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BIOSCIENCE – VOLUME II

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Chapter -1

Animal Physiology

Blood and Hemostasis

1. BLOOD

Blood is a highly differentiated connective tissue made up of cellular elements and an extracellular matrix. The cellular elements—referred to as the **formed elements**—include red blood cells (RBCs), white blood cells (WBCs), and cell fragments called platelets. The extracellular matrix, called plasma, makes blood unique among connective tissues because it is fluid. Our large, complex bodies need blood to deliver nutrients to and remove wastes from our trillions of cells. The heart pumps blood throughout the body in a network of blood vessels.

The Characteristics of Blood

Blood is opaque fluid due to the presence of the various cells, proteins, organic, inorganic substances. Hemoglobin in blood gives it a red appearance. In general blood is limited to the circulation, including the heart and the pulmonary and systemic blood vessels. Blood contributes around 6 to 8% of the total body weight of a healthy adult.

The blood Volume: The **blood volume** is normally 4.5 to 5.5 L in females and 5.0 to 6.0 L in men. The comparative high volume of blood in human males is due to high levels of testosterone which increases the rate of hematopoiesis.

Density and viscosity of blood: **Density (specific gravity)** of blood depends on the number of blood cells present and the composition of the plasma. Density of blood is approximately 1.050 g/mL which is more than water (0.99 g/mL). The density of individual blood cells varies according to cell type and ranges from 1.115 g/mL for erythrocytes to 1.070 g/mL for certain leukocytes. The **viscosity** of blood is determined by the resistance in the direction of flow. The blood is 3.5 to 5.5 times more viscous than water. T

Blood plasma and the serum: Plasma is the clear, yellowish-colored liquid portion of blood that remains after

red blood cells, white blood cells, platelets and other cellular components are removed through centrifugation. When blood is allowed to clot or coagulate, the suspending liquid is referred to as **serum**. Serum contains almost the same composition as plasma except blood clotting factor VIII (**Anti-hemophilic factor**)

Components of Plasma: The major component of plasma is water (93%) and various other dissolved solutes, including proteins, lipids (fats), carbohydrates, amino acids, vitamins, minerals, hormones, wastes, cofactors, gases, and electrolytes (**Table 1**). The solutes of plasma play important roles in homeostasis for example, maintaining normal plasma pH and osmolality.

Synthesis of plasma proteins: Most of the plasma proteins (albumin, fibrinogen, as well as 50-80% of the globulin) are synthesized in the liver **except gamma globulin**, which is synthesized in **lymphoid tissue**. In certain diseased conditions such as **liver cirrhosis**, production of plasma protein decreases, which results in low colloidal pressure in blood which leads to water loss from blood to the tissue and causes edema of tissue. The different proteins in plasma perform special functions shown in **table 2**.

Table 1: Composition of plasma

Class	Substance
Cations	Sodium (Na ⁺)
	Potassium (K ⁺)
	Calcium (Ca ⁺⁺)
	Magnesium (Mg ²⁺)
	Iron (Fe ³⁺)
	Copper (Cu ²⁺)
Anions	Chloride(Cl ⁻)
	Bicarbonate(HCO ₃ ⁻)
	Lactate
	Phosphate(HPO ₄ ²⁻ /H ₂ PO ₄ ⁻)
Protein	Total
Sugar	Glucose

Other	Creatinine
	Blood urea
	Alkaline phosphatase
	Acid phosphatase
	Cholesterol

Table 2: Plasma protein and their function

Protein	Function
Albumin	Maintenance of osmotic pressure Carrier for free fatty acids Maintenance of pH (buffering)
γ-Globulin	Humoral Immunity
Transthyretin (Prealbumin)	Transport of thyroid hormone and retinol
Transferrin	Iron transport
Ceruloplasmin	Copper transport
C-reactive Protein	Acute phase reactant Inflammation
Haptoglobin	Hemoglobin Transport
Hemopexin	Heme binding and transport

Functions of Blood

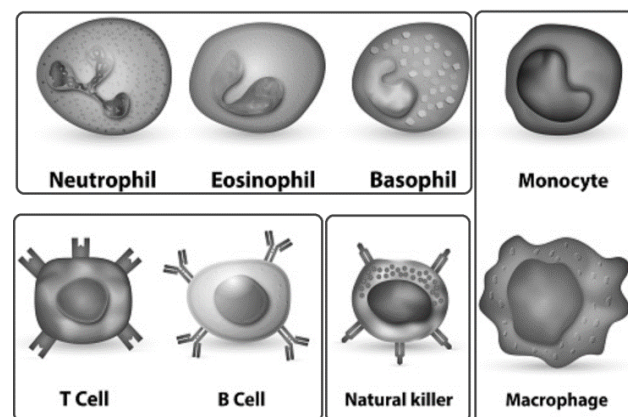
The cellular components of blood and plasma may work alone or work together to perform their functions which includes

- **Transport** of material from one part of the body to another.
- **Immunity** provided by blood is the defense for the body against disease.
- **Hemostasis**, the arrest of blood loss.
- **Homeostasis** is the mechanism by which the blood stabilized the internal environment

Types of Blood Cells

Blood contains three types of cells - **Erythrocytes (red blood cells)**, **leukocytes (white blood cells)** and **platelets (thrombocytes)**. Different types of cells in blood can be separated on the basis of density (**density gradient**) or on the basis of surface markers by fluorescence activated cell sorting (**FACS**).

Erythrocytes are the most abundant cells of blood and about 4 to 6 million cells present in each microliter (μL). While the number of leukocytes is 4,500 to 10,000 and the platelets are 150,000 to 400,000 per μL . Leukocytes can be further divided into subtypes on the basis of different functional uniqueness, capabilities and morphological differences (**Figure 2**).

**Figure 1:** Types of leukocytes in blood and tissues.

The normal circulating levels of different cell types of blood are listed in **table 3**. Polymorphonuclear neutrophils are maximum - 40 to 75% of total leukocytes. These cells actively phagocytose to the invading microorganisms and destroy them. Another polymorphonuclear cells are **eosinophils** and **basophils** that account only 1 to 6% of total leukocytes in blood and participate in allergic hypersensitivity reactions.

Table 3: Circulating blood cell levels

Blood Cell Type	Approximate Normal Range
Erythrocytes (cell/μL)	
Men	4.3-5.9 $\times 10^6$
Women	3.5-5.5 $\times 10^6$
Leukocytes (cell/μL)	4500-11000
Neutrophil	4000-7000
Lymphocytes	2500-5000
Eosinophil	100-1000
Monocytes	0-500
Basophils	0-100
Platelets (cells/μL)	150,000-400,000

Some mononuclear cells, such as **monocytes** and **lymphocytes**, are 20 to 50% of the total leukocytes. Lymphocytes are involved in production of antibodies, phagocytosis, antigen presentation, and cytokine production against invading pathogens. Under different

pathological conditions the leukocyte number and their relative proportion of subtypes can be different. For example, during infection the absolute neutrophil count frequently increases. Similarly when allergic individuals are exposed to allergens the count of eosinophil increases. In AIDS and few viral infections the lymphocyte count declines. Thus, besides a **blood cell count**, the **differential analysis** of leukocyte subtypes can give important diagnostic clues of a disease.

Erythrocytes: Erythrocytes are the most abundant cells in blood. The diameter of erythrocytes is about 7 μm with a maximum thickness of 2.5 μm . The shape of the erythrocyte is biconcave disks. It lacks a nucleus to optimize its surface area and increase the efficiency of gas exchange.

Hemoglobin is an oxygen-carrier red protein pigment, present in erythrocytes. The hemoglobin contains a **globin** protein and **heme groups** (iron-carrying portion). This complex protein with 64.5 kDa molecular weight have four polypeptide chains: two α -globin molecules each of 141 amino acids and two variable type of chain ($\beta, \gamma, \delta, \epsilon$) depending on developmental stage, each having 146 amino acid residues (**Figure 3**).

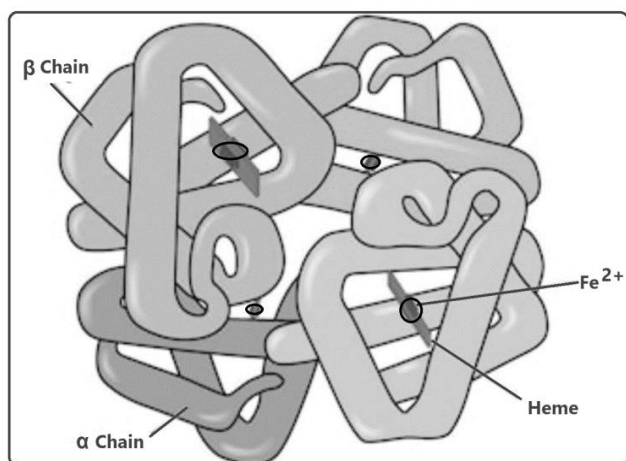


Figure 3: Each molecule of hemoglobin possesses four polypeptide chains, each containing iron bound to its heme group.

Subtypes of hemoglobin in humans: Human erythrocytes may contain four types of hemoglobin molecules on the basis of their polypeptide composition: embryonic, fetal, and two variable types found in adults. Most prevalent **adult hemoglobin**, **HbA- $\alpha_2\beta_2$** , contains two α chains and

two β chains. **HbA₂- $\alpha_2\delta_2$** , makes up about 1.5 to 3% of total hemoglobin in an adult. Earlier in the embryonic development, **embryonic hemoglobin is composed of $\alpha_2\epsilon_2$ chains**. Then the production of ϵ chains stops at about the third month of fetal development. During intrauterine life (fetal stage) the **fetal hemoglobin ($\alpha_2\gamma_2$)** is the major hemoglobin component. During infancy the level of this hemoglobin in circulating blood cells decreases rapidly and reaches a concentration of 0.5% in adults.

Oxy-hemoglobin and reduced hemoglobin: Reason for the difference in the color of blood in arteries and veins is presence of oxyhemoglobin or reduced hemoglobin. The oxygen-saturated form of hemoglobin is **Oxyhemoglobin (HbO₂)** (bright red in colour) which carries oxygen from the lungs to tissues and releases it. After releasing oxygen, HbO₂ becomes **reduced hemoglobin (Hb)**-bluish-red in colour.

A particular structural gene having five different loci controlled the production of each type of globin chain. Almost over 550 types of variant hemoglobin molecules are known due to the mutations in these five loci, majority of them with unknown clinical significance. The nature of mutations in globin genes may be a single substitution, deletion of the codons, or gene rearrangement as a result of unequal crossing over between homologous chromosomes. For example, **Sickle-cell anemia**, patient contains sickle-cell hemoglobin (HbS) which differs from normal adult hemoglobin because of the substitution of a single amino acid in each of the two β chains at 6th position (glu $\rightarrow$ val). The reason is the transversion type of mutation which alters the 6th codon from GAG codon to GUG.

Megakaryocyte and platelets: Large, multilobed promegakaryocytes of bone marrow develop into megakaryocytes. The **megakaryocytes** are the precursor cells that shed off their membranous extension in the form of a disk-like, irregular shaped structure called **Platelets**.

Megakaryocytes shed platelets in the **sinusoids** of bone marrow and then the platelets are released into the blood, where they participate in hemostasis. The release of platelets from megakaryocytes is stimulated by several factors, including the hormone **thrombopoietin**.

Thrombopoietin is released when the number of circulating platelets decreases. Platelets have amorphous

nuclei. Their size is one fourth to one third the size of erythrocytes. Platelets contain intracellular granules, in which physiologically important proteins are stored. These proteins (physiological mediators) are secreted when the platelets are activated during coagulation.

White blood cells: White blood cells (leukocytes) are also known as "**WBC's**". White blood cells are made in the bone marrow but they also divide in the blood and lymphatic systems. They are commonly amoeboid (cells that move or feed by means of temporary projections, called pseudopods (false feet), and escape the circulatory system through the capillary beds.

Granulopoiesis is the process by which white blood cells develop. The myeloid series has the most characteristic cell lineage. The different types of WBC's are Basophils, Eosinophils, Neutrophils, Monocytes, B- and T-cell lymphocytes. Neutrophils, Eosinophils, and Basophils are all **granular leukocytes** and are named on the basis of their appearance after staining with polychromatic dyes, such as **Wright's stain**.

Neutrophils: Neutrophils are the first to act when there is an infection and are also the most abundant white blood cells. Neutrophils fight bacteria and viruses by phagocytosis which means they engulf pathogens that may cause infection. The lifespan of a Neutrophil is only about 12-48 hours. They are phagocytic cells and respond instantly to microbial invasion by detecting foreign proteins or changes in host defense network proteins. Neutrophils provide an efficient defense against pathogens that have gotten past physical barriers such as the skin.

Eosinophils: Eosinophils are not common in the circulation but are easily recognized on stained blood films. Eosinophils are chemotoxic and kill parasites. They exhibit a metabolic burst when activated. Eosinophils participate in defense against certain parasites, and they are involved in allergic reactions. Infection with parasites often results in a sustained overproduction of eosinophils.

Basophils: Basophils store and synthesize **heparin (anticoagulant) and histamine (vasodilator)**. Mediators released from basophils increase local blood flow and facilitate the transport of other leukocytes at the site of infection, allergic reactivity or other forms of hypersensitivity.

Monocytes: Monocytes develop from promonocytes and lymphocytes develop from prolymphocytes. Monocytes are the biggest of the white blood cells and are responsible for rallying the cells to defend the body. Monocytes carry out phagocytosis and can leave blood vessels and mature in long lived phagocytic cells called **macrophages**.

Lymphocytes: Lymphocytes help with our immune response. There are two Lymphocytes: the B- and T- cell. B-Lymphocytes produce antibodies that find and mark pathogens for destruction. T-Lymphocytes kill anything that they deem abnormal to the body.

2. ERYTHROPOIESIS

Erythropoiesis is a complex physiological process to maintain oxygen level in the body through production of red blood cells. During erythropoiesis multipotent HSCs become committed to the red-cell lineage. It involves highly specialized functional differentiation and gene expression.

Site for Erythrocyte Production

All blood cells are derived from hematopoietic stem cells. Hematopoietic stem cells are an undifferentiated mass of cells capable of producing precursors (progenitors) of any of the blood cells. Stem cells have a tendency to maintain their number (self-renewal). The stem cell divides into two daughter cells, one remains a pluripotent stem cell (maintains undifferentiated condition) and the other becomes committed to a particular developmental pathway. The major site of erythrocyte production after birth is the soft interior of bones called bone marrow, specifically the "red" bone marrow. However, the RBC production sites change during the course of development

- Early embryonic stage : **Yolk sac**
- Middle trimester(Fetus): **Liver, spleen and lymph**
- After birth: **Red bone marrow** is the major site for production of RBC.

In humans, bone marrow of almost all bones continuously produces RBC up to the age of first five years. But, after that, the pool of hematopoietic stem cells (HSC) decreases rapidly at most bone marrow sites. Around the age of 20, bone marrow of tibia and femur stop producing erythrocytes. However, after the age of 20 years, RBCs are produced in the bone marrow of membranous bones such

as vertebrae, sternum and ribs. The cellularity of HSC in bone marrow declines with age.

Hematopoietic Growth Factor (HGF)

Some transcription factors and Hematopoietic growth factor (HGF) control the decision of formations of different types of blood cells. Committed progenitor (stem) cell proceeds into two branches -**lymphoid stem cells** which give rise to the lymphocytes, NK cells and dendritic cells and the second one is **myeloid stem cells**, the progenitors of all the other cells.

Hematopoietic growth factor (HGF) includes various proteins, hormones and paracrine agents stimulate the proliferation and differentiation of the variety of progenitor cells at several points and direct them to proceed further in a particular lineage. **Erythropoietin** hormone and other HGF are listed in **Table 4**.

The functioning of the HGFs is very complex because (1) huge variety of HGF, (2) produced by a variety of cell types throughout the body, and (3) HGFs often makes use of other actions in addition to stimulating blood-cell production.

Table 4: Reference table of Major Hematopoietic Growth Factor (HGFs)

Name	Fate of progenitor cell
Erythropoietin	Erythrocytes
Colony-stimulating factors (CSFs)	Granulocytes and (example: Granulocyte CSF), monocytes (M-CSF)
Interleukins (example: interleukin 3)	Various leukocytes
Thrombopoietin	Platelet (from Megakaryocyte)
Stem cell factor (SCF)	Many blood cell type

Regulation of Erythrocyte Production

Rate of erythrocyte production (erythropoiesis) is regulated by a hormone called **erythropoietin**.

Kidney synthesizes erythropoietin: During hypoxic conditions such as during inadequate pumping of blood by the heart, lung disease, anemia, and exposure to high altitudes results into increase in level of a transcription

factor called **hypoxia-inducible factor-1 (HIF-1)**. HIF-1 activates several genes including that of erythropoietin by binding to **hypoxia response element** present in the erythropoietin gene. This eventually leads to induction of **erythropoietin** synthesis. As the demand for oxygen supply increases, the amount of erythropoietin secretion increases due to renal hypoxia. The major stimulants for erythropoietin production are **norepinephrine**, **epinephrine** and **prostaglandins**. Increased level of erythropoietin increases the rate of erythrocytes production

Removal or destruction of both of the kidneys results in anemia as other than kidney the 10 percent of erythropoietin produced mainly in the liver is not at all sufficient to produce enough of erythrocytes. Other than the above erythropoietin stimulating agents, **testosterone**, the male sex hormone is also involved in stimulating erythropoietin secretion. Due to this **males have slightly higher hemoglobin** concentration and RBC number than females.

Differentiation of Red Blood Cells

Red blood cells are formed from precursor blast cells in the bone marrow by a process termed as **erythropoiesis**. Precursor blast cell generates hemoglobin and loses its nuclei and organelles during its differentiation into erythrocytes. However, young erythrocytes still possess few ribosome's and when stained give a web-like or reticular appearance, therefore it is also referred as reticulocytes. As the erythrocytes mature they lose the ribosomes and it is then that they leave bone marrow and enter the circulation (**Figure 5**).

If you find many reticulocytes in the blood flow, then it means that there is some unusual rapid erythrocyte production. Since erythrocytes lack nuclei or any other organelles, making them difficult to maintain their normal structure, so that the average lifespan of an RBC is approximately 120 days. It suggests that about 1% of total RBC in humans is destroyed and replaced every day.

Erythrocyte production requires: amino acids, lipids, carbohydrates, iron and certain growth factors such as folic acid and vitamin B₁₂.

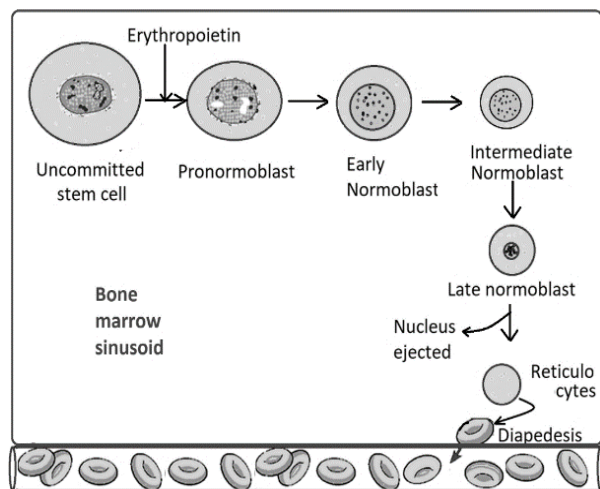


Figure 5: Erythrocyte production in healthy individuals occurs in marrow sinusoids. The committed stem cells differentiate along the erythrocyte lineage, forming normoblast (erythroblast), reticulocytes and finally mature erythrocytes, which enter the bloodstream by process of diapedesis.

Erythrocyte Destruction

Life of an erythrocyte is 120 Days. The old erythrocytes either break in the bloodstream or are engulfed by macrophages in the spleen. The site of erythrocyte destruction is **spleen** and **liver**. Hemoglobin released from lysed erythrocytes either binds to a protein present in plasma known as **haptoglobin**, or gets broken down to globin and heme. Heme gets eventually carried by another plasma protein called **hemopexin**. Both haptoglobin and hemopexin are cleared from the circulation by macrophages in the liver. In macrophages, the globin portion gets catabolized into amino acids by various proteases and heme is broken to release iron (Fe^{3+}) which gets further reduced to **biliverdin**, giving plasma its green color.

Pathological abnormalities of RBCs

Changes in red cell appearance occur in a variety of pathological conditions. Nucleated red cells are normally not seen in peripheral blood because their nuclei are lost before they move from the bone marrow into the blood. However, they appear in many blood and marrow disorders, and their presence can be of diagnostic significance.

Normoblasts are observed in peripheral blood during several types of anemias, especially when there is high

demand for new erythrocytes. If it is found in peripheral blood of seriously ill patients then it is a sign of preceding death often by several hours. **Megaloblast** is seen in peripheral blood due to folic acid deficiency or during pernicious anemia.

Anemia is a condition when blood is not efficiently transport oxygen because of following reasons –

1. Lack of total number of erythrocytes
2. Low concentration of hemoglobin
3. Both of the above two

A nucleotide substitution (mutation) in hemoglobin gene causes alteration in one of the amino acids in the hemoglobin peptide chain, leading to **sickle-cell anemia**. This causes problems generally carrying low oxygen concentration. Mutated hemoglobin molecules interact with each other, change their shape to sickle cell or any other deformed structure and form a fiber like meshwork that can block the capillaries, this results in damage of capillaries with consequent tissue damage and pain.

Microcytic hypochromic anemia: When there is excessive blood loss due to injury or any other reason, a person normally cannot absorb sufficient iron from the intestines to balance the loss. Under such conditions the size of red cells are much smaller than normal and with low amounts of hemoglobin leads to microcytic anemia.

Normocytic Anemia: Normocytic normochromic anemia is the type of anemia in which the circulating RBCs are the same size (normocytic) and have a normal red color (normochromic). Most of the normochromic, normocytic anemias are a consequence of other diseases such as chronic disease (inflammation, neoplasia), renal failure, endocrine failure (hypothyroidism, hypopituitarism), marrow failure (pure red-cell aplasia, Aplastic anemia, Infiltration), acute blood loss, and polymyalgia rheumatica. A nutritional deficiency disease that is characterized by normocytic anemia associated with deficiency of **pyridoxine** (vitamin B6), and has a material basis in decreased intake, malabsorption, increased clearance or breakdown, and certain medications (e.g. isoniazid).

Aplastic anemia: It is due to non-functioning of bone marrow. For Example, during the radiotherapy or chemotherapy for treatment of cancer stem cells of the bone marrow are damaged which leads to anemia in a few

weeks. If a high amount of erythrocyte is synthesized in the body then it leads to problems in flow of blood through blood vessels, a condition referred to as **polycythemia** (occurs in high-altitude dwellers).

3. HEMOSTASIS

Hemostasis is the physiological process that stops bleeding at the site of an injury in small vessels—arterioles, capillaries, venules while maintaining normal blood flow elsewhere in the circulation. In contrast, bleeding due to injury in a medium or large artery can't be regulated by the physiological system. There are two main components of hemostasis. **Primary hemostasis** refers to platelet aggregation and platelet plug formation. Platelets are activated in a multifaceted process (see below), and as a result they adhere to the site of injury and to each other, plugging the injury. **Secondary hemostasis** refers to the deposition of insoluble fibrin, which is generated by the proteolytic coagulation cascade. This insoluble fibrin forms a mesh that is incorporated into and around the platelet plug. This mesh serves to strengthen and stabilize the blood clot. These two processes happen simultaneously and are mechanistically intertwined. For both of these processes megakaryocytes are required.

Blood Coagulation

Blood coagulation or clotting is a slow process and proceeds after the platelet plug formation. It occurs locally to the platelet plug formed and acts as a dominant hemostatic defense. During clotting blood is transformed into a solid gel referred to as a clot or thrombus and mainly consisting of polymers of fibrin protein. Clotting supports platelet plug and solidifies the blood of the wound. Clotting occurs by a cascade of events initiated upon vessel injury or damage (**Figure 9**).

In these series of events, inactive plasma protein gets cleaved proteolytically exposing its active site and becomes activated. Upon activation these plasma proteins act as enzymes or cofactors for other enzymes and catalyze the next event or reaction of the cascade. The activated platelets display some exclusive phospholipids known as **platelet factor** (PF) that function as a cofactor in the steps assisted by bound clotting factors.

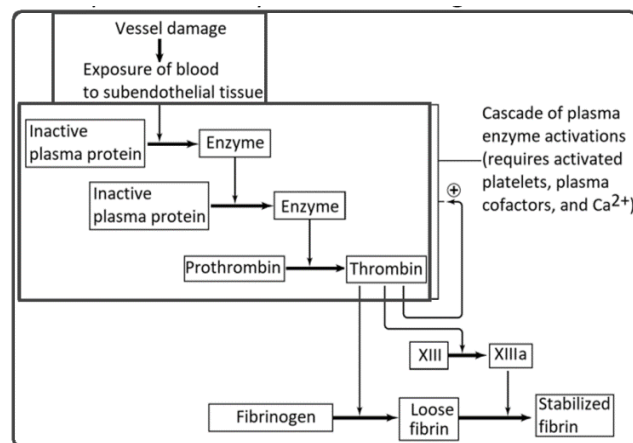


Figure 9: The clotting pathway.

Fibrinogen (Factor I) is a soluble glycoprotein produced by the liver and present abundantly in plasma. It is composed of two trimers $(\alpha_A\beta\gamma)_2$, with each trimer composed of three different polypeptide chains namely α_A , β , and γ . Two $\alpha_A\beta\gamma$ trimers joined together by 29 inter- and intra-chain disulfide bonds. The $(\alpha_A\beta\gamma)_2$ is transferred to the Golgi where it is glycosylated, hydroxylated, sulfated, and phosphorylated to form the mature fibrinogen glycoprotein that is secreted into the blood.

Thrombin (Factor II) is the main serine protease enzyme of the coagulation pathway with multiple key activities. However, the primary function of thrombin is to cleave fibrinopeptides (FP) A and B from the α and β chains of the fibrinogen molecule, triggering spontaneous fibrin polymerization and the beginning of the fibrin clot. FPA and FPB are measurable in plasma and serve as a marker of thrombin activation.

Thrombin amplifies the coagulation mechanism by activating cofactors V and VIII and factor XI by a positive feedback mechanism that serves to generate more thrombin. Thrombin also activates factor XIII, which forms covalent bonds between the D domains of the fibrin polymer to cross-link and stabilize the fibrin clot (discussed in detail subsequently). Thrombin initiates aggregation of platelets. Thrombin bound to thrombomodulin activates the protein C pathway to suppress coagulation, and it activates TAFI to suppress fibrinolysis.

Thrombin therefore plays a role in coagulation (fibrin), platelet activation, regulation of coagulation activation (protein C), and controlling fibrinolysis (TAFI). Prothrombin, the inactive precursor to thrombin, is

synthesized by the liver in a **vitamin K-dependent reaction** and is released into the circulation.

In vivo thrombin is formed from prothrombin as a result of activation of both the intrinsic and extrinsic pathways of the coagulation cascade. Thrombin catalyzes splitting of fibrinogen into several other polypeptides. These remnant polypeptides bind to each other forming a loose mesh of fibrin that later gets stabilized due to the action of enzymes, known as factor XIIIa that form covalent cross-linkages. Factor XIIIa is formed by plasma protein factor XIII by an enzymatic reaction catalyzed by thrombin. For each event of the cascade plasma calcium is required. Apart from inducing formation and stabilization of fibrin, thrombin also positively exerts a feedback effect on its own formation. It does so by activating the upstream functioning enzymes and the platelets. Thus, initial thrombin formation catalyzes more of its formation in subsequent time.

In clotting reactions, numerous erythrocytes and various other cells are trapped in the fibrin meshwork, fibrin is the essential component of the clot, and clotting can occur in the absence of all cellular elements except platelets. The activation of platelets is necessary because on the surface of these platelets several cascade reactions take place. Besides platelet activation mechanisms, thrombin is another important stimulator of platelet activation.

The activated platelets display specific plasma-membrane receptors that bind with several clotting factors, allowing the reactions on the surface of platelets. A particular phospholipid is also displayed on activated platelets, called platelet factor (PF), which act as a cofactor in the steps intervened by the bound clotting factors. Plasma calcium is required at various steps of clotting reactions. However, the low plasma concentration of calcium can never get enough to cause clotting defects for the reason that before such low concentrations were reached, death would occur from muscle paralysis or cardiac arrhythmias. Conclusively it could be said that for clotting, platelet activation, aggregation and thrombin activation is most important.

Table 5: Names and synonyms of the substances involved in blood clotting pathways.

Clotting factor	Other name	Pathways of Activation
I	Fibrinogen	Common
II	Prothrombin	Common
III	Thromboplastin (Thrombokinase)	Extrinsic
IV	Calcium	All
V	Pro-accelerin, Labile factor, Accelerator globulin	Extrinsic and Intrinsic
VII	Proconvertin (Serum Prothrombin Conversion Acceleration or SPCA)	Extrinsic
VIII	Antihemophilic factor (AHF)	Intrinsic
IX	Plasma thromboplastin component (PTC) Christmas factor, Antihemophilic factor B	Intrinsic
X	Stuart Power factor thrombokinase	Extrinsic and intrinsic
XI	Plasma thromboplastin antecedent (PTA) Antihemophilic factor C.	Intrinsic
XII	Hagemen factor, Glass factor	Intrinsic
XIII	Fibrin stabilizing factor (FSF),]Loki Lorand factor.	Common

The upstream events to thrombin activation include two sequential pathways, merging finally in thrombin activation. The pathways are the intrinsic pathway and the extrinsic pathway. According to their name, for intrinsic pathways everything necessary is present in the blood, whereas for extrinsic pathways a cellular element outside the blood is needed (**Figure 10**). In the intrinsic **pathway** as the first plasma protein factor XII comes in contact to exposed collagen, it gets proteolytically cleaved and activated into factor XIIa by many plasma proteins not mentioned in Figure 9. Similar phenomenon occurs when the blood is taken in a glass tube (Glass acts as collagen and activates blood coagulation; however, a silicone coating on glass vessels decreases the contact activation hence, rate of blood coagulation). Factor XIIa catalyzes proteolytic cleavage of factor XI to factor XIa (active form), which further activates factor IX to factor IXa. Factor IXa activates factor X to factor Xa, which finally converts prothrombin to thrombin. Factor VIIIa serves as a cofactor for factor XIa. Hemophilia a genetic disorder occurs due to absence of Factor VIII or in some cases factor IX.

The **extrinsic pathway** gets initiated by a protein known as tissue factor which is present on the outer plasma membrane of various tissue cells (fibroblasts and other cells in the walls of blood vessels below the endothelium). Upon damage these endothelial cells get exposed to blood plasma protein, particularly plasma protein factor VII that gets bound to tissue factor. Once bound this factor gets activated to factor VIIa which catalyzes activation of factor X and factor IX. Factor IX generates a positive feedback loop by inducing activation of more factors X by initiating an intrinsic pathway.

In summary, clotting gets activated by either activation of factor XII or tissue factor–factor VIIa complex. These two paths merge at factor Xa. Thrombin activated by both of these pathways activates factors XI and VIII in the intrinsic pathway and factor Va that serves as a cofactor for factor Xa.

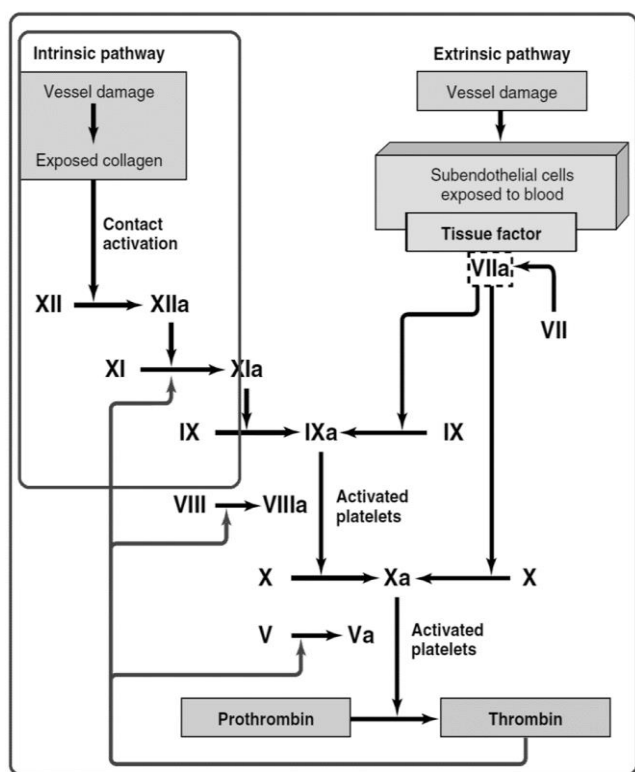


Figure 10: Two clotting pathways—called intrinsic and extrinsic—merge and can lead to the generation of thrombin.

Sequential activation of intrinsic and extrinsic pathways -

- (1) Coagulation usually is initiated by the activation of tissue factor (extrinsic pathway) and factor XII (for intrinsic path). Accordingly Thrombin is initially

produced by an extrinsic pathway, too small in amounts to generate a sustainable coagulation.

- (2) Initial thrombin produced, through a positive-feedback loop increases its amount by acting on the intrinsic pathway, that is, activating factors XI, VIII and platelets.

So, activation of extrinsic pathway and thrombin eliminates the need of factor XII mediated activation. Thrombin also facilitates conversion of prothrombin to thrombin by activating factor V and platelets.

Liver is an essential organ in terms of maintenance of clotting reaction. A liver failure or damage can result in serious bleeding problems. This is because the liver is the site for production of many plasma clotting factors and the bile salts produced in the liver are essential for intestinal absorption of lipid-soluble vitamin K (**Figure 11**). Vitamin K is important for the production of prothrombin and several other clotting factors.

Actions of Thrombin

Pro-coagulant:

- Stimulate platelet activation
- Cleaves fibrinogen to fibrin
- Activate clotting factor XI, VIII, V, XIII

Anticoagulant

- Activate protein C, Which inactivates clotting factor VIIIa and Va

Factors resisting clot formation

Three different mechanisms regulate anticlotting. A defect in any of these three mechanisms would lead to abnormal high risk of clotting also known as hypercoagulation.

1. **Tissue factor pathway inhibitor (TFPI):** it's a plasma protein secreted by mainly endothelial cells and acts during the very initial phase of coagulation. It binds to tissue factor–factor VIIa complex and inhibits generation of factor Xa. Due to this anticoagulant mechanism only a small amount of thrombin is produced by extrinsic pathways.
2. **Thrombin** – thrombin initiates the second anticoagulant mechanism by binding to a receptor present on endothelial cells known as **thrombomodulin**. As it binds to this receptor all of its

clot-producing effects dilute. Thrombin further binds another plasma protein, **protein C**. Finally thrombin with protein C and another plasma protein inactivates factors VIIIa and Va. (**Figure 12**). Thus, we saw earlier that thrombin directly activates factors VIII and V, and now we see that it indirectly inactivates them via protein C.

3. **Antithrombin III** – it is a plasma protein that gets activated when it comes in contact with heparin, a protein present in endothelial cells. Antithrombin III inactivates thrombin and other clotting factors.

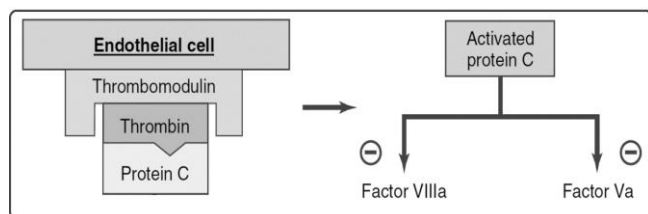


Figure 12: Thrombin indirectly inactivates factors VIIIa and Va via protein C.

The Fibrinolytic System

Fibrin meshwork that forms the clot is not permanent and is to be dissolved until there is permanent repair of the vessel. Clot is removed by the fibrinolytic (or thrombolytic) system (**Figure 13**) which is the principal effector of clot removal. During the clotting reaction the **plasminogen** or **pro-fibrinolysin**, a protein plasminogen activator, is bound to fibrin throughout the clot just like other plasma proteins. Plasminogen remains in inactivated form until **tissue plasminogen activator (t-PA)** released from injured tissues and vascular endothelium that converts plasminogen into **plasmin** (after the clot has stopped the bleeding). Plasmin resembles trypsin in being a proteolytic digestive enzyme. It digests the fibrin fibres, other protein coagulants such as fibrinogen, Factor V, Factor VIII, prothrombin, and Factor XII. t-PA is a very weak enzyme and its catalyzing ability increases only when it gets bound to fibrin. Thus fibrin initiates its own dissolution process.

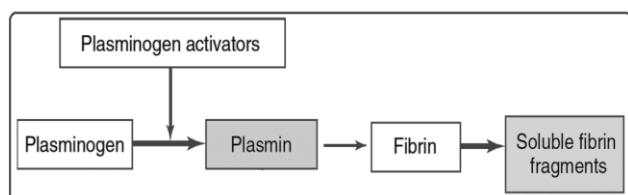


Figure 13: Basic fibrinolytic system.

Menstrual fluid besides blood consists of cervical mucus, vaginal secretions, and endometrial tissue. It lacks the proteins hemoglobin, prothrombin, thrombin, and fibrinogen. Additionally, menstrual blood contains less iron and blood platelets than venous blood. It is now generally accepted that menstrual blood doesn't clot due to the absence of **fibrinogen and fibrin** and that this is due to a fibrinolytic activity in the discharge. The fibrinolytic enzyme (**plasmin, fibrinolysin**) normally exists in the blood as a precursor (plasminogen, profibrinolysin). The transformation of plasminogen into plasmin is brought about by activators. **Table 7** below represents the anti-clotting of endothelial cells.

Table 7: Anticlotting Roles of Endothelial Cells

Action	Result
Normally provide an intact barrier between the blood and sub endothelial connective tissue	Platelet aggregation and the formation of tissue factor- factor VIIa complex are not triggered
Synthesize and release PGI ₂ and Nitric oxide	These inhibit platelets activation and aggregation
Secrete tissue factor Pathway Inhibitor	Inhibit the ability of tissue factor- factor VIIa complex to generate factor Xa
Bind thrombin (via Thrombomodulin), which then activate protein C	active protein C inactivates clotting Factor VIIIa and V
Display heparin molecule on the surface of their plasma membrane	Heparin binds antithrombin III and this molecule then activates thrombin and several others clotting factors
Secrete tissue plasminogen activator	tissue plasminogen activator catalyze the formation of plasmin, which dissolves clots

Anti-Clotting Drugs

Anti-Clotting drugs are used to prevent and treat ailments such as myocardial infarction or heart attack. Myocardial infarction is due to damage of endothelial cells. Such damage triggers the clotting as well as hinders the normal anticlotting functions of endothelial cells. For example, atherosclerosis hinders the ability of endothelial cells to secrete nitric oxide.