كلية الطب البشري
Faculty of Medicine

Hematopoietic System-2024

Physiology Lectures (L1-L6)

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Lecture 1

Blood is part of the extracellular fluid. It is continuously circulating in blood vessels throughout the cardiovascular system through the heart's pumping action. It constitutes 8% of the body weight.

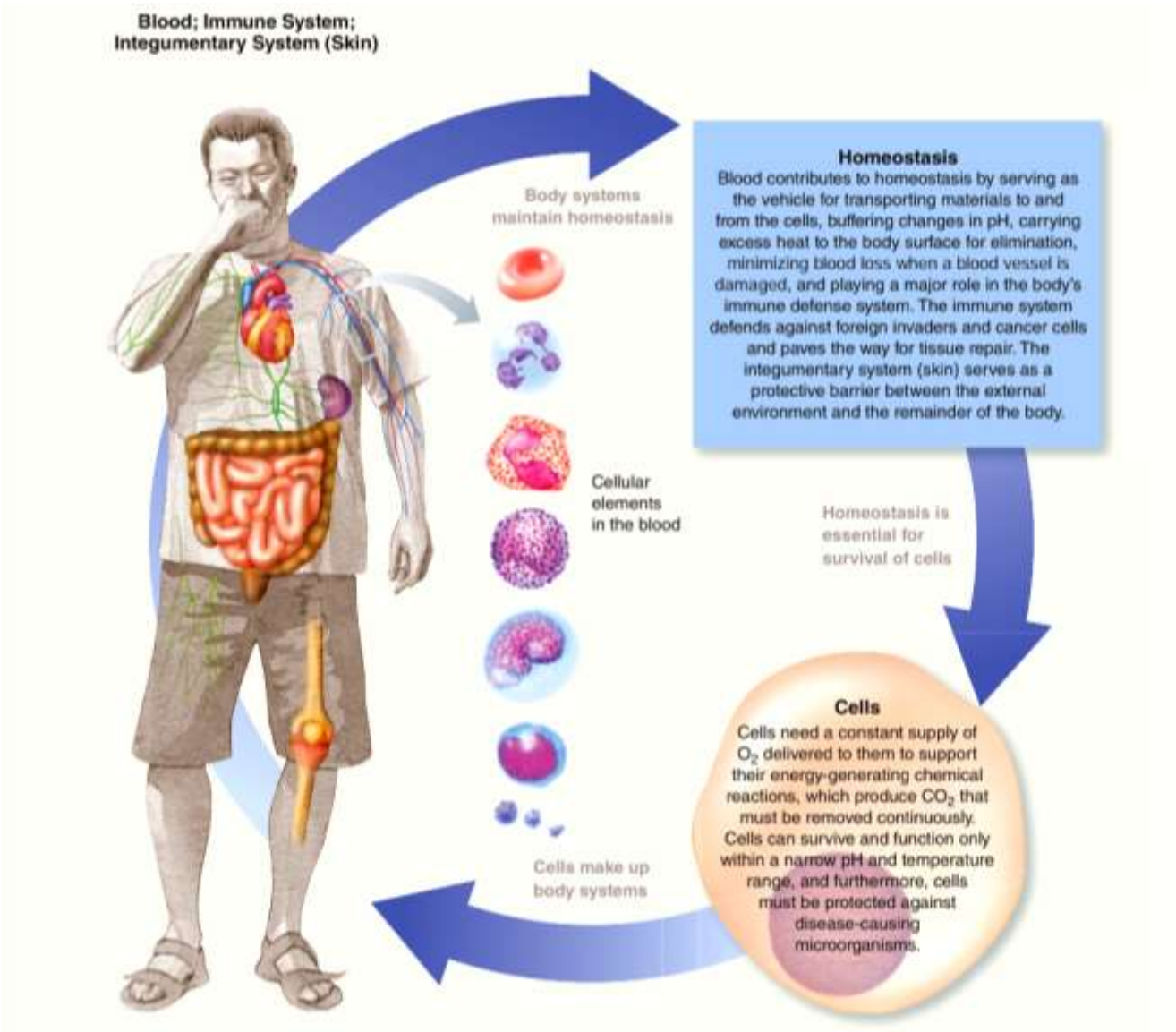
Composition of Blood

Blood is composed of two parts:

1. ***Plasma:*** This is the fluid part of blood. It constitutes 55% of blood.
2. ***The cellular part*** includes red blood corpuscles (RBCs), white blood cells (WBCs), and platelets. It constitutes 45% of blood.

Functions of blood:

1 – Transport.
2 – Immune Function
3 – Haemostasis [Stoppage of bleeding]
4 – Homeostasis [Keeping body environment constant].



PLASMA

It is a clear yellow fluid.

Its volume is about 3.5 L (5% of body weight).

It clots on standing. The remnant is called serum.

Composition of Plasma

1-Water: 92%

2-Organic substances: Plasma proteins, Lipids, glucose, amino acids, vitamins, enzymes, and waste products.

3-Inorganic constituents: (Na^+, Cl^-, HCO_3^-).

4-Blood gases: O_2, CO_2 and N_2.

Plasma Proteins

There are many types of plasma proteins in the blood. The most important types include:

Type	Concentration (g/dl)
1. Albumin	3.5-5
2. Globulin (α,β,γ)	2.5
3. Fibrinogen	0.4
4. Prothrombin	0.01

Sites of Formation of Plasma Proteins:

-Albumin, fibrinogen, and prothrombin are synthesized in the liver.

-Globulins: 50% are synthesized in the liver, and 50% (γ globulin) are synthesized in the plasma cells of the reticuloendothelial system (RES), a diffuse system of cells presents in the liver, spleen, lymph nodes, and bone marrow.

Albumin/Globulin Ratio (A/G):

The A/G ratio is 1.5 to 2.5:1.

It decreases in:

1-Liver diseases, such as liver cirrhosis and infective hepatitis, since the liver does not produce sufficient albumin.

2-Kidney diseases, e.g., nephrosis, as albumin, with its small molecular size, is lost in the urine.

3-Infections: due to increased γ globulin.

Functions of Plasma Proteins:

1. Osmotic Function (mainly by albumin): The total osmotic pressure of plasma is about 5000 mmHg:

*plasma proteins cause 25 mmHg pressure. It is known as the colloidal osmotic pressure or oncotic pressure.

*The remaining pressure is caused by crystalloids, e.g., Na^+, Cl^-, HCO_3^-, and is called the *crystalloid osmotic pressure.*

-Plasma proteins have a weaker osmotic effect, but they are more important because they cannot diffuse through the capillary membrane. Therefore, they are kept inside blood vessels and tend to draw water from interstitial fluid (ISF) into capillaries. The colloidal osmotic pressure regulates blood volume by regulating fluid exchange between ISF and blood.

Edema: It is the presence of abnormally large amounts of fluid in the intercellular tissue spaces of the body (It is excessive accumulation of fluid in the tissues). Hypoalbumeneamia is one of the causes of edema.

2. Transport function: Albumin and globulin (α and β) act as carriers for some substances, e.g., hormones, vitamins, lipids, and minerals. They prevent their loss of urine.

3. Defensive function: γ-globulins are also called immunoglobulins (antibodies). They defend the body against microorganisms and their toxins.
4. Blood clotting function: Prothrombin and fibrinogen are essential for this process.
5. Viscosity: Whole blood is 3-5 times as viscous as water, while plasma is 1.5 times as viscous. Viscosity is responsible for peripheral resistance that maintains arterial blood pressure. Fibrinogen contributes most to plasma viscosity due to its large size and elongated shape.
6. Capillary function: Plasma proteins are required for normal capillary permeability because They partially block capillary pores.
7. Buffer function: Any buffer system consists of a weak acid and a strong base.
In an alkaline medium (blood pH is alkaline: 7.4), plasma proteins form proteinic acid and sodium proteinate. So, they act as a buffer system: Therefore, plasma proteins maintain the pH of blood constant at 7.4 despite the addition of acids or alkalis. They constitute 15% of the buffering power of blood.
8. Function as a source of tissue proteins: Plasma proteins act as *labile protein stores* for tissue proteins since they are dynamic structures in continuous turnover.

RED BLOOD CORPUSCLES (RBCs)

Shape and Size of RBCs

- RBCs are non-nucleated biconcave discs

RBCs Count: In adult males: 4.5–6 million/mm^3 In adult females: 4–5.5 million/mm^3

- It is higher in newborns and athletes at high altitudes and decreased in old age.

Structure of RBCs

- RBCs have no nuclei and are therefore called *corpuscles*.

RBCs have a biconcave shape (The peripheral proteins like spectrin, ankyrin, and actin on the inner surface of the membrane help maintain the shape of the RBC). The biconcave shape has the following advantages: It has a large surface area and enhances cell flexibility allowing erythrocytes to be squeezed into tiny capillaries without rupture. Also, it results in minimal tension on the membrane when the cell volume increases in venous blood due to the transport of CO_2.

- The most important content of RBCs is Hb. K^+ is the principal intracellular cation, and carbonic anhydrase (CA) is an enzyme present in RBCs, which is essential for the transport of CO_2. No mitochondria exist in RBCs; therefore, they derive their energy from anaerobic glycolysis.

Characteristics of human red cells.

		Male	Female
Hematocrit (Hct) (%)		47	42
Red blood cells (RBC) (10^6/µL)		5.4	4.8
Hemoglobin (Hb) (g/dL)		16	14
Mean corpuscular volume (MCV) (fL)	$= \frac{\text{Hct} \times 10}{\text{RBC } (10^6/\mu L)}$	87	87
Mean corpuscular hemoglobin (MCH) (pg)	$= \frac{\text{Hb} \times 10}{\text{RBC } (10^6/\mu L)}$	29	29
Mean corpuscular hemoglobin concentration (MCHC) (g/dL)	$= \frac{\text{Hb} \times 100}{\text{Hct}}$	34	34

RBCs Indices (reflect the functional characteristics of RBCs)

Hematocrit value (Hct)=Packed cell volume (PCV):
The percentage of the blood, by volume, that is occupied by RBCs. {46% (40–50%) for adult male and 42% (37–47%) for adult female).

Hemoglobin (Hb): It is the red oxygen-carrying pigment of RBCs Hb content is the number of grams of hemoglobin in 100 ml (dl) of blood:
- in adult male: 15-16 g/dl
- in adult female: 13-14 g/dl

Mean Corpuscular volume (MCV): Average volume of single RBC.
Mean Corpuscular hemoglobin (MCH): Average amount of Hb /single RBC.
Mean Corpuscular hemoglobin concentration (MCHC): s the average concentration of hemoglobin in a given volume of packed red blood cells.

Lecture 2

Structure of Hemoglobin: Hemoglobin is made up of 4 subunits; each is formed of a polypeptide chain and heme. The four polypeptide chains are collectively called globin. Heme is an iron protoporphyrin in which iron is in the ferrous state (Fe^{2+}).

Reactions of Hemoglobin:

1-The dynamics of the reaction of hemoglobin with O2 make it a particularly suitable O2 carrier. Hemoglobin is a protein comprised of four subunits, each containing a **heme** moiety attached to a polypeptide chain. In normal adults, most hemoglobin molecules contain two α and two β chains. Heme is a porphyrin ring complex that includes one atom of ferrous iron. Each of the four iron atoms in hemoglobin can reversibly bind one O2 molecule. The iron stays in the ferrous state, so the reaction is **oxygenation** (not oxidation). It has been customary to write the reaction of hemoglobin with O2 as
Hb + O2 ⇄ HbO2. Because it contains four deoxyhemoglobin (Hb) units, the hemoglobin molecule can also be represented as Hb4, and it actually reacts with four molecules of O2 to form Hb4O8.

The oxygen–hemoglobindissociation curve relates the percentage saturation of the O2-carrying power of hemoglobin (abbreviated as SaO2) to the PO2. Due to the T–R configuration interconversion, the curve has a characteristic sigmoid shape. Combination of the first heme in the Hb molecule with O2 increases the affinity of the second heme for O2, and oxygenation of the second increases the affinity of the third, and so on, so that the affinity of Hb for the fourth O2 molecule is many times that for the first.

Factors that decrease the affinity between oxygen and HB shift the curve to the right, while factors that increase the affinity between oxygen and Hb cause a curve shift to the left.
The affinity of Hb for oxygen is decreased by (=**R**elease of oxygen =shift to the **R**ight):
a.Hydrogen ions, as they compete with oxygen for deoxygenated Hb.
b. Rise of temperature.
c. 2, 3-diphosphoglycerate (2, 3-DPG) concentration (2,3-DPG is very plentiful in red cells. It is formed from 3- phosphoglyceraldehyde, a glycolysis product via the Embden–Meyerhof pathway. It is a highly charged anion that binds to the β chains of deoxyhemoglobin.

2-Hemoglobin combines ***with carbon dioxide*** to form carbaminohemoglobin. This is one of the ways by which the carbon dioxide added to the blood at the tissues is transported to the lungs.

3-Hemoglobin reacts ***with carbon monoxide*** (CO) to form carboxyhemoglobin. CO combines with iron and displaces oxygen; thus, it prevents hemoglobin from carrying oxygen. The affinity of hemoglobin for CO is 200 times that of oxygen.

4-When hemoglobin is exposed ***to strong oxidizing agents***, the Fe^{2+} iron is changed to ferric iron, and the hemoglobin is changed to dark-colored methemoglobin, which cannot carry oxygen. Small amounts of methemoglobin are normally formed but are reduced by *NADH-metHb reductase* back to hemoglobin.

Types of Hemoglobin:

Adult hemoglobin (HbA): In normal adult human hemoglobin (hemoglobin A), the two polypeptides are α chains and β chains. Thus, hemoglobin A is designated α2β2.

Not all the hemoglobin in the blood of normal adults is hemoglobin A. less than 3% of the total Hb is HbA2, in which β chains are replaced by δ chains (α2δ2).

Small amounts of hemoglobin A derivatives closely associated with hemoglobin A represent glycated hemoglobin. One of these, hemoglobin A1c (HbA1c), has glucose attached to the terminal valine in each β chain and is of particular interest because it increases in the blood of

patients with poorly controlled diabetes mellitus and is measured clinically as a marker of the progression of that disease and the effectiveness of treatment.

Fetal hemoglobin (=HbF): The blood of the human fetus normally contains fetal hemoglobin (hemoglobin F). Its structure is like that of hemoglobin A except that γ chains replace the β chains; that is, hemoglobin F is α2γ2. The cause of this greater affinity is the poor binding of 2,3-DPG by the γpolypeptide chains that replace β chains in fetal hemoglobin. Fetal hemoglobin is normally replaced by adult hemoglobin soon after birth. Hemoglobin F is, therefore, critical to facilitate the movement of O2 from the maternal to the fetal circulation.

Other types: In young embryos, there are Gower 1 hemoglobin and Gower 2 hemoglobin. Switching from one form of hemoglobin to another during the development

Functions of RBCs

A. Functions of Hemoglobin:

1-The transport of oxygen from lungs to tissues and carbon dioxide (involving CA enzyme reaction) from tissues to lungs is the most important function of RBCs. Carbonic anhydrase in RBCs converts the carbon dioxide taken up by them:

2-Hemoglobin is an important buffer. It has six times more buffering power than plasma proteins. Therefore, it can buffer H^+ inside RBCs (formed during CO_2 transport) and can carry CO_2 with minimal change in pH. Hemoglobin-carrying CO_2 (deoxyHb) is a stronger buffer than hemoglobin-carrying O_2 since deoxyHb dissociates less (i.e., it forms a weaker acid = a stronger buffer).

B. *Functions of Membrane:*

1-As the membrane of RBCs is plastic, it allows changes in the volume of RBCs (increased volume in venous blood) with minimal change in tension on the membrane. It allows RBCs to squeeze through narrow capillaries.

2-The biconcave shape of RBCs is suitable for the diffusion of gases, as it gives a maximum surface area in relation to the size of RBCs.

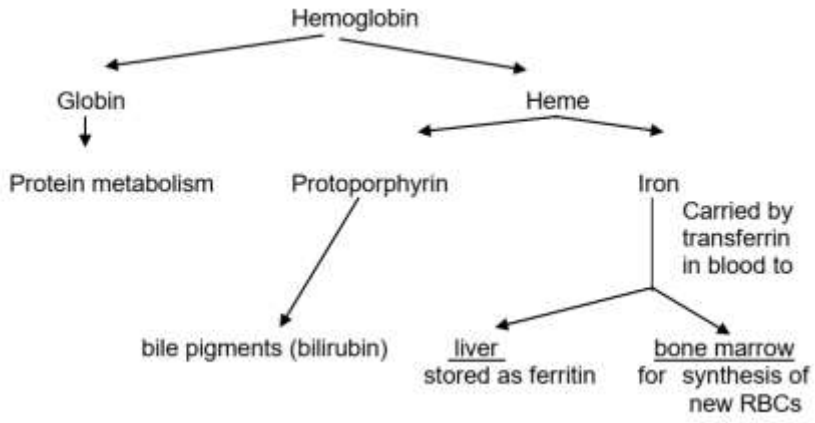
3-The membrane of RBCs keeps hemoglobin inside. If hemoglobin becomes free, it passes to plasma and will:

- Pass to kidneys → block renal capillaries → renal failure.
- Increasing blood viscosity → increases blood pressure, and the work done by the heart may cause heart failure.

Life Span and Fate of RBCs

The life span of RBCs is 120 days. Old RBCs have fragile walls, which rupture easily when RBCs pass through very narrow blood vessels, especially in the spleen.

- Hemoglobin is rapidly captured by the cells of the RES and broken into:



ERYTHROPOIESIS

***Definition*:** It is the process of formation of new RBCs.

***Sites of Erythropoiesis*:**

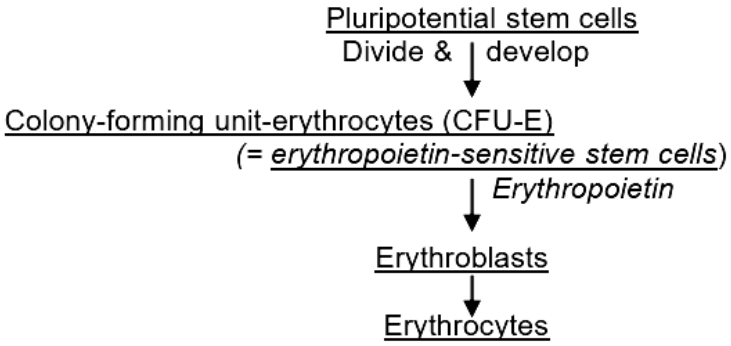
- In the fetus: RBCs are formed in the liver and spleen.
- After birth: RBCs are formed in the red bone marrow of long bones.
- By the age of 20: The red bone marrow in long bones becomes replaced by fatty tissue and cannot produce RBCs.
- After the age of 20: The bone marrow of flat membranous bones, such as ribs, vertebrae, pelvis, sternum, and skull, produce RBCs.

Lecture 3

ERYTHROPOIESIS (continue):

Stages of Differentiation of RBCs:

Pluripotential stem cells in the bone marrow can develop into any blood cells.



Reticulocytes are the immediate precursor of RBCs, following their release to the bloodstream they mature within 1-2 days into RBCs. Reticulocytes contain a small amount of basophilic material, mainly remnants of the Golgi apparatus and mitochondria. They normally make less than 1-2 % of all RBCs. Used to estimate the degree of effective erythropoiesis. Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure.

Factors Affecting Erythropoiesis:

I. Role of Erythropoietin and Oxygen Supply to the Tissues:

There is an increased rate of production of RBCs in conditions associated with hypoxia, such as:

1. Hemorrhage: hypoxia is due to the loss of RBCs.
2. High altitude: hypoxia is due to decreased O_2 tension in atmospheric air.
3. Athletes: athletes have a relative oxygen deficiency since they have higher oxygen requirements than normal.
4. Heart failure: hypoxia is due to decreased blood flow in peripheral vessels.
5. Lung diseases: hypoxia is due to decreased oxygen diffusion from the lungs to the blood. Tissue hypoxia stimulates the release of a hormone called “erythropoietin."

Erythropoietin hormone:

Sources: In adults: 85% are produced in the kidneys and 15% in the liver. Therefore, anemia develops in kidney diseases and nephrectomy, as the liver cannot compensate for the erythropoietin deficiency.

-***Mechanism of action of erythropoietin:*** It accelerates all steps of erythropoiesis as it stimulates mRNA synthesis. It also shortens the maturation time of RBCs in the bone marrow.

-Stimulation of secretion:

-Hypoxia is the main stimulus.

-Conditions that increase Oxygen hemoglobin affinity increase Erythropoietin secretion like Alkalosis or other causes of oxy-Hb dissociation curve to be shifted to the left.

-Cobalt salts and androgens.

-Beta-adrenergic agonists and adenosine.

II. Diet:

A. Proteins: High-biological value proteins (containing all essential amino acids) of animal origin are needed for normal erythropoiesis.

B. Iron:

Functions of iron:

1-Formation of Hb in RBCs and myoglobin in muscles.

2-Co-factor for some oxidation enzymes, e.g., catalase, peroxidase, and cytochrome oxidase.

Total body iron: about 4 g.

Iron absorption, transport, and storage:

-Most dietary iron is ferric (Fe^{3+}), which is reduced by **stomach HCl** & **vitamin C** (ascorbic acid) to ferrous (Fe^{2+})→ more readily absorbed.

-Phytic acid (in cereals), oxalates, & phosphates prevent iron absorption as they form insoluble complexes.

-Iron absorption occurs in the **upper part of the small intestine** by an active process→ carried by **Transferrin** in blood and transported to the bone marrow to form Hb and to the muscles to form myoglobin.

-Excess iron is stored in the **liver & spleen** as **ferritin**.

-Ferrous iron is transported into the enterocytes by divalent metal transporter 1 (DMT1) present at the apical membrane of these cells. The iron that is not reduced in the stomach and reaches the duodenum in the F3+ form will still be reduced by a reductase enzyme associated with DMT1 before it can be transported by DMT1 into the enterocyte.

-Heme can be transported into the enterocytes by the specific heme carrier protein (HCP1) present in the apical membrane of the enterocytes. Iron is released from heme by the action of the heme oxygenase enzyme inside the enterocytes.

-Inside the enterocyte, iron transported by DMT1 and HCP1 has one of two fates, ***depending on body requirements:*** If the body stores of iron are replete, and there is no increased rate of erythropoiesis, most of the iron inside the enterocytes is stored in the form of ferritin. Because duodenal enterocytes' lifespan is very short (approximately 3-4 days), this intracellular ferritin iron is quickly lost into the intestinal lumen as the aging enterocytes are sloughed off and excreted in stools. If, on the other hand, there is increased demand by the body, then most of the iron inside the enterocytes is transported out of the cells at their basolateral border to reach the bloodstream. The export of iron out of the enterocytes at the basolateral border occurs through an iron export protein called ferroportin. While Fe2+ is transported out of the enterocytes, it is oxidized to Fe3+ form by the action of the ferrioxidase enzyme. This enzyme is present on the basolateral border of the enterocytes in association with ferroportin.

-Iron (Fe3+) delivered by the enterocytes to plasma binds to a plasma transport protein called transferrin. Transferrin molecule has two binding sites for iron. Normally, transferrin in plasma is 35% saturated with iron.

-Transferrin delivers iron to different cells in the body.

Regulation of iron absorption: Human body does not have mechanisms to regulate iron excretion. Therefore, we depend on mechanisms that regulate iron absorption and regulate the release of recycled iron from macrophages to maintain iron homeostasis. These regulatory mechanisms for iron absorption and recycling involve the following:

Role of dietary iron: Excess iron in food decreases the DMT1 on enterocytes, thus decreasing iron absorption. This is sometimes referred to as *"the mucosal block*."

Role of Hepcidin: Hepcidin is a 25-amino acid hormone secreted by the liver. Hepcidin is a major regulator of intestinal iron absorption and iron release by macrophages.

Actions of hepcidin: Hepcidin binds to iron export protein ferroportin in duodenal enterocytes, macrophages, and liver cells. The degradation of ferroportin molecules follows such binding. This leads to the Inhibition of intestinal absorption of iron, the Inhibition of the release of recycled iron from macrophages, and the Inhibition of the release of iron from the liver and other store sites.

Factors affecting hepcidin secretion:

-Hypoxia and erythropoietin hormone decrease hepcidin secretion. In this way, iron absorption and release are increased to supply the increased demand by accelerated erythropoiesis for iron.

-Iron loading increases hepcidin secretion by the liver.

-Inflammation increases hepcidin secretion. This explains why anemia is a common complication of many inflammatory diseases.

Effect of iron deficiency: Iron deficiency anemia.

Causes of iron deficiency:

1-Decreased iron intake in the diet

2-Failure of iron absorption, which may be due to:

-Partial gastrectomy (insufficient HCl secretion)

-Diseases of the upper small intestine

-Vitamin C deficiency

-Too much Phytic acid, oxalates, and phosphates in the diet

3-Chronic blood loss: It results in iron deficiency, as the iron stores are insufficient and dietary iron cannot compensate for the amount of iron lost. It occurs in:

- Excessive bleeding during menstruation in females
- Bleeding peptic ulcer and piles.
- Parasitic infestation.

C. Vitamins:

All vitamins are essential for erythropoiesis, esp. vitamin C, B_{12}, and folic acid.

Vitamin B_{12} (Cyanocobalamine = Extrinsic Factor)

Functions of vitamin B_{12}:

a. Vitamin B_{12} is essential for transforming mRNA into DNA, i.e., for the synthesis of DNA and nuclear maturation in RBCs. Therefore, vitamin B_{12} is also known as the *maturation factor*.

b. It is also essential for the metabolism of the myelin sheath of nerves.

Absorption of vitamin B_{12}:

- The parietal cells of the fundus of the stomach secrete a glycoprotein known as *intrinsic factor*. It unites with vitamin B_{12} (*extrinsic factor*) to be protected from digestion by the GIT enzymes.
- It is absorbed from the terminal ileum and passes to blood.

Causes of vitamin B_{12} deficiency:

a. absence of intrinsic factor due to atrophy of the gastric mucosa. The anemia, which develops due to the absence of intrinsic factors, is known as *pernicious anemia*.

b. Liver diseases: as they result in defective storage of the vitamin.

c. disease or surgical resection of the terminal ileum.

d. Very rarely, there is deficient vitamin B_{12} in the diet.

Effect of vitamin B12 deficiency:

a. failure of nuclear maturation and division of erythroblasts in the bone marrow. Therefore, erythroblasts increase in size and develop into *megaloblasts* and megalocytes. They are larger in size, contain a larger amount of hemoglobin, and have a shorter life span than erythrocytes. Therefore, the anemia, which develops due to vitamin B_{12} deficiency, is also called *megaloblastic* or *Macrocytic anemia*.

b. Neurological symptoms:

Since vitamin B_{12} is essential for the metabolism of the myelin sheath of nerves, its deficiency causes neurological manifestations.

2. Folic Acid: Folic acid is needed for DNA synthesis. Therefore, it is required for the division and maturation of RBCs.

Effect of folic acid deficiency:

Folic acid deficiency causes failure of maturation of RBCs and the development of macrocytes, resulting in Macrocytic anemia.

Causes of folic acid deficiency:

a.Dietary deficiency of folic acid
b. GIT diseases interfere with folic acid absorption.
c. Cytotoxic drugs (antifolates) used in the treatment of cancer.

D. Trace Elements:

Copper is a co-factor for Hb synthesis but does not enter into its formation.

Cobalt: It stimulates erythropoiesis and enters in vitamin B_{12} formation.

E. Hormones:

Several hormones increase the rate of erythropoiesis, including Thyroxin, Androgens, and Glucocorticoids.

F-Healthy Bone Marrow:

- Since the bone marrow is the site of erythropoiesis, it must be healthy for normal RBCs production.
- Bone marrow may be destroyed by: X-rays, atomic radiation, drugs, and malignant tumors.
- Bone marrow destruction leads to a decrease in all types of blood cells, i.e., RBCs, WBCs, and platelets. This condition is called aplastic anemia.

G. Healthy Liver:

The liver is important for erythropoiesis because:

- It forms the globin part of hemoglobin
- It stores vitamin B_{12} and iron, which are essential for erythropoiesis
- It produces 15% of erythropoietin.

Lecture 4

BLOOD GROUPS

Blood groups are genetically determined antigens present on the membranes of red cells. These antigens can be detected by reactions with the corresponding antibodies in plasma.

ABO system:

-The cell membrane of RBCs has either A or B antigens.

-A antigens are present on RBCs of 40% of the population, and B antigens are present on RBCs of 10% of the population. In comparison, both are present on RBCs of 5% and absent in RBCs of 45% of the population. Thus, there are four groups of people according to the presence or absence of antigens A and B on RBC membranes.

Antibodies against red cell agglutinogens are called **agglutinins.** Antigens very similar to A and B are common in intestinal bacteria and possibly in foods to which newborn individuals are exposed. Therefore, infants rapidly develop antibodies against the antigens not present in their own cells. Thus, type A individuals develop anti-B antibodies, type B individuals develop anti-A antibodies, type O individuals develop both, and type AB individuals develop neither

The reaction between antigens on RBCs and the corresponding antibodies in plasma results in the agglutination of RBCs, so the antigens are called agglutinogens, and the antibodies are agglutinins.

Genotype	Blood group (Phenotype)	Agglutinogen	Agglutinin
AA.AO	A	A	Anti-B
BB.BO	B	B	Anti-A
AB	AB	AB	---
OO	O	O	Anti-A, anti-B

- O is a universal donor. - AB is a universal recipient.

Rh Blood type: D factor

- This is an antigen on the RBC membrane of 85% of the population who are said to be Rh-positive. It was first discovered in the Rhesus monkey.
- Rh antibodies are formed in the plasma of an Rh-negative person if he is transfused with Rh-positive blood, and the person, in this case, is sensitized to the Rh factor. So if that person receives Rh-positive blood again, agglutination and hemolysis of the RBCs results.
- Rh-positive person never forms anti-D antibodies whether he receives Rh-positive or Rh-negative blood.

Importance of Rh factors: -

1- Erythroblastosis Fetalis: (hemolytic disease of the newly born). When an Rh-positive male marries an Rh-negative female, the fetus will be Rh-positive. During delivery, many Rh-positive fetal red cells enter the mother's circulation, and anti-D agglutinins of the immunoglobulin G type are formed in the mother's blood, which is now sensitized to the D antigen. When the Rh-negative sensitized mother becomes pregnant again with an Rh-positive fetus, the antibodies (IgG) in her blood cross the placenta to the fetus leading to agglutination and hemolysis of fetal RBCs. Usually, the first baby escapes the damage, but the next babies are affected. The affected baby

is severely anemic and jaundiced at birth due to excessive bilirubin formation. The blood-brain barrier of the fetus is not well developed; bilirubin reaches the brain causing damage, a condition called kernicterus. In more severe conditions, the baby is born dead.

-The first baby may be affected if a previous transfusion

already sensitizes the Rh-negative mother with Rh-positive blood.

Prevention:

1-Rh negative female should never receive Rh-positive blood

2-When an Rh-negative female delivers Rh positive baby, anti-D antibodies are given to her immediately after delivery to neutralize the D antigen of the Rh-positive fetal red cells that entered her blood, thus preventing sensitization of the mother.

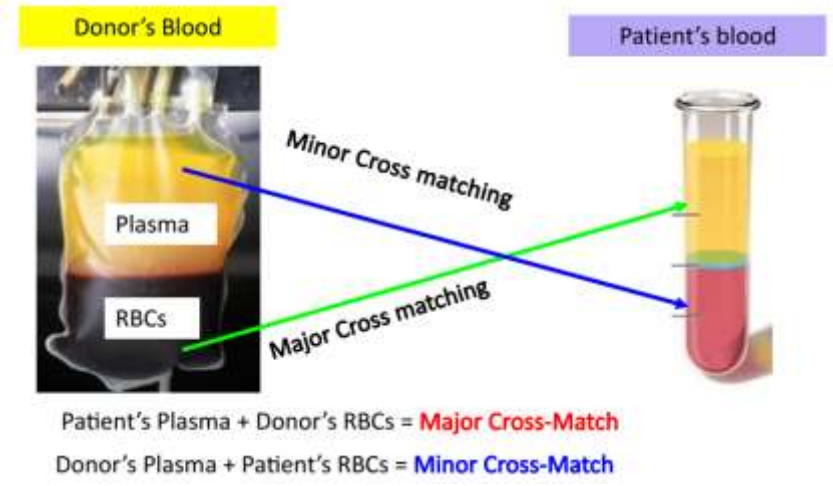
If an Rh-negative person is transferred with Rh-positive blood, he will produce agglutinins against the Rh factor (D antigen). If, after some time, this person is transfused again with Rh-positive blood, agglutination occurs.

ABO incompatibility is the most common maternal-fetal blood group incompatibility and the most common cause of hemolytic disease of the newborn (HDN). ABO incompatibility is often seen in newborns with type A blood because of the higher frequency of type A compared to type B in most populations. ABO incompatibility in newborns generally presents as neonatal jaundice in contrast to the severe intrauterine or neonatal hemolytic anemia associated with Rh sensitization, clinically important neonatal anemia due to ABO incompatibility occurs infrequently. The major clinical issue with HDN due to ABO incompatibility is jaundice.

Several reasons have been proposed to account for the lack of intrauterine hemolysis due to ABO incompatibility. These include less well-developed A and B antigens on fetal red blood cells to stimulate maternal antibody production and the ubiquitous distribution of A and B antigens in other tissues resulting in fewer antibodies that cross the placenta to bind to antigens on fetal red cells. The most important reason that ABO incompatibility does not cause hydrops fetalis (intrauterine fetal death) is that the naturally occurring anti-A and anti-B antibodies are IgM and do not cross the placenta. Less than 1% of mothers with type O have clinically significant anti-A or anti-B antibodies, that is, IgG.

Importance of blood group determination:

Blood transfusion. Normally donor's red cells agglutinate with the corresponding antibodies of the recipient's plasma. The reverse rarely occurs due to the dilution of the donors' agglutinins in the large volume of the recipient's blood. It is important to do a cross-matching test by adding the donor's blood to the recipient's serum before blood transfusion.



When incompatible blood is transfused, the mismatched transfusion reaction occurs immediately. The reaction is primarily due to the agglutination of the donor's red cells followed by their hemolysis. This is called acute hemolytic transfusion reaction. Usually, it occurs due to ABO incompatibility. The severity of the reaction depends on the degree of hemolysis.

The complications of mismatched transfusion are:

1. Shivering and fever (febrile reactions) usually occur
2. Hemoglobinemia and hemoglobinuria
3. Hemolytic jaundice
4. Acute renal failure. Renal failure occurs due to: blocking the renal tubules and damaging the tubules, and the release of toxic substances from the lysed red cell causes renal vasoconstriction5.
5. Hyperkalemia (due to the release of potassium ions from red cells). This may cause cardiac arrest in diastole.

Balance must be maintained between the rate of cell production and that of red cell loss from the circulation; imbalance results in either decreased red cell mass (anemia) or increased red cell mass (polycythemia).

ANEMIA

Definition: It is a decrease in the oxygen-carrying capacity of the blood, which may be due to: Decreased number of RBCs or decreased hemoglobin content of the blood.

Classification and Causes of Anemia:

Using the erythrocyte parameters mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Anemias can be classified: according to cell volume (MCV):
Microcytic, normocytic, or macrocytic. According to the ratio of Hb Concentration /erythrocyte count (MCH): hypochromic, normochromic, or hyperchromic.

1. **Microcytic Hypochromic Anemia (Iron Deficiency Anemia)**: In this type of anemia, the size of RBCs is smaller than normal (Microcytic), and their hemoglobin content is less than normal (hypochromic). It is caused by iron deficiency.

2. Macrocytic Anemia (Megaloblastic Anemia): In this type of anemia, the size of RBCs is larger than normal. It is caused by vitamin B_{12} or folic acid deficiency.

3. Normocytic Normochromic Anemia: In this type of anemia, the size of RBCs and their Hb content are normal, but their number is decreased.

-Causes of normocytic normochromic anemia:

Acute Blood Loss (Hemorrhagic Anemia): In sudden and rapid hemorrhage, the body replaces plasma within 1-3 days, but bone marrow cannot replace RBCs that quickly. Therefore, RBCs become diluted in plasma. RBCs count returns to normal within 3-4 weeks.

Bone Marrow Depression (Aplastic Anemia): Depression of the bone marrow will decrease all blood elements (RBCs, WBCs, and platelets). It may be due to : exposure to X-rays and atomic irradiation, malignancy or viral infection and drugs.

Excessive Breakdown of RBCs (Hemolytic Anemia): may be due to intrinsic or extrinsic factors.

- *Intrinsic factors: as in sickle cell anemia and G6PD deficiency.*
- *Extrinsic factors: as in*
  a. Infections, e.g., streptococci and malaria
  b. Chemical poisons, e.g., benzene derivatives
  c. Incompatible blood transfusion
  d. Snake venom.

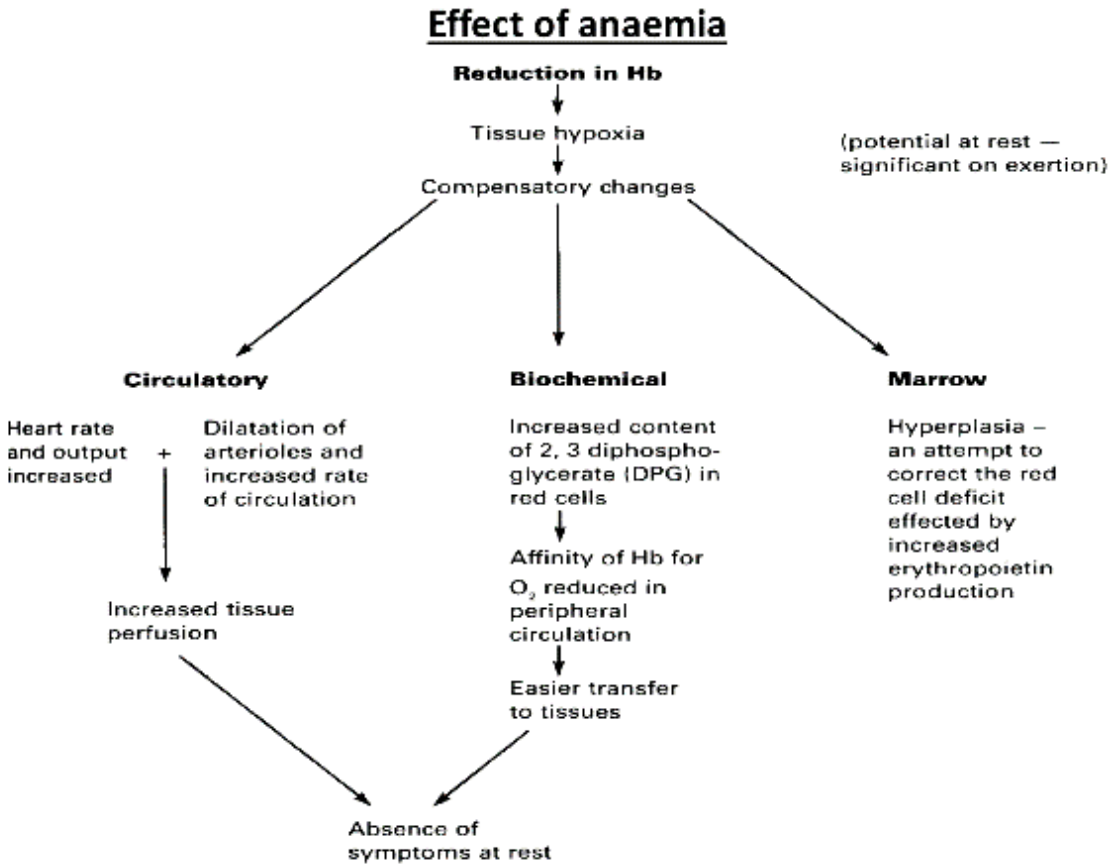
NB/

Conditions associated with an increase in reticulocytes:

• Hemolytic anemias: Immune hemolytic anemia, RBC membrane defects, Sickle cell diseases,
• Following hemorrhage
• Following treatment of anemias •

Conditions associated with a decrease in reticulocytes:

• Iron deficiency anemia
• Aplastic anemia



POLYCYTHEMIA:

It represents an increase in the number of red cells. It exists in two main forms:

A-Absolute Polycythemia:

1. ***The primary form***, also called polycythemia vera, is a clonal neoplastic disorder of hematopoietic stem cells.
2. ***The secondary forms*** are conditions of increased red cell production that usually occur due to increased erythropoietin secretion. In the primary form, the cause of the disease is the abnormality of hemopoietic stem cells characterized by uncontrolled proliferation of cells of erythroid, granulocytic, and megakaryocytic series, increasing all forms of formed elements of blood. In secondary forms, the cause of the disease is excess erythropoietin secretion that increases red cell production (mostly without an increase in granulocytes and platelets).

B-Relative Polycythemia:

The relative or apparent polycythemia is not true polycythemia but a spurious increase in red cells due to dehydration.

PLATELETS (THROMBOCYTES)

- Platelets are small, non-nucleated, granulated bodies.
- The normal platelet count is 300,000/mm^3. Decreased platelet number is called thrombocytopenia.
- The diameter of platelets is about 2-4 μm.

Formation of Platelets: Platelets are formed in the bone marrow from megakaryocytes.

Structure of Platelets

A. Platelet Membrane:

- It contains receptors for collagen, von Willebrand factor, and fibrinogen.
- It has a glycoprotein coat containing phospholipids, which form:
  - Platelet factor 3 (PF3) (helps blood clotting)
  - Platelet-activating factor (PAF) (activates phospholipase C).
- The membrane invaginates to form an open canalicular system, i.e., a large surface area for the uptake of extracellular calcium and release of intracellular materials.

B. Platelet Cytoplasm:

The cytoplasm contains many active substances:

1. Beneath the membrane:
   a. a skeleton of microtubules, which maintain the shape of platelets.
   b. Contractile proteins: actin, myosin, and thrombosthenin, which allow platelets to contract and change their shape.
2. Intracellular organelles: Remnants of Golgi apparatus and endoplasmic reticulum Mitochondria for the synthesis of ATP and ADP, Lysosome containing hydrolytic enzymes.
3. Glycogen granules for energy production
4. Enzymes for the synthesis of prostaglandins from phospholipids of platelet membrane. Prostaglandins are local factors that mediate vascular and local tissue reactions.
5. Two types of granules:
   a. *dense granules:* they contain non-protein substances (ADP, serotonin, calcium)
   b. *alpha granules:* they contain proteins (Some clotting factors, platelet-derived growth factor (PDGF) that helps growth of endothelium i.e., wound healing)

HEMOSTASIS

It means stoppage of bleeding from an injured blood vessel. The hemostatic process consists of the following:

A. Vasoconstriction of injured blood vessel
B. Temporary platelets plug formation by platelet reactions.
C. Blood clot formation to stabilize the temporary platelet plug.
D. Limitation reaction to dissolve clot after wound healing.

I. Local Vasoconstriction:

Injury to the blood vessel is immediately followed by its constriction. This reduces the blood flow from the vessel and allows platelets to adhere at the site. The vasoconstriction may be so strong that it completely obliterates the lumen of the injured vessel. Vasoconstriction is due to the following:

1. Nervous reflexes: initiated by pain sensation from the traumatized vessel.

2. Local myogenic contraction: due to direct damage of the blood vessels. The degree of myogenic contraction is proportional to the amount of damage, i.e., a longitudinal cut in a vessel causes less spasm than a transverse cut.

3. Chemical substances: serotonin and thromboxane A_2 liberated from platelets cause vasoconstriction.

II. Formation of Temporary Platelet Plug: (Platelets reactions)

When a blood vessel is injured, platelets form a mechanical plug to seal the injury site. The platelet plug can stop blood loss if the injury is small. The platelet reactions in hemostasis include:

1-Platelet adhesion: Normally, platelets do not adhere to healthy blood vessels. However, when a blood vessel is cut, subendothelial collagen and von Willebrand factor are exposed, and platelets adhere to them by their membrane receptors.

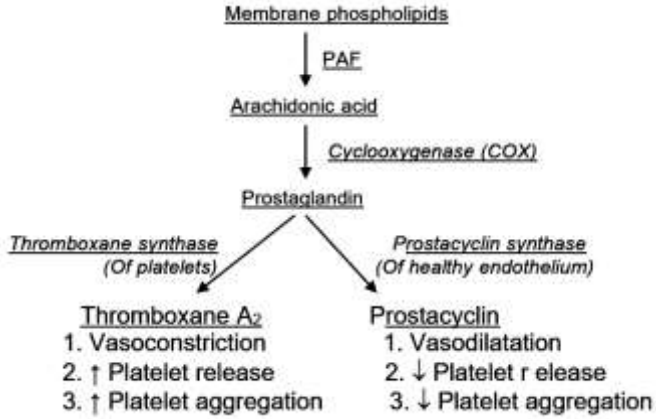
2-Platelet activation: The adhesion of platelets to collagen and VWF activates the platelets: they swell, change their shape, put out pseudopodia, stick to other platelets, and their contractile proteins contract forcefully, causing the release of the platelet granules.

3-Release reaction: When the contents of dense and α granules are released, they go into action:

Platelet-derived growth factor (PDGF) stimulates the growth of the endothelial lining of blood vessels, helping their repair.

Platelet-activating factor (PAF) is produced from membrane phospholipids of platelets. It activates a chain of reactions that ultimately lead to the formation of arachidonic acid from membrane phospholipids. Arachidonic acid is then converted by cyclooxygenase to prostaglandin. In platelets, the enzyme thromboxane A_2 synthase converts prostaglandin to thromboxane A_2, which causes vasoconstriction, and helps the release reaction and platelet aggregation.

Simultaneously, in the walls of healthy blood vessels, the enzyme prostacyclin synthase acts on prostaglandins, resulting in prostacyclin formation. Prostacyclin is a powerful vasodilator that inhibits platelet aggregation and release reaction. As its actions are opposite to those of thromboxane A_2, prostacyclin keeps the platelet plug localized to the injury site.



NB Aspirin inhibits cyclooxygenase. It, therefore, decreases the synthesis of both thromboxane A_2 and prostacyclin. The endothelial cells can start to produce new cyclooxygenase within a few hours, while platelets cannot. Therefore, the daily intake of small amounts of aspirin reduces clot formation and prevents myocardial infarctions.

4-Platelet aggregation: Released ADP and thromboxane A_2 cause platelet aggregation at the injury site. Platelet aggregation activates more and more platelets, leading to more release reactions and liberating more ADP and thromboxane A_2. This self-propagating process forms a platelet plug that closes the blood vessel.

5-Platelet procoagulant activity: Platelet release and aggregation result in the exposure of platelet factor 3 (PF3) on the platelet membrane. PF3 helps to start blood coagulation by activating some clotting factors.

6-Platelet fusion: Aggregated platelets undergo irreversible fusion. This is produced by the high concentration of ADP and platelet enzymes.

Lecture 5

III. Blood Coagulation (Blood Clotting): The clotting factors are plasma proteins, mostly (β-globulins). They are proteolytic enzymes, which are present in blood in an inactive form. When activated, they activate other inactive enzymes in cascade reactions, which end in clot formation. Clotting factors were given numbers to simplify the description of the clotting mechanisms. They are given an "a" when they are activated.

Clotting factors are categorized into three groups:

1- Fibrinogen group:

- I , V, VIII & XIII(13 = 8+5 ,1) . -Activated by thrombin. -Not present in serum.

2-Prothrombin Group:

-II, VII, IX & X(1972) . -Need vitamin K for synthesis -Prothrombin is not present in serum.

3-Contact Group:

-XI and XII. -Present in serum

The Clotting Mechanism: The loose platelet plug changes to a definitive blood clot by conversion of soluble fibrinogen to insoluble fibrin.

Fibrinogen forms loose fibrin which becomes a dense fibrin clot by forming cross-linkages. This reaction is catalyzed by factor XIII and Ca^{2+}.

- Fibrinogen ⟶ fibrin monomer + 2 pairs of polypeptide chains

- Fibrin monomers polymerize ⟶ loose mesh of fibrin

- Fibrin $\xrightarrow[XIII + Ca^{2+}]{\text{Cross linkages}}$ tight fibrin clot

The conversion of fibrinogen to fibrin is catalyzed by thrombin, which is formed from prothrombin in the presence of active factor X.

- Prothrombin $\xrightarrow{Xa}$ thrombin

Either intrinsic or extrinsic pathways activate factor X.

A. Intrinsic Pathway

- This system is called intrinsic, as the phospholipids involved in the reactions arise from platelets (PF3), i.e., it is present in plasma.
- Initiation of the pathway may occur either:

i. In vivo: by contact of blood with subendothelial collagen of the damaged vessel.

ii. In vitro: by contact of blood with:

- electronegative charged wet surfaces, e.g., a glass of a test tube.
- collagen fibers

1. Any of the previously mentioned factors activates factor XII.
2. XIIa activates factor XI, which in turn activates IX.
3. IXa forms a complex with VIIIa and activates factor X in the presence of phospholipids (PL) and Ca^{2+}.

B. Extrinsic Pathway

- This system is called extrinsic as it requires the presence of phospholipids from outside blood vessels.
- It is initiated only in vivo by factor III: tissue thromboplastin (TPL) released from damaged tissues.

- TPL activates factor VII, which directly activates factor X in the presence of Ca^{2+}, TPL, and PL, and indirectly through the activation of factor IX.

Common Part in Both Pathways:

- Xa (activated by intrinsic and extrinsic pathways) catalyzes the conversion of prothrombin to thrombin in the presence of factor V, PL, and Ca^{2+}.
- Finally, thrombin transforms soluble fibrinogen into insoluble fibrin.
- Thrombin in the presence of Ca^{2+} also activates factor XIII, which stabilizes the fibrin clot. Platelets, blood cells, and plasma become entangled in the clot.
- Contraction of the platelets in the fibrin mesh causes clot retraction and squeezes serum out.
- The serum is devoid of fibrinogen, prothrombin, and factors V, VIII, and XIII which become consumed during clotting.

Important Notes

-***Von Willebrand factor (vWF)*** is a glycoprotein crucial to primary hemostasis through platelet and subendothelial collagen adhesion, and the intrinsic coagulation cascade, through factor VIII stabilization. It resides in the plasma, subendothelial matrix, and storage granules within endothelial cells and platelets.

- During primary hemostasis, vascular injury exposes vWF bound to subendothelial collagen. Then, glycoprotein 1b (GP1b) receptors on the surface of nearby platelets adhere to the exposed vWF, triggering platelet activation and a cascade of events which includes the release of platelet storage granule content such as vWF from alpha granules and the recruitment of more platelets to form a plug at the site of damaged endothelium.
- Plasma vWF supports the intrinsic coagulation cascade by stabilizing factor VIII, thereby increasing its circulating half-life. During the intrinsic coagulation pathway, thrombin cleaves the factor VIII binding site with vWF, allowing the release (activation) of factor VIIIa to continue the clotting process. By serving as a carrier for factor VIII, vWF influences the common coagulation pathway and the generation of thrombin and fibrin.
- The extrinsic pathway is very rapid (15 sec.), while the intrinsic pathway is slow (1-6 min.).
- Injury of a blood vessel will trigger both the intrinsic system (by collagen) and the extrinsic system (by TPL).
- In the test tube, clotting occurs only by the intrinsic system (glass or addition of collagen).
- In intravenous thrombosis, blood clotting occurs via the intrinsic system, which is initiated by the exposure of clotting factors to collagen.

- Thrombin functions:

- activates fibrinogen to fibrin.
- activates the other factors of the fibrinogen group (V, VIII, and XIII)
- accelerates the actions of factors IX, X, and XI
- accelerates the formation of more thrombin from prothrombin (positive feedback).
- Accelerates platelet aggregation and fusion.

Therefore, as soon as a small amount of thrombin is formed, the clotting reactions are markedly enhanced by thrombin, and the clot continues to grow until this process is stopped by limiting reactions.

Anticlotting Mechanisms = Limiting Reactions

The tendency of blood to clot is balanced in vivo by limiting reactions that prevent blood clotting in healthy blood vessels and break down any clots already formed.

A. General limiting reactions:

1. Smooth vascular endothelium prevents activation of platelets & factor XII.
2. Rapid blood flow removes activated clotting factors and inactivates them in the liver. So, slow blood flow favors intravascular thrombosis.

3. Heparin is a natural anticoagulant present in the blood.

B. Specific limiting reactions:

1. Thromboxane A_2 and prostacyclin: The formation of thromboxane A_2 at the site of blood vessel injury allows clot formation, while the synthesis of prostacyclin by healthy endothelium prevents the spread of the blood clot to neighboring healthy areas and obstruction of the lumen of blood vessels.
2. Antithrombin III: This circulating inhibitor of blood coagulation binds to active factors II, IX, X, XI, and XII, blocking their activity. This binding is facilitated by heparin.
3. The Fibrinolytic System:

-Thrombomodulin is produced by most endothelial cells. This protein binds thrombin to form the Thrombomodulin-thrombin complex, which activates protein C.

-Activated protein C (APC) causes:

- Inactivation of factors Va and VIIIa, and
- Inactivation of the inhibitor of tissue plasminogen activator (tPA)=(TPA-I), increases the formation of plasmin.

-Plasmin (fibrinolysin) lyses fibrin and fibrinogen, forming fibrinogen degradation products (FDP), inhibiting thrombin.

Anticoagulants:

These are substances used to prevent blood clotting.

A. In vitro anticoagulants: They prevent blood coagulation outside the body.

1. Removal of Ca^{2+} ions:
   - Oxalates precipitate Ca^{2+} ions as calcium salts,
   - Citrates (used in blood transfusions) bind Ca^{2+} ions by deionizing them.
2. Silicon-coated tubes prevent the activation of factor XII.
3. Addition of heparin.

B. In vivo anticoagulants: They prevent blood clotting inside the body.

	Heparin	***Dicumarol***
Origin	Mast cells and basophils	Plant
Mode of Action	Facilitates the action of Antithrombin III (***Inactivates*** II, IX, X, XI, XII)	Competitive Inhibition of vitamin K on its receptors in the liver → inhibits ***the formation*** of II, VII, IX, X.
Site of Action	In vivo and in vitro	only in vivo
Onset	Rapid	Slow
Duration	Short	Long
Administration	intravenous (IV) and subcutaneously (SC)	Orally
Antidote	Protamine sulfate 1% Fresh blood transfusion	Vitamin K Fresh blood transfusion

Hemostatic Function Tests

1. Blood count and blood film

2. Bleeding time: It is the time needed for bleeding to stop without blood clotting. The normal bleeding time is 1-3 minutes, depending on platelet count and function. It is prolonged in thrombocytopenic purpura.

3. Tests for blood coagulation

a-Clotting time: It is the time needed for blood to clot. Normally, it is 3-10 minutes at 37^{o}C. *It is prolonged in* disorders such as vitamin K deficiency, hemophilia, and liver diseases.

b-Prothrombin time: A blood sample is collected in a tube containing citrate or EDTA to chelate any calcium and thus inhibit coagulation, and then the cells are removed by centrifugation. After the cells are removed, excess calcium is added with an excess of thromboplastin to anticoagulated plasma to initiate coagulation. A normal PT is 11.0–12.5 seconds. A PT greater than 20 seconds is indicative of a coagulation deficit.The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different batches of the manufacturer's tissue factor used in the reagent to perform the test.

c-International normalized ratio (INR): The INR was devised to standardize the results.

Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an international reference tissue factor. The ISI is usually between 1.0 and 2.0.

$$\mathrm{INR} = \left(\frac{\mathrm{PT_{test}}}{\mathrm{PT_{normal}}}\right)^{\mathrm{ISI}}$$

The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system used.

A high INR level, such as INR=5, indicates a high chance of bleeding, whereas if the INR=0.5, there is a high chance of having a clot. The normal range for a healthy person is 0.9–1.3, and for people on warfarin therapy, 2.0–3.0. However, the target INR may be higher in particular situations, such as those with a mechanical heart valve.

d- Activated Partial Thromboplastin Time test (aPTT)): a test performed to investigate bleeding disorders and to monitor patients taking an anticlotting drug such as heparin which inhibits factors X and thrombin, while activating anti-thrombin. The partial thromboplastin time is the time it takes for a clot to form, measured in seconds. Normally, the sample will clot in 35 seconds.

e-Prothrombin concentration: normally > 70 %.

Abnormalities of Hemostasis:

A-Bleeding:

1. Thrombocytopenic purpura: It is due to a decreased platelet count below 50,000/mm^3. It is characterized by the presence of subcutaneous hemorrhages. The bleeding time is prolonged.

2. Vitamin K deficiency:

-Vitamin K is essential for forming the prothrombin group (II, VII, IX, and X) in the liver.
-Vitamin K is continuously formed by the intestinal flora.
-Vitamin K is fat-soluble and requires bile for its absorption.
-Causes of vitamin K deficiency: (associated with prolonged clotting time)

- ✓ Absence of intestinal flora: in newborns, prolonged oral antibiotics.
- ✓ Absence of bile: in obstructive jaundice.
- ✓ Block of its receptors in the liver by dicumarol.

3. Hemophilia:This is a congenital sex-linked disease carried on the X chromosome. It is recessive and is transmitted by females to their male sons. It is characterized by severe bleeding, even after mild trauma. Joint damage (hemophilia arthopathy) is the most common complication of bleeding in hemophilia. There are three types of hemophilia:

*Hemophilia A: due to the absence of factor VIII (85% of cases)
*Hemophilia B: due to deficiency of factor IX (10% of cases)
*Hemophilia C: due to deficiency of factor XI (5% of cases).
There is prolonged clotting time.

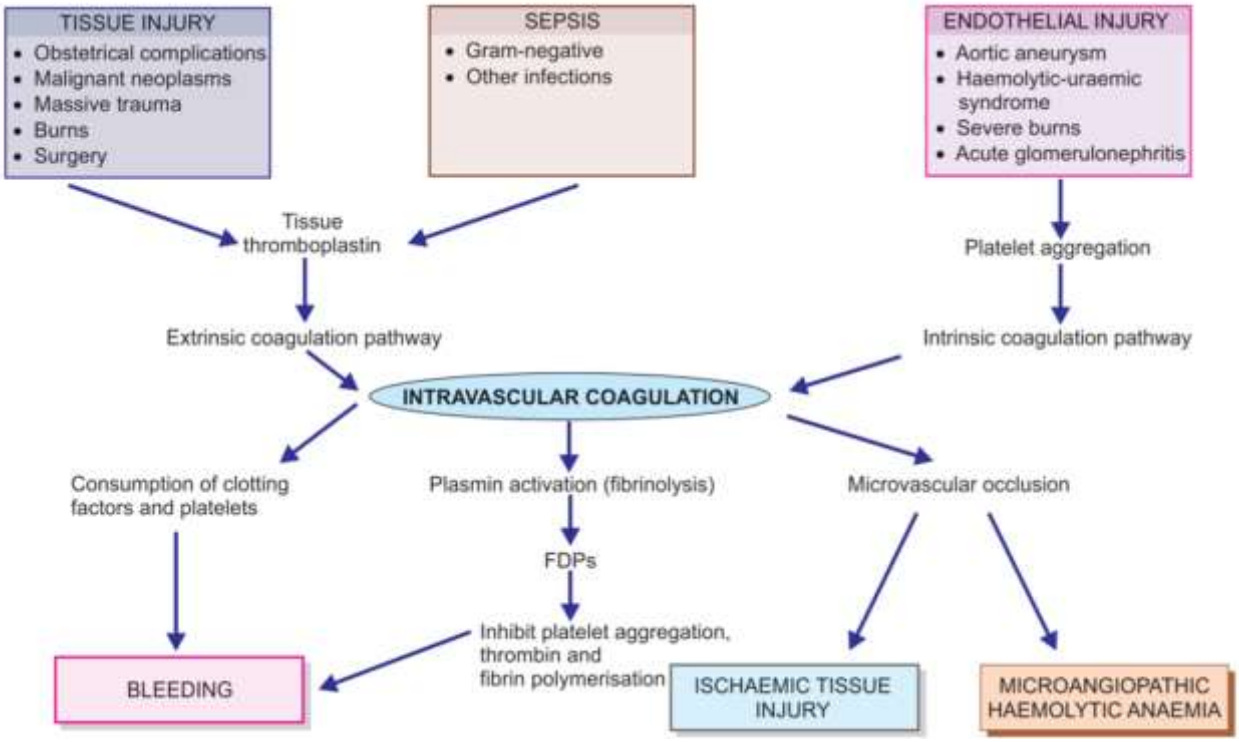
B-Clotting (Thromboembolic Conditions):

Slow blood flow [long bed rest, varicose veins, atherosclerosis]

C. Disseminated Intravascular coagulation (DIC):

DIC is a clinicopathological syndrome in which there is **widespread intravascular coagulation** that occurs due to procoagulants that are introduced into or produced by blood circulation.

1. The procoagulant activity overcomes the natural anti-coagulant mechanisms. This is also called ***consumption coagulopathy*** or ***defibrination syndrome.***
2. This is a hemorrhagic disorder in which diffused intravascular coagulation results in defects of hemostasis.
3. In this disease, **coagulation factors and platelets are overutilized.** This results in bleeding.
4. The most common procoagulant stimulus is the ***tissue factor (tissue thromboplastin) exposure to the blood,*** that activates extrinsic pathway of coagulation



Lecture 6

WHITE BLOOD CELLS (WBCs)
LEUKOCYTES

The total leukocytic count is 4,000–11,000/mm^3 of blood.

Functions of Leukocytes

Leukocytes are responsible for the body's defense against pathogenic organisms and their toxins.

The matured cells are released into circulation and remain in circulation for a few hours before they enter the tissues (circulation pool): At rest, many leucocytes, especially neutrophils, adhere to the endothelial lining of blood vessels, which is ***called the margination pool of leucocytes***. In addition, leucocytes circulate in the blood ***(the active circulation pool)***.

Disruption of margination causes leucocytosis: Leucocytes adhere to the inner lining of the blood vessel, called margination. In exercise and other conditions of increased hemodynamics, leukocytosis occurs mainly due to the disruption of the margination of leucocytes.

TYPES OF LEUKOCYTES:

A. Granular Leukocytes:

1. *Neutrophils:* (60-70% of WBCs)

-They represent the first line of defense against invading organisms.

- They perform their function by:

a. Margination: Neutrophils attach to the walls of capillaries.

b. Diapedesis: Neutrophils squeeze themselves through the pores of capillaries and pass into tissue spaces.

c. Amoeboid movement: This movement allows neutrophils to reach invading organisms.

d. Chemotaxis: Breakdown products of inflamed tissues and bacterial toxins attract neutrophils to the infected area.

e. Phagocytosis: This is the most important property of neutrophils. They can ingest invading bacteria and necrotic tissue by engulfing them (endocytosis). It results in the formation of a phagocytic vacuole. The neutrophil granules release their contents into the phagocytic vacuoles, killing the bacteria.

- In more severe infections, pus is formed. It consists of necrotic tissue, dead neutrophils, and dead macrophages.

2. Eosinophils: (2-6% of WBCs)

Eosinophils increase in parasitic infestations. They kill too large parasites to be engulfed by releasing toxic substances.

-They also increase during allergic conditions.

-Eosinophils are weakly phagocytic and show chemotaxis.

3. Basophils: (0-1% of WBCs)

- Basophils are similar to mast cells. They contain histamine (increases capillary permeability) and heparin (naturally occurring anticoagulant).

B. Agranular Leukocytes:

1. Monocytes: (2-8% of WBCs)

-They are the largest type of leukocytes.

-They enter the blood from the bone marrow and circulate for 72 hours. Then they enter the tissues & become ***tissue macrophages***. The tissue macrophage system was earlier known as the reticuloendothelial system.
-Monocytes migrate by amoeboid movement in response to chemotactic stimuli to the sites of inflammation soon after neutrophils.
-They phagocytose bacteria, dead neutrophils, and remnants of destroyed tissues.

Few monocytes are transformed into highly specialized mononuclear cells called ***dendritic cells:*** They play an important role in antigen processing and presentation to the T cells.

2. Lymphocytes: (20-30% of WBCs)
-They are formed in lymphoid tissues and enter the circulation via the lymphatic vessels.
-Lymphocytes play an important role in the body's defense through the immune system.

IMMUNITY
The body can resist almost all types of organisms and toxins that tend to damage tissues and organs.

Antigens:

- These are substances that can induce an immune response and react specifically with its products.
- Foreign organisms or toxins possess certain chemical structures that are specific to them and different from all other compounds. Such chemical structures are known as "antigenic determinants."
- Their molecular weight is more than 10,000.
- Antigens usually have many antigenic determinants, which may differ from each other or be repeated molecular structures.
- Antigenic determinants may be present on cell membranes or may be free, e.g., bacterial toxins.

Immune responses broadly involve two steps:
1. Recognition of pathogen or the foreign material
2. Reactions or responses to eliminate it. The responses are called immune responses. Largely, immune responses are of two types:
1. Innate or nonadaptive response (Innate immunity).
2. Acquired or adaptive response (Acquired immunity).
There are two types of immunity: innate (non-specific) and acquired (specific) immunity.

I. INNATE IMMUNITY = NON-SPECIFIC IMMUNITY
Innate immunity is composed of non-specific mechanisms which defend the body against invasion by most organisms. It is neither specific for particular infectious agents nor improved by repeated encounters with the same agent. It includes the following mechanisms: i.e.,
1. Resistance of the skin to invasion by organisms.
2. Destruction of organisms swallowed by HCl and digestive enzymes in the stomach.
3. Phagocytosis of the bacteria by granulocytes and tissue macrophages.

II. ACQUIRED IMMUNITY = SPECIFIC IMMUNITY
The body can recognize the invading agent and develop a powerful specific immune response against it. It is carried out in 2 ways:

A. Cellular Immunity (Cell-Mediated Immunity)
Formation of activated cytotoxic T-lymphocytes, which can circulate in the blood, detect and attack the antigen specifically.

- This type of immunity is produced by the activated cytotoxic T lymphocytes.

- It occurs when the body is exposed to viruses, fungi, a few bacteria (e.g., the tubercle bacillus), cells from other individuals (tissue transplants), or tumor cells.
- Cytotoxic T cells cannot recognize free antigens. They are activated when their T cell receptor "sees" the antigen to which they are specific in association with the MHC class I protein:
  1. The antigen may be ingested by an antigen-presenting cell (macrophages, dendritic cells, and B cells), partially digested, and presented on its cell membrane coupled with the MHC class I protein. In addition, the antigen-presenting cells also release interleukin-1 (IL-1), which activates T-lymphocytes.
  2. T-helper cells are activated when they recognize the antigen in association with MHC class II proteins on the surface of antigen-presenting cells. The T-helper cells secrete interleukins that activate T cytotoxic cells.

MHC Antigens

Major histocompatibility complex or MHC antigens are self-antigens that help in identifying and rejecting the foreign antigens:

1. They are also called ***HLA antigens*** (human leucocyte associated antigens), as they were first identified on the membrane of leucocytes. However, afterward they were found to be present on the surface of all the body cells except in red cells (remember, red cells contain blood group antigens).
2. Like blood group antigens they are chemically glycoproteins. They are made up of **α and β subunits.**

Mechanism of Action of MHC Antigens

The proteins in the cells are continuously broken down to their peptide fragments. MHC I molecules pick up the peptide fragments containing 8–10 amino acids, whereas MHC II molecules pick up peptides containing 13–17 amino acids:

1. When a peptide fragment of a self protein is picked up by the MHC antigen and expressed on the surface of the APC along with MHC proteins, T cells ignore it.
2. However, when the peptide fragment is of a foreign protein, T cells recognize it and get activated that induce cell- mediated immunological responses.

- When cytotoxic T cells are activated, they proliferate and differentiate into clones of:
  1. Activated cytotoxic T cells: They destroy the antigen by:
  -Directly attacking the cell expressing the antigen by inserting pore-forming molecules (perforins) into the membrane, causing cell lysis.
  -Secreting substances that attract macrophages to the site of the antigen so that it is phagocytosed and killed.
  2. Memory T cells remain dormant inside the body, and on second exposure to the same antigen, these cells proliferate more rapidly and powerfully than T lymphocytes.

B. Humoral Immunity

- B-lymphocytes are responsible for humoral immunity.
- Humoral immunity mainly acts against bacterial infections.
- B-lymphocytes are activated by the antigen when they come into direct contact with it.

B-lymphocytes have membrane-bound antibodies on their surface, which act as receptors for antigen recognition.

- Activated B-lymphocytes proliferate, forming a clone of B-lymphocytes specific to this antigen. These cells differentiate into:

1. Plasma cells, which contain a well-developed endoplasmic reticulum. The plasma cells secrete antibodies specific to the antigen. Secreted antibodies circulate in the blood. When they encounter the antigen, they combine with it and destroy it. There are five types of antibodies: IgG, IgA, IgM, IgD, and IgE

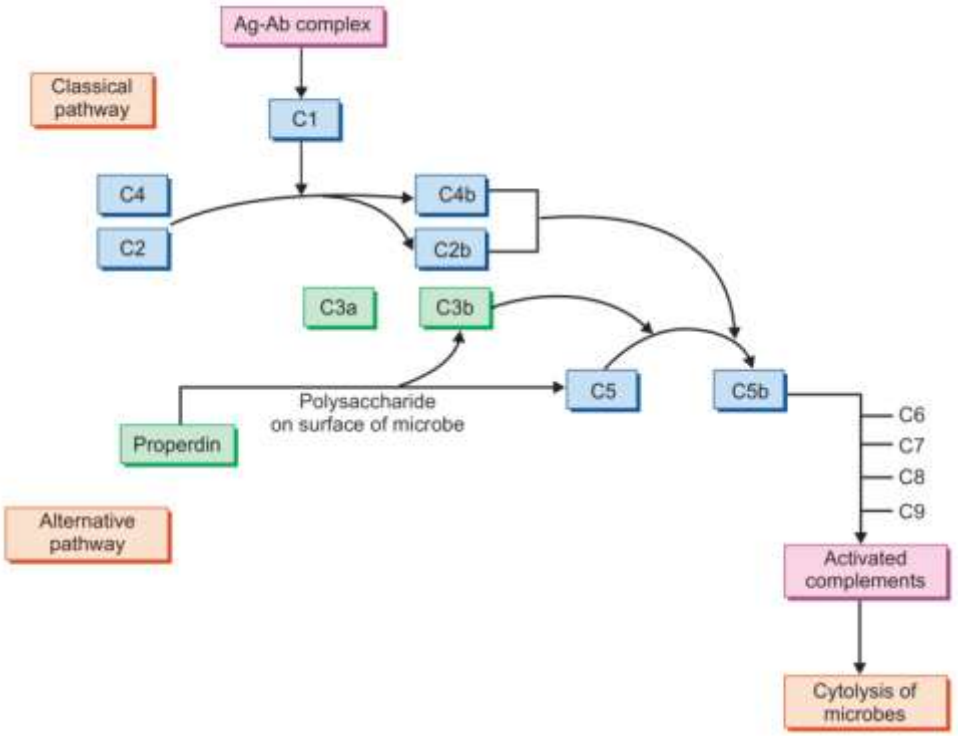
2. Memory B cells, which remain dormant until the antigen reenters the body. Then they launch a more rapid and more powerful secondary immune response.

Mechanism of Action of Antibodies:

A.Direct attack of the antigen: Binding of antigen to its antibody causes:

1. Agglutination of bacteria, i.e., the antigens clumps together.
2. Precipitation of the antigens, i.e., when soluble antigens are bound to antibodies, they become insoluble and precipitate.
3. Lysis: Antibodies directly attack the cell membrane of bacteria, causing their rupture.
4. Opsonization: Some antibodies coat the microorganisms and make them "tasty" for Phagocytosis by neutrophils and macrophages.

B. activation of the complement system: There is a group of plasma proteins designated as complement proteins as they complement the effects of antibodies in destroying antigens.



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