Hemostasis

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.

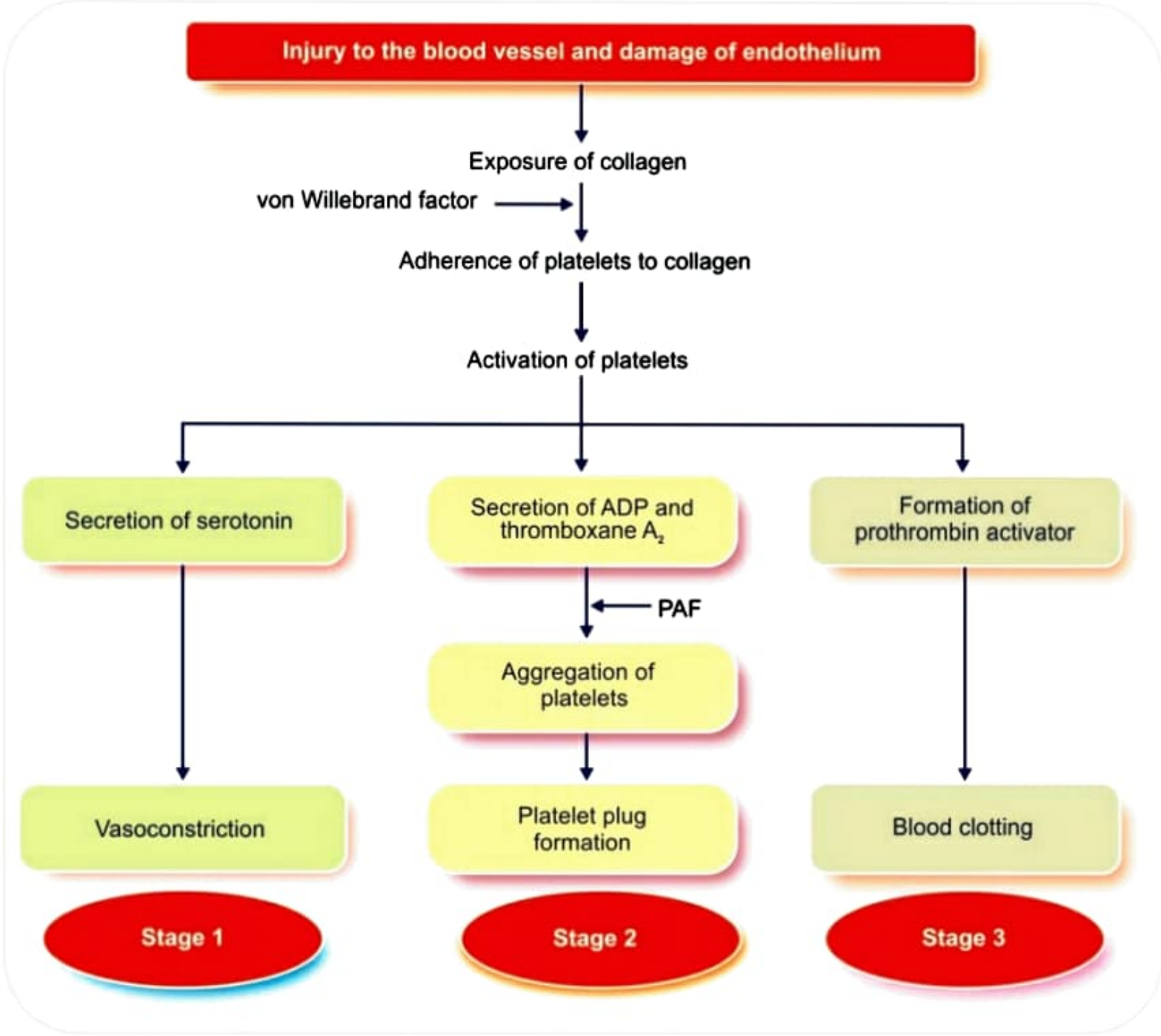

FIGURE 19.1: States of hemostasis. ADP = Adenosine diphosphate; PAF = Platelet-activating factor.

Coagulation of Blood

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

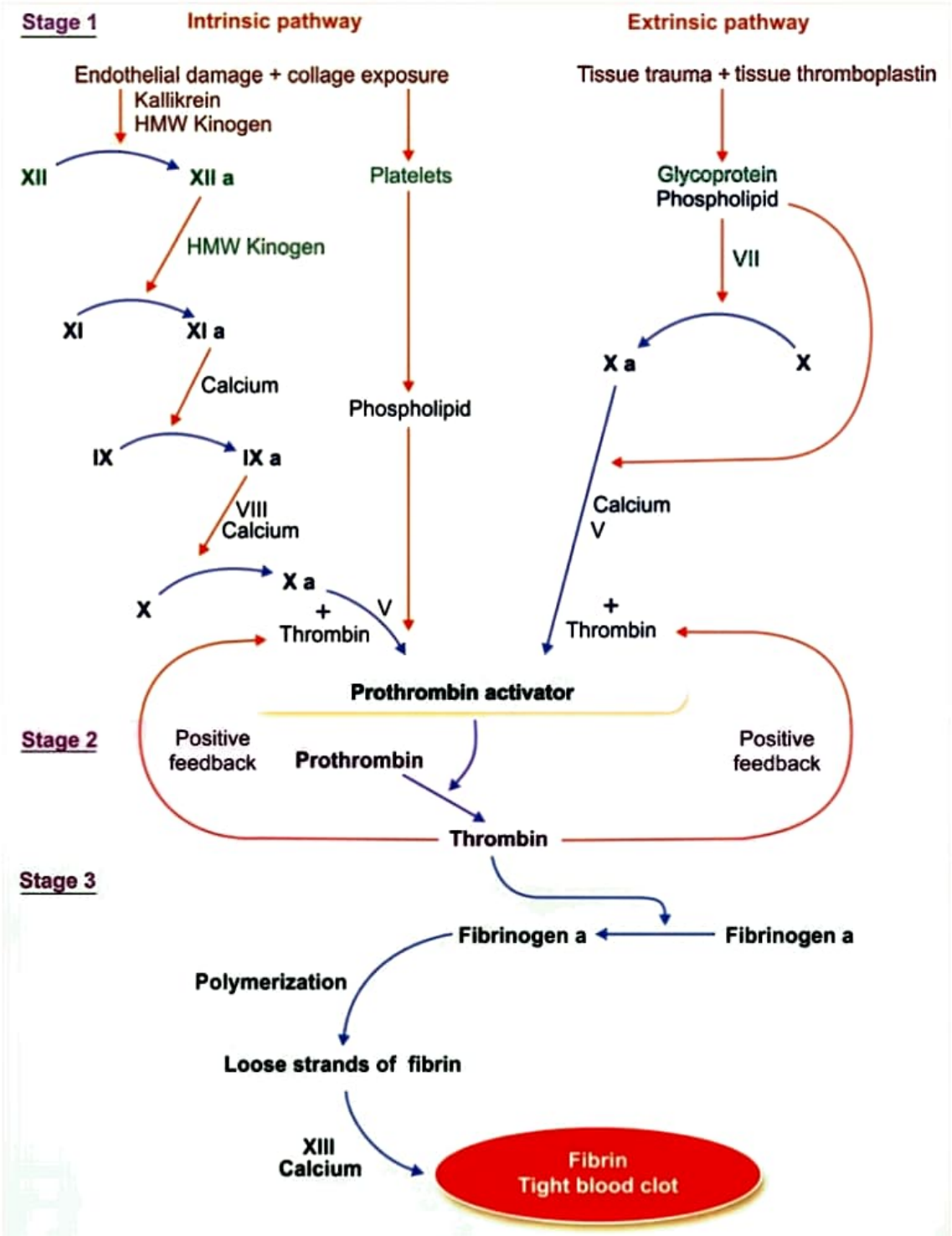

FIGURE 20.1: Stages of blood coagulation. a = Activated, + = Thrombin induces formation of more thrombin (positive feedback); HMW = High molecular weight.

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.

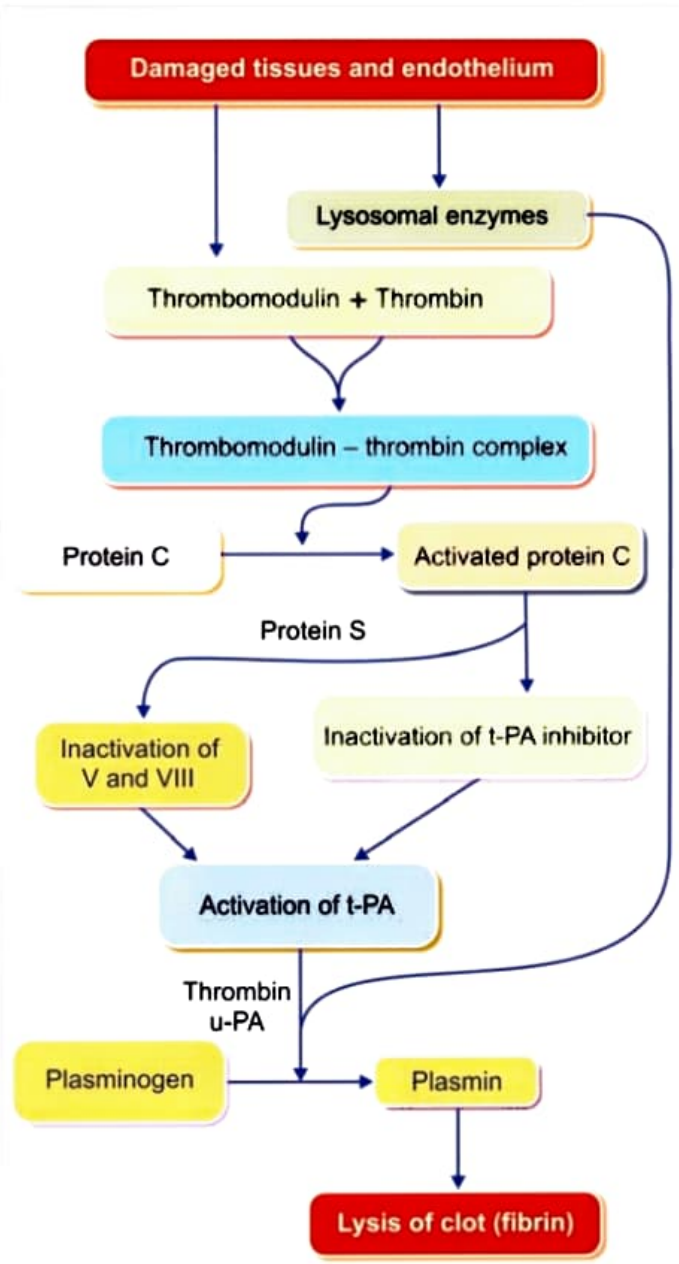

FIGURE 20.2: Fibrinolysis. t-PA = Tissue plasminogen activator, u-PA = Urokinase plasminogen activator.

■ 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

■ 2. COUMARIN DERIVATIVES

Warfarin and dicoumoral are the derivatives of coumarin.

■ 4. OXALATE COMPOUNDS

■ 5. CITRATES

■ 3. EDTA

■ 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.

■ 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.

■ APPLIED PHYSIOLOGY

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.

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.