iii. Aplastic and pernicious anemia
iv. Chickenpox
v. Smallpox
vi. Splenomegaly
vii. Scarlet fever
viii. Typhoid
ix. Tuberculosis
x. Purpura
xi. Gaucher's disease.

2. *Thrombocytosis*

Increase in platelet count is called thrombocytosis.

Thrombocytosis occurs in the following conditions:

i. Allergic conditions
ii. Asphyxia
iii. Hemorrhage
iv. Bone fractures
v. Surgical operations
vi. Splenectomy
vii. Rheumatic fever
viii. Trauma (wound or injury or damage caused by external force).

3. *Thrombocythemia*

Thrombocythemia is the condition with persistent and abnormal increase in platelet count. Thrombocythemia occurs in the following conditions:

i. Carcinoma
ii. Chronic leukemia
iii. Hodgkin's disease.

4. *Glanzmann's Thrombasthenia*

Glanzmann's thrombasthenia is an inherited hemorrhagic disorder, caused by structural or functional abnormality of platelets. It leads to **thrombasthenic purpura** (Chapter 20). However, the platelet count is normal. It is characterized by normal clotting time, normal or prolonged bleeding time but defective clot retraction.

Hemostasis

Chapter 19

DEFINITION

Hemostasis is defined as arrest or stoppage of bleeding.

STAGES OF HEMOSTASIS

When a blood vessel is injured, the injury initiates a series of reactions, resulting in hemostasis. It occurs in three stages (Fig. 19.1):

1. Vasoconstriction
2. Platelet plug formation
3. Coagulation of blood.

VASOCONSTRICTION

Immediately after injury, the blood vessel constricts and decreases the loss of blood from damaged portion. Usually, arterioles and small arteries constrict. Vasoconstriction is purely a local phenomenon. When the blood vessels are cut, the endothelium is damaged and the collagen is exposed. Platelets adhere to this collagen and get activated. The activated platelets secrete serotonin and other vasoconstrictor substances which cause constriction of the blood vessels. Adherence of platelets to the collagen is accelerated by von Willebrand factor. This factor acts as a bridge between a specific glycoprotein present on the surface of platelet and collagen fibrils.

PLATELET PLUG FORMATION

Platelets get adhered to the collagen of ruptured blood vessel and secrete adenosine diphosphate (ADP) and thromboxane A_2. These two substances attract more and more platelets and activate them. All these platelets aggregate together and form a loose temporary platelet plug or temporary hemostatic plug, which closes the ruptured vessel and prevents further blood loss. Platelet aggregation is accelerated by platelet-activating factor (PAF).

COAGULATION OF BLOOD

During this process, the fibrinogen is converted into fibrin. Fibrin threads get attached to the loose platelet plug, which blocks the ruptured part of blood vessels and prevents further blood loss completely. Mechanism of blood coagulation is explained in the next chapter.

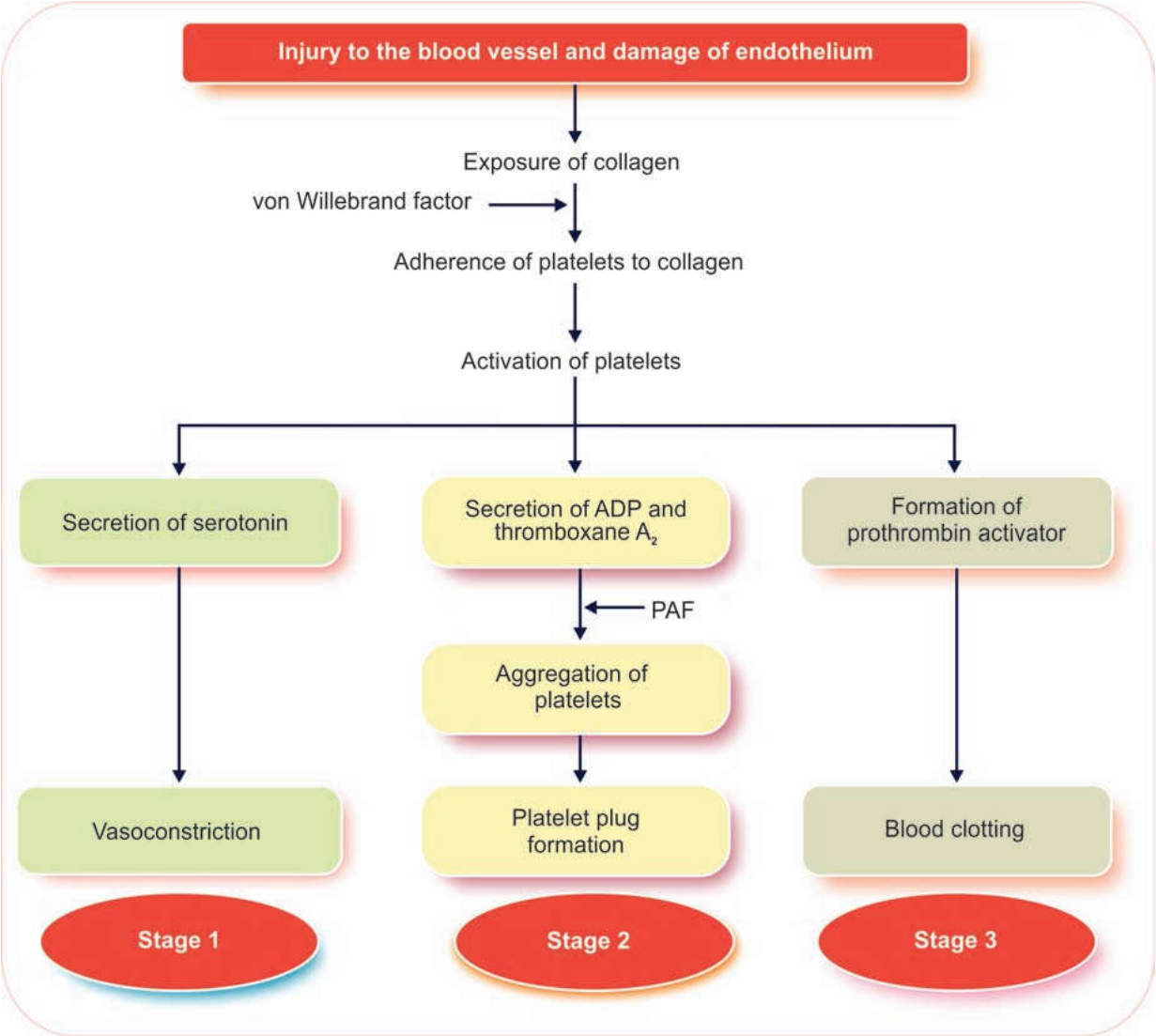

FIGURE 19.1: States of hemostasis. ADP = Adenosine diphosphate; PAF = Platelet-activating factor.

Coagulation of Blood

Chapter 20

- DEFINITION
- FACTORS INVOLVED IN BLOOD CLOTTING
- SEQUENCE OF CLOTTING MECHANISM
- BLOOD CLOT
- ANTICLOTTING MECHANISM IN THE BODY
- ANTICOAGULANTS
- PHYSICAL METHODS TO PREVENT BLOOD CLOTTING
- PROCOAGULANTS
- TESTS FOR BLOOD CLOTTING
- APPLIED PHYSIOLOGY

DEFINITION

Coagulation or clotting is defined as 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

Coagulation of blood occurs through a series of reactions due to the activation of a group of substances. Substances necessary for clotting are called clotting factors.

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 factor (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

In general, blood clotting occurs in three stages:
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. 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 (see below).

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 (Fig. 20.1).

■ 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

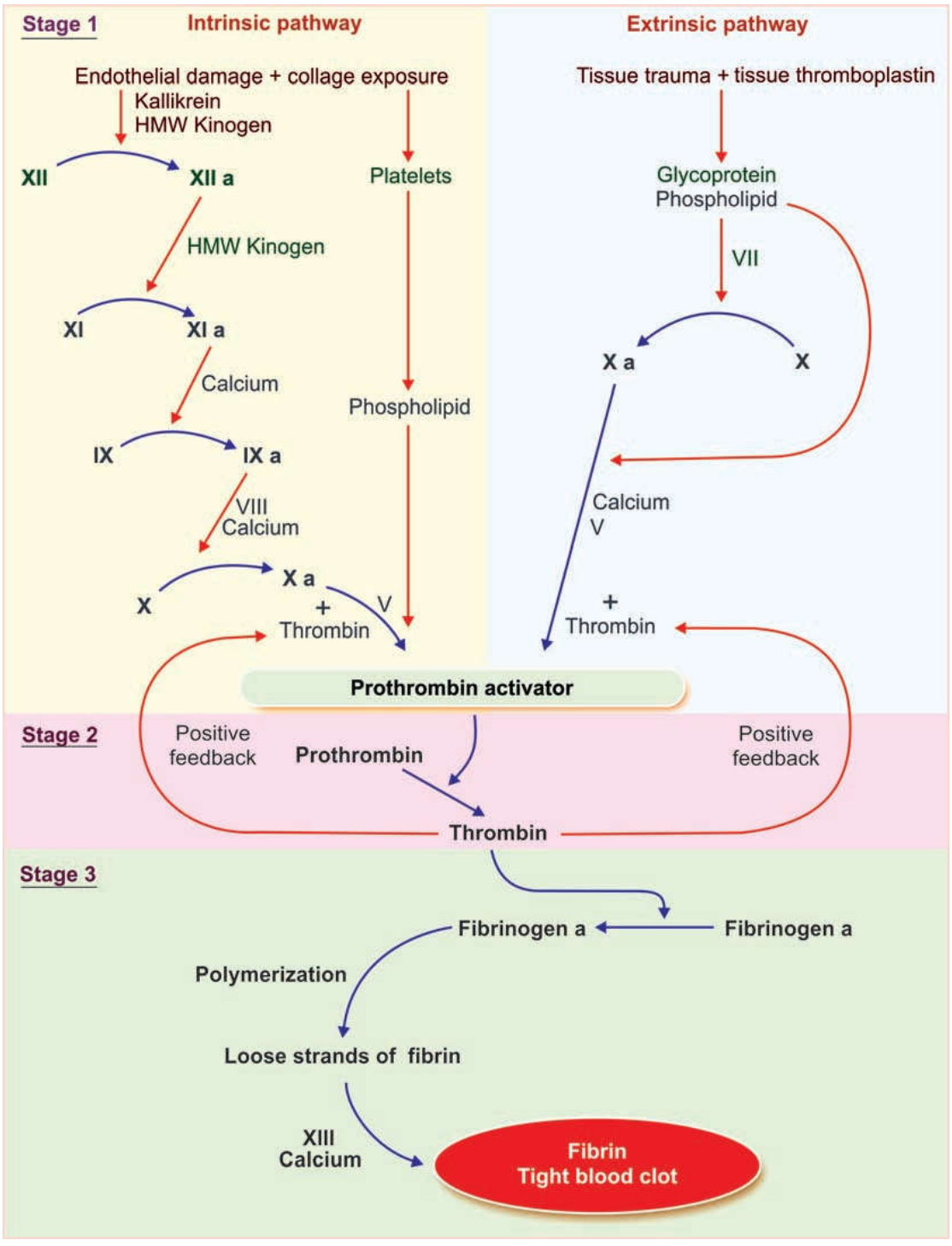

FIGURE 20.1: Stages of blood coagulation. a = Activated, + = Thrombin induces formation of more thrombin (positive feedback); HMW = High molecular weight.

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 (Fig. 20.1). All the tight fibrin threads are aggregated to form a meshwork of **stable clot.**

BLOOD CLOT

DEFINITION AND COMPOSITION OF 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 thrombosthenin 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.

Formation of Plasmin

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 (Fig. 20.2).

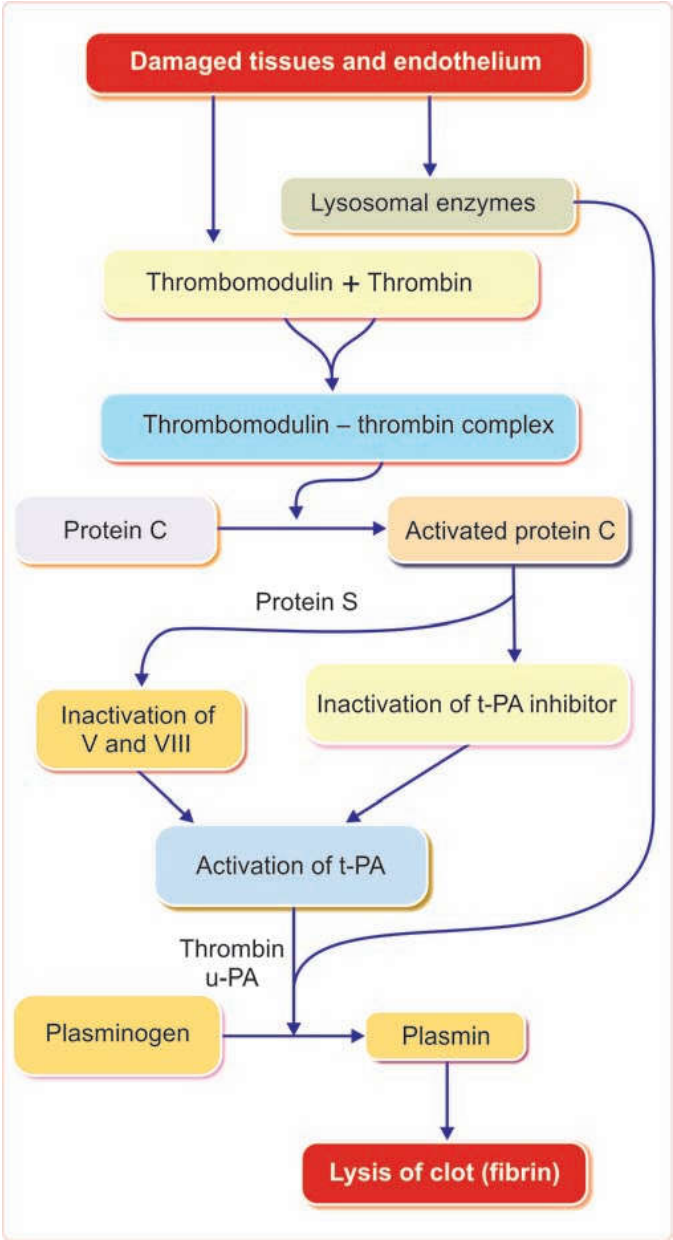

FIGURE 20.2: Fibrinolysis. t-PA = Tissue plasminogen activator, u-PA = Urokinase plasminogen activator.

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.

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. Commercial heparin is prepared from the liver and other organs of animals. Commercial preparation is available in liquid form or dry form as sodium, calcium, ammonium or lithium salts.

Mechanism of Action of Heparin

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).

Uses of Heparin

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.

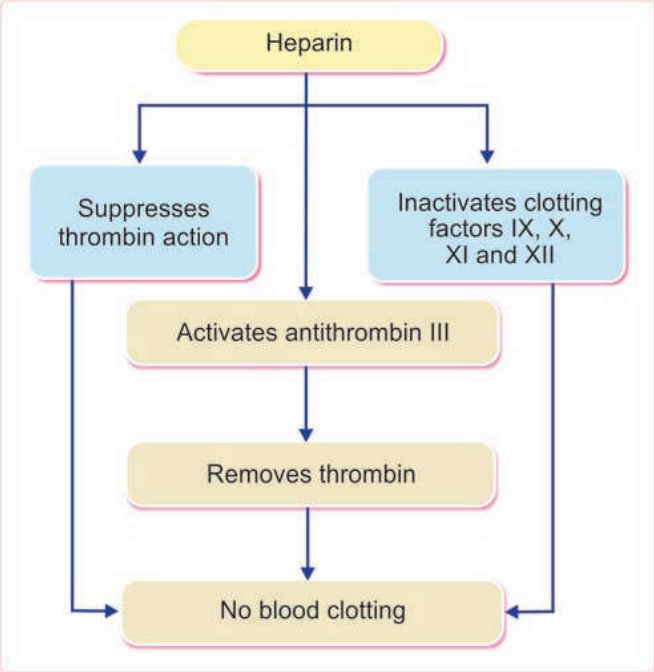

FIGURE 20.3: Mechanism of action of heparin

Uses

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 (Na_2 EDTA).
ii. Tripotassium salt (K_3 EDTA).

Mechanism of Action

These substances prevent blood clotting by removing calcium from blood.

Uses

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

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.

Uses

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

Coagulation of blood is postponed or prevented by the following physical methods:

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

Sosium 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.

TESTS FOR BLOOD CLOTTING

Blood clotting tests are used to diagnose blood disorders. Some tests are also used to monitor the patients treated with anticoagulant drugs such as heparin and warfarin.

1. Bleeding time
2. Clotting time
3. Prothrombin time
4. Partial prothrombin time
5. International normalized ratio
6. Thrombin time.

BLEEDING TIME

Bleeding time (BT) is the time interval from oozing of blood after a cut or injury till arrest of bleeding. Usually, it is determined by Duke method using blotting paper or filter paper method. Its normal duration is 3 to 6 minutes. It is prolonged in purpura.

CLOTTING TIME

Clotting time (CT) is the time interval from oozing of blood after a cut or injury till the formation of clot. It is usually determined by capillary tube method. Its normal duration is 3 to 8 minutes. It is prolonged in hemophilia.

PROTHROMBIN TIME

Prothrombin time (PT) is the time taken by blood to clot after adding tissue thromboplastin to it. Blood is collected and oxalated so that, the calcium is precipitated and prothrombin is not converted into thrombin. Thus, the blood clotting is prevented. Then a large quantity of tissue thromboplastin with calcium is added to this blood. Calcium nullifies the effect of oxalate. The tissue thromboplastin activates prothrombin and blood clotting occurs.

During this procedure, the time taken by blood to clot after adding tissue thromboplastin is determined. Prothrombin time indicates the total quantity of prothrombin present in the blood.

Normal duration of prothrombin time is 10 to 12 seconds. It is prolonged in deficiency of prothrombin and other factors like factors I, V, VII and X. However, it is normal in hemophilia.

PARTIAL PROTHROMBIN TIME OR ACTIVATED PROTHROMBIN TIME

Partial prothrombin time (PPT) is the time taken for the blood to clot after adding an activator such as phospholipid, along with calcium to it. It is also called activated partial prothrombin time (APTT). This test is useful in monitoring the patients taking anticoagulant drugs.

It is carried out by observing clotting time after adding phospholipid, a **surface activator** and calcium to a patient's plasma. Phospholipid serves as **platelet substitute.** Commonly used surface activator is **kaolin.**

Normal duration of partial prothrombin time is 30 to 45 seconds. It is prolonged in **heparin or warfarin therapy** (since heparin and warfarin inhibit clotting) and deficiency or inhibition of factors II, V, VIII, IX, X, XI and XII.

■ INTERNATIONAL NORMALIZED RATIO

International normalized ratio (INR) is the rating of a patient's prothrombin time when compared to an average. It measures extrinsic clotting pathway system.

INR is useful in monitoring impact of anticoagulant drugs such as warfarin and to adjust the dosage of anticoagulants. Patients with atrial fibrillation are usually treated with warfarin to protect against blood clot, which may cause strokes. These patients should have regular blood tests to know their INR in order to adjust warfarin dosage.

Blood takes longer time to clot if INR is higher. Normal INR is about 1. In patients taking anticoagulant therapy for atrial fibrillation, INR should be between 2 and 3. For patients with heart valve disorders, INR should be between 3 and 4. But, INR greater than 4 indicates that blood is clotting too slowly and there is a risk of uncontrolled blood clotting.

■ THROMBIN TIME

Thrombin time (TT) is the time taken for the blood to clot after adding thrombin to it. It is done to investigate the presence of heparin in plasma or to detect fibrinogen abnormalities. This test involves observation of clotting time after adding thrombin to patient's plasma. Normal duration of thrombin time is 12 to 20 seconds. It is prolonged in heparin therapy and during dysfibrinogenimia (abnormal function of fibrinogen with normal fibrinogen level).

■ APPLIED PHYSIOLOGY

■ 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. Damage of skin while falling or extraction of a tooth may cause excess bleeding for few weeks. Easy bruising and hemorrhage in muscles and joints are also common in this disease.

Causes of hemophilia

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.**

Types and causes of purpura

Purpura is classified into three types depending upon the causes:

i. *Thrombocytopenic purpura*

Thrombocytopenic purpura 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*

Thrombasthenic purpura 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*

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. *Thrombus*

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 (Chapter 67).

Area of tissue that undergoes infarction is called infarct. Infarction commonly occurs in heart, brain, lungs, kidneys and spleen.