HEMATOLOGY

(HEM-210)

Human Anatomy and Embryology

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Level: 3

2025-2026

LYMPHATIC SYSTEM

SPLEEN, THYMUS GLAND AND LYMPH NODES

Learning Objectives:

1. Describe anatomy of lymphatic system; definitions, end of lymphatic system, and sites of body have not lymph vessels.
2. -Describe anatomy of the spleen
3. Describe anatomy of the thymus gland
4. Describe anatomy of the lymph nodes
5. What is the applied anatomy of the lymph nodes?

Lymphatic System

- Essentially a drainage system.
- There is no circulation.
- Essential for immunologic defences of the body against bacteria and viruses, absorption and transport of dietary fat.

Lymphatic (Lymphoid) Tissues:

- Type of connective tissue that contains large amounts of lymphocytes

Sites that produce lymphocytes:

1. Thymus
2. Lymph nodes
3. Spleen
4. Lymphatic nodules aaggregated in walls of digestive tract.
6. Myeloid tissue in red bone marrow

Lymphatic Vessels:

- Tubes that assist cardiovascular system in removal of tissue fluid from tissue spaces of the body then return the fluid to the blood.

Lymphatic vessels are present in all tissues and organs of the body EXCEPT:

1. Central nervous system

2. Eyeball
3. Internal ear
4. Epidermis of the skin
5. Cartilage
6. Bone

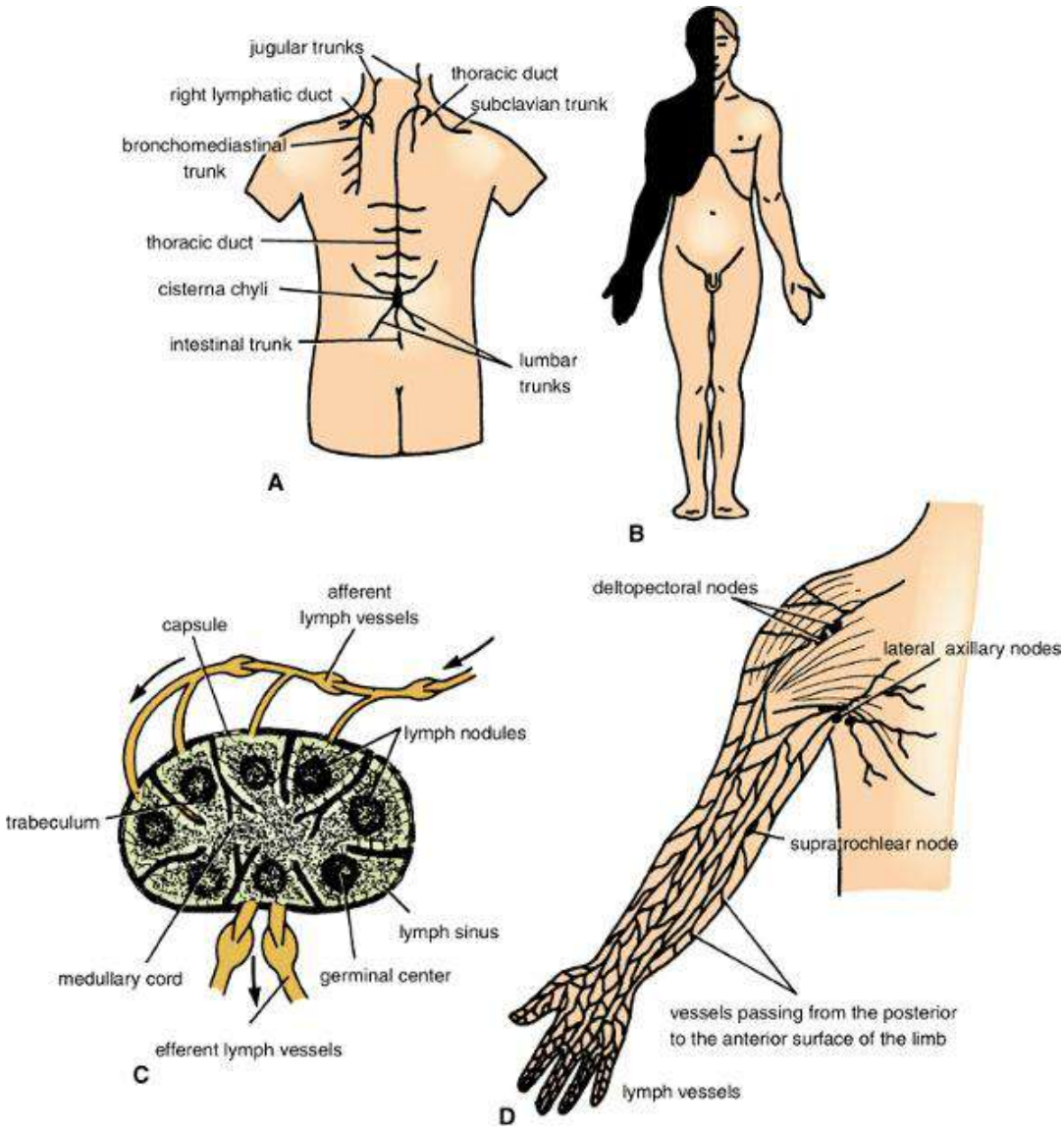


Figure 1: A. The thoracic duct and right lymphatic duct and their main tributaries. **B.** The areas of body drained into thoracic duct (clear) and right lymphatic duct (black). **C.** General structure of a lymph node. **D.** Lymph vessels and nodes of the upper limb. (Snell, 2019).

Lymph: Tissue fluid once it enters a lymphatic vessel.

Lymph Capillaries: Network of fine vessels that drain lymph from the tissues

- They are drained by small lymph vessels, which unite to form large lymph vessels.
- Lymph vessels have beaded appearance because of presence of valves along their course.

Lymph Nodes: Small masses of lymphatic tissue located along course of lymphatic vessels through it lymph is filtered on its way to venous system.

Afferent Lymph Vessels: Lymph vessels carry lymph to a lymph node.

Efferent Lymph Vessels: Lymph vessels that transport lymph away from a lymph node.

Right Lymphatic Trunk: Ends into junction of right venous angle.

- Drains lymph from body's right upper quadrant.

Thoracic Duct: Drains lymph from remainder of the body.

- Ends into left venous angle (angle between left internal jugular and left subclavian veins).

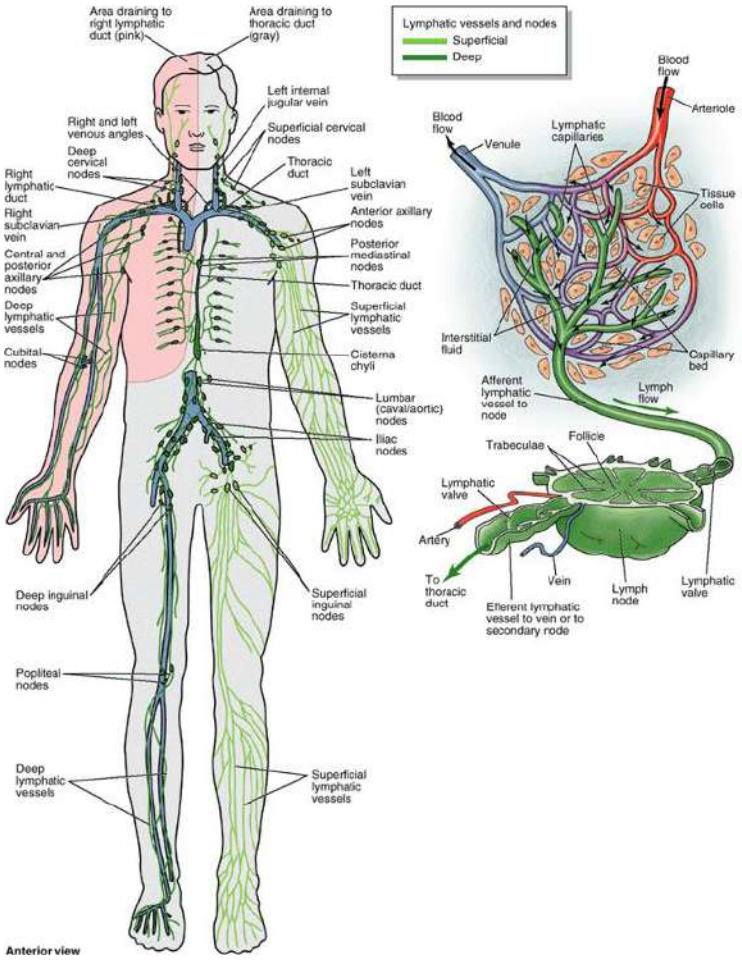


Figure 2: Lymphatic system. Black arrows indicate flow of interstitial fluid out of blood vessels and (absorption) into the lymphatic capillaries. (Moore, et al., 2023).

THORACIC DUCT

- About 45 Cm long.
- Has many valves and beaded appearance.
- Main lymphatic duct in the body.

Drains lymph vessels from all the body except:

1. Right side of head and neck.
2. Right upper limb.
3. Right side of chest.
4. Upper surface of right lobe of the liver.

Beginning:

- In the abdomen.
- Behind right crus of diaphragm.
- As dilated sac; cisterna chyli.
- At level of L1, 2.

Course and relations of thoracic duct:

- Ascends through aortic opening of diaphragm, on right side of descending thoracic aorta.
- Crosses median plane behind oesophagus to reach its left border.

At lower border of body of 4th thoracic vertebra (sternal angle):

- Runs upward along left edge of oesophagus to enter root of neck.
- Bends laterally behind carotid sheath and in front vertebral vessels.

At level of 7th cervical vertebra:

- Turns downward in front of phrenic nerve.
- Crosses 1st part of left subclavian artery.

End of thoracic duct:

- In the neck.
- Beginning of left brachiocephalic vein.

Thoracic duct at root of the neck receives:

1. Left jugular lymph trunk.
2. Left subclavian lymph trunk.
3. Left broncho-mediastinal lymph trunk.

Thoracic duct conveys to blood lymph from:

1. Lower limbs
2. Pelvic cavity
3. Abdominal cavity
4. Left side of thorax
5. Left side of head and neck
6. Left upper limb

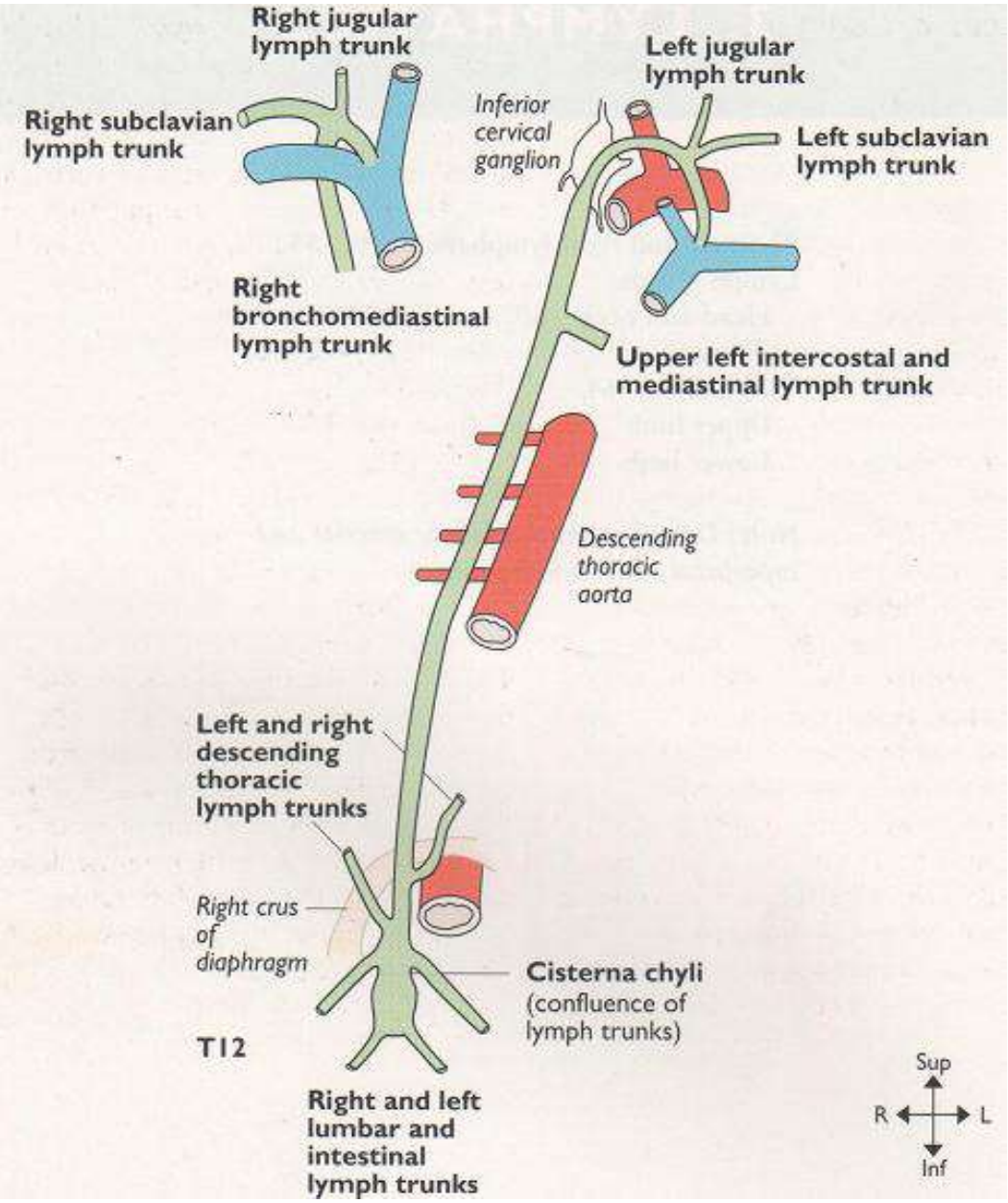


Figure 3: Thoracic duct; beginning, end and relations.

RIGHT LYMPHATIC TRUNK

- About ½ inch (1.25 Cm) long.

Formed by union of:

1. Right jugular lymph trunk.
2. Right subclavian lymph trunk.
3. Right broncho-mediastinal lymph trunk.

Drains:

1. Right side of head and neck.
2. Right upper limb.
3. Right side of thorax.

End: Beginning of right brachiocephalic vein.

SPLEEN (1, 3, 5, 7, 9, 11)

- 1 inch thick, 3 inches wide, 5 inches long, 7 ounces weight and 9-11 ribs relation.
- Reddish in colour.
- The largest single mass of lymphoid tissue in the body.
- Oval in shape.
- Lies just below left half of diaphragm close to the 9^{th}, 10^{th} and 11^{th} ribs.
- The long axis: Lies along shaft of the 10^{th} rib.
- Its lower pole extends forward only as far as mid-axillary line.
- Can not be palpated on clinical examination.
- Has notched anterior border.
- Surrounded by peritoneum which passes from its hilum as gastrosplenic ligament to greater curvature of the stomach (carrying short gastric and left gastroepiploic vessels).
- Peritoneum also passes to left kidney as lienorenal ligament (carrying splenic vessels and tail of pancreas).

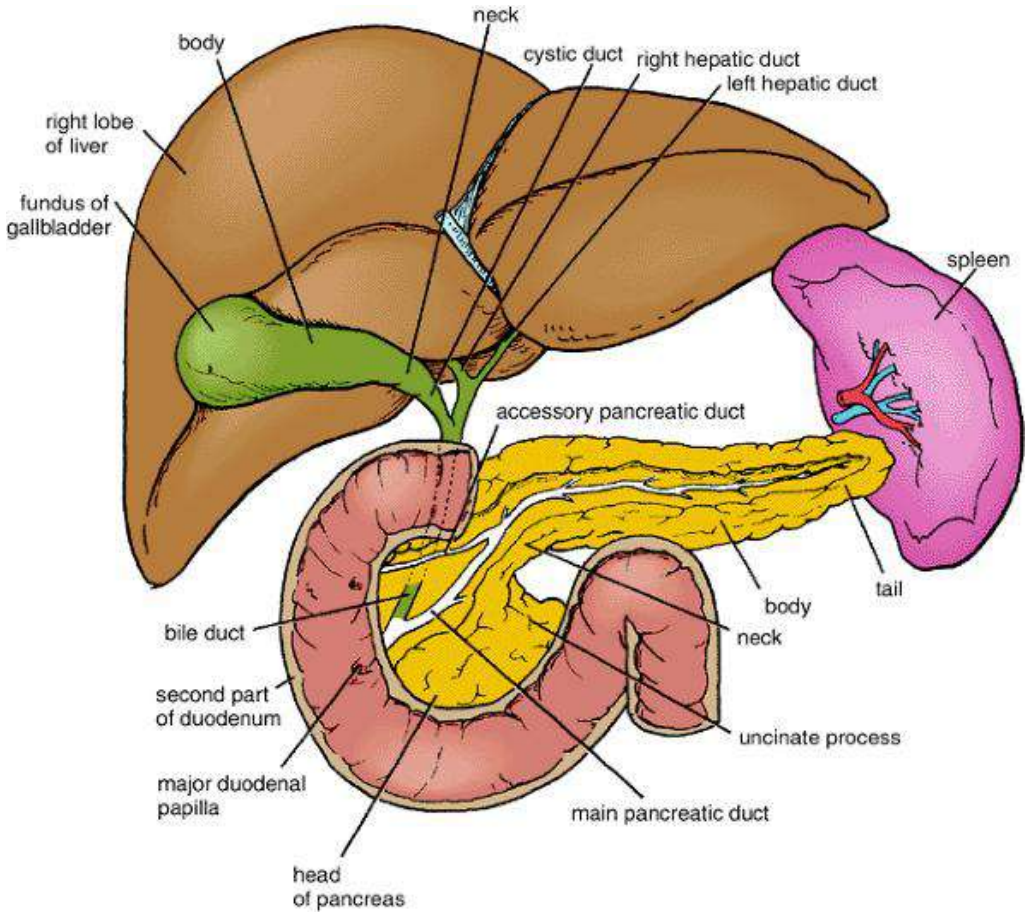


Figure 5: Relation of spleen to rail of pancreas. (Snell, 2019).

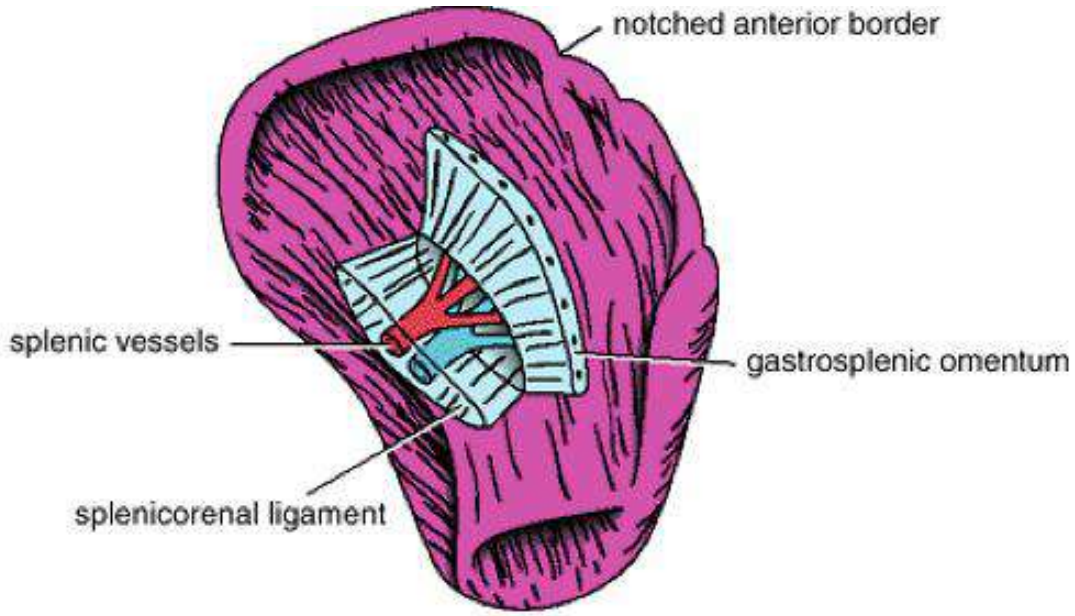


Figure 6: Morphology of the spleen. (Snell, 2019).

Relations of Spleen:

Anteriorly (visceral surface):

1. Stomach.
2. Tail of pancreas.
3. Left colic flexure.
4. Left kidney.

Posteriorly (Diaphragmatic Surface) of Spleen:

1. Diaphragm
2. Left pleura (left costo-diaphragmatic recess)
3. Left lung
4. Left 9th, 10th and 11th ribs
5.

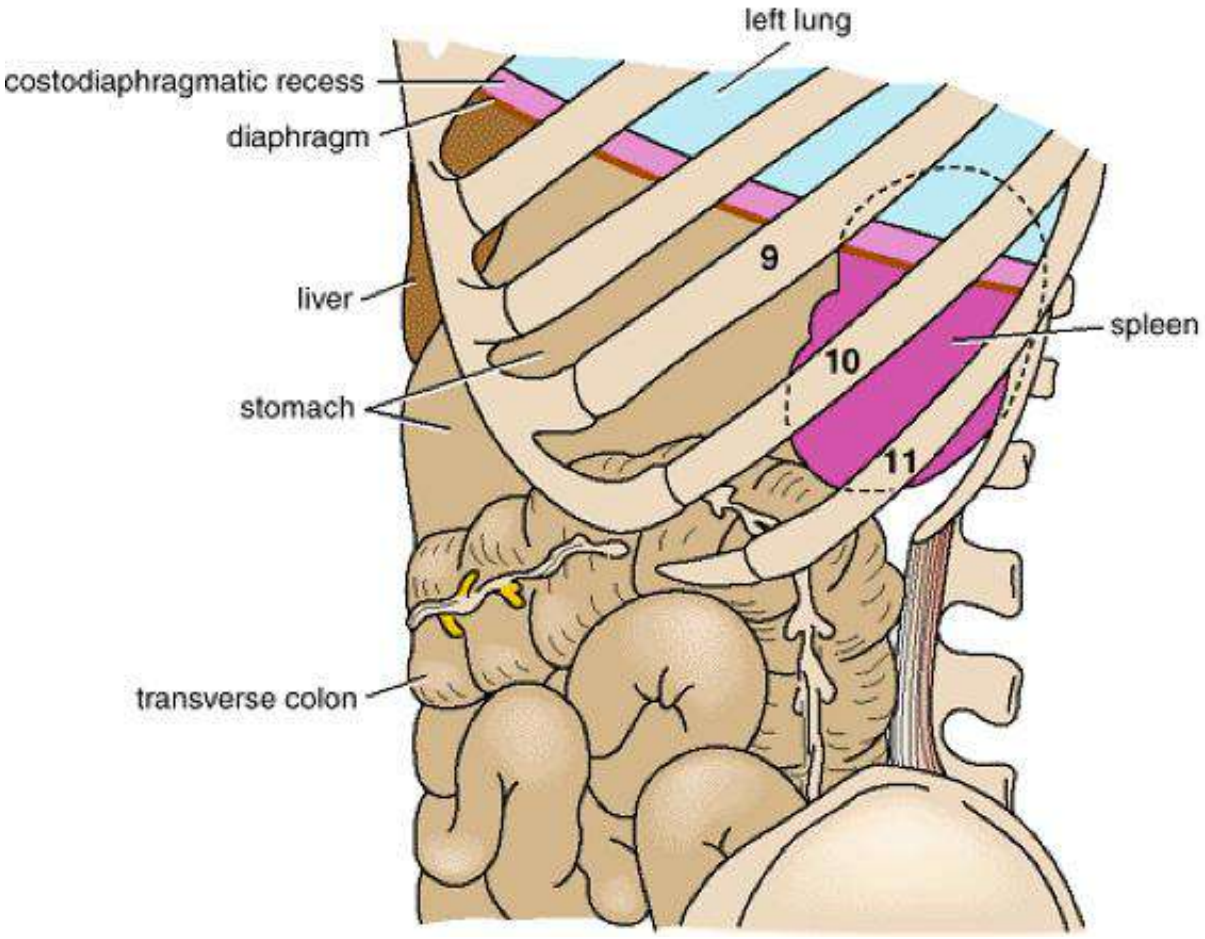


Figure 7: Relations of the spleen. (Snell, 2019).

Blood, Lymph and Nerve Supply of the Spleen:

Arteries: Celiac trunk, splenic artery: enter spleen at the hilum

Veins: Portal vein

- Splenic vein leaves the hilum and runs behind tail and body of pancreas to join superior mesenteric vein behind neck of pancreas to form portal vein.

Lymph Drainage: Lymph vessels emerge from hilum and pass through lymph nodes along course of splenic artery and drain into celiac nodes.

Nerve Supply: Accompany splenic artery and derived from celiac plexus.

THYMUS GLAND

- Primary lymphoid organ.
- Flattened, bilobed structure, no main hilum.
- Lying between sternum and pericardium in anterior mediastinum.

Thymus gland in newborn infant:

- Has largest size relative to size of the body.
- Extend up into superior mediastinum front great vessels into root of the neck.
- Continues to grow until puberty but later undergoes involution.
- Pink, lobulated appearance.
- Site for development of T (thymic) lymphocytes (immunity).

Arterial supply of thymus gland:

1. Inferior thyroid artery. 2. Internal thoracic artery. 3. Superior thyroid artery.

Venous drainage of thymus gland:

1. Left brachiocephalic vein. 2. Internal thoracic veins. 3. Inferior thyroid veins.

Lymphatic drainage of thymus gland: No afferent lymphatics

Efferent vessels end into following nodes:

1. Brachiocephalic 2. Tracheobronchial 3. Parasternal

Nerve supply of thymus gland:

1. Sympathetic chain via cervico-thoracic (stellate) ganglion and vagus nerve (ANS).
2. Branches from phrenic and descending cervical nerves to its capsule (sensory).

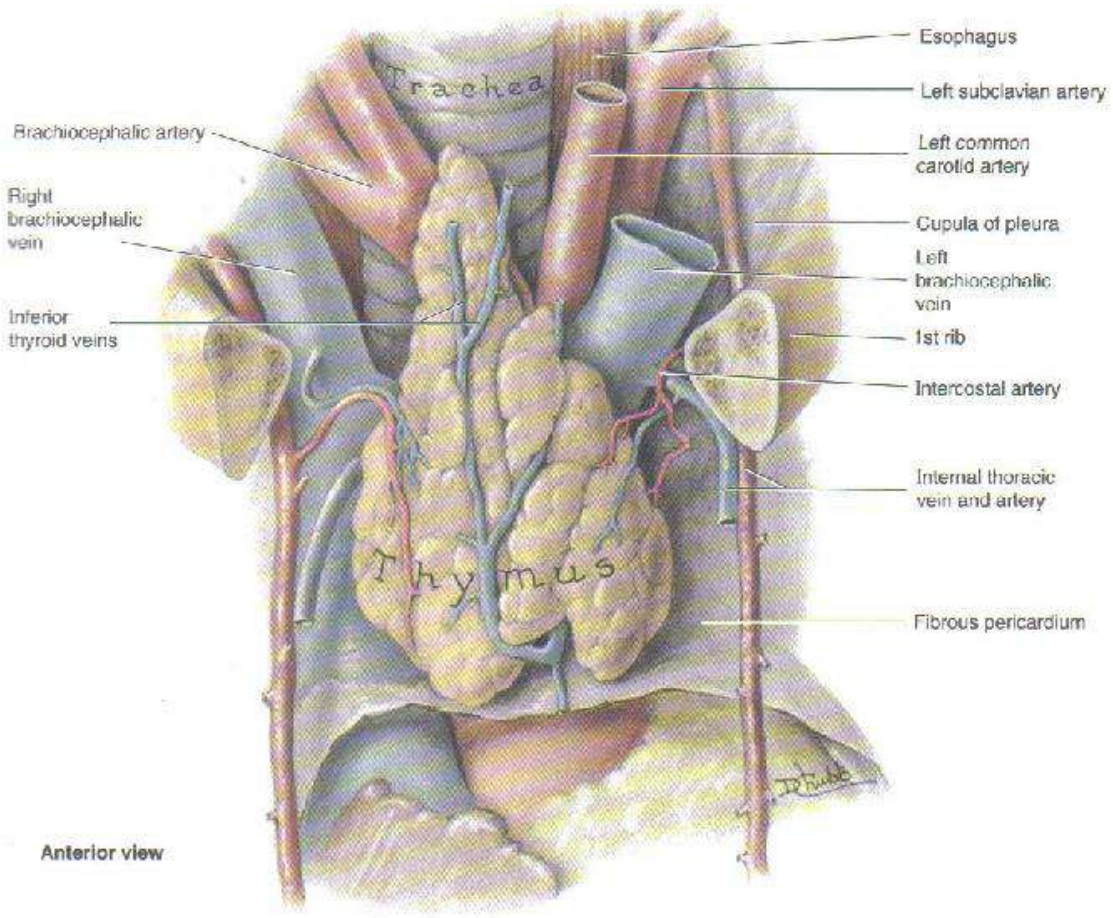


Figure 8: Relations of the thymus gland. (Moore, et al., 2023).

LYMPH NODES

- Encapsulated centres of antigen presentation and lymphocyte activation, differentiation and proliferation.
- Generate mature, antigen-primed, B and T cells, and filter particles, including microbes, from the lymph by the action of numerous phagocytic macrophages.
- Normal young adult body contains up to 450 lymph nodes, of which 60–70 are found in the head and neck, 100 in the thorax and as many as 250 in the abdomen and pelvis.
- Lymph nodes are particularly numerous in the neck, mediastinum, posterior abdominal wall, abdominal mesenteries, pelvis and proximal regions of the limbs (axillary and inguinal lymph nodes).
- The greatest number lie close to the viscera, especially in mesenteries.

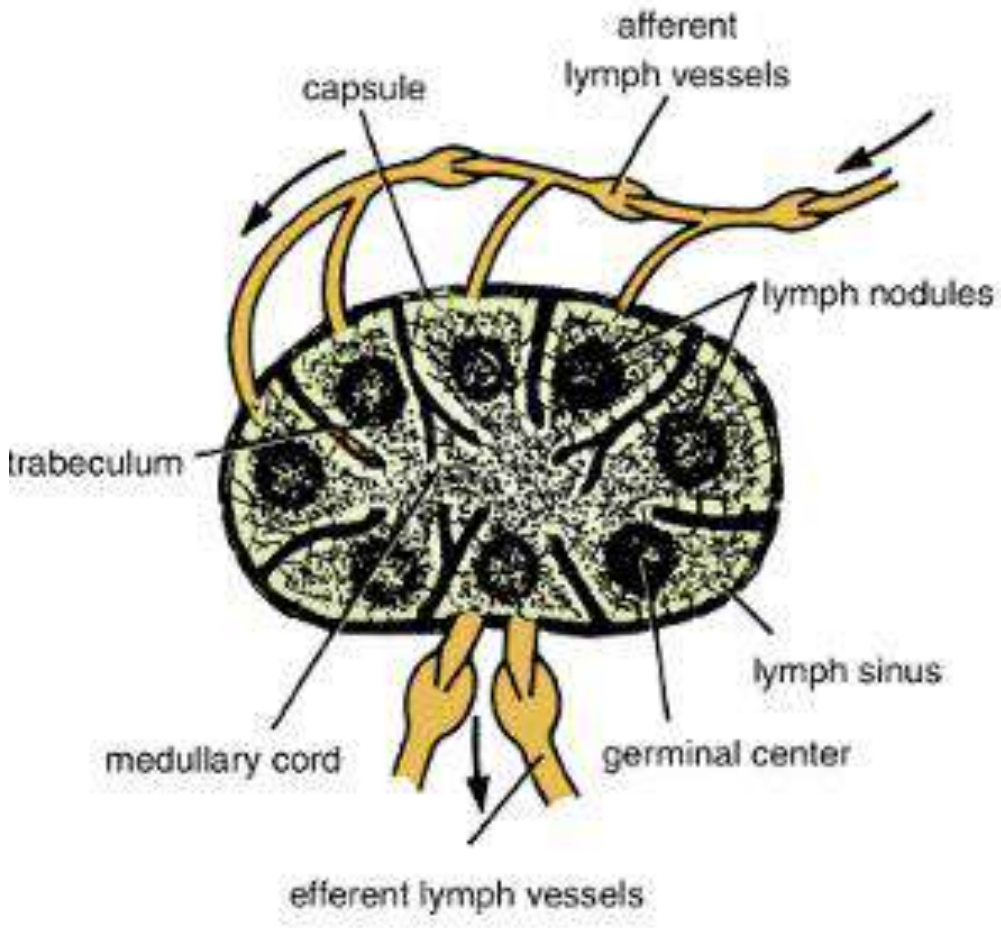


Figure 9: Lymph nodes, afferent and efferent lymph vessels. (Snell, 2019).

Lymphatic and Vascular Supply:

Lymph Vessels:

- Carry lymph to a lymph node through the cortex are referred to as afferent vessels; and that transport it away from a node through the hilum are efferent vessels.

Arteries and veins:

- Pass through the hilum, giving off straight branches which traverse the medulla, and sending out minor branches.
- In the cortex, arteries form dense arcades of arterioles and capillaries in anastomosing loops, eventually returning to highly branched venules and veins.
- Veins leave a node through its principal trabeculae and capsule and drain them and the surrounding connective tissue.

Lymph Nodes (Applied):

- May swell as the result of metastases, or primary tumour.
- For this reason, lymphatic drainage of all major organs of the body, including the skin, should be known.

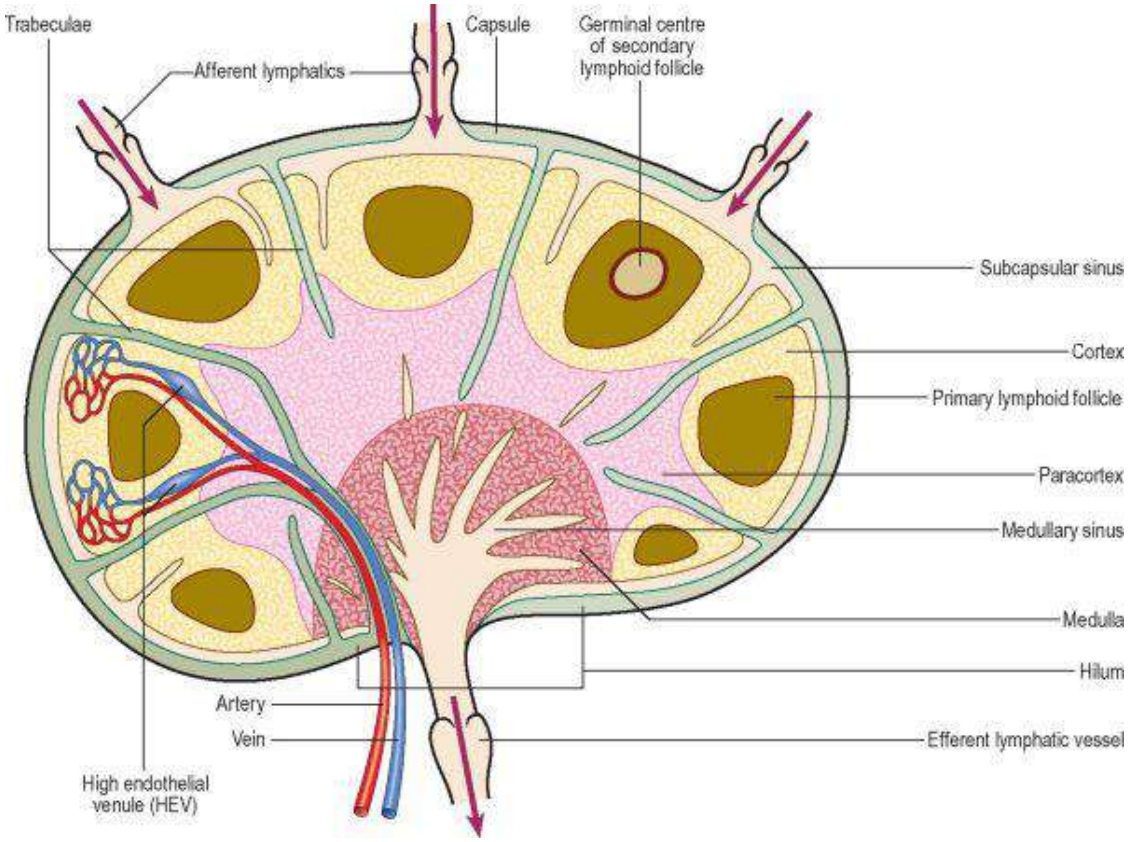
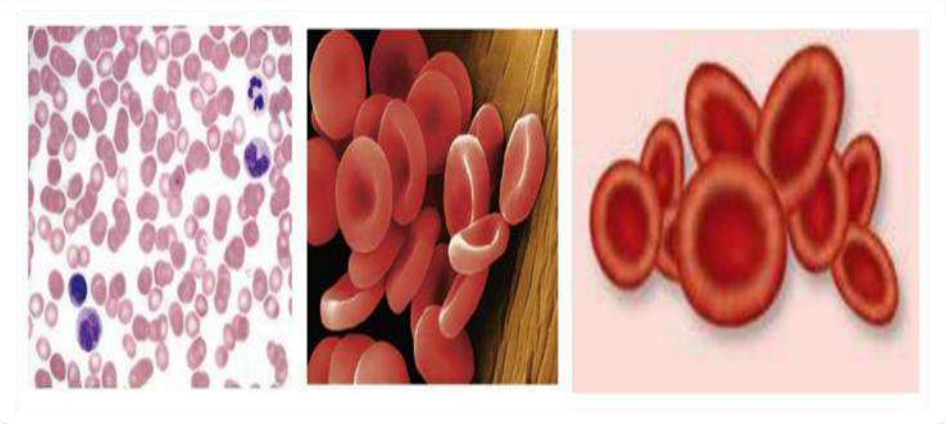


Figure 10: Lymph nodes, afferent and efferent lymph vessels, arteries and veins.

References:

1. Snell, R. S. (2019): Clinical Anatomy by Regions (Anatomy) 9th Edition. Lippincott Williams & Wilkins.
2. Moore, Keith L. and Dalley A. F. (2023): Clinically Oriented Anatomy, 9th Edition, Lippincott Williams & Wilkins.
3. KAPLAN Medical USMLE Step 1 Lecture Notes Anatomy (2021).



BLOCK HEM-210

Haematology - Lymphatic System

Histology and cell biology department

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Level: 2

2025-2026

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BLOCK HEM-210 Haematology - Lymphatic System

Lecture (1)

Learning Objectives

After this lecture, students should be able to:

- Describe the cytological characteristics of Blood Elements (Red Blood Cells, White Blood Cells, Platelets).
- Differentiate between the different types of White Blood Cells.
- Differentiate between Red Blood Cells and White Blood Cells.

Blood

Blood is a specialized type of Connective Tissue formed of blood cells in a fluid matrix (plasma).

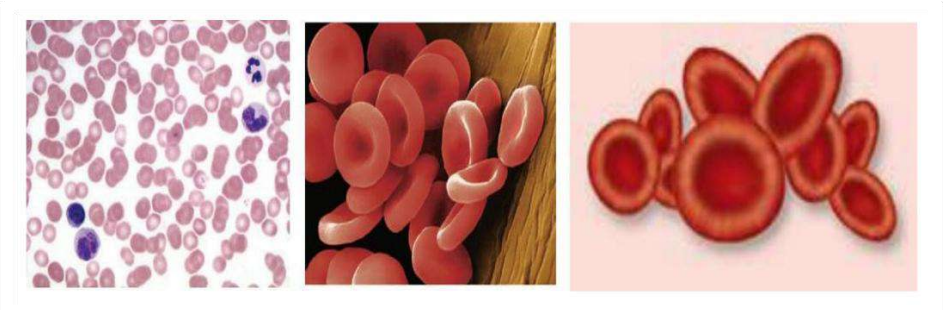
- **Blood cells (45%)** are of three types:
    - Erythrocytes (Red Blood Cells): 4-6 million per cubic millimeter.
    - Leucocytes (White Blood Cells): 4-11 thousand per cubic millimeter.
    - Thrombocytes (platelets): 150-400 thousand per cubic millimeter.
- **Plasma (55%)** is formed of water, gases, inorganic substances, organic substances, hormones and enzymes. The volume of blood is about 5 liters.

Functions of the Blood

- Transport of oxygen, nutrients and hormones to tissues.
- Removal of carbon dioxide and waste product from tissues.
- Regulation of body temperature.
- Regulation of acid-base balance.
- Protection against infections.

Red Blood Corpuscles (Red Blood Cells) or Erythrocytes

- **Light Microscopy:** they are rounded biconcave discs (with pale thin center and dark thick periphery).
- **Electron Microscopy:** they are corpuscles and not true cells (as they have neither nuclei nor organelles) so cannot divide.



Adaptation of Red Blood Corpuscles for Their Function

1. **They contain free spaces for:**
    - Hemoglobin (33%): to combine easily with oxygen and carbon dioxide.
    - Enzymes (1%): Hemoglobin reductase (for oxygen) and carbonic anhydrase (for carbon dioxide).
2. **The cell membrane is:**
    - Biconcave (to increase their surface area for gas exchange).
    - Formed of lipoprotein (to be highly selective for oxygen and carbon dioxide exchange).
    - Elastic and flexible (to allow their squeeze inside narrow capillaries).

- Osmotic pressure of Red Blood Cells and plasma is isotonic (0.9% saline) and their life span is about 4 months.
- Old Red Blood Cells are phagocytosed in liver and spleen (after that iron is reused while pigments are excreted).

Normal Number of Red Blood Cells

- In Female: 4.5-5 million per cubic millimeter (due to menstruation and hormones).
- In Male: 5-5.5 million per cubic millimeter.

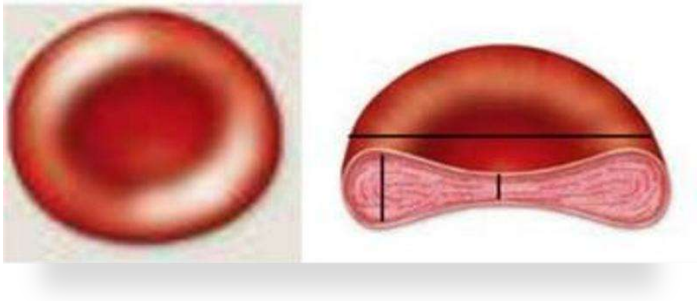
Abnormalities

- **Anemia** (Red Blood Cells less than 4 million per cubic millimeter): it has many types:
    - **Deficiency anemia:** due to deficiency of iron, vitamin B12, copper, proteins.
    - **Hemolytic anemia:** due to destruction of Red Blood Cells which may be:
        - **Congenital:** Abnormal cell membrane (spherocytosis). Decrease of G6PD enzymes (favism). Abnormal Hemoglobin F (thalassemia). Abnormal Hemoglobin S (sickle cell anemia).
        - **Acquired:** incompatible blood transfusion, malaria, toxins.
    - **Hemorrhagic anemia:** due to hemorrhage from wound, nose, menses, piles.
    - **Aplastic anemia:** due to bone marrow depression by radiation, drugs.
- **Polycythemia** (Red Blood Cells more than 6 million per cubic millimeter): occurs with hypoxia (decreased oxygen) whether physiological (high altitude and exercise) or pathological (lung disease or heart disease).

Size of Red Blood Cells

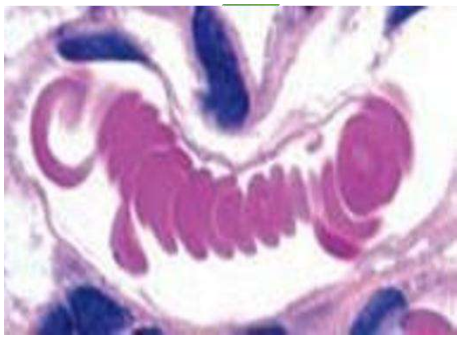
- **Normal size:** diameter 6-9 (average 7.5) micrometers. Central thickness 0.75 micrometers. Peripheral thickness 2.6 micrometers.
- **Abnormal size:**
    - Increased diameter (macrocytic anemia).
    - Decreased diameter (microcytic anemia).

- Different diameters (anisocytosis).



Shape of Red Blood Cells

- **Normal shape:** rounded biconcave non-nucleated discs showing rouleaux appearance (as rows of coins).



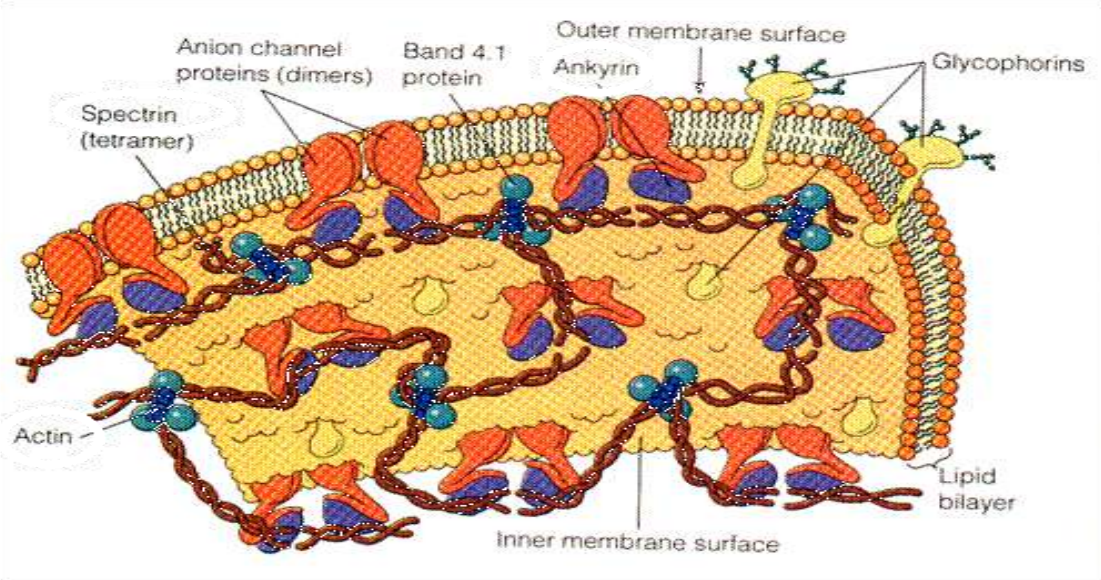
- **Abnormal shape:** Rounded biconvex (sphero-cytosis). Oval (ovalo-cytosis). Pear-shaped (poikilo-cytosis).

Color of Red Blood Cells

- **Normal color:** hemoglobin is greenish yellow (if unstained) and acidophilic (if stained by Leishman stain).
- **Abnormal color:**
  - Increased hemoglobin (hyper-chromic Red Blood Cells).
  - Decreased hemoglobin (hypo-chromic anemia).
  - Central hemoglobin (target cell anemia).

Additional Notes on Red Blood Cells

1. The biconcave disc appearance increases the surface area by about 25% that increases the respiratory efficiency.
2. Erythrocytes are normally quite flexible, which permits them to bend and adapt to the small diameters and irregular turns of capillaries. Several cytoskeletal proteins (ankyrin, spectrin, and actin) stabilizes the membrane, maintains the Red Blood Cell's shape, and provides its elasticity.
3. The glycosylated extracellular domains of integral proteins in the Erythrocyte cell membrane (glycophorins) include antigenic sites for the ABO blood typing system. Another type of antigen is present in 85% of individuals which is the Rhesus antigen (D antigen) and is called Rhesus positive, while the remaining 15% have no antigen and are called Rhesus negative.



Blood Groups

Blood groups are important for blood transfusion and for medico-legal applications. There are 4 main types of blood groups:

1. **Group A:**
    - Genotype: A1A1, A2A2, A1O or A2O.
    - Phenotype: antigen A on Red Blood Cells and anti-B antibody in plasma.
2. **Group B:**
    - Genotype: BB or BO.
    - Phenotype: antigen B on Red Blood Cells and anti-A antibody in plasma.
3. **Group AB:**
    - Genotype: A1B or A2B.
    - Phenotype: antigens A and B on Red Blood Cells without antibodies in plasma (universal recipient).
4. **Group O:**
    - Genotype: OO.
    - Phenotype: no antigens on Red Blood Cells (universal donor) with anti-A and anti-B antibodies in plasma.

There are other less important blood subgroups as MNSs, P, Duffy, Lewis, Kell and Rhesus.

Blood Type	Donate Blood To	Receive Blood From
A+	A+ AB+	A+ A- O+ O-
O+	O+ A+ B+ AB+	O+ O-
B+	B+ AB+	B+ B- O+ O-
AB+	AB+	Everyone
A-	A+ A- AB+ AB-	A- O-
O-	Everyone	O-
B-	B+ B- AB+ AB-	B- O-
AB-	AB+ AB-	AB- A- B- O-

Rhesus (RH) Factor

Rhesus factor is an antigen present on Red Blood Cells of 85% of individuals (Rhesus positive persons). It is important for females because a positive fetus may die by hemolysis (erythroblastosis fetalis) if his negative mother was sensitized by previous exposure to positive Red Blood Cells.

White Blood Cells (Leucocytes)

- White Blood Cells are true cells (as they have nuclei, organelles and inclusions).
- They are colorless (devoid of hemoglobin) but appear white when packed together.
- They have amoeboid movement to penetrate capillaries and perform their phagocytic function in Connective Tissue.

Number of White Blood Cells

- **Normal number:** White Blood Cells count 4-11 thousand per cubic millimeter and around 16 thousand per cubic millimeter at birth.
- **Abnormal number:**
    - **Leucopenia** (White Blood Cells less than 4 thousand per cubic millimeter): occurs with typhoid fever, influenza viral infection, some drugs and radiation.
    - **Leukocytosis** (White Blood Cells more than 11 thousand per cubic millimeter): which may be physiological (pregnancy, newborn, cold bath) or pathological (acute and chronic infections).

Types of White Blood Cells

According to types of granules, leucocytes are classified to:

1. **Granular leucocytes** (with specific and non-specific granules): basophils, eosinophils and neutrophils.
2. **Non-granular leucocytes** (with only non-specific granules): monocytes and lymphocytes (T and B).

Differential Leucocytic Count

The percentage of each type of leucocytes relative to total leucocytic count is:

- **Basophils:** 00 - 0.75 %
- **Eosinophils:** 01 - 03 %
- **Neutrophils:** 57 - 67 %
- **Monocytes:** 3 - 7 %
- **Lymphocytes:** 25 - 33 %

	count (/ ^{3}mm)	diameter (µm)	life span	nucleus	specific granules
RBCs	4-6 million	7.5 µ	4 months	absent	absent
platelets	150-400 th.	2-4 µ	5-10 days	absent	present
WBCs	4-11 th.	6-18 µ	day-years	present	may be

Differential leucocytic count (percentage of each type of leucocytes relative to total leucocytic count):

basophils	00 -0.75 %	12-15 µ	months	S-shape	histamine
eosinophils	01-03 %	12-16 µ	1-2 weeks	bi-lobed	histaminase
neutrophils	57-67 %	12-15 µ	1-4 days	2-5 segments	collagenase
monocytes	3-7 %	14-18 µ	days-years	kidney-shape	non
lymphocytes	25-33 %	06-18 µ	hours-ys	1 segmnt	non
▪ small	▪ 15-20 %	▪ 06-09 µ		▪ dark	
■ large	05-10 %	09-18 µ		■ pale	

T-lymphocytes count 60-80 % of all lymphocytes and can life 2 years while o B-lymphocytes count 25-30 % of all lymphocytes and can life 3 months

Counting of Blood Cells

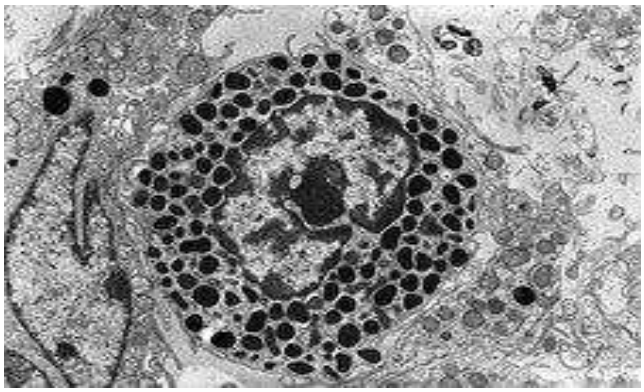
- Counting of Red Blood Cells, White Blood Cells and platelets are made by Haemocytometer that is formed of: diluting pipette of Red Blood Cells, diluting pipette of White Blood Cells and counting slide.
- Differential leucocytic count is made by Examination of blood film (stained by neutral Leishman stain).

Basophils (0-0.75% of Leucocytes)

Concerned with allergy production.

- **Diameter:** 12-15 micrometers. **Life span:** months.
- **Light Microscopy:** Nucleus of basophil is single, irregular and S-shaped.

- **Electron Microscopy Granules:**
    1. Primary non-specific azurophillic granules (primary lysosomes).
    2. Secondary specific basophilic large granules as that of mast cells (containing histamine, heparin and eosinophil chemotactic factor). These granules mask nucleus and can be stained by Giemsa.

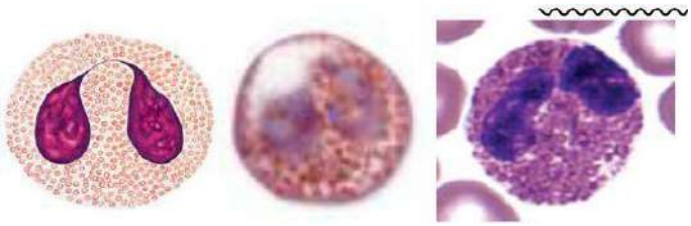


- **Functions:** Production, storage and secretion of histamine (capillary vasodilator released during allergy), heparin (anticoagulant) and eosinophil chemotactic factor (that attract eosinophils to terminate allergy).
- **Basophilia** (increase number of basophils): It occurs in allergic diseases, parasitic infestations, liver cirrhosis.

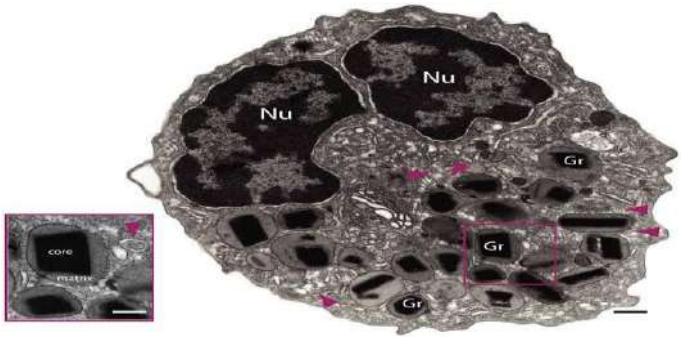
Eosinophils (1-3% of Leucocytes)

Concerned with allergy termination.

- **Diameter:** 12-16 micrometers. **Life span:** 1-2 weeks.
- **Light Microscopy:** Nucleus of eosinophil is single, horseshoe and bilobed.



- **Electron Microscopy Granules:**
    1. Primary non-specific azurophillic granules (primary lysosomes).
    2. Secondary specific acidophilic large granules (containing histaminase and sulphatase).

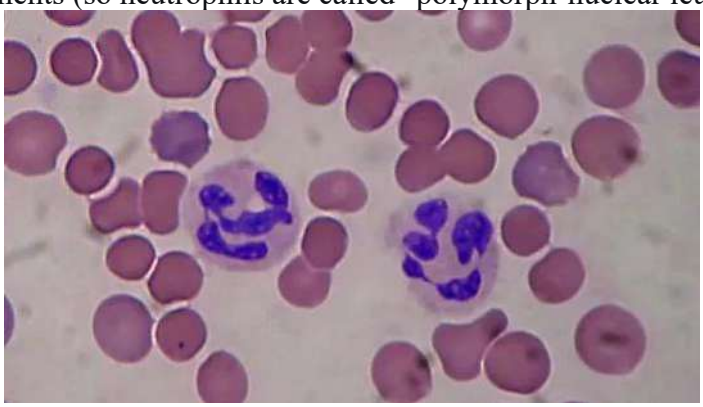


- **Functions:**
    - Attracted to allergic sites (by eosinophil chemotactic factor of basophils or mast cells) to terminate allergy by destruction of histamine and phagocytosis of allergic antigen-antibody complexes.
    - Inactivate and kill parasitic larvae.
- **Eosinophilia** (increase number of eosinophils): It occurs in allergic diseases and parasitic infestations.
- **Eosinopenia** (decrease number of eosinophils): It occurs with cortisone therapy that inhibits bone marrow.
- **Note:** Eosinophils are present under skin and mucosa of respiratory, intestinal and genital tracts.

Neutrophils (57-67% of Leucocytes)

Concerned with non-specific immunity.

- **Diameter:** 12-15 micrometers. **Life span:** 1-4 days.
- **Light Microscopy:** Nucleus of neutrophil is single and formed of connected 2-5 segments (so neutrophils are called "polymorph-nuclear leucocytes").

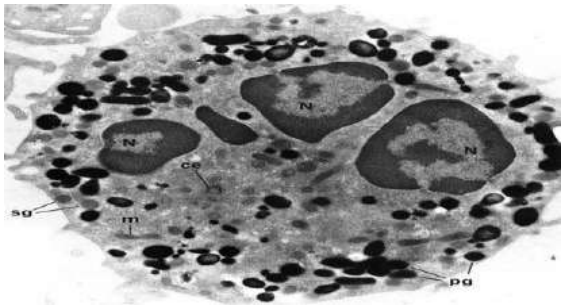


- **Classification:**
    - By Schilling count: immature with non-segmented nuclei (4%) and mature with segmented nuclei (96%).
    - By Arneth count: class I, II, III, IV and V with 1, 2, 3, 4 and 5 segments in their nuclei respectively.

Class I	1 segment	5%
Class II	2 segments	35%
Class III	3 segments	41%
Class IV	4 segments	17%
Class V	5 segments	2%

Arneth classification of neutrophils

- **Electron Microscopy Granules:**
    1. Primary non-specific azurophillic granules (primary lysosomes).
    2. Secondary specific pale large granules (containing bactericidal phagocytin, bacteriostatic lactoferrin, collagenase and alkaline phosphatase).



- **Functions: Non-specific immunity (first line of defense):**
    1. They leave blood vessels and enter Connective Tissue (through amoeboid movement) to phagocytose bacteria by pseudopodia.
    2. They destruct bacteria by proteolytic enzymes present in granules (that dissolve bacterial proteins).
    3. They attract monocytes to inflamed areas to remove pus (dead neutrophils, dead bacteria and tissue debris).
    4. They stimulate bone marrow (to form neutrophils).
    5. They secrete trephine substance (that performs healing).
- **Neutrophilia** (increase number of neutrophils): It occurs in appendicitis, tonsillitis, abscess.
- **Neutropenia** (decrease number of neutrophils): It occurs in typhoid fever, drugs, radiation.

Monocytes (3-7% of Leucocytes)

Concerned with non-specific immunity.

- **Diameter:** 14-18 micrometers. **Life span:** 3 days in blood, 3 months in Connective Tissue proper and years outside them.
- **Light Microscopy:** They have large pale kidney-shaped nuclei with pseudopodia.

- **Electron Microscopy:** Few organelles with well-developed Golgi apparatus and many azurophilic granules (lysosomes).
- **Functions:**
    - Change to macrophage (and other mononuclear phagocytic cells) to phagocytose small foreign bodies.
    - Fuse together to form giant cells (as osteoclasts) to phagocytose large foreign bodies.
- **Monocytosis** (increase number of monocytes): It occurs in chronic infections as syphilis, malaria, tuberculosis.

Lymphocytes (25-33% of Leucocytes)

Concerned with specific immunity.

According to Size:

- **Small inactive lymphocytes (15-20% of leucocytes):**
    - Diameter: 6-9 micrometers (like Red Blood Cells).
    - Light Microscopy: they have dark rounded nuclei with pale scanty cytoplasm (forming a thin rim around nucleus).
    - Electron Microscopy: few organelles with many free ribosomes and microvilli.
- **Large active lymphocytes (5-10% of leucocytes):**
    - Diameter: 9-18 micrometers.
    - Light Microscopy: they have pale rounded nuclei with clear nucleolus and dark abundant cytoplasm.
    - Electron Microscopy: many mitochondria, many rough endoplasmic reticulum and well developed Golgi apparatus.

Function of Lymphocytes: Specific immunity (which is the second line of defense mechanism).

Note: Lymphocytes can pass through oral epithelium and appear in oral cavity as "salivary corpuscles".

Types of Lymphocytes

1. **B-lymphocytes (25-30% of circulating small lymphocytes):**
    - **Life span:** 3 months.
    - They are bursa dependent as they develop in bursa of Fabricius (of birds) and in bone marrow (of human).
    - **Origin and Function:** Originate from B-type colony forming cells of bone marrow → differentiate to B-lymphoblasts then B-lymphocytes → acquire surface receptors → migrate to peripheral lymphoid organs as small inactive cells.
    - **Activation:** Antigens are picked by T-helper lymphocytes → delivered to small B-lymphocytes → activation to:
        - Plasma blasts → plasma cells → antibodies (gamma globulins) → primary humoral immunity.
        - B-memory cells → rapid response after further exposure to the same antigen → secondary humoral immunity.
2. **T-lymphocytes (60-80% of circulating small lymphocytes):**
    - **Life span:** 2 years.
    - They are thymus dependent (require thymus gland for development and maturation).
    - **Origin and Maturation:** Originate from T-type colony forming cells of bone marrow → differentiate to T-lymphoblasts → migrate to cortex of thymus gland → proliferate, differentiate to T-lymphocytes, acquire T-cell receptors → migrate as small inactive cells to thymus-dependent zones of lymph nodes and spleen.
3. **Functions of Activated T-Lymphocytes:**
    - **T-cytotoxic cells (T-killer cells):** Perform primary cellular immunity (secrete perforins which lyse foreign cells).
    - **T-memory cells:** Perform secondary cellular immunity against secondary infection.
    - **T-helper cells:** Help in humoral immunity of B-lymphocytes.
    - **T-suppressor cells:** Suppress both humoral and cellular immunity.
    - **T-lymphokines producing cells:** Secrete hormone-like factors (Interferon, cytotoxic factor, chemotactic factor, colony stimulating factor, mitogenic factor).
4. **Natural Killer Lymphocytes (5% of circulating lymphocytes):**
    - **Life span:** 3 months.
    - They have non-B non-T receptors and can kill some tumor cells and infected cells.
    - **Lymphocytosis** (increase number of lymphocytes): It occurs in chronic diseases (as pertussis, tuberculosis, syphilis) and in leukemias.

Comparison Between Red Blood Cells and White Blood Cells

Feature	Red Blood Cells	White Blood Cells
Types	One type	Five types
Number	4-6 million/cubic mm	4-11 thousand/cubic mm
Size	6-9 (7.5) micrometers	6 to 18 micrometers
Shape	Biconcave discs with rouleaux appearance	Spherical without rouleaux appearance
Color	Greenish yellow	Colorless
Osmotic Pressure	Liable to hemolysis	Resist hemolysis
Light Microscopy	Corpuscles: no nuclei, no cell organelles, contain hemoglobin	True cells: contain nuclei, contain cell organelles, not contain hemoglobin
Function	Carrying O2 and CO2 inside blood vessels (no amoeboid movement)	Phagocytosis outside blood vessels (have amoeboid movement)
Development	In red bone marrow	In red bone marrow and in lymphatic tissues
Site	In blood	In blood, in Connective Tissue and in lymphatic tissues
Life span	4 months	Few days to many years

Lecture 2

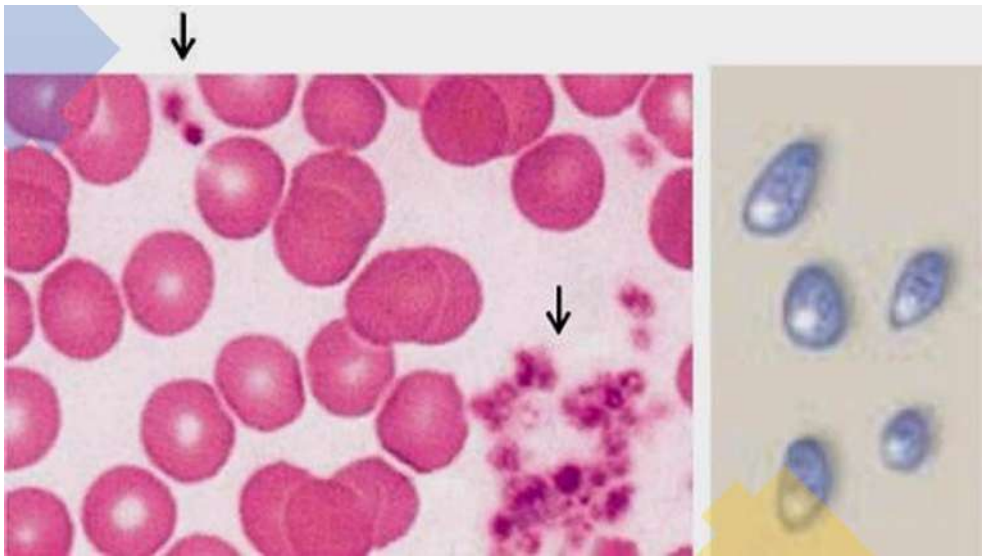
Learning Objectives

After this lecture, students should be able to:

- Describe the platelets.
- Describe the constituents of bone marrow
- Differentiate between 2 types of bone marrow, red and yellow.

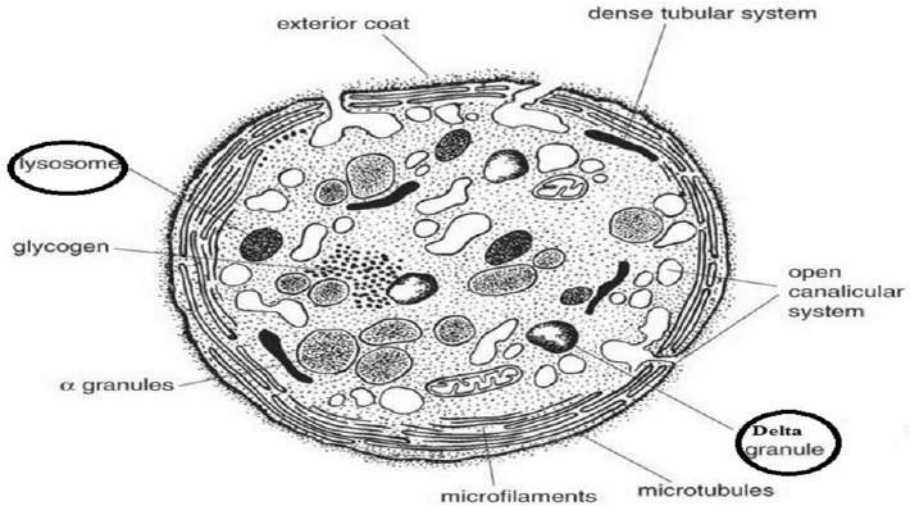
Blood Platelets or Thrombocytes

- Platelets are not true cells (as they have no nuclei and cannot divide).
- **Number:** 150-400 thousand per cubic millimeter.
- **Diameter:** 2-4 micrometers.
- **Life span:** 5-10 days.
- **Light Microscopy:** Small rounded plates formed of peripheral pale hyalomere and central basophilic granulomere.

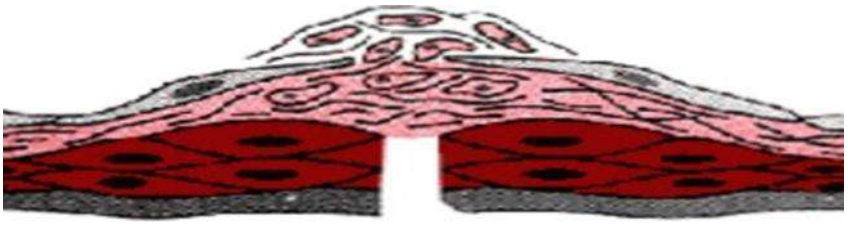


- **Electron Microscopy Structure:**
    - **Peripheral Hyalomere:**
        - Marginal bundles (of microtubules and microfilaments).
        - Open canalicular system (invaginations of cell membrane).
        - Irregular tubular system (endoplasmic reticulum that stores calcium ions).
    - **Central Granulomere:**
        - Alpha granules (300-500 nm): large, contain fibrinogen, coagulation factors, platelet factor 4, platelet-derived growth factor.
        - Beta granules (mitochondria).

- Delta granules (250-300 nm): medium-sized, contain histamine, serotonin, ATP, ADP.
- Lambda granules (lysosomes): contain hydrolytic enzymes.
- Glycogen granules.



- **Functions:**
  1. Blood agglutination: to form white thrombus (platelets + fibrin).
  2. Blood coagulation: to form red thrombus (platelets + fibrin + Red Blood Cells).
  3. Secretion of serotonin (vasoconstrictor).
  4. Clot retraction by contractile microfilaments.

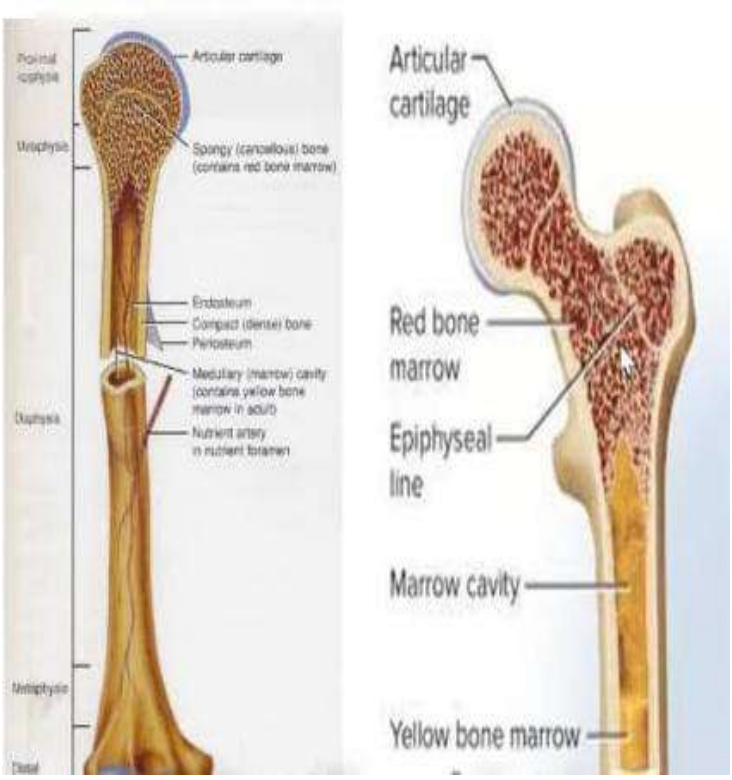


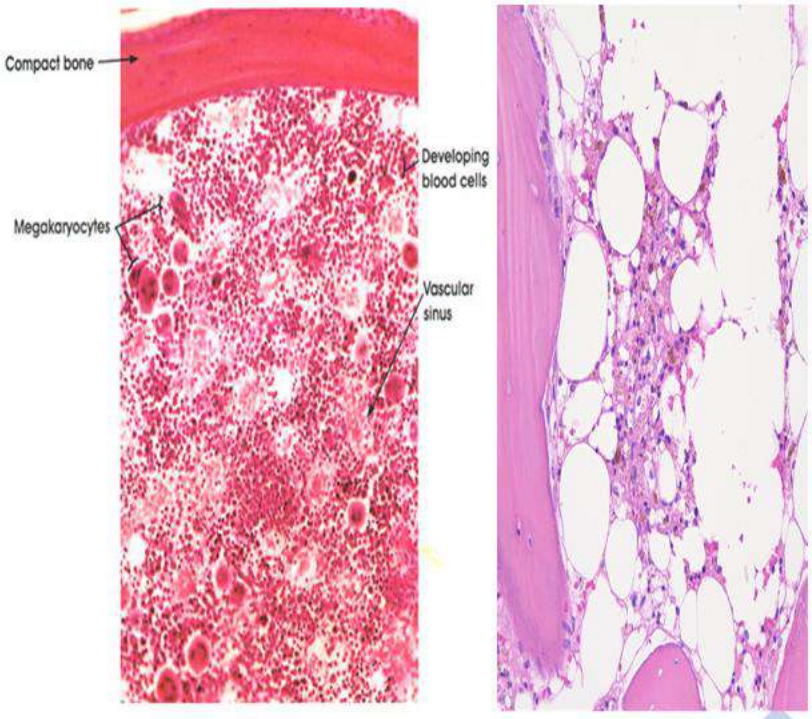
- **Thrombocytopenia** (decrease number of platelets) causes increase in bleeding time.

Bone Marrow

Blood cells are developed in:

1. **Myeloid tissue (bone marrow):** which may be red active bone marrow or yellow inactive bone marrow.
   - **Red active bone marrow:** Present in all bones up to 7 years and in flat bones of adults.
   - **Yellow inactive bone marrow:** Present in long bones of adults and acts as a reserve (may change to red bone marrow in severe blood loss).





2. **Lymphoid tissue:** Lymph node, spleen, tonsils and thymus.

Histological Structure of Red Active Bone Marrow

It is formed of:

- **Stroma** of fixed Connective Tissue cells (reticular cells, fat cells, fixed macrophages, pericytes, endothelial cells and osteogenic cells).
- **Blood sinusoids.**
- **Mature and immature blood cells.**

Note: Myeloid / Erythroid ratio (immature leucocytes / immature erythrocyte) = 5/1 (because life span of Red Blood Cells is greater 5 times than life span of White Blood Cells).

Functions of Red Active Bone Marrow

1. Production of blood cells (depending on stem cells and erythropoietin hormone).
2. Phagocytosis of old Red Blood Cells by bone marrow macrophages.
3. Storage of iron in bone marrow macrophages.

Haematopoiesis

All blood cells are formed in the red active bone marrow from mother stem cells named "pluri-potential hematopoietic stem cells".

- These pluri-potential stem cells give rise to 2 types of stem cells:
    1. **Myeloid stem cells:** can differentiate to erythrocytes, thrombocytes, granulocytes or monocytes.
    2. **Lymphoid stem cells:** can differentiate to lymphocytes only.
- Myeloid stem cells give rise to progenitor cells named "colony forming cells" which are small rounded basophilic cells (8-10 micrometers) with small rounded pale nuclei.
- Progenitor cells differentiate to blasts, pro-cytes, cytes then mature cells.

haemo-poiesis	RBCs	platelets	granulocytes	monocytes	lymphocytes
(1) progenitors	erythrocyte-CFCs	megakaryocyte-CFCs	granulocyte-CFCs	monocyte-CFCs	lymphocyte-CFCs
(2) blasts	3 erythro-blasts	megakaryo-blasts	myelo-blasts	mono-blasts	B or T lymphobastes
(3) pro-cytes	normoblasts	pro-megakaryocytes	pro-myelocytes	pro-monocytes	B or T pro-lymphocytes
(4) cytes	reticulocytes	megakaryocytes	2 BEN myelocytes		B or T small lymphocytes
(5) mature cells	erythrocytes	platelets	BEN	monocytes	B or T large lymphocytes

Erythropoiesis (Development of Red Blood Cells)

- **Depends on:** progenitor cells, erythropoietin hormone, iron, folic acid, vitamin B12 and amino acids.
- **Duration:** 7 days.

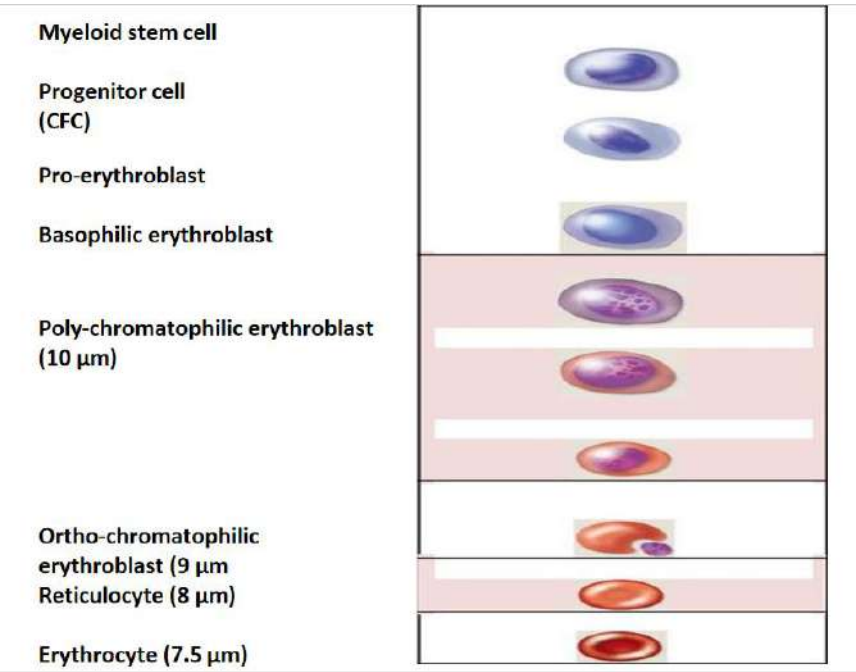
- **Stages:**
    1. Pluri-potential hematopoietic stem cells → Myeloid stem cells.
    2. Progenitor cells (Erythrocyte-Colony Forming Cells).
    3. **Blasts:** Pro-erythroblast (12 micrometers, 2 nucleoli) → Basophilic erythroblast (11 micrometers, no nucleoli) → Polychromatophilic erythroblast (10 micrometers, hemoglobin appears).
    4. **Pro-cytes:** Orthochromatophilic erythroblast "normoblast" (9 micrometers, characterized by extrusion of pyknotic nucleus).
    5. **Cytes:** Reticulocyte (8 micrometers, basophilic reticulum of polyribosomes).
    6. **Mature:** Erythrocyte (7.5 micrometers, filled with hemoglobin, no nucleus).
- **Note:** Reticulocytes increase in peripheral blood (>1% of Red Blood Cells) during hemorrhage or hemolysis.

Changes During Erythropoiesis

1. Cell and nuclear volumes decrease, until the nucleus is finally extruded.
2. Gradual decrease in polyribosomes (basophilia) with simultaneous increase in hemoglobin (eosinophilia).
3. Mitochondria and other organelles gradually disappear.

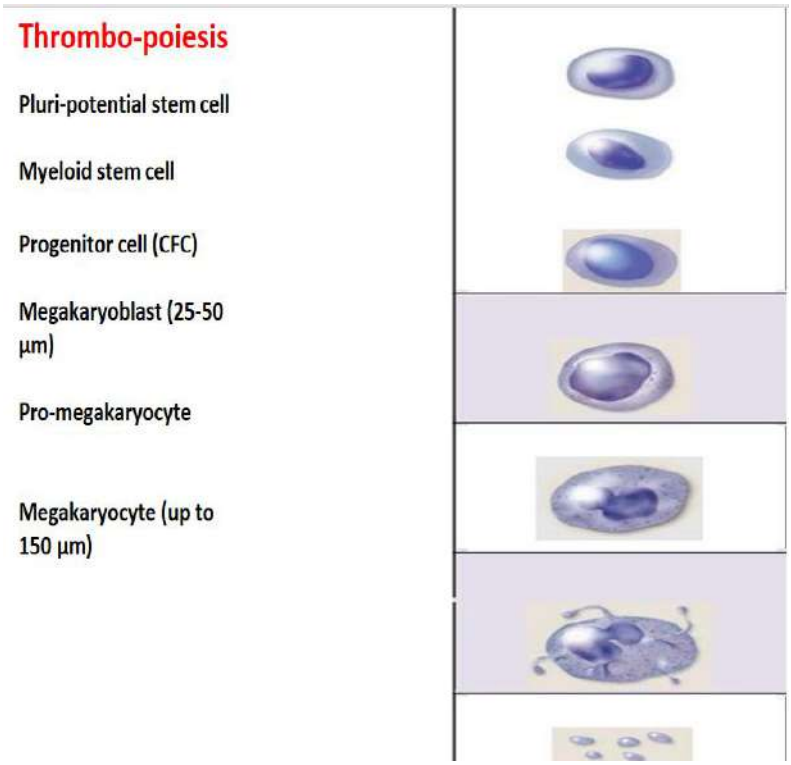
The development of red blood corpuscles (erythrocytes) takes the following stages:

1- Pluripotent Hemopoietic Stem Cells
2- Myeloid stem cells for erythrocytes.
3- CFU-erythrocytes (CFU-E)
4-blast: Proerythroblast.
Basophil erythroblast.
Polychromatophil erythroblast.
5- procyte :ortho-chromatophilic erythroblasts “normoblasts”
6- reticulocyte
7- Erythrocyte (the mature RBCs)

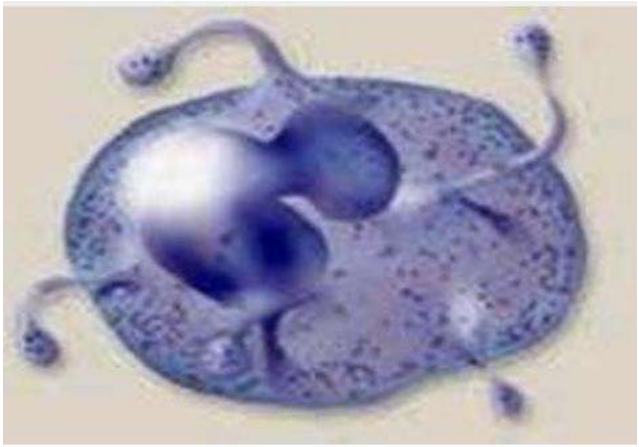


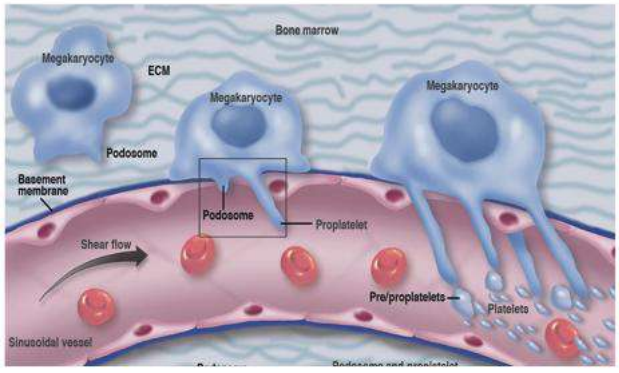
Thrombopoiesis (Development of Platelets)

- **Stages:**
    1. Pluripotent stem cells.
    2. Myeloid stem cells.
    3. Colony forming unit for megakaryocytes.
    4. Megakaryoblasts.
    5. Megakaryocytes.
    6. Blood platelets.



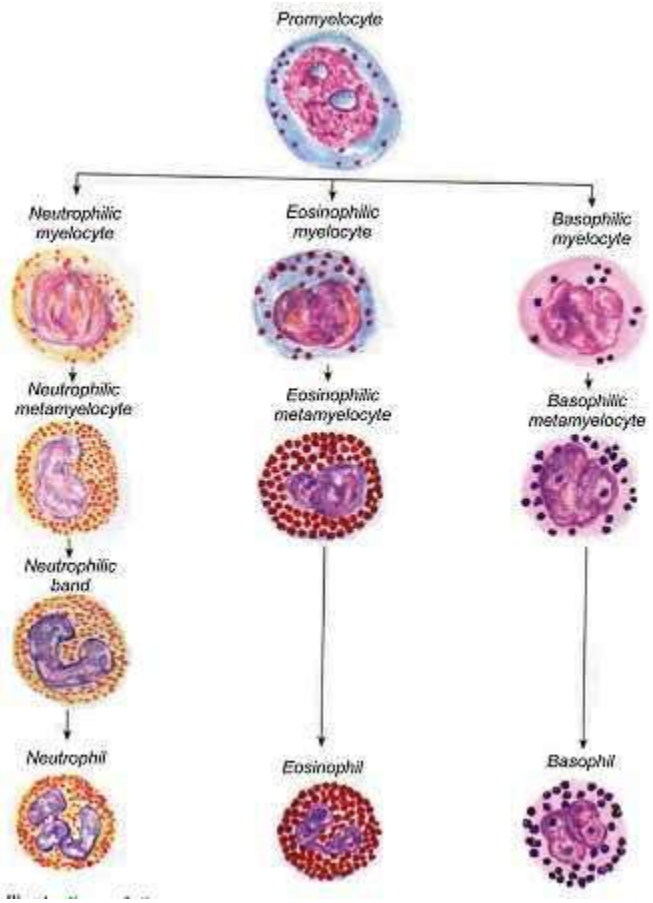
- **Megakaryocytes:**
    - Giant cells, up to 150 micrometers in diameter, with large, irregularly lobulated polyploid nuclei.
    - Cytoplasm contains numerous organelles and extensive Golgi apparatus which forms platelet granules.
    - Extend long, branching pseudopodia-like projections called proplatelets which pinch off to form platelets.
- Each megakaryocyte produces a few thousand platelets then undergoes apoptosis.





Granulopoiesis (Development of Granulocytes)

- **Stages:**
    1. Pluripotent Hemopoietic Stem Cells.
    2. Myeloid stem cells for granulocytes.
    3. Colony forming unit for granulocyte series.
    4. Myeloblast.
    5. Promyelocyte.
    6. Myelocyte.
    7. Metamyelocyte.
    8. Mature Granular Leucocyte (Basophil, Eosinophil, Neutrophil).



- **Note:** Neutrophilic metamyelocytes ("juvenile" with kidney-shaped nucleus) and band neutrophils (with bend-rod nucleus) increase with acute infection (>2% of neutrophils in peripheral blood).

Agranulopoiesis (Development of Monocytes and Lymphocytes)

- **Monocytopoiesis Stages:**
    1. Pluripotent stem cells.
    2. Myeloid stem cells for monocytes.
    3. Colony forming unit for monocyte series.
    4. Monoblast.
    5. Promonocyte.
    6. Monocyte.
- **Lymphocytopoiesis Stages:**
    1. Pluripotent stem cells.
    2. Lymphoid stem cells.
        - For B-cells: remain in bone marrow.
        - For T-cells: migrate to thymus cortex.
    3. Lymphoblasts.
    4. Lymphocytes.

Lecture 3 : Lymphatic System

Learning Objectives

After this lecture, students should be able to:

- Describe the cytological characteristics of lymphocytes.
- Define and classify lymphatic tissue: diffuse and nodular.
- Describe the structure of lymphatic organs; Primary as thymus gland.
- Describe the structure of lymphatic organs; Secondary as lymph node.
- Describe the structure of lymphatic organs; Secondary as spleen.

The Lymphatic System

The lymphatic system consists of:

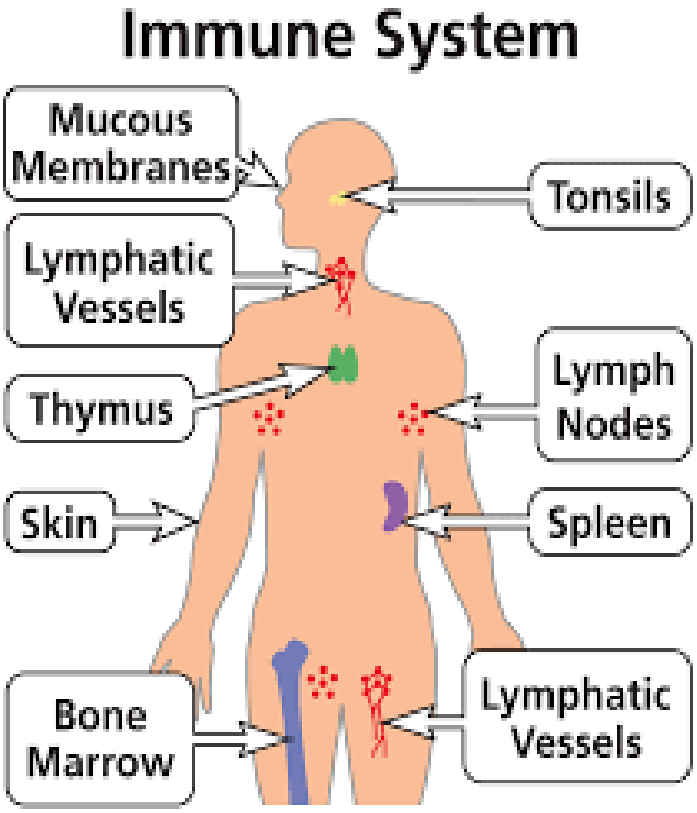
1. **Lymphatic Vessels (Lymphatics):** It is a network of lymph capillaries and lymph vessels which return the lymph to systemic circulation.
2. **Lymphatic (Lymphoid) Organs:**
    - **Primary Lymphoid Organs** (the thymus and bone marrow).
    - **Secondary Lymphoid Organs** (the lymph nodes, the spleen, and lymphoid tissue found in the mucosa of the digestive, respiratory, or urogenital mucosae which is collectively known as mucosa-associated lymphoid tissue).

Lymphocytes are the definitive cell type of the lymphatic system.

Lymphoid Organs and Immune System

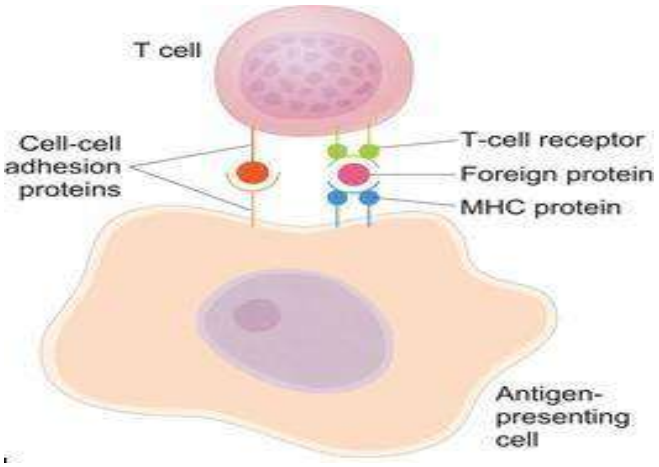
The immune system provides defense or immunity against infectious agents. Histologically this system consists of a diverse population of leukocytes located within every tissue of the body and lymphoid organs. Lymphatic vessels connect parts of the system to the blood vascular system.

Immune System: Innate and Adaptive Immunity



1. **Non-specific (Innate) Immunity:** Represents the first line of defense against microbial invasion. It consists of:
    - Physical barriers (e.g., the skin and mucous membrane).
    - Chemical defenses (e.g., low pH).
    - Various secretory substances (e.g., in saliva).
    - Phagocytic cells (e.g., macrophages, neutrophils).
    - Natural killer cells.
2. **Specific (Adaptive) Immunity:** Targets specific invaders. During adaptive immune responses, specific B and T lymphocytes become activated to destroy invading organisms.

Cellular Elements of Immune System



- **Lymphocytes:** Are three types: B cells, T cells, and Natural Killer cells.

- **Supporting Cells:** Interact with lymphocytes and play important roles in the presentation of antigen to lymphocytes (Antigen presenting cells).

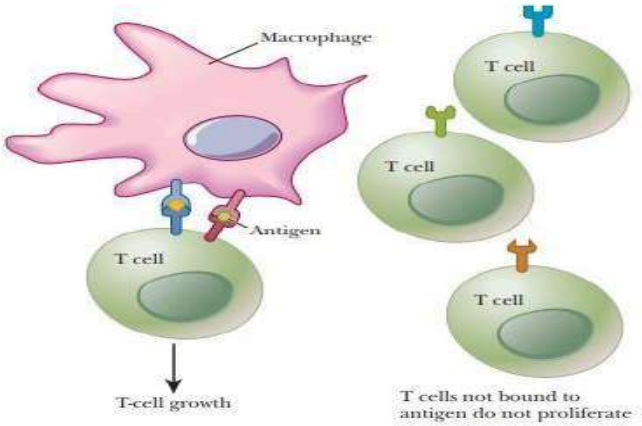
Differences between T and B Lymphocytes

	T lymphocyte	B lymphocyte
Origin	**-Derived from bone marrow. Then migrate to the thymus for completion of maturation.**	**-Derived from bone marrow, and undergo maturation in the bone marrow. Then they migrate to non-thymic lymphoid organs.**
Type of immunity	**-Cellular immunity (Cell mediated immune response). T-Lymphocytes react and kill tumor cells and virus infected cells**	**-Humoral immunity (Antibody-mediated immune response). B- lymphocytes become activated and change into plasma cells which produce antibodies (immunoglobulins).**

Antigen-Presenting Cells

They phagocytose and process antigens into molecular fragments and present them to the lymphocytes. They include:

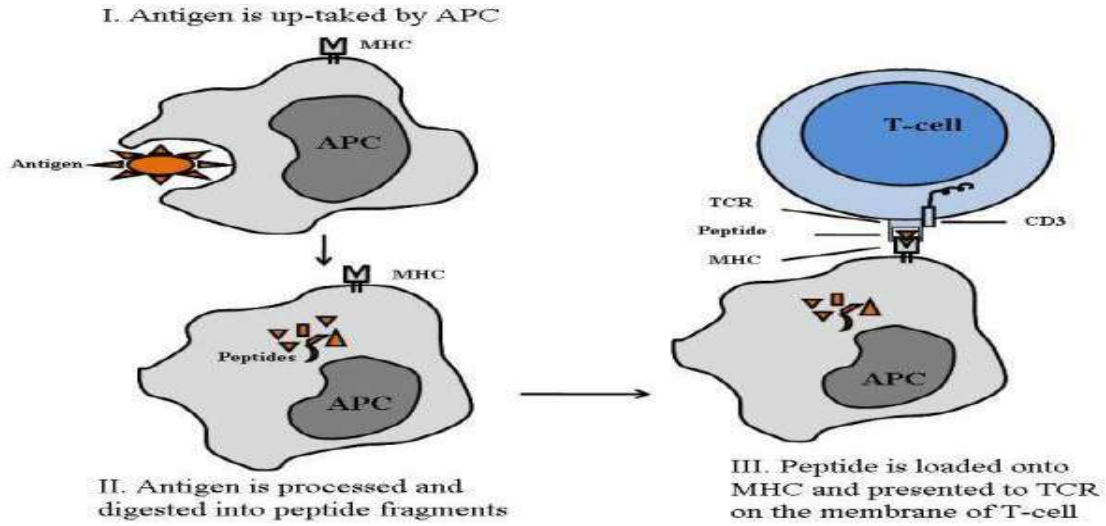
- Macrophages.
- Epidermal Langerhans cells (skin).
- Microglia (Central Nervous System).
- Kupffer's cells (liver).



Functions:

1. **Phagocytosis and Endocytosis:** APCs engulf pathogens, foreign particles, or cellular debris.
2. **Antigen Processing:** They break down the ingested material into smaller peptide fragments.

3. **Antigen Presentation:** These peptide fragments are displayed on the cell surface bound to **Major Histocompatibility Complex (MHC)** molecules.
4. **Activation of Lymphocytes:** By presenting antigens, APCs activate T lymphocytes, which then orchestrate a targeted immune response.



Lymphoid Organs

Lymphatic organs serve as sites where lymphocytes proliferate, differentiate, and mature. In addition, in the thymus, bone marrow, and gut-associated lymphatic tissue, lymphocytes are "educated" to recognize and destroy specific antigens. These are immunocompetent cells that can distinguish between "self" (molecules normally present within an organism) and "non-self" (foreign molecules—i.e., those not normally present).

The Lymphatic System

The lymphatic system is composed of lymphatic vessels and the lymphoid organs.

- **Primary Lymphoid Organs** are the bone marrow and thymus.
- The **Secondary Lymphoid Organs** include the lymph nodes, mucosa-associated lymphoid tissue, and spleen.

Lymphoid Tissue

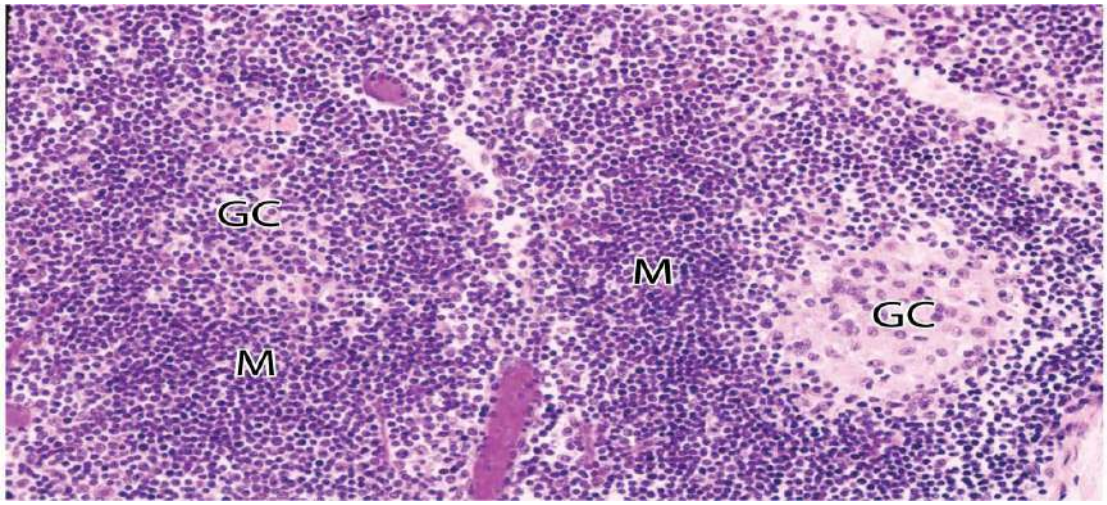
Lymphoid tissue is usually reticular connective tissue filled with large numbers of lymphocytes, supported by a reticular fiber network of type III collagen. Lymphoid tissue can be either:

- **Diffuse:** Within areas of loose connective tissue.
- **Nodular (Follicular):** Surrounded by capsules forming discrete (secondary) lymphoid organs.

Lymphatic Nodule (Follicle):

The lymphatic nodules or follicles are rounded circumscribed masses of cells composed mainly of B lymphocytes, present within the diffuse lymphatic tissue. The nodules may be termed primary or secondary.

- **The Primary Nodule** is a rounded mass of packed small lymphocytes.
- **The Secondary Nodule** is formed of a central pale area called the **germinal center** and a peripheral dark zone **(mantle)**. The germinal center is pale in staining because most of its cells are large and their cytoplasm is abundant and pale in staining.
- **The Mantle** is dark due to the intense staining of the nuclei of the closely packed small lymphocytes.



Functions of the Nodules:

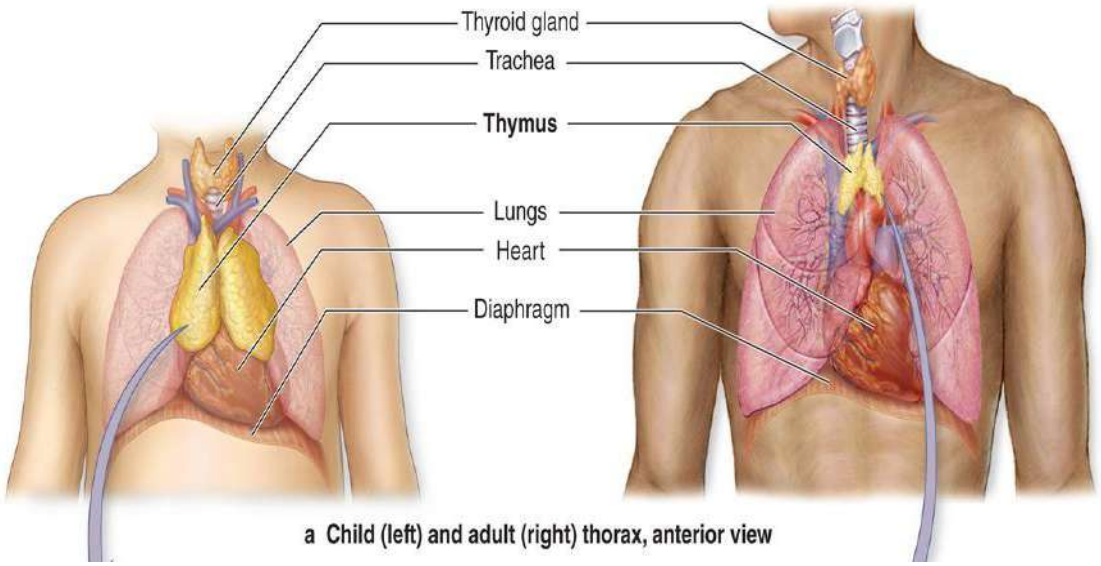
- The nodules produce lymphocytes.
- They have phagocytic activity due to the presence of macrophages. The macrophage system filters the lymph (as in the lymph nodes) and the blood (as in the spleen).
- They produce antibodies due to their contents of plasma cells.

Sites of Lymphatic Nodules:

1. Lymphatic nodules are present in the lamina propria of the digestive and respiratory passages, at the periphery of the white pulp of the spleen and in the lymph nodes.
2. Also the tonsils, Peyer's patches and the appendix are very rich in lymphatic nodules.

Thymus

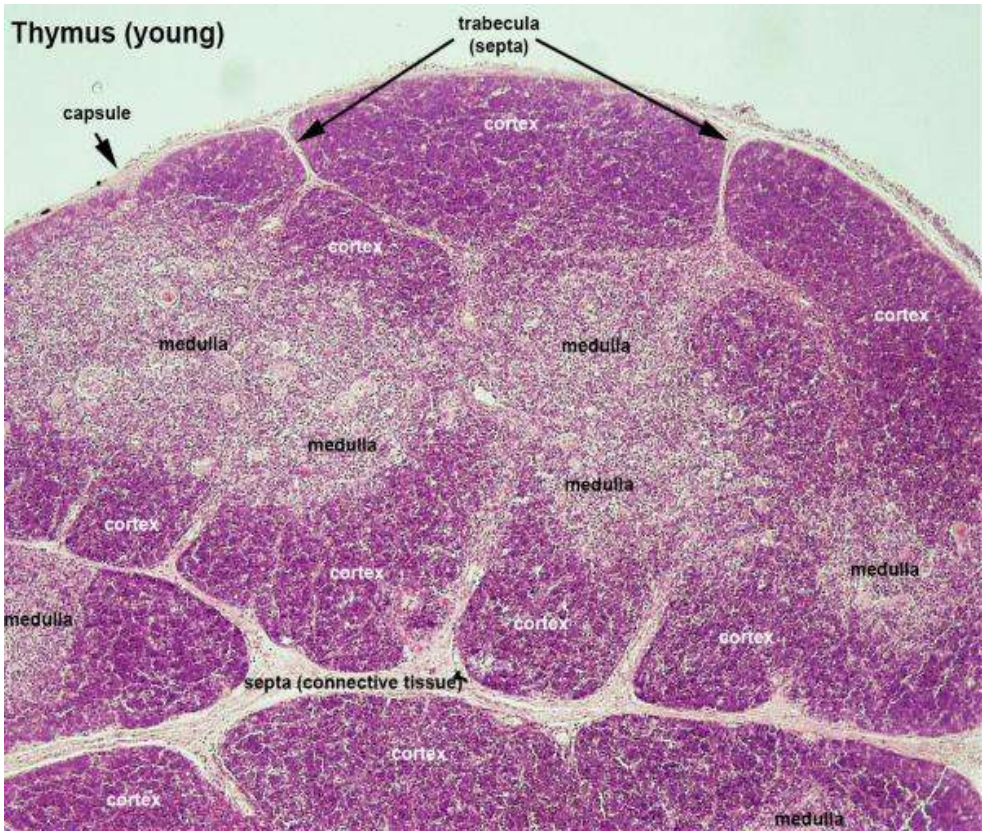
A bilobed structure in the mediastinum, fully formed and functional at birth. Remains large and very active in T-cell production until puberty, during which it normally undergoes involution, with decreasing lymphoid tissue mass and cellularity and reduced T cell output.



a Child (left) and adult (right) thorax, anterior view

Histological Structure of the Thymus

1. **Stroma:**
    - Capsule.
    - Trabeculae.
2. **Parenchyma:**
    - The parenchyma is divided into incomplete lobules. Each thymic lobule is composed of a peripheral dark zone known as the **cortex** and a central light zone called the **medulla**.



The Parenchyma:

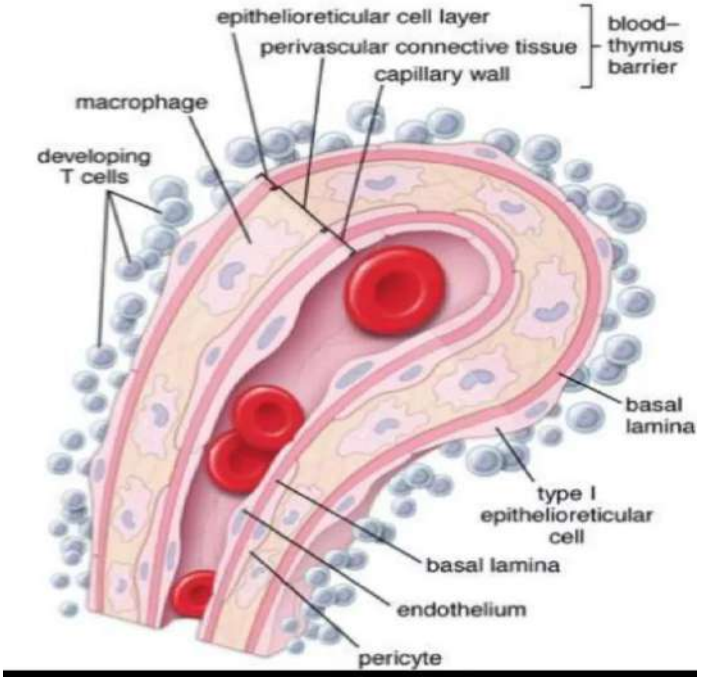
The cortex of each lobule appears much darker than the medulla because of the presence of large number of T-lymphocytes (thymocytes). The cortex also contains macrophages and **thymic epithelial cells**.

Thymic Epithelial Cells:

Have certain features of both epithelial and reticular cells. They are stellate cells with light staining oval nuclei resting on a basement membrane. Thymic epithelial cells form sheaths around the blood vessels, creating a barrier to the entry of antigenic material into the thymic parenchyma. This is known as the **blood-thymus barrier**.

Blood-Thymus Barrier

- **Location:** In the cortex of thymus.
- **Function:** It protects developing T cells from circulating antigens in the blood.
- **Components:**
    1. Continuous endothelium of blood capillaries.
    2. Thick basal lamina.
    3. Thymic epithelial cells joined by desmosomes and occluding junctions.



The Medulla

Has the same cell population as the cortex, with a larger number of thymic epithelial cells. The medulla contains **Hassall's corpuscles** which are characteristic of this region. They consist of concentrically arranged flattened thymic epithelial cells that become filled with keratin filaments and degenerate. They are of unknown function.

Thymus Dependent Zones

Are areas in the secondary lymphoid tissue which contain mainly T-lymphocytes. It includes:

- Paracortical zone of lymph nodes.
- Periarterial lymphatic sheath in white pulp of spleen.

Lecture 4

Learning Objectives

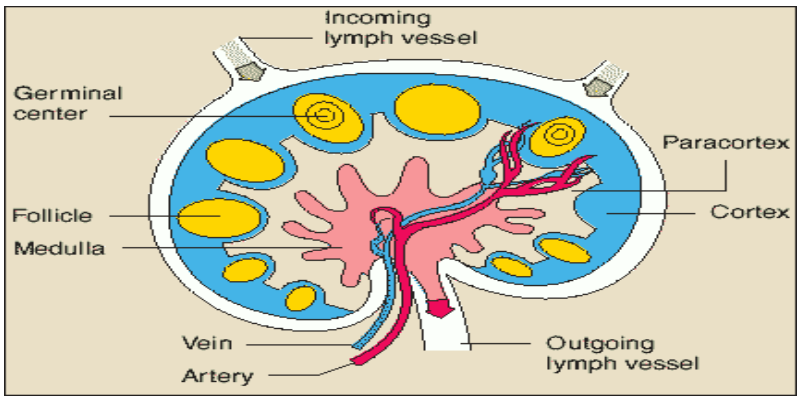
After this lecture, students should be able to:

- Describe the structure of lymphatic organs; Secondary as lymph node.

Describe the structure of lymphatic organs; Secondary as spleen.

Lymph Node

Lymph nodes are small encapsulated structures present in the path of lymph vessels to filter lymph. Each lymph node has a convex surface perforated by afferent lymph vessels and a concave surface where efferent lymph vessels leave the lymph node.



Histological Structure of Lymph Node

The lymph node is structurally formed of a stroma and a parenchyma.

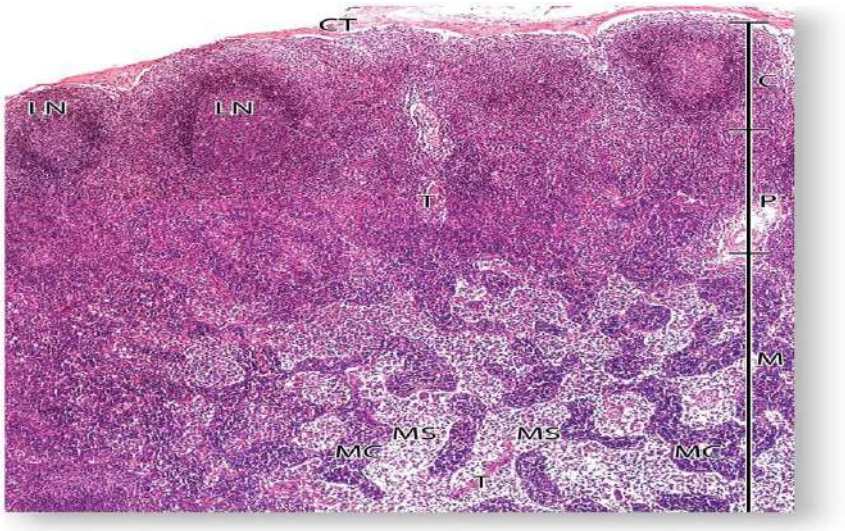
The Stroma:

Is formed of a dense irregular collagenous connective tissue capsule. The capsule sends trabeculae that extend into the substance of the node, subdividing the outer region of the node into incomplete compartments. A network of reticular connective tissue forms the framework of the entire lymph node.

The Parenchyma

Is formed of lymphocytes of all types, plasma cells, dendritic cells, macrophages, and antigen presenting cells. All of these cells are arranged in 3 regions: **cortex, paracortex and medulla**.

- **Cortex:** Formed of lymphatic nodules (Primary and secondary lymphatic nodules).
- **Paracortex:** Is the region of the lymph node between the cortex and the medulla. It contains mostly T lymphocytes (thymus dependent zone of lymph node).
- **Medulla:** In the medulla, lymphocytes are arranged in irregular cords **(medullary cords)**. These cords contain lymphocytes, plasma cells and macrophages surrounded by medullary lymph sinuses.

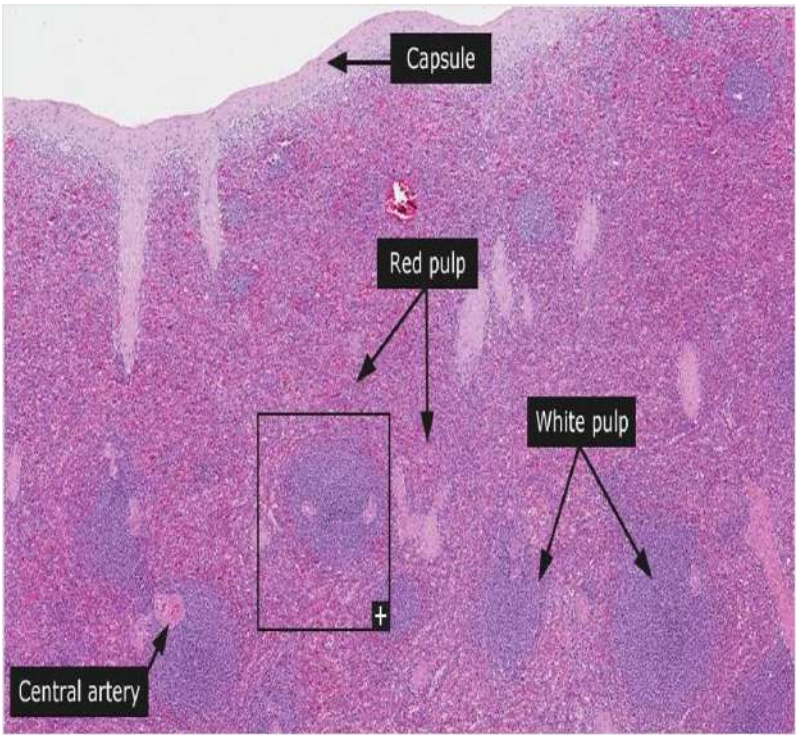


Spleen

The spleen is the largest lymphoid organ which filters blood.

Histological Structure:

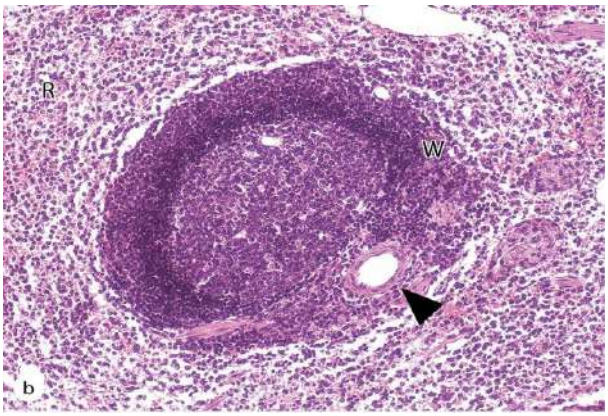
- **Stroma:** The spleen is covered by a connective tissue capsule which sends short irregular connective tissue septa. The muscle fibers are prominent in the spleen of some animals in which it serves as a blood store.
- **Parenchyma:** The spleen is formed of **white pulp and red pulp**.



White Pulp of Spleen

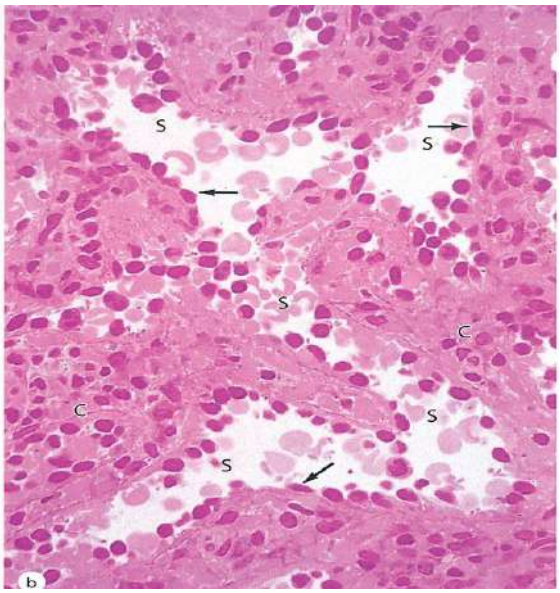
It is formed of two parts:

1. **The Periarterial Lymphatic Sheath** which surrounds the central artery. It is formed of T lymphocytes (thymus dependent zone of spleen).
2. **Splenic Nodules.**



Red Pulp

It consists of **splenic sinusoids** and **splenic cords** which contain T and B lymphocytes, macrophages and many blood cells (erythrocytes, platelets, and granulocytes).

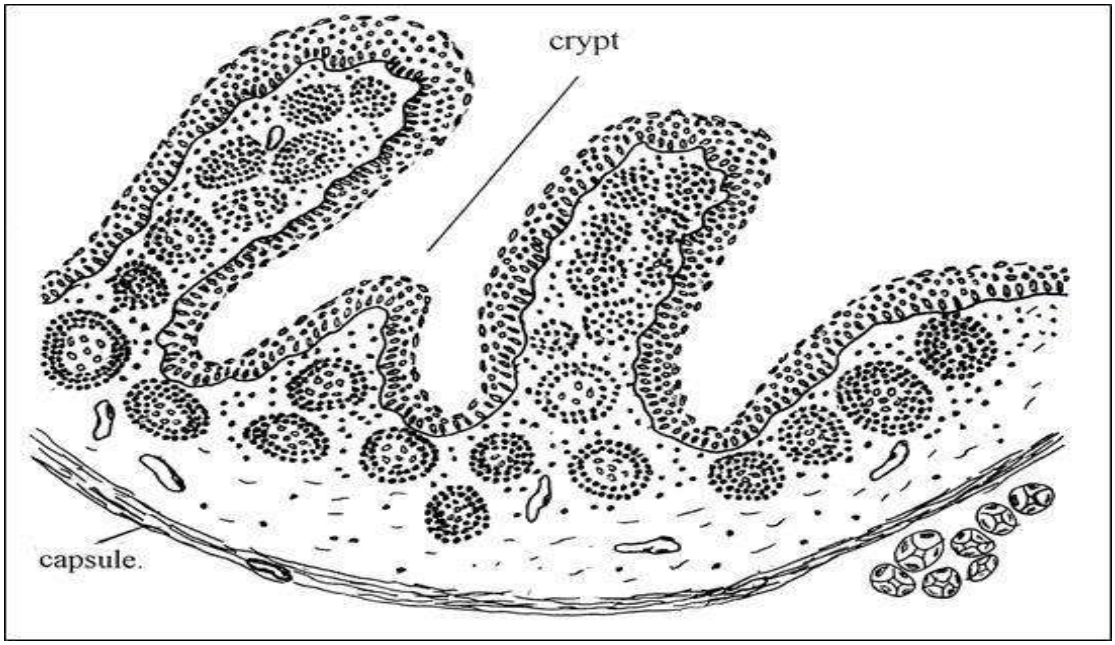


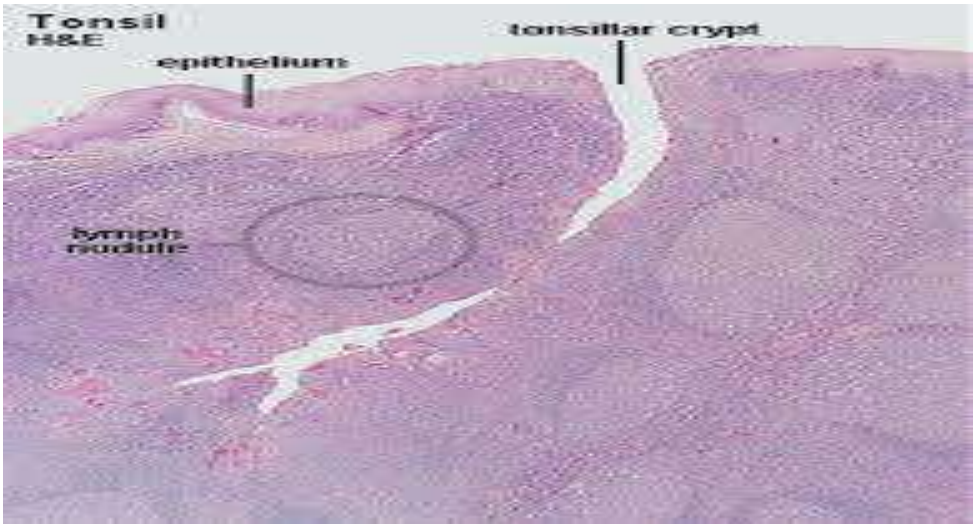
Tonsils

Each palatine tonsil is isolated from the surrounding connective tissue by a dense fibrous capsule (incompletely encapsulated).

The superficial aspect of the tonsils is covered by a stratified squamous non-keratinized epithelium that dips into the 10-12 deep crypts that invaginate the tonsilar parenchyma.

The parenchyma of the tonsil is composed of numerous lymphoid nodules, many of which have germinal centers indicative of B cell formation.





References

1. Junqueira's Basic Histology (Text and Atlas), 15th Edition, 2018.
2. Elsevier's Integrated Histology, 1st Edition, 2007.

HEM-210

Physiology

Plasma and Red Blood cells

ILOs

1- Identify the composition and functions of the blood,

2- Clarify contents of plasma and functions of plasma proteins

3- Demonstrate functions of Red blood cells and its hemoglobin content.

4- Identify the red blood cells, their functions.

5- The erythropoiesis and factors affecting it and types of anemia and polycythemia.

Blood is a vital fluid circulate within Cardio Vascular System (CVS), and its volume is 5600ml.

Blood Functions:

1- Transport function (glucose, O2, CO2).

2- Defensive function (WBCs, anti-bodies).

3- Hemostatic function (stop bleeding).

4- Homeostatic function (keeps the composition of the tissue fluid constant).

Blood Composition:

45% cells: 1- Red blood corpuscles (RBCs).
2- White blood cells (WBCs).
3- Platelets

55% plasma: 1- Water 90 %.
2- Inorganic substances (Na, Cl).
3- Org anic substances (protein, lipid, glucose).
4- Gases (CO_2, O_2).

PLASMA PROTEINS

Concentration: 7.2 gm /dl.

Composition:

1- Albumin : its concentration 3.5 – 5 g/dl.

2- Globulin : its concentration 2.5 g/dl.

3- Fibrinogen : its concentration 0.4 g/dl.

4- Prothrombin: its concentration 0.01 g/dl.

Site of formation:

All types of plasma protein are formed in liver **except** 50% of Globulin formed in plasma cells.

Albumin – Globulin ratio (A/G ratio)

It is a ratio between albumin and globulin concentration.

- Normally = 1.2 – 1.6.
- It decreases in liver and kidney diseases.

Functions of plasma proteins:

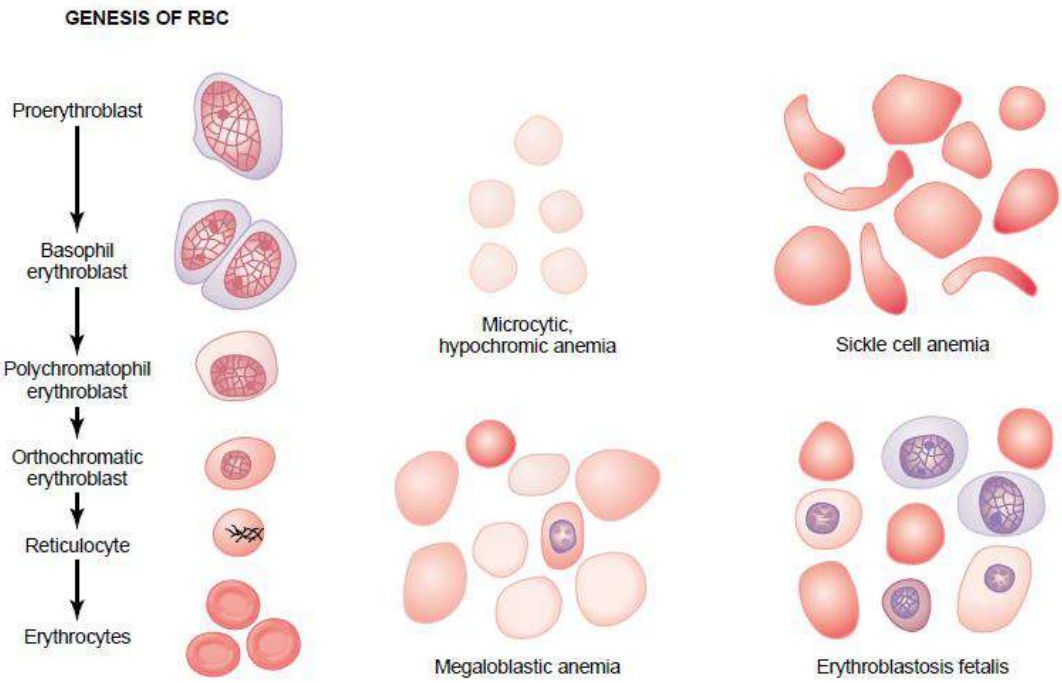
A- Specific functions:

1- **Osmotic function** it I s function of albumin where water withdrawn from tissue to plasma by osmotic pressure of albumin (28 mmHg).
2- **Defensive function** it is function of gamma globulin while alpha and beta globulin have transport function.
3- **Viscosity of the blood** it is function of fibrinogen, the importance of this viscosity is to maintain arterial blood pressure.
4- **Clotting of the blood** it is function of fibrinogen and Prothrombin.

B- Nonspecific functions:

1- Plasma proteins act as a **carrier** for important elements of the blood (vitamins, hormones).
2- **Buffer function**: plasma proteins adjust PH of blood at 7.4. Buffering function of plasma protein represent 15% of buffering power of blood.
3- **Diet reserve**: plasma proteins act as a source for rapid replacement of tissue protein.
4- **Capillary permeability:** plasma proteins control movement of substances across capillaries (in and out) through the pores.

RED BLOOD CORPUSCLES (RBCs)

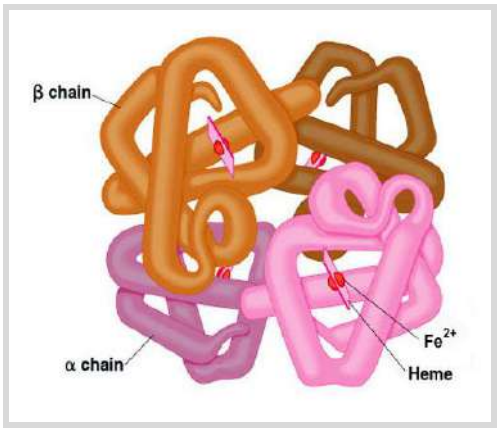


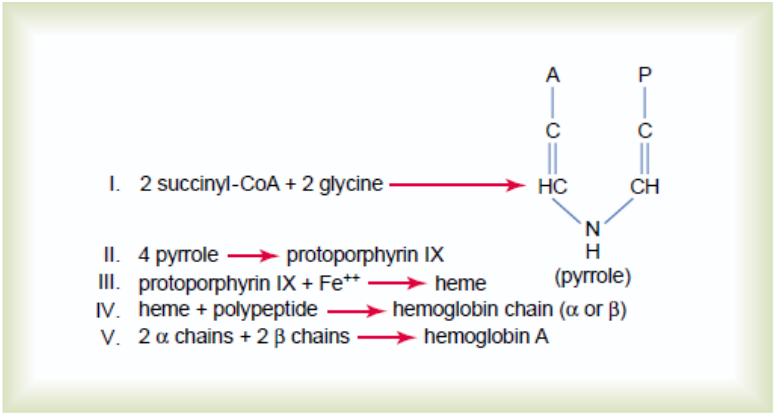
Genesis of normal red blood cells (RBCs) and characteristics of RBCs in different types of anemias.

RBCs are the **highest** concentration of cells in the body.

- In male = 5.5 million/mm^3.
- In female = 4.8 million/mm^3.
- RBCs are **non nucleated, biconcave** shape to provide large surface area for transport and to enhance cell flexibility.
- RBCs **life span** = 120 day.

Hemoglobin (Hb)





- Hemoglobin is a red pigment present on RBCs
- Hemoglobin content:

  In male = 15- 16 gm/dl

  In female = 13-14 gm/dl

  In infant = 19 gm/dl

Hb reactions:

1- Hb reacts with O_2 to form oxyhemoglobin (O_2 react with iron in ferrous state).

2- Hb reacts with CO_2 to form carbamino hemoglobin.

3- Hb react with CO to form carboxyhemoglobin (Hb affinity to CO is 200 time O_2).

4- Oxidation to form methamoglobin where ferrous iron is oxidized to ferric iron so it can't carry O_2.

5- Glycosylation to form glycosylated Hb.

Hb functions:

1- Transport O_2 from lung to tissue.

2- Transport CO_2 from tissue to lung.

3- Buffering function to adjust pH of blood (Hb buffering 6 times more powerfull than plasma protein buffer).

Hematocrite value or packed cell volume:

Definition: it is the percentage ratio of RBCs volume to the total blood volume

Normal value: 46 % for adult male & 42 % for adult females.

Changes in HV:

- It is higher in

(A)Physiological:1- Venous blood than arterial blood.

2- Newly born infants

3- Peoples at higher altitude.

(B) Pathological: in dehydration & polycythemia.

- It is less in:

(A)Physiological: female

(B) Pathological:1- anemia.

2- over hydration.

Erythropoiesis

It is process of formation of RBCs. Erythropoiesis takes place in:

Fetus > liver and spleen.

Children > bone marrow.

Adult > end of long bone.

Above 20 year > membranous bone.

Factors affecting erythropoiesis:

1-Healthy bone marrow

2- Liver and Kidney

3- Oxygen supply to tissue

4- Hormones

5- Diet

1- Healthy bone marrow is essential for formation of normal RBCs as it is site of formation. Destruction of bone marrow leads to anemia known as aplastic anemia which is (normocytic normochromic anemia). Bone marrow may be destroyed by drugs, radiation, and tumors.

2- Liver and kidney are essential for formation of normal RBCs as both are considered to be site of formation of erythropoietin hormone (15% liver – 85% kidney) also liver is considered to be site of storage of iron and B12 .

3- O2 supply to tissue is one of the most important factors in formation of RBCs

decrease O_2 supply will lead to stimulation of formation of RBCs through increase Erythropoietin hormone. Decrease O_2 supply occurs in heart and lung disease, high altitude and haemorrhage.

4- Hormones

erythropoietin – androgen – cortisone

and thyroid hormone are essential for formation of RBCs.

5-Diet

Diet must contain vitamins as folic acid and B12, metals as iron, copper and cobalt and protein for formation of RBCs.

Erythropoietin hormone

site of formation:

Fetus > liver

Adult > 15% liver and 85% kidney

Erythropoietin stimulated by :

1- Hypoxia

2-Alkalosis

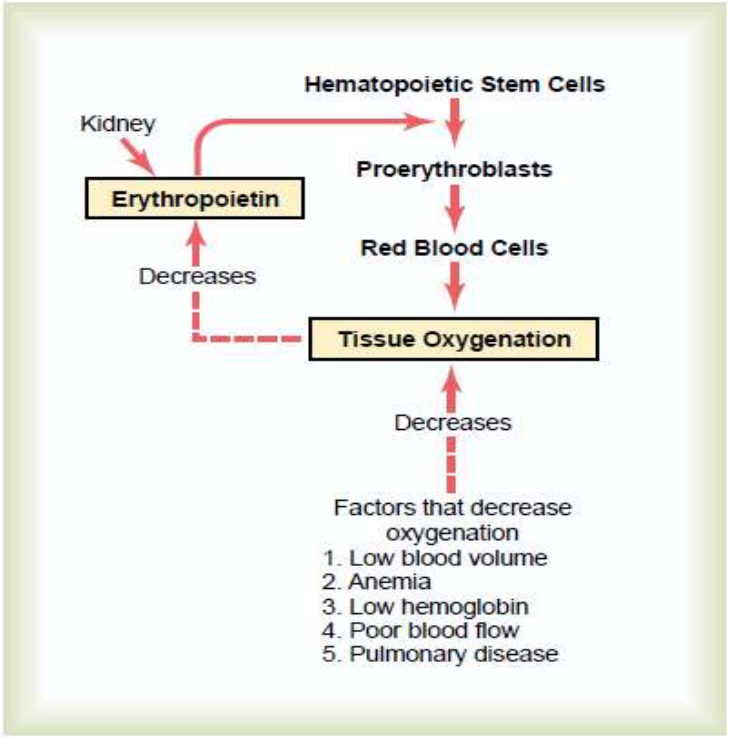
3- Androgen

4- Adenosine

5- Cobalt salt

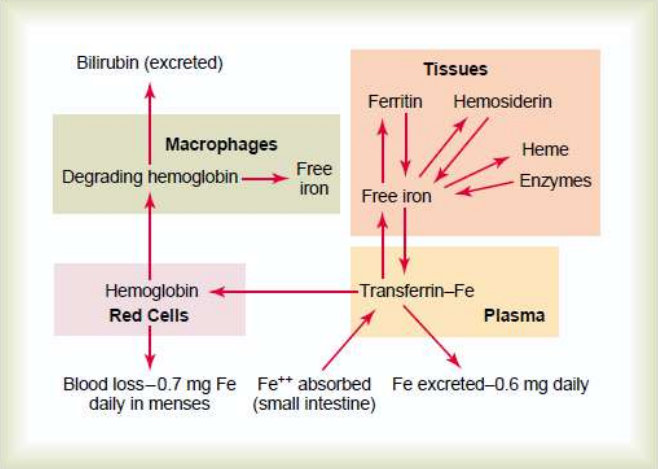
6- Catecholamine

Erythropoietin hormone accelerates all stages of erythropoiesis and that is why in renal failure patient develops anemia .



Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

Iron absorption



1- Iron absorbed in ferrous state while iron in diet is ferric.
2- Reduction of ferric to ferrous occurs by gastric Hcl and ascorbic acid (vitamin C).
3- Iron absorbed mainly in upper part of small intestine (duodenum).
4- Part of iron is delivered to mitochondria.
5- Remaining part is either combined with apoferritin (in intestine) or carried in plasma on transferrin.
6- Iron combined with apoferritin is changed to ferritin which is main storage of iron.
7- Iron transported in blood bound mainly to transferrin to all part of body and stored in liver as ferritin.
8- Deficiency in iron is due to decrease iron intake or decrease iron absorption or chronic blood loss lead to microcytic anemia.

NB: apoferritin is present in intestine and liver.

B_{12} absorption

1- Intrinsic factor secreted by gastric gland (parietal cell).
2- Intrinsic factor combines with vitamin B12 for protection and transport of B_{12}.
3- Vitamin B_{12} absorbed from lower part of small intestine (ileum).
4- Vitamin B_{12} enter mucosal cell with Intrinsic factor by pinocytosis.
5- Inside cell vitamin B_{12} set free in order to be absorbed to blood where it bound to transcobalamineII to every part in the body and stored in liver.
6- Deficiency in vitamin B_{12} may be due to decrease in vitamin B_{12} absorption lead to anemia known as macrocytic anemia.

	Iron	**B_{12}**
Function	important for formation of hemoglobin and myoglobin	- DNA formation - Cell division - Cell maturation - Formation of myelin sheath
Storage	in liver	in liver
Requirement	0.6 mg/day	5 μg/day
Site absorption	upper part of small intestine	lower part of small intestine
Deficiency lead to	microcytic anemia	macrocytic anemia
Need	Hcl and vitamin C for reduction of ferric iron to ferrous	intrinsic factor for protection from Hcl

Anemia

Anemia is a decrease in number of RBCs, hemoglobin content or both.

Anemia is considered when RBCs count → < 4.5 million in males &
< 3.9 million in females
& Hb content → < 13.5 gm % in males
< 11.5 gm % in females.

Blood indices:

1-Mean corpuscular Hb (MCH) = amount of Hb in single RBC =

$$\frac{\text{Hb content X 10}}{\text{RBC count in million}}$$

- Normally, it is 25-32 picogram.
- Values less than 25 picogram are called hypochromic.

2-Mean corpuscular volume (MCV) = volume of single RBC =

$$\frac{\text{Hematocrit value X 10}}{\text{RBC count in million}}$$

- Normally, it is 80 -95 μ^3
- Values less than 80 are called microcytes and values more than 95 are called macrocytes.

Anemia is classified according to blood indices into:

1-Normocytic normochromic anemia : i.e normal blood indices

2-Microcytic hypochromic anemia : i.e lower blood indices

3-Macrocytic anemia : i.e. higher blood indices.

1-Normocytic normochromic anemia:

Causes:

a- Aplastic anemia: decrease RBC synthesis due to bone marrow inhibition by: antibiotic – malignant tumor – irradiation.

b- Hemolytic anemia: excessive hemolysis of RBC due to :

i- Intrinsic disorders of RBCs:

- Membrane disorders: e.g. spherocytosis where cells are spherical, small & fragile
- Hemoglobin disorders: e.g. sickle cell anemia where RBCs contain abnormal Hb S which precipitate at low oxygen tension & hemolysis
- Enzyme disorders: e.g. decrease glucose-6-phosphate dehydrogenase which reduces ferric to ferrous. Oxidants as aspirin cause hemolysis.

NB : Favism : hemolysis on eating beans.

ii- Extrinsic disorders:

- Antibody causing hemolysis e.g. erythroblastosis foetalis
- Bacterial toxins
- Chemicals e.g. benzene derivatives.
- Drugs e.g anti convulsant & anti malarial.

c- Acute blood loss: (Acute haemorrhage).

2- <u>Microcytic hypochromic anemia:</u>

Small RBC with low Hb content inside caused by iron deficiency

Causes of iron deficiency anemia:

a- Decrease dietary intake: → starvation as in children & pregnancy where there is increase in their needs.

b- Decrease iron absorption as in:

- Gastrectomy where HCl is absent
- Small intestine diseases
- Vitamin C deficiency
- increase phosphate & phytate where they form insoluble salts with iron.

c- Chronic blood loss: as in piles, peptic ulcer & ankylostoma.

N.B. Tea decrease iron absorption because it contains tannic acid & theophylline

<u>3- Macrocytic anemia:</u>

Due to decrease vit B_{12} or folic acid → decrease DNA → decrease proliferation of erythroblasts → megalocytes which are macorcytes.

Causes of folic acid deficiency:

- Decrease intake in diet
- Increase demands as in pregnancy
- Deficient absorption as in intestinal diseases
- Antifolate drugs used in treatment of cancer

Causes of vit B_{12} deficiency:

- Deficient absorption as in intestinal diseases.
- After gastrectomy due to absence of intrinsic factor.
- Liver diseases.
- Deficiency of vit B_{12} in diet which is rare.

Pernicious anemia:

- It is a familial disease of elderly & more common in women
- It is an autoimmune disease- There is an immune reaction against gastric parietal cells leading to achlorhydia and absent intrinsic factor.
- There is degeneration of posterior and lateral column of spinal cord leading to neurological manifestations.

Treatment of anemia:

1- In each type, try to treat the cause:

a- In iron deficiency : give ferrous salts by mouth, in severe cases give iron by injection.

b- In pernicious anemia: give B_{12} by injection through the whole life.

c- Macrocytic anemia due to folic acid deficiency is treated by folic acid.

2- In severe cases, blood transfusion is needed

Polycythemia

It means increase number of RBCs

Types:

a- Primary (polycythemia Vera): it is due to tumor of bone marrow.

b- Secondary: in cases of hypoxia as in obstructive lung diseases & high altitudes.

Effects:

1) Increase blood viscosity → increase resistance to blood flow →decrease venous return.
2) Increase resistance → blood pressure tends to rise.
3) Cyanosis due to increase reduced hemoglobin.

Platelets & Hemostasis (physiology).

ILOs

1- Demonstrate platelets and its normal platelet count and its functions
2- Identify the hemostasis & its mechanisms,
3- The blood coagulation and coagulation factors, the mechanism of blood coagulation & Compare between intrinsic and extrinsic Pathway.
4- Demonstrate different clotting factors
5- Identify the platelets and their functions.
6- Categorize the disorders of bleeding

Hemostasis

It is prevention of blood loss after injury and is done by the following mechanisms:

1- Vascular spasm i.e. vasoconstriction.
2- Platelet reactions and temporary hemostatic plug formation.
3- Formation of blood clot.

1-Vascular spasm: it results from:

a- Nervous reflexes which are initiated by pain from ruptured vessels.
b- Myogenic spasm of vessel due to trauma.
c- Local factors: as serotonin, thromboxane A_2 (released from platelets) .

2-Platelets

- Platelets count = 150,000 – 300,000 / mm^3
- Platelets are non-nucleated oval in shape.
- Platelets formed in bone marrow.
- Platelets structure:

1- Platelet membrane: contain PF3 important for blood clotting and also contain collagen important for formation of plug.
2- cytoplasm structures:
    - contractile protein
    - microtubules
    - mitochondria
    - enzymes
    - granules

→ dense granules

→ alpha granules

Role of platelets in hemostasis (Functions of platelets):

1- Platelet adhesion

platelet adhere to subendotheial collagen in presence of:

I- glycoprotein layer of platelet

II- von- willbrand factor

2-Platelet activation

platelet swell, change in shape and put out pseudopodia.

Platelet activation is stimulated by:

I – ADP

II- thrombin

3-Platelet release

Platelet release its contents

Platelet release 3 important substances:

I – Serotonin which produce vasoconstriction

II – ADP which stimulate aggregation

III- Thromboxane A2 which produce vasoconstriction and aggregation

4-Platelet aggregation

Platelets stick to each other

Platelet aggregation stimulated by:

I - Thromboxane A2

II- ADP

5-Platelet procoagulant (Help coagulation through PF3)

From the membrane of platelet PF3 exposed which form ideal surface for concentration of clotting factor.

6-platelet Fusion

platelet fuses to each other in presence of ADP

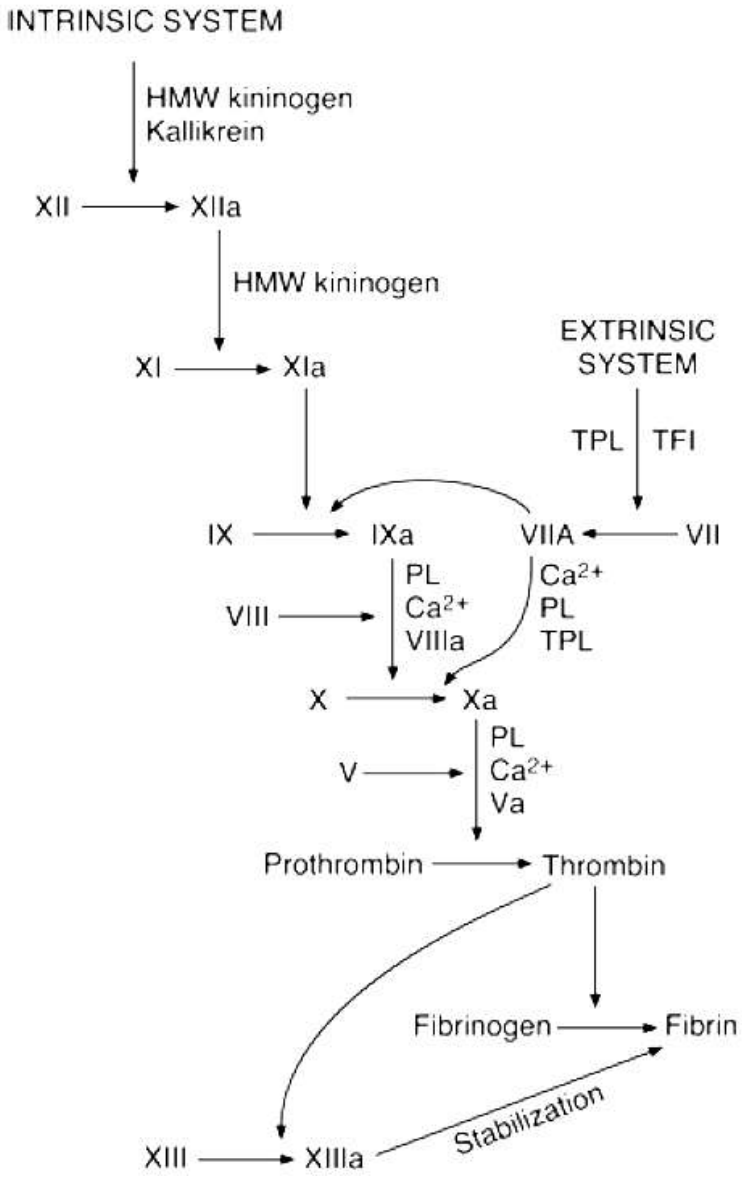
Clotting mechanisms

- Clotting factors are present in plasma in an inactive form.
- Clotting mechanisms concern with activation of an inactive clotting factor lead finally to formation of fibrin clot.

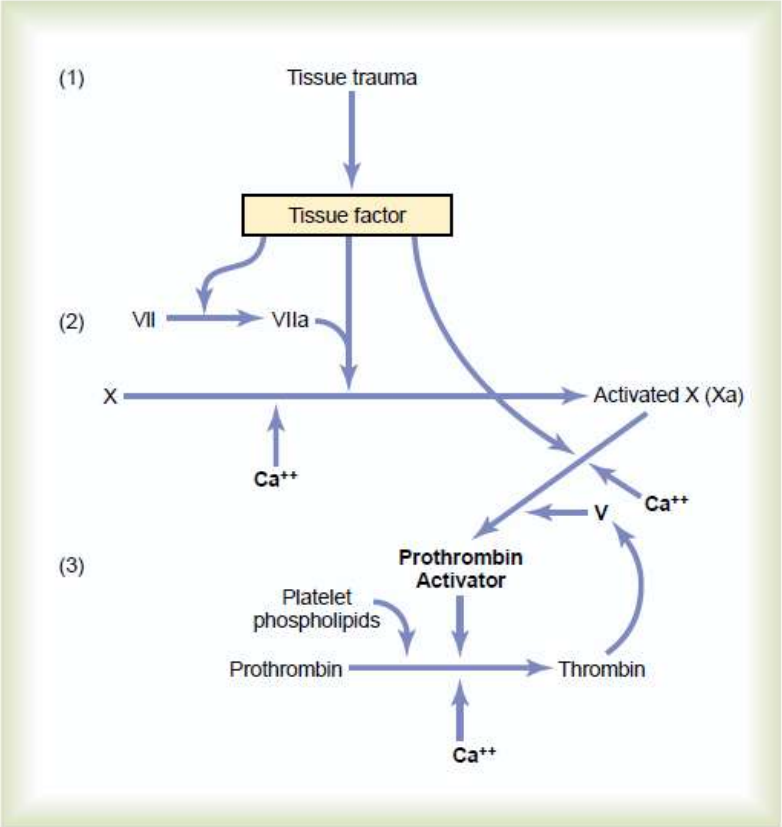
Fibrin formation occur through 2 pathways:

1- Intrinsic pathway

2- Extrinsic pathway



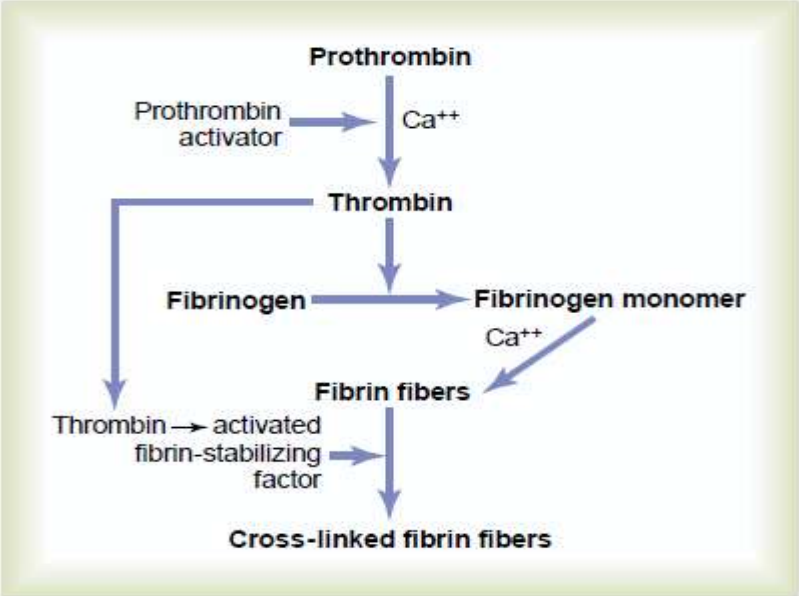
Extrinsic pathway



1- Extrinsic path way start by release of thromboplastin from injured tissue.
2- Thromboplastin activates factor VII.
3- Factor VII active activate factor X.
4- Factor X active convert prothrombin to thrombin in presence of Ca, platelets and factor V.
5- Thrombin converts fibrinogen to fibrin monomer.
6- Fibrin monomer converted to fibrin clot by active factor XIII and Ca.

Intrinsic pathway

1- Collagen fiber activate factor XII this activation is helped by high molecular weight kininogen and plasma kallikrein.
2- Active factor XII activate factor XI.
3- Active factor XI activate factor IX.
4- Active IX, active VIII, Ca and platelets form complex activate factor X.
5- Factor X active convert prothrombin to thrombin in presence of Ca, platelet and factor V.
6- Thrombin converts fibrinogen to fibrin monomer.
7- Fibrin monomer converted to fibrin clot by active factor XIII and Ca.



Schema for conversion of prothrombin to thrombin and polymerization of fibrinogen to form fibrin fibers.

Important notes:

1- When blood vessel ruptures, blood clotting is initiated by both systems simultaneously.
2- The extrinsic system is very rapid (15 sec) & very extensive, while the intrinsic is slower (1-6 min).
3- Ca^{++} is required for promotion of all steps except the first 2 steps of the intrinsic way. Ca^{++} level rarely falls to level that affect clotting.
4- There is a link between intrinsic & extrinsic pathway: activated factor VII(extrinsic) & factor IX (intrinsic) activates factor X (common pathway).
5- Vitamin K is important for activation of factors II, VII, IX and X.

Anti-clotting mechanisms

Functions:

1- Limit tendency of blood to clot

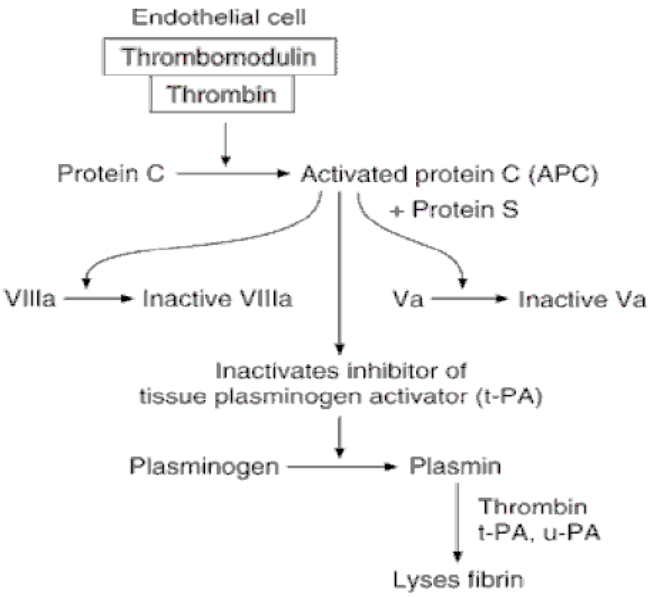
2- Break down already formed blood clot

Mechanism:

1- general

2- specific

general	Specific
a) Vascular endothelium is smooth and not suitable for formation of plug or clot.	a) Anti thrombin III inactivate active factor IX-X-XI and XII.
b) Liver remove and inactivate activated clotting factor.	b) Prostacyclin inhibit platelet aggregation and limit platelet plug.
c) Heparin.	c) Fibrinolytic system.
	d) Protein C, Protein S. • inactivate factors V & VIII • Inactivate inhibitor of tissue plasminogen activator • Increase plasmin formation



Fibrinolysis:

Is formed from its inactive precursor plasminogen by the action of tissue plasminogen activator (TPA) and thrombin. Plasmin lyses fibrin and fibrinogen into fibrinogen degradation products (FDP) which inhibit thrombin.

Anti-coagulants

Anti-coagulants are substance that prevent blood clotting

	In vitro	In vivo
1	Oxalate salt	Heparin
2	Citrate salt	Dicumarol
3	Silicon	
4	Heparin	

	Dicumarol	**Heparin**
1- Source	Plant source	Basophils, mast cells & liver
2-Intake	Oral (by mouth)	Injection intravenous i.v. & intramuscular i.m.
3- Onset & duration	Slow onset & long duration	Rapid onset & short duration
4- Chemistry	Similar to vitamin K	Sulphated mucopolysaccharide
5- Site of action	Only in vivo	In vivo & In vitro

6- Action	Competes with vitamin K. So, inhibits its utilization by liver (competitive inhibition). It decreases synthesis of factors II, VII, IX, X, prtn C & prtn S.	-It increases antithrombin III activity. - It prevents activation of factor IX - It has a clearing action→it increases lipase enzyme which clears blood from lipids after meals.
7- Antidote	Vitamin K	Protamine Sulphate 1% (basic protein)

Abnormalities of Hemostasis

(1)Vitamin K deficiency:

-Vitamin K is important for synthesis of factors II, VII, IX & X by liver

-It is synthesized by bacterial flora of intestine (it is advisable to delay circumcision one month after birth).

-Its deficiency leads to deficiency of factors II, VII, IX & X.

-Causes of its deficiency:

a- Sterility of intestine as in:

1- Newly born infants.

2- Long treatment with antibiotics

b- Decrease absorption as in:

1- Obstructive jaundice

2- Fat malabsorption because vit K is fat soluble vitamin

c- Liver diseases.

d- Anticoagulants: which act by competitive inhibition with vit. K

(2)Hemophilia:

Hereditary, congenital, sex linked recessive disease carried by female, transmitted always to male (carried on X chromosome).

- It is 3 types:

  Hemophilia A due to deficiency 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).

- It is characterized by severe prolonged bleeding on mild trauma.

- Clotting time is prolonged.

(3) Thrombocytopenic purpura:

- Characterized by subcutaneous hemorrhages which are called petichae
- It is due to decrease platelets number (bleeding occurs if it is below 50000/mm^3.
- Bleeding time is prolonged.

(4)Thromboembolism:

Clot is formed inside blood vessels as in atherosclerosis & after operation.

(5) DIC (Disseminated intravascular clotting):

Excessive bleeding & clot formation which may occur in intrauterine fetal death, repeated blood transfusion or repeated renal dialysis.

Blood groups

ILOs

1- Identify blood types (ABO system and Rh factor)

2- Mention importance of blood goups.

3- Identify complication of incompatible blood transfusion.

ABO system:

- The cell membrane of RBCs contains mucopolysaccharide substances called antigens. Two types of antigens are known: A antigen & B antigen.
- People are classified into 4 groups according to antigen (agglutinogen) on RBC membrane; the plasma contains antibodies (agglutinins) against the absent antigen.

Group	A	B	AB	O
%	40	10	5	45
Antigen	A	B	A & B	-
Antibody	Anti-B	Anti-A	-	Anti-A & Anti-B

- Blood group A : A antigen is present
- Blood group B : B antigen is present
- Blood group AB : A & B antigens are present
- Blood group O : Neither A nor B antigens are present.
- If an antigen is present in RBCs and the plasma contains its corresponding antibodies → agglutination → hemolysis.
- The antigens are called agglutinogen and the antibodies are called agglutinins.

Importance:

1-Medicolegal importance (Disputed Parenthood):

Inheritance of blood groups is by 2 antigens from both father & mother

The A & B antigens are dominant, while the O one is recessive.

Blood group is a good negative test in disputed parenthood.

2-Blood transfusion:

In incompatible blood transfusion, the donor RBCs is agglutinated by recipient plasma, as the donor's plasma is diluted by the recipient blood.

*Group O is universal donor, because there is no agglutinogen.

*Group AB is universal recipient, because there is no agglutinin

*Cross matching test: should be done before blood transfusion in which the recipient plasma is mixed with donor's RBCs, and recipient RBCs is mixed with donor plasma, If no agglutination→transfusion is done.

Rh factor (D factor):

- It is a system composed of C, D, E antigens
- It is first discovered in blood of Rhesus monkey
- D is the most antigenic component.
- 85 % of people are Rh positive i.e. have D antigen.
- 15 % of people are Rh negative i.e. have no D antigen.
- Normally Rh + Ve & Rh – Ve have no anti-D
- Negative Rh persons forms anti D if antigen D is transformed to them.
- Positive Rh never form anti D, whether receives Rh +Ve or Rh -Ve

Importance:

1) Erythroblastosis Foetalis: (Rhesus hemolytic disease of the newly born)

The disease occurs if:

- An **Rh negative female** is married from an **Rh positive male** & she carries an Rh positive fetus
- At delivery of this first baby (which will be born normal), little fetal blood leaks into maternal circulation.
- Mother will produce **anti-D** agglutinins (IgG)
- During next pregnancy, maternal agglutinins (IgG) cross the placenta causing fetal hemolysis leading to :
    - **Anemia** of fetus
    - **Jaundice**, increase bile pigments which cross the undeveloped blood brain barrier and deposit in basal ganglia (Kernicterus).

- The first baby is affected in case of maternal sensitization by
  - Previous Rh + Ve blood transfusion
  - Fetal maternal hemorrhage during pregnancy.
- The disease can be prevented by:

- Avoiding Rh + Ve blood transfusion to Rh – Ve females.
- Anti-D antibodies are given to neutralize the Rh +Ve fetal cells and prevent maternal sensitization.

*If baby is born alive, he would be treated by exchange transfusion with blood group O Rh negative.

N.B. No fetal complication regarding ABO system because ABO antibodies cannot cross the placenta (IgM).

2) Repeated blood transfusion:

- If **Rh – Ve persons** is transfused with **Rh +Ve blood**, he will produce agglutinins against Rh factor.
- If this person is transfused (later on) with Rh +Ve blood, agglutination occurs.

Blood transfusion:

Indications:

1) To restore whole blood as in haemorrhage.
2) To restore one element: RBCs, WBCs & platelets.
3) Erythroblastosis foetalis.

Precautions:

1) Compatible. 2) free from contamination. 3) High Hb content.
4) Free from disease. 5) Fresh i.e. less than 2 weeks storage.

Storage: Blood is stored at $-4^{o}C$ not more than 2 weeks.

The following is added to it: →citrate as an anticoagulant
→Dextrose as nutrient for cells
→Citric acid to reduce PH

Storage will lead to: →Decrease platelets
→Decrease dextrose
→Decrease factor 7, 8 & 9
→Increase, potassium & lactic acid

Complications:

1) **Mechanical:** air or fat embolism
2) **Physiological**: excess transfusion → overloading →heart failure.
- Pyrogenic reaction → fever.
3) **Infective**: infective hepatitis, malaria, AIDS.

4) **Incompatibility:** Transfusion with incompatible blood leads to clumping & hemolysis of given RBCs leading to:

a- Blockage of blood capillaries:

This occurs by clumping RBCs leading to →backache and joint pain.

Blocking of coronary vessels leads to → angina pain

b-Intravascular hemolysis leads to :

1. Shock due to release of histamine and vasodilators-→drop of arterial blood pressure.
2. Liberation of K+ (hyperkalemia)→cardiac arrhythmia.
3. Liberation of Hemoglobin which:
   a. is broken to bilirubin leading to yellow coloration of skin and mucous membrane (jaundice)
   b. Leads to blockage of renal tubules as it is filtered by renal glomeruli →leading to renal failure.

White blood cells (leukocytes)

ILOs

1- Enumerate different types of WBCs, normal count and demonstrate functions of each type.
2- Recognize the different lymphatic organs
3- Explain the functions of lymphatic system. Clarify reticulo-endothelial system and their general functions.

White blood cells (WBCs) are the mobile units of the body defensive system

They work in two different ways to prevent diseases:

1) Phagocytosis
2) Formation of antibodies & sensitized lymphocytes.

Site of formation:

In bone marrow: granulocytes, monocytes & few lymphocytes.

In lymphoid tissue: lymphocytes & plasma cells.

Count: 4000 – 11000/mm^3.

Granulocytes: They have granules in their cytoplasm, include:

Neutrophils: 50-70 % of WBCs
Eosinophils: 1-4 %
Basophils: 0.4 %

Agranulocytes: They have no granules in their cytoplasm, include:

Lymphocytes: 20 – 40%
Monocytes: 2-8 %

Function of leukocytes:

(1) Eosinophils:

a) Attack parasites, that are to large to be engulfed by phagocytosis
b) They produce leukotriene in allergic diseases.
c) They are involved in mucosal immunity e.g. GIT, respiratory system, lower urinary tract.
d) Eosinophils migrate into blood clots where they produce profibrinolysin which when activated will digest blood clot.
e) They are weak phagocytes & they show chemotaxis.

(2) Basophils:

a) Contain histamine, heparin & leukotriene.
b) Responsible for immediate type of hypersensitivity reactions (allergy).
c) Mast cells are similar to basophils but found in tissues as epithelial surfaces & in areas rich in connective tissue.

(3) Neutrophils (mature cells)

a) Half-life is 6 hours.
b) They ingest & kill bacteria.

(1) Lymphocytes:

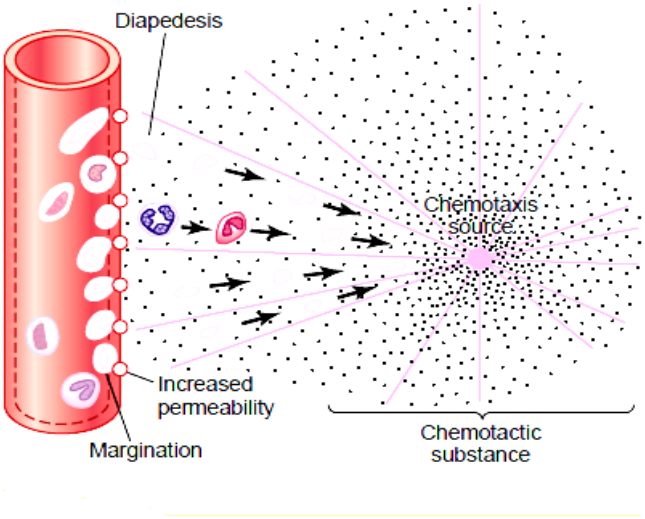
a) They are formed in lymph nodes, thymus & spleen.
b) They enter blood stream via lymphatics.
c) Their life span: few days to several months.
d) They are the key cells of immunity.

(2) Monocytes: …..immature cells

a) Little defense mechanism.
b) Have amoeboid movement & pass to area of inflammation after neutrophils.
c) Circulate for 72 hours & then enter the tissues.

- In the tissues, they become macrophages which have high defense mechanism e.g. Kupffer cells of liver, osteoclast in bone.

The inflammatory response:



Movement of neutrophils by *diapedesis* through capillary pores and by *chemotaxis* toward an area of tissue damage.

- It is response of living tissue to injury like infection, trauma & chemicals.
- It is characterized by local vasodilatation causing redness & increase vascular permeability causing accumulation of fluid and swelling (edema).
- The cells involved are neutrophils & monocytes.

Steps

Margination: WBCs stick to the inflamed capillary wall.

Diapedesis: WBCs squeeze themselves through capillary pores.

Amoeboid movement: through tissue to reach bacteria.

Chemotaxis: Positive: attraction of cells towards bacterial toxins & site of inflammation by chemotactic agents e.g. Complement & leukotriene

Negative: repulsion of cells away from bacteria.

Opsonization: preparation of bacteria to be tasty to phagocytes.

- Opsonins are immunoglobulin G (IgG) & complement proteins.
- Opsonins coat bacteria then bind to receptors on phagocyte cell membrane and then engulfed by Phagocytosis.

- A phagocytic vacuole is then formed with which lysosomes fuse and release enzymes to kill bacteria.

N.B

- **Leukocytosis:** increase WBCs to 20.000 as in acute inflammation e.g. tonsillitis.
- **Leucopenia:** decrease WBCs as in exposure to irradiation & drugs.

- **Leukemia:** malignant disease of Bone marrow→increase number of WBCs

Immunity

It is the ability of the human body to resist microorganisms or toxins. It is 2 types:

(**1) Nonspecific (innate) immunity**: not directed to specific organisms.

(2) Specific (acquired) immunity: directed to a specific organisms….

The two systems interact & function together.

(1) Nonspecific (innate) immunity:

1-Rapid

2-Non specific i.e. does not need special recognition of foreign cell

3-Innate i.e. does not depend on previous exposure.

4-It includes the following ….

1. Mechanical & chemical barriers.

A- Epithelium:
- Covers the skin
- Lines gastrointestinal, genitourinary & respiratory tracts.

B- Mucus: in respiratory tract which traps dust & pathogenic organisms.

C- Acid: secreted by stomach.

2. Non specific cellular mechanism:

A) Microphages: →neutrophils & Eosinophils.

B) Macrophages:→monocytes & tissue macrophages: act by phagocytosis & produce cytokines.

C) Natural killer cells (NK cells)

i. Non T non B lymphocytes.

ii. Large lymphocytes.

iii. Can destroy malignant cells & cells infected by virus & are considered the 1st line of defense against virus.

iv. Not specific to specific virus or tumor.

3. Nonspecific humoral mechanism:

A) Lysozymes: mucopolysaccharide that dissolve bacteria.

B) Interferon's (IFN): proteins released from virus infected cells

-They are α , β & Υ IFN

i. Alpha: - antiviral & increase activity of NK cells

- Used in treatment of cancer

ii. Beta: antiviral

iii. Gama: increase the activity of macrophages

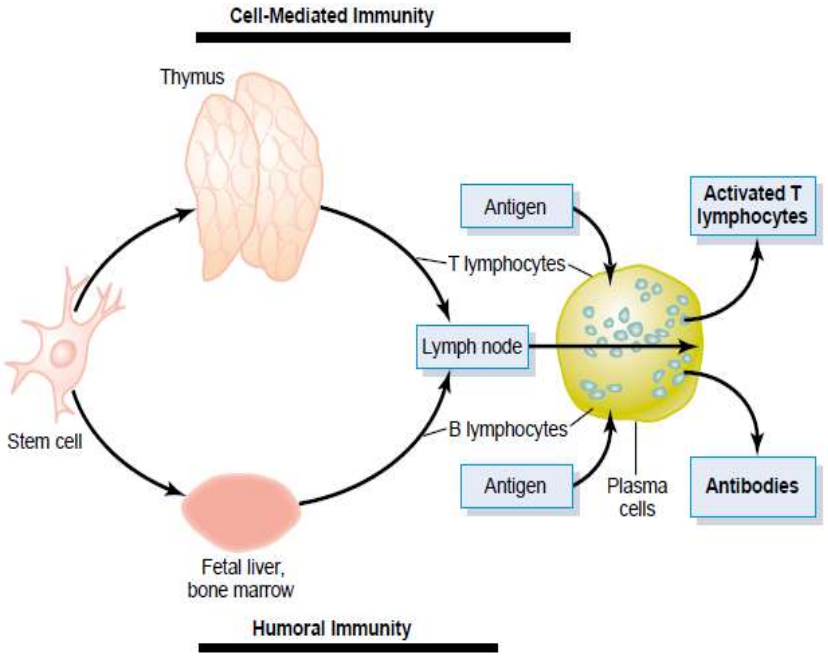
C) Properdin system: A complex system of proteins that can activate complement

D) Acute phase proteins: formed in liver during acute inflammation & tissue destruction. They are anti-inflammatory proteins e.g. C-reactive proteins.

E) Complement system:

-A system of 11 plasma protein enzymes (C1→C9) & C9 is 3 subunits.

-They mediate humoral & cellular immunity.



Formation of antibodies and sensitized lymphocytes by a lymph node in response to antigens. This figure also shows the origin of *thymic* (T) and *bursal* (B) lymphocytes that respectively are responsible for the cell-mediated and humoral immune processes.

(2)Specific or acquired immunity:

It is the ability of immune system to respond to foreign molecules called antigens.

Antigen (Ag): It is a substance which stimulates immune response and reacts specifically with antibodies, characterized by being:

1. Foreign.
2. Usually protein, polypeptide, may be carbohydrate or fat.
3. Large M.W. > 10.000.
4. Has a determinant group which is responsible for specification of Antigen.

Specific immunity is divided into:

(A) Humoral immunity: involves B-lymphocytes and antibody formation. It is the major defense against bacteria.

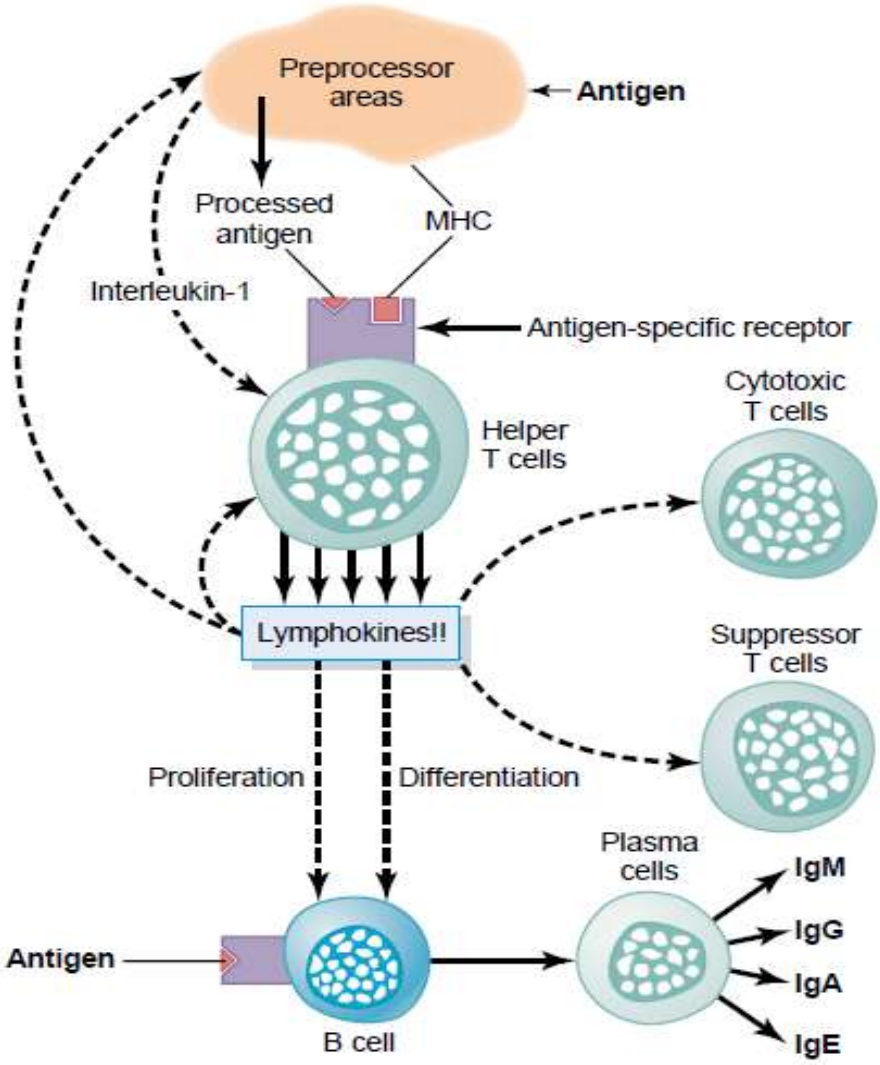
(B) Cell mediated immunity: requires generation of T-lymphocytes. It is the defense against virus, fungi, delayed allergic reactions, tumors and rejection of transplanted tissues.

Cell-Mediated Immune Response:

1. On exposure to antigen, specific T lymphocytes are activated.
2. Memory T- lymphocytes are formed leading to rapid response on second exposure to same Ag i.e. secondary response.

Types and functions of activated T-lymphocytes:

They are classified according to cell surface markers or functional activity into:



Regulation of the immune system, emphasizing a pivotal role of the *helper T cells*. MHC, major histocompatibility complex.

Helper T-lymphocytes (Th)-CD4:

a- They are the most numerous cells

b- They recognize the Ag accompanied by MHC-II.

c- They are 2 types Th-1 and Th-2:

- Th-1: -Secrete interleukin-2 (IL-2) and δ interferon-Stimulate growth and proliferation of cytotoxic and suppressor T cells.
- Th-2: secrete IL-4, IL-5, IL-6 which stimulate growth and differentiation of B-cells & antibody production.

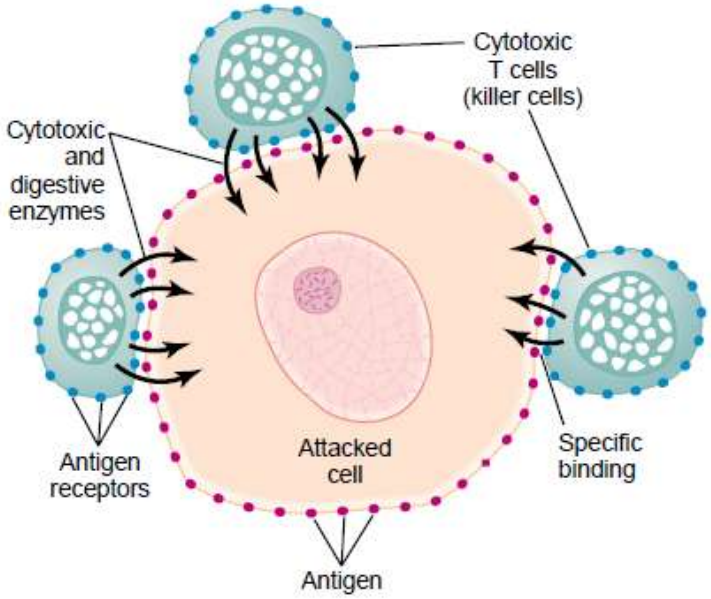
Cytotoxic T- lymphocytes (TC)-CD8:

a- They recognize Ag accompanied by MHC-I

b- Any pathological protein will be presented with MHC-I & stimulate TC.

c- Functions:

- They are direct attack cells & secrete hole-forming proteins called perforins that produce holes in target cells.
- They are important defense against viral infection & cancer cells.
- They are responsible for rejection of transplants of foreign tissues & delayed allergic reactions.



Direct destruction of an invading cell by sensitized lymphocytes (cytotoxic T cells).

Suppressor T–lymphocytes (Ts)-CD8:

Functions:

- Suppress activities of both B & T lymphocytes to provide an important negative feedback mechanism.

Humoral Immune response

[1] B lymphocytes recognize Ag via antibodies on their surface.

[2] B lymphocytes differentiate into:

a) **Plasma cells** which have well developed granular endoplasmic reticulum which forms large quantities of antibodies (immunoglobulins).

b) **Memory B cells** which on second exposure to the same antigen produce secondary response which is much more rapid and much more potent response than primary response. . Memory cells persist in the body for months or years.
NB: T helper cells (Th2) are needed for full activation of B-lymphocytes through productions of IL-4,5 & 6

Primary & Secondary antibody response:

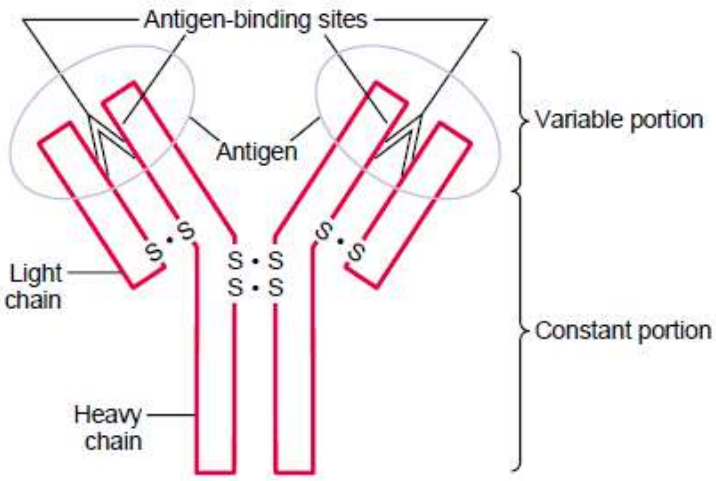
1) **Primary response**: Following an initial exposure to foreign antigen, antibodies start to appear in the plasma after 8 days incubation period, reach a high level in few weeks then decline rapidly. Antibodies formed are IgM
2) **Secondary response**: On exposure to the same antigen, the concentration of the antibody produced is much higher than the first exposure. Antibodies formed are IgG

Antibodies

-Antibodies are gamma globulins called immunoglobulins (IGs).

-They have MW between 90.000 &150.000 .

Types of antibodies: Immunoglobulins (IG) M A G E D



Structure of the typical IgG antibody, showing it to be composed of two heavy polypeptide chains and two light polypeptide chains. The antigen binds at two different sites on the variable portions of the chains.

	IgG	**IgM**
Structure	Monomer: 2 sites for antigen binding	Pentamer: 10 sites for antigen binding
M.W	Lowest molecular weight	Highest molecular weight
Functions	1) Passive immunity 2) Rh antibody are IgG, so can cross the placenta 3) Complement activation 4) Responsible for secondary response 5) Act as Opsonins activating phagocytosis,Produces major antiviral, antibacterial activity	1) Active immunity 2) ABO antibody are IgM, so not cross the placenta 3) Complement activation 4) Responsible for primary response

IgA (Secretory immunoglobulins):

1. Monomer, dimer or trimmer.
2. Found in body secretions (mucous, saliva, tears, milk & colostrum).
3. It protects against superficial infection e.g. eye , nasopharynx and also in urinary tract.

4. It is first line at mucosal level against viral infection.

IgD:

1. It is responsible for antigen recognition by B cells.
2. It is present as an antigen receptor in B- lymphocytes.

IgE:

1. It binds to specific receptors on basophils & mast cells.
2. If reacts with antigen, it leads to release of histamine, heparin & leukotriens.
3. It plays a role in allergic condition & parasitic infestation.

Functions of antibodies:

Antibodies bind antigens & cause their destruction by:

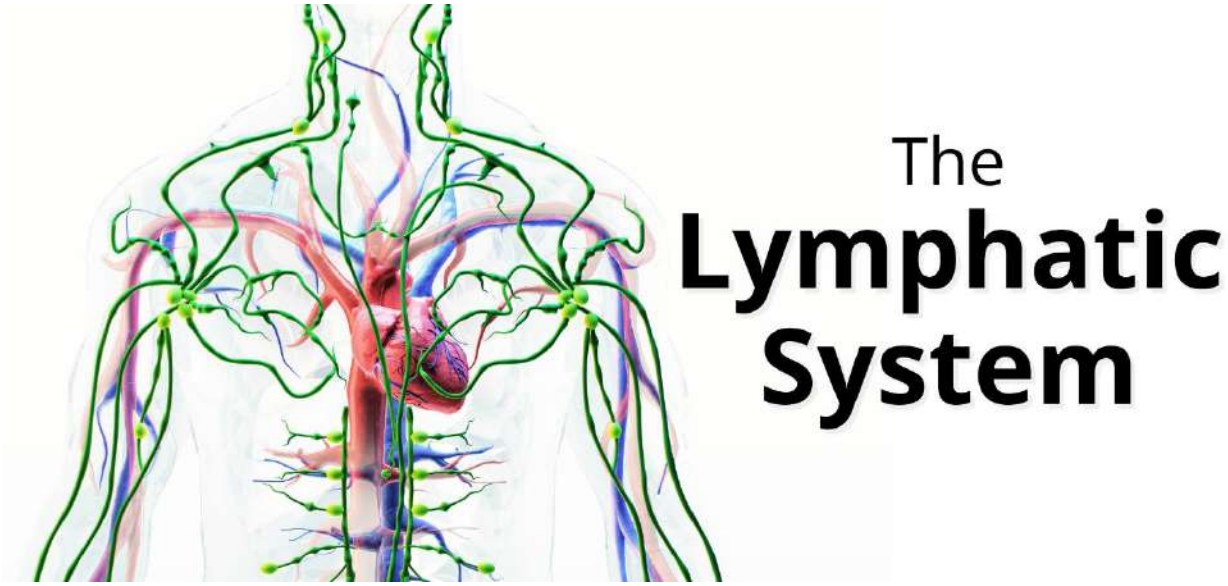
1. Agglutination of the antigen.
2. Neutralization of the toxic site of the antigen.
3. Opsonization
4. Activation of Natural killer (NK) cells.
5. Activation of the complement system by the classic pathway
6. -The structure of the antibody is complementary to its antigen (Key & its lock)
7. -The interaction may be one or more of the above mentioned effects.

- **Acquired immune deficiency Syndrome (AIDS)**

HIV virus → - Decrease T helper
- Decrease T cytotoxic
- Decrease B cells
- There is loss of immune function.

- **Autoimmune disease:**

- Normally immune system distinguishes between self & non self-cells.
- Sometimes, antibodies are formed against self-constituents e.g. insulin dependent diabetes in which antibodies is formed against Pancreas B cells.



Pathology département

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INDEX

LYMPHATIC SYSTEM & LYMPHOID NEOPLASMS

PART 1: LYMPHATIC SYSTEM & LYMPH NODE PATHOLOGY

Learning Objectives

- Recognize lymphadenitis types
- Identify reactive follicular hyperplasia
- Classify lymphoid tissue tumors
- Mention the subtypes of Hodgkin lymphoma.
- Describe the different types of Hodgkin lymphoma with its clinical presentation, histopathologic features, immunophenotyping of Hodgkin giant cells and prognosis of Hodgkin lymphoma
- Describe a subset of Non-Hodgkin lymphomas that are clinically important
- Small lymphocytic lymphoma/ chronic lymphocytic leukemia
- Follicular lymphoma
- Mantle cell lymphoma
- Extra-nodal marginal zone lymphoma (MALT lymphoma)
- Diffuse large B cell lymphoma
- Burkitt lymphoma

Lymphoid Tissue Distribution

- Lymph nodes

- Bone marrow

- Thymus

- Spleen

- Nasopharynx, tonsils, Peyer's patches

- Appendix and bronchus-associated tissue

Lymphoid Organs Classification

Primary Lymphoid Organs:

- Bone marrow, thymus
- Yolk sac, fetal liver

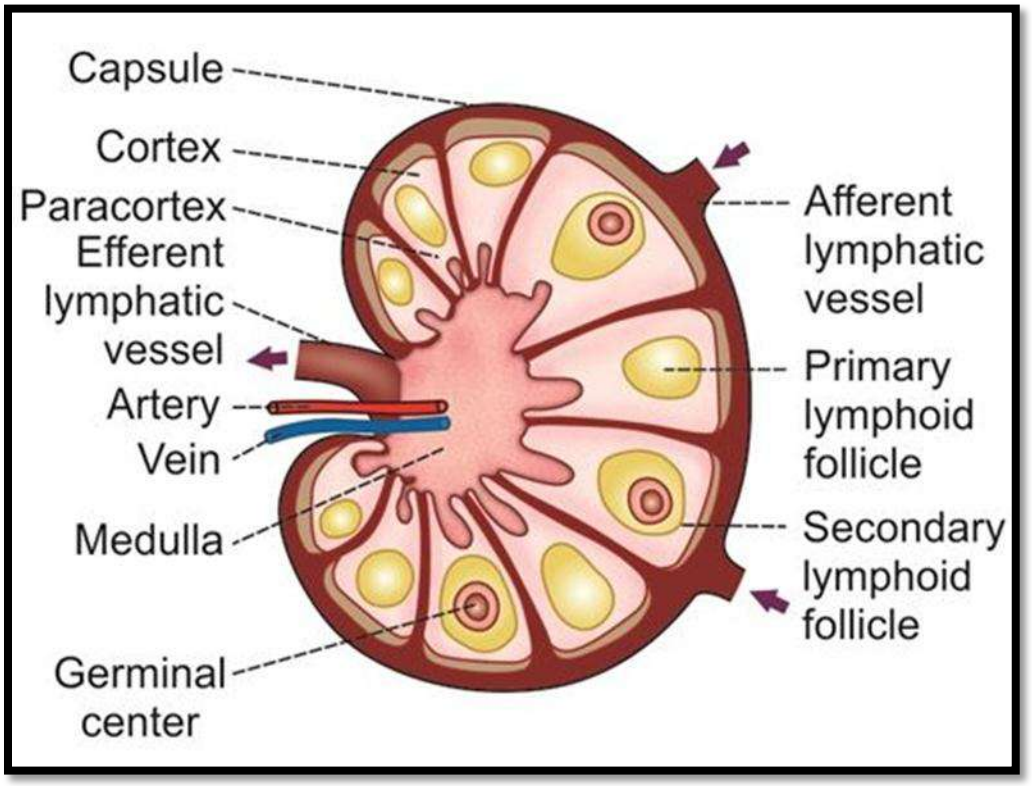
Secondary Lymphoid Organs:

- Lymph nodes, spleen
- Tonsils, adenoid
- Peyer's patches, appendix

Histology of Lymph Nodes

- Capsule, subcapsular sinus
- Cortex (B cell zone)
- Paracortex (T cell zone)
- Medullary sinuses/cords
- Afferent/efferent lymphatics
- Primary/secondary follicles

Histological diagram of a lymph node showing cortex, paracortex, medulla, follicles, and sinuses.



Schematic cross-section of a lymph node highlighting histological zones.

Lymph Node Diseases Classification

1. Reactive lymphoid hyperplasia
2. Inflammatory diseases (lymphadenitis)
3. Tumors of lymph nodes:
   - Lymphoma
   - Metastatic carcinoma

Lymphadenitis

Definition: Inflammation of lymph nodes resulting in swelling and tenderness.

Classification:

1. Acute Lymphadenitis:

- Bacterial
- Viral

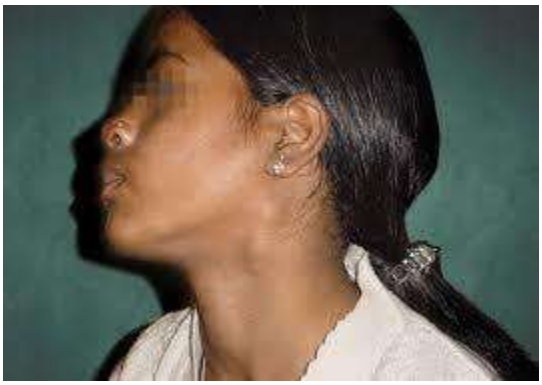
2. Chronic Lymphadenitis:

- Non-specific
- Specific (Granulomatous):
  - Tuberculosis (TB)
  - Sarcoidosis
  - Toxoplasmosis
  - Crohn's disease
  - Cat scratch disease

Acute Bacterial Lymphadenitis

- Occurs in LNs draining acute inflammation (e.g., tonsillitis)
- Rich in neutrophils
- Gross: enlarged, soft, red, tender
- May progress to abscess

2: Gross image of an acutely inflamed lymph node showing redness and swelling.

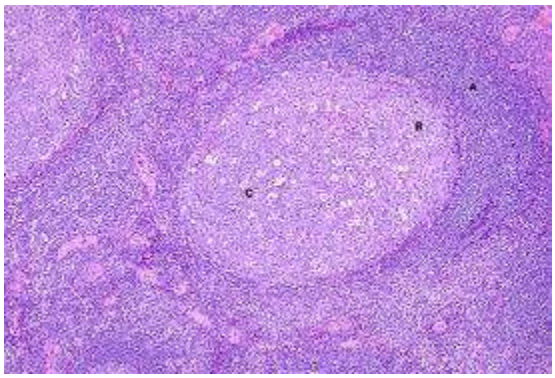


Acute bacterial lymphadenitis with cardinal signs of inflammation.*

Acute Viral Lymphadenitis

- Commonest: Infectious mononucleosis (EBV)

- Microscopic: Paracortical hyperplasia



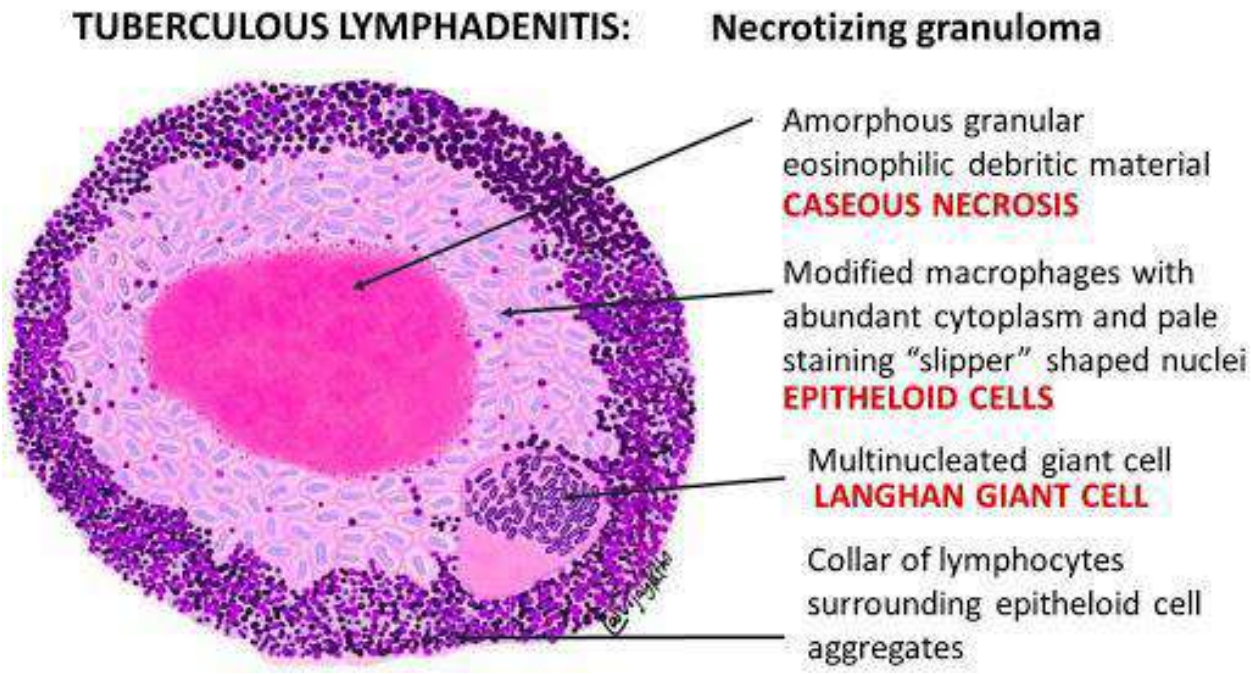
Chronic Granulomatous Lymphadenitis

Tuberculous Lymphadenitis:

- Early: Firm, non-adherent nodes, grayish-white cut surface

- Late: Matted LNs with caseous necrosis

- Microscopic: Caseating granulomas with epithelioid cells, Langhans giant cells



Sarcoidosis:

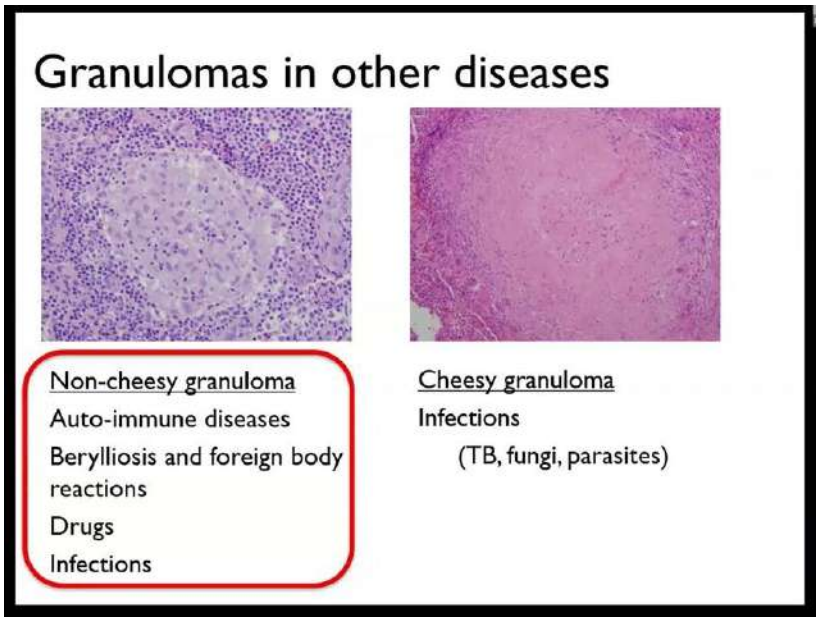
- Non-caseating "naked" granulomas

- May contain Schaumann & asteroid bodies

Cat Scratch Disease:

- Stellate necrotizing granulomas with neutrophilic microabscesses

: Comparison of granulomatous inflammation in TB (caseating) and sarcoidosis (non-caseating).



Reactive Lymphoid Hyperplasia

- Non-neoplastic enlargement due to immune response

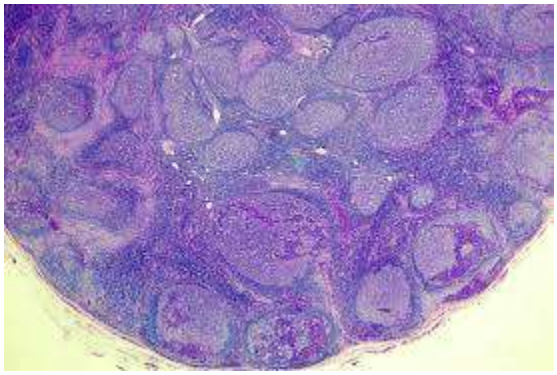
- Types:

Follicular hyperplasia (B-cell stimulation)

Paracortical hyperplasia (T-cell stimulation, e.g., SLE, viral infections)

Sinus hyperplasia (histiocyte distension)

4: Histology of reactive follicular hyperplasia showing secondary follicles with germinal centers and tingible body macrophages.



Reactive follicular hyperplasia with prominent germinal centers.

PART 2: HODGKIN LYMPHOMA (HL)

Definition

- Malignant tumor of lymphoid tissue characterized by:
  - Reed-Sternberg (RS) cells
  - Polymorphic inflammatory background

Epidemiology

- ~30% of all lymphomas
- Bimodal age distribution
- Contiguous spread
- ~50% EBV-associated

Clinical Features

- Painless lymphadenopathy (cervical/supraclavicular)
- Systemic symptoms: fever, night sweats, weight loss

Gross Pathology

- Early: Enlarged, firm, discrete nodes
- Late: Matted, fixed mass

Microscopic Features

- Loss of normal architecture
- RS cells and variants:
  - Classic RS cells (mirror-image nuclei)
  - Mononuclear variant

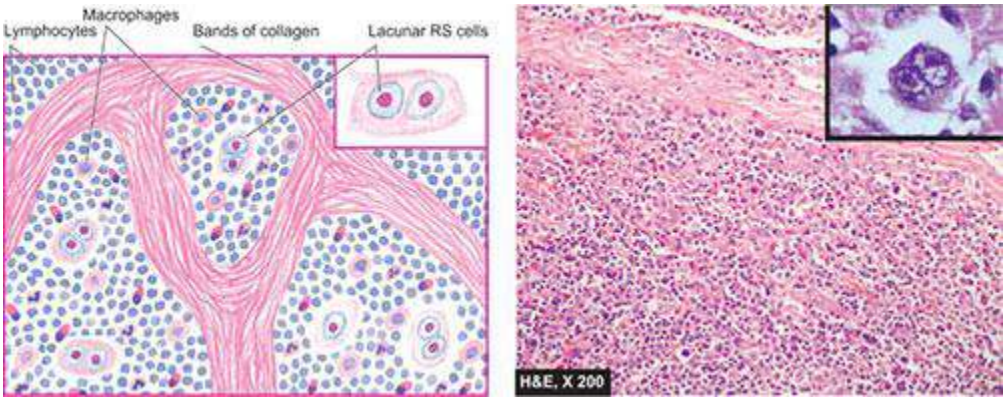
- Lacunar cells (nodular sclerosis)

- Popcorn cells (nodular lymphocyte-predominant)

- Inflammatory background

5: Photomicrograph of a classic Reed-Sternberg cell with “owl-eye” nuclei.

Classic binucleated Reed-Sternberg cell in a background of mixed inflammatory cells.



Histological classical Hodgkin lymphoma.*

Classification of Hodgkin Lymphoma

- Classical HL (95%):

  - Nodular sclerosis (65%)

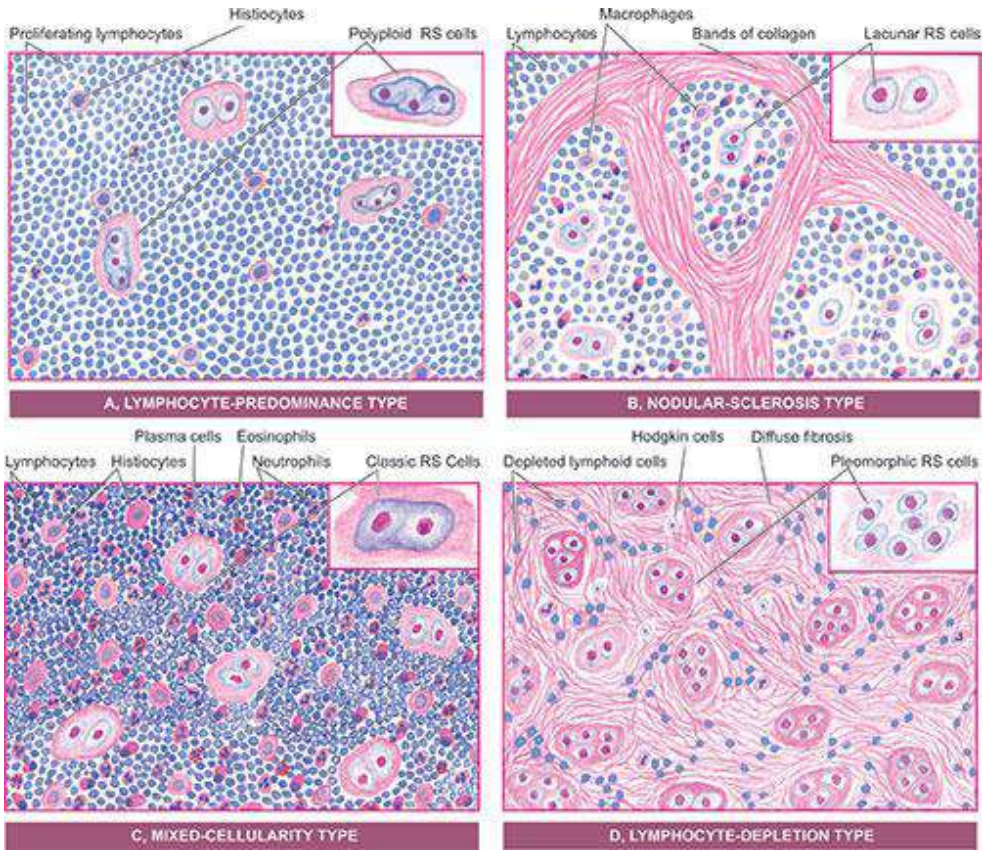
  - Mixed cellularity (20–25%)

  - Lymphocyte-rich (5%)

  - Lymphocyte depletion (<5%)

Nodular lymphocyte-predominant HL (5%)

6: Side-by-side histology of nodular sclerosis HL (collagen bands) and mixed cellularity HL (diffuse infiltrate).



Histological subtypes of classical Hodgkin lymphoma.*

Immunophenotyping

- RS cells: CD15+, CD30+

Staging & Prognosis

- Stage I:One LN group

- Stage II: ≥2 LN groups, same side of diaphragm

- Stage III: Both sides of diaphragm

- Stage IV: Extranodal spread

- Prognosis depends on subtype and stage.

PART 3: NON-HODGKIN LYMPHOMA (NHL)

Definition

- Malignant lymphoid tumors of B- or T-cell origin
- Nodal or extranodal

Features vs. Hodgkin Lymphoma

- Heterogeneous group
- Non-contiguous spread
- Frequent extranodal presentation
- Generally worse prognosis than HL

Classification Schemes

1. Working Formulation (morphology-based)

- Low, intermediate, high grade

2. WHO Classification (cell-of-origin based)

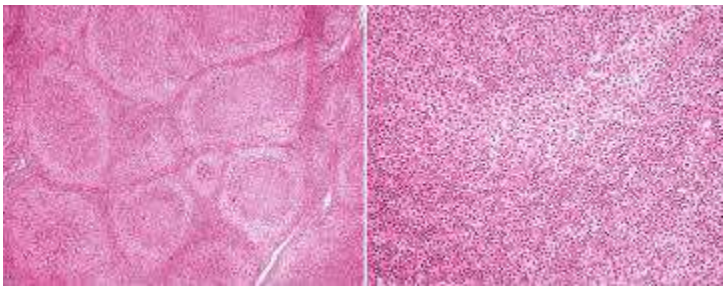
- B-cell lymphomas
- T-cell lymphomas

Selected NHL Subtypes

1. Follicular Lymphoma:

- Nodular architecture
- BCL2+ (differs from reactive hyperplasia)
- CD19+, CD20+

7: Histology of follicular lymphoma showing crowded, monomorphic follicles without mantle zones.



Follicular lymphoma with effaced architecture and neoplastic follicles.

2. Diffuse Large B-Cell Lymphoma (DLBCL):

- Large cells, diffuse pattern

- Aggressive

- CD20+

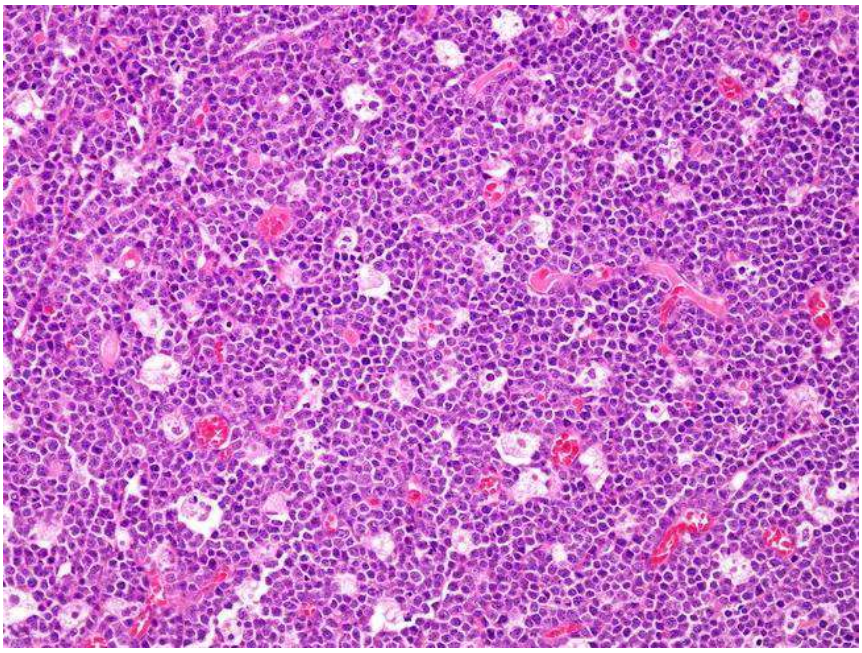
3. Burkitt Lymphoma:

- “Starry-sky” appearance (macrophages with debris)

- High mitotic rate

- Endemic (EBV+) vs. sporadic

8: Burkitt lymphoma histology showing a “starry-sky” pattern.



Burkitt lymphoma with tingible-body macrophages imparting a starry-sky appearance.

4. Mantle Cell Lymphoma:

- CD5+ B-cell lymphoma

- Often involves extranodal sites

5. MALT Lymphoma:

- Extranodal marginal zone lymphoma

- Often gastric (H. pylori-associated)

- Indolent course

6. Anaplastic Large T-Cell Lymphoma:

- Large, pleomorphic cells

- CD30+

Diagnosis of Lymphoma

- Clinical suspicion (persistent lymphadenopathy)
- Histology (architectural effacement, atypical cells)

Immunohistochemistry (essential for subtyping):

- B-cell markers: CD20
- T-cell markers: CD3
- Specific markers: CD10 (Burkitt), BCL2 (follicular), CD30 (ALCL, HL)

Metastatic Carcinoma in Lymph Nodes

- Replacement by epithelial tumor cells
- Important for TNM staging

Generalized Lymphadenopathy:

Causes

- Inflammatory (infectious, autoimmune)
- Neoplastic (lymphoma, metastasis, leukemia)
- Drug-induced
- Reactive hyperplasia

TABLE 1: Comparison of Hodgkin vs. Non-Hodgkin Lymphoma

	HL	NHL
Incidence	+/- 30 % of all lymphomas	More common
Age incidence	Bimodal	Increase with age
Neoplastic cells	RS cells or its variants	B cells or T cells
Background cells	Numerous reactive cells	No or rare reactive cells
Progression	Often localized to a single group of LNs	Tend to involve more than one group of LNs
Spread	Usually contiguous spread.	Usually non-contiguous spread.
Peri-nodal extension	Less frequent peri-nodal extension	Frequent peri-nodal extension
Extra nodal extension	Extension of extra-nodal sites is uncommon.	Extension to extra-nodal sites is common.
Prognosis	Generally better than NHL (based on stage)	Generally worse than HL (based on stage)

References

-HARSH MOHAN Text book of pathology seventh edition

-PRINCIBLES OF SYSTEMIC PATHOLOGY By GAMAL NADA, M.D.

Professor of Pathology Faculty of Medicine Cairo University

Hematology: Blood & lymph system

(HEM-210)

Handout

Pharmacology

Level: 2

2025-2026

Prepared by

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Index

Lecture 1

ANTICOAGULANTS, THROMBOLYTICS & ANTIPLATELET DRUGS

I- ANTICOAGULANTS

Classification: The most recent classification of anticoagulants based on their **Mechanism of action:**

1. Indirect thrombin inhibitors:

a) Heparins (unfractionated heparin 'UFH' & Low molecular weight heparin "LMW") .

b) Synthetic pentasaccharide: e.g., fondaparinux.

2. Direct thrombin inhibitors : e.g., Argatroban, Dabigatran, Hirudin and bivaluridin.

3. Direct factor Xa inhibitors: e.g., Betrixaban, rivaroxaban, apixaban and edoxaban.

4. Vitamin K antagonist : e.g., Warfarin.

1- INDIRECT THROMBIN INHIBITORS

A) UNFRACTIONATED HEPARIN - Stored with histamine in mast cells, strong acidic and strong negative charge.

Mechanism of action: – It activates antithrombin III by changing its conformational state causing fast inactivation of

thrombin and factor Xa equally leading to inhibition of coagulation factors. – The effect of heparin is non-predictable and so need monitoring using aPTT (activated

partial thromboplastin time). It acts in vivo and in vitro.

Pharmacokinetics:

1- It is not absorbed orally due to high negative charge and large molecular size.

3. It can be used by I.V. infusion with immediate effect and duration of action 3-5 h;

also, it can be used by S.C. injection.

4. It is not used I.M. as may cause painful hematoma.

5. t½ is 1.5 h, metabolized in liver and excreted as metabolites by the kidney.

Laboratory monitoring:

- By measuring activated partial thromboplastin time, aPTT.

Administration:

– I.V. infusion by initial bolus injection of 5000 units followed by 700-2000 units / hour. – S.C. injection can be used for long-term management of patients with pregnancy. – Alternatively, prophylactic low dose of 5000 units every 8-12 h is given preoperative to reduce the risk of postoperative deep venous thrombosis and fatal pulmonary embolism.

Side effects:

1- Bleeding: Dose-dependent & needs dose adjustment based on aPTT monitoring.

2- Heparin-Induced Thrombocytopenia (HIT- immune-based reactions): In about

0.5 % patients after 5 days of starting drug therapy. If happen, heparin must be

stopped and use of direct thrombin inhibitor as hirudin and bivalirudin or fondaparinux.

3- Hypersensitivity reactions: UFH taken from Pork or cattles. 4. Osteoporosis &

alopecia

Antidotes of heparin toxicity:

Protamine sulphate is given by slow I.V.,(1 mg can antagonize 100 units of heparin).

B) <u>LOW-MOLECULAR WEIGHT (LMW) HEPARINS</u>

Members and pharmacological properties:

 Enoxaparin (Clexan; S.C.), nadroparin and dalteparin: they differ from heparin in:

1. They are fragments of unfractionated heparin with low molecular weight.

2. Promote inhibition of factor Xa by antithrombin with little effect on thrombin.

3. Have longer t½ , so they are used S.C. once or twice / day.

4. They have high bioavailability and predictable anticoagulant effect, so no need for routine lab monitoring or dose adjustment.

5. They have lesser side effects as thrombocytopenia, osteoporosis and bleeding.

6. Their effect is incompletely neutralized by protamine sulphate.

7. They are monitored by antifactor Xa activity but not by aPTT.

Indications of heparins (UFH & LMWH):

1- Treatment of deep venous thrombosis or pulmonary embolism.

 Heparin is used firstly because of its rapid effect and at the same time warfarin is given.

 Heparin is given for 4-5 days till the onset of the full effect of warfarin which is continued for 3-6 months.

2- Prevention of venous thromboembolism in high risk patients: as after orthopedic or gynecological surgery.

3- Heparins can be used in the following conditions:

a) Initial management of unstable angina and acute MI.

b) During and after coronary angioplasty or stent placement.

c) Treatment of disseminated intravascular coagulation (DIC).

d) During surgery requiring cardiopulmonary bypass.

e) To prevent occlusion of haemodialysis machine.

Contraindications of Heparins:

1- Patients with hypersensitivity to the drug

2- Active bleeding and hemophilia

3- Significant thrombocytopenia (platelet count is necessary)

4- Visceral carcinoma

5- Uncontrolled hypertension & intracranial hemorrhage

6- Advanced hepatic or renal disease.

7- Active tuberculosis & ulcerative lesions of the gastrointestinal tract.

8- Patients who have recently had surgery of the brain, spinal cord, or eye

C) FONDAPARINUX - - -

Synthetic pentasaccharide binding strongly to antithrombin with high specific activivity against factor Xa i.e., inhibits factor Xa like LMWH.

Has long half-life (15 h) allowing once daily dosage.

No cross reactivity with heparin-antibodies, so it is recommended in HIT.

2- VITAMIN K- ANTAGONIST

 Warfarin sodium (Dendivan or Marivan)R.

 It is the widely used cumarin (dicumarol is a second cumarin derivative).

Mechanism of action:

 It acts only in vivo by by inhibiting the enzyme vitamin K epoxide reductase which is responsible for synthesis of vitamin K-dependent coagulation factors (II, VII, IX and X).

 With delayed onset because the effect depends on the t ½ of the clotting factors (3 days).

Pharmacokinetics:

1- Complete absorption after oral or parenteral use (oral (F) is near 100%), but parenteral use does not alter the speed of anticoagulant effect.

2- High binding to plasma proteins (99%), so it has low Vd.

3- Delayed onset of action (after 1-3 days) till metabolism of the already present clotting factors.

4- t½ is 40 h., duration of action is 2-5 days (due to enterohepatic circulation + long t ½ + long time needed for resynthesis of clotting factors).

5- Metabolized in liver and kidney to inactive metabolites and excreted in urine and stool.

Laboratory control:

By measuring prothrombin time PT and calculation of INR (1-1.5).

The drug is better to be given at bedtime to allow monitoring of a peak effect in the next morning. It is given 10 mg at bed time for 3 days, then 5 mg / day as maintenance dose.

Side effects:

1- Bleeding: is the most serious one.

2- Osteoporosis due to deficiency of Vit K

3- Teratogenic effect: as it crosses placental barrier causing teratogenic effect (as bone defect, bleeding of fetus and abortion).

4- Hypersensitivity reactions (fever and dermatitis) and alopecia.

5- Skin necrosis and purple toe syndrome (painful bluish discoloration of sides and planter surfaces of toes), which is decreased by elevation of legs, it appears after 3-8 weeks of treatment, may be due to release of cholesterol emboli from atheroma.

6- GIT manifestations: anorexia, nausea, vomiting, diarrhea and abdominal cramps.

Reversal of anticoagulant activity (Antidote):

In cases of mild bleeding: stop the drug + vitamin K1 (Phytonadion) orally is given.

In cases of severe bleeding: vitamin K1 by slow I.V. (to avoid hypotension) and fresh frozen plasma (rich in clotting factors) are given.

Drug interaction of warfarin (conditions that affect warfarin activity)

(1) Conditions that decrease the effect: - Increase in the clearance by enzyme inducers as phenobarbitone and phenytoin.

- Vitamin K administration or Hypothyroidism.

- Decrease the absorption by cholestyramine or malabsorption.

- Hereditary resistance due to anomalous of vitamin K epoxide reductase.

(2) Conditions that increase the effect:

a) Decrease in the clearance by enzyme inhibitors as cimetidine, metronidazole……..

b) Hyperthyroidism by increasing the metabolism of the clotting factors, so increase the sensitivity to warfarin.

c) Vitamin K deficiency due to inadequate intake or killing of bacterial flora by antibiotics as cephalosporins.

d) Displacement of warfarin from plasma proteins binding as by NSAIDs.

e) Low concentration of coagulation factors due to hepatic dysfunction or CHF

f) Advanced age (over 60 years).

g) Aspirin by inhibition of platelet function.

Indications of warfarin:

1- Prevention of progression or recurrence of deep venous thrombosis (for 3

months) or pulmonary embolism (for 6 months) after initial course of heparin.

2- Prevention of systemic embolization in patients with:

- Acute myocardial infarction.

- Prosthetic heart valves.

- Dilated cardiomyopathy.

- Chronic atrial fibrillation.

3- Prevention of venous thromboembolism in high risk patients as after orthopedic or gynecological surgery.

Contraindications for warfarin:

1) In patients with high risk for bleeding tendency (similar as heparins):

- Severe hepatic disease.

- Recent head trauma.

- History of GIT bleeding.

- Peptic ulcer.

- Subacute bacterial endocarditis.

- Recent major surgery.

- Haemophilia.

- Uncontrolled hypertension.

2) During pregnancy due to teratogenic effect.

3) Hepatic and renal dysfunction as there is a need for adjustment of the dose.

3- DIRECT THROMBIN INHIBITOR

1- Oral direct thrombin inhibitor: e.g., Dabigatran etexilate mesylate (Pradaxa) is the only oral thrombin inhibitor approved by FDA till 2018.

 Predictable anticoagulant effect (no monitoring requirement).

 Rapid therapeutic effect compared to warfarin.

 No need for platelet monitoring (no thrombocytopenia).

 Fewer drug interactions in comparison with warfarin.

 Dabigatran antidote is available (idarucizumab) developed at 2015 under the trade name “praxbind”.

 Used in heparin-induced thrombocytopenia.

2- Parentral drugs: e.g., Hirudin, Argatroban, Lepirudin (Refludan), Bivalirudin (Angiomax):

 Hirudin is a specific, irreversible thrombin inhibitor from leech saliva that is now available in recombinant form as lepirudin.

 Its action is independent of antithrombin, which means it can reach and inactivate fibrin bound thrombin in thrombi

4- ORAL DIRECT FACTOR Xa INHIBITOR

1- Betrixaban : the oldest one of this group: - The only anticoagulant that is not cleared by the kidneys and so no dose adjustment in renal

impairment. - Has a rapid onset of action - Has a half-life that supports once-daily dosing. - No need for monitoring “unlike warfarin or unfractionated heparin”. - Has been developed with an antidote; PRT4445 (portola), a universal Factor Xa inhibitor

antidote).

2- Rivaroxaban, apixaban & edoxaban: the recent drugs approved.

 Require no monitoring

 Their half-lives are shorter than warfarin (used once daily) but with rapid onset of

action.

 With appropriate oral bioavailability.

 Used for prevention of embolic stroke and in patients with AF, following orthopedic surgery and DVT.

 Antidote (Andexanet alfa) which is factor Xa is available and used to reverse their toxic action.

II- ANTIPLATELET DRUGS

- Thrombi may be arterial or venous:

 Arterial thrombus (White thrombus): rich in platelets.

 Venous Thrombi (Red thrombus): rich in fibrin. - Thrombus may block arteries or veins → partial or complete obstruction → MI, pulmonary embolism, cerebral stroke or DVT.

- Agents generated within the platelets that increase their aggregation include:Prosaglandins (Thromboxane A2), Adenosine diphosphate (ADP), and glycoprotein IIb/IIIa (GP IIb/IIIa) receptors. Increase platelet aggregation may enhance arterial thrombi formation. - Inhibition of thromboxane A2 formation, inhibition of ADP and blocking GP IIb/IIIa receptors prevent platelet aggregation.

Classification of antiplatelets drugs by mechanism of action:

a) Inhibition of thromboxane A2 formation (aspirin)

b) Inhibition of ADP induced platelet aggregation (clopidogrel, prasugrel, ticlopidine),

c) Blockade of glycoprotein IIb/IIIa receptors of platelets (abciximab, tirofiban, and eptifibatide).

d) Other drugs: e.g., Dipyridamole and cilostazol are additional antiplatelet drugs.

(1) **ASPIRIN IN LOW DOSE** - It acts by irreversible blocking of platelet cyclooxygenase enzyme leading to decrease in the synthesis of thromboxane A2 (platelet aggregation and vasoconstrictor). - - - -

Its effect lasts till synthesis of new platelet with new COX (after 7-10 days).

Aspirin must be used in low dose (75-325 mg / day) as the large dose causes also decrease in prostacyclin (PGI2) which has antiplatelet effect.

It is used as prophylactic against thrombosis in coronary and cerebral vessels in atherosclerotic patients.

It is also used in patient with myocardial infarction, unstable angina, coronary by pass graft and artificial heart valve.

Drawbacks of Aspirin (low dose):

Risk of gastrointestinal adverse events (ulceration and bleeding)

Aspirin intolerance

It is not very effective antithrombotic when used alone after stent placement and so used in combination with clopidogrel

Lack of response in some patients (aspirin resistance).

Very important notes for low dose aspirin:

1. Aspirin must be stopped (7-14 days) before surgical operation why? – To avoid increased blood loss if taken before surgery.

2. NSAIDs e.g., Ibuprofen, if taken concomitantly with, or 2 hours prior to aspirin, can obstruct the access of aspirin to the serine residue of COX 1, so can antagonize the platelet inhibition by aspirin. – Therefore, aspirin should be taken at least 30 minutes before other NSAIDs as ibuprofen or at least 8 hours after ibuprofen.

3. COX-2 inhibitors (Coxibs e.g., celecoxib) do not have antiplatelet effects and may contribute to cardiovascular events by shifting the balance of chemical mediators in favor of thromboxane A2 (prothrombotic). – So patients taking coxibs still need low-dose aspirin for cardiovascular protection.

4. Aspirin is used as initial prophylaxis in case of acute MI with plavix.

(2) TICLOPIDINE & CLOPIDOGREL (Plavix)

Mode of action:

Irreversibly inhibit the binding of ADP to its receptors on platelets → inhibiting ADP pathway.

Clopidogrel is a prodrug which require bioactivation by CYT P450.

Drawbacks:

1- Prolonged bleeding for which there is no antidote.

2- Inhibition of cytochrome P450 → interferes with the metabolism of drugs such as phenytoin, tolbutamide, warfarin, fluvastatin, and tamoxifen if taken concomitantly.

3- Neutropenia, thrombocytopenia, and aplastic anemia) limit ticlopidine usefulness (hematological abnormalities are much less with clopidogrel. So, clopidogrel is preferred over ticlopedine.

4- Food interferes with the absorption of ticlopidine but not with clopidogrel.

Uses:

Using Plavix (300 mg loading dose, 75 mg/day maintenance dose) is now golden therapy to prevent coronary stent occlusion (usually combined with aspirin).

In combination Plavix with aspirin are used to prevent MI and stroke.

Unstable angina (acute coronary syndrome)

NEW ADP RECEPTOR ANTAGONISTS:

Prasugrel: More rapid onset of action than clopidogrel and Irreversible inhibitor of the ADP.

Ticagrelor: More rapid onset of action than clopidogrel but acts as reversible ADP receptor inhibitor.

(3) GLYCOPROTEIN ILb/IIIa RECEPTOR BLOCKERS

The plateklet GP IIb/IIIa receptors function as a receptor for final pathway of platelet aggregation

e.g., Abciximab, Tirofiban, Eptifibatide.

They block GP IIb/IIIa receptor of platelet preventing the binding of fibrinogen and von Willbrand factor to its receptors on the platelets, so inhibit platelet aggregation.

They are used I.V. in angioplastic coronary intervention and acute coronary syndromes.

With short half-lives, so must be used with continuous I.V. infusion. Oral formulations are not yet available (Aug 2018).

(4) Other drugs

a) Dipyridamol: -It is a vasodilator and inhibitor for platelet aggregation. -Acts as phosphodiesterase inhibitor and blocking of adenosine uptake (adenosine acts on A2-receptors, stimulating platelet adenyl cyclase causing increase in cAMP). -Usually used with aspirin to prevent cerebrovascular ischemia.

b) Cilostazol: -New phosphodiesterase inhibitor that has vasodilator and antiplatelet effect and used mainly in treatment of intermittent claudication.

III- THROMBOLYTIC DRUGS (FIBRINOLYTICS)

They are drugs that rapidly dissolve the already formed intravascular thrombi and clot in acutely occluded vessel by catalysing the formation of plasmin from its precursor plasminogen.

Members:

a) Streptokinase: it is lytic protein (not enzyme) synthesized by streptococci

b) Urokinase: human enzyme synthesized by the kidney that converts plasminogen into active plasmin .

c) Tissue plasminogen activator: e.g. Alteplase, Reteplase and Tenecteplase.

They are prepared by recombinant techniques. They prefer binding to plasminogen that adher to fibrin more than systemic fibrin (fibrin specific).

Mechanism of action:

They act by activation of plasminogen to plasmin.

The formation of plasmin may occur in circulation (with non-specific drugs) or localized to fibrin surface (with specific drugs).

Streptokinase and urokinase are fibrin non-specific because they act on fibrin in both clots and systemic circulation (drawback).

T-PA are fibrin specific.

Therapeutic uses:

- by I.V. infusion or by bolus I.V. injection in the following:

a- Acute myocardial infarction:

They are used only in patients with S-T segment elevation and their age is less than 75 years and present within 6-12 h of the onset of symptoms (early intervention is better).

Low dose of aspirin improves the efficacy of thrombolytic therapy.

Recent studies suggest that angioplasty with or without stent placement is

superior to thrombolytic therapy.

b- Pulmonary embolism:

- They accelerate resolution of emboli within the first 24 hours (standard heparin

therapy).

c- Acute deep venous thrombosis.

d- Acute arterial thrombosis.

e- Acute ischemic stroke (used only in thrombotic or embolic stroke).

Summary of use of thrombolytic in acute MI - Although catheterization and placement of a stent (medicated or non

medicated) is the standard therapy of acute MI, thrombolytic therapy including: streptokinase, urokinase, and tPA as Alteplase, Reteplase and Tenecteplase is still very important where stent or catheterization is not readily available.

- The proper selection of patients for thrombolytic therapy is critical. Patients with ST-segment elevation and bundle branch block on ECG have the best outcomes.
- Greatest benefit for thrombolytic therapy when it is given early, within
6 hours after symptomatic onset of acute MI.
- Thrombolytic drugs reduce the mortality of acute MI & adjunctive drugs such as aspirin, heparin, BBs, and ACEIs reduce mortality even further.

Contraindications of thrombolytics:

- Patients who are at high risk of bleeding as internal bleeding, hemorrhagic stroke, uncontrolled hypertension, surgery or trauma in the last 2 months

Side effects:

1- Hemorrhage is the most serious complication of all thrombolytic drugs.

- It is more common with the fibrin non-specific group due to systemic formation of plasmin that causes lysis of clotting factors and lysis of fibrin in physiological thrombi at sites of vascular injury.

2- Allergic reactions with fever, urticaria and hypotension, more with streptokinase because of its antigenic properties.

Treatment of bleeding by toxic dose of fibrinolytics:

It is treated by either by aminocaproic acid or tranexamic acid

IV-DRUGS USED IN BLEEDING DISORDERS

1- Vitamin K1 (phytonadione) & Vitamin K2 (Menaquinone)

 Used in warfarin toxicity and also in hemorrhagic disordres of neonates.

2- Plasma fractions

 Recominant factor VIIa.

 Desmopressin acetate: increase factor VII activity

 Cryoprecipitate. -They are used in bleeding particularly with hemophilia.

3- AMINOCAPROIC ACID

 It is a fibrinolysis antagonist.

 It acts by blocking of the binding of plasmin to fibrin. It used by I.V. injection.

Therapeutic uses:

i. It is used to control bleeding caused by thrombolytic therapy.

ii. Adjunctive therapy in hemophilia.

iii. Prophylaxis for rebleeding from intracranial aneurysms.

iv. Decrease postsurgical GIT bleeding and postprostatectomy bleeding.

v. Decrease bladder bleeding secondary to radiation or drug-induced cystitis.

Side effects:

It may cause intravascular thrombosis, hypotension, myopathy, abdominal discomfort, diarrhea and nasal stuffiness.

Contraindications:

Disseminated intravascular coagulation and upper genitourinary bleeding.

4- TRANEXAMIC ACID -It is analog of aminocaproic acid and used as fibrinolytic antagonist. -It acts by blocking of the binding of plasmin to fibrin. It used by I.V. injection.

References

- Lippincott® Illustrated Reviews: Pharmacology South Asian Edition.
- Basic and Clinical Pharmacology 16th Edition.

HEM-210

Microbiology and immunology lecture handout

By

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Bacterial Lymphadenitis and Blood Related Infections

Objectives By the end of this lecture, the student will be able to:

- Memorize the general features and major virulence factors of some important bacteria causing lymphadenitis.
- Demonstrate laboratory diagnosis of some important bacteria causing lymphadenitis.
- List etiologies of bacteremia and septicemia.
- Demonstrate laboratory diagnosis of bacteremia and septicemia.
- Memorize infection causes of fever of unknown origin.

Important Bacteria Causing Lymphadenitis

- *Staphylococci*
- *Bartonella*
- *Francisella tularensis*
- *Streptococci*
- *Mycobacterium*
- *Yersinia pestis*
- *Brucella*
- *Treponema pallidum*

BRUCELLA

Distinguishing Features

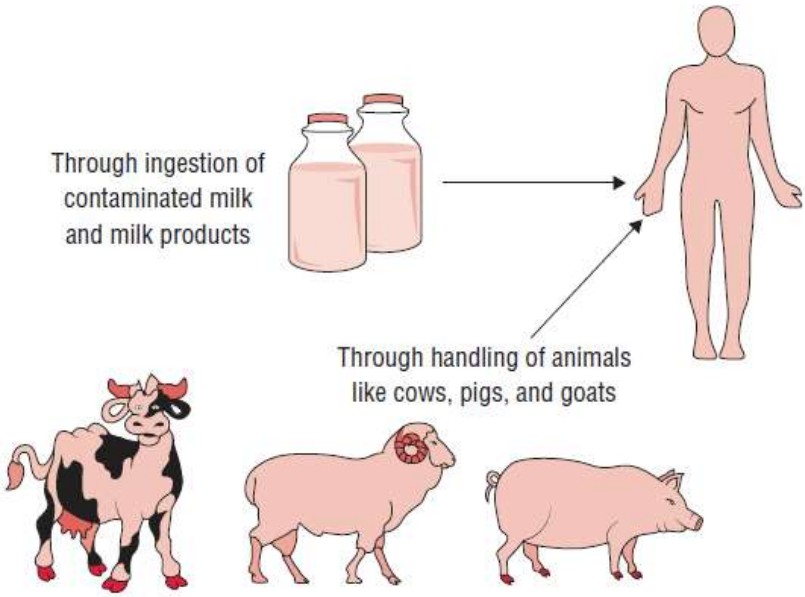
- Small gram-negative rods, aerobic
- Facultative intracellular
- Zoonosis (infection transmissible from vertebrate animals to humans)
- Culture is hazardous
- Potential bioterrorism agent
- Reservoir: domestic livestock

Species of Medical Importance

- Brucella abortus: cattle
- Brucella melitensis: goats
- Brucella suis: pigs
- Brucella canis: dogs

Transmission

- Unpasteurized dairy products
- Direct contact with the animal (e.g., work in slaughterhouse)
- Ingestion of contaminated milk and milk products
- Handling of animals like cows, pigs, and goats

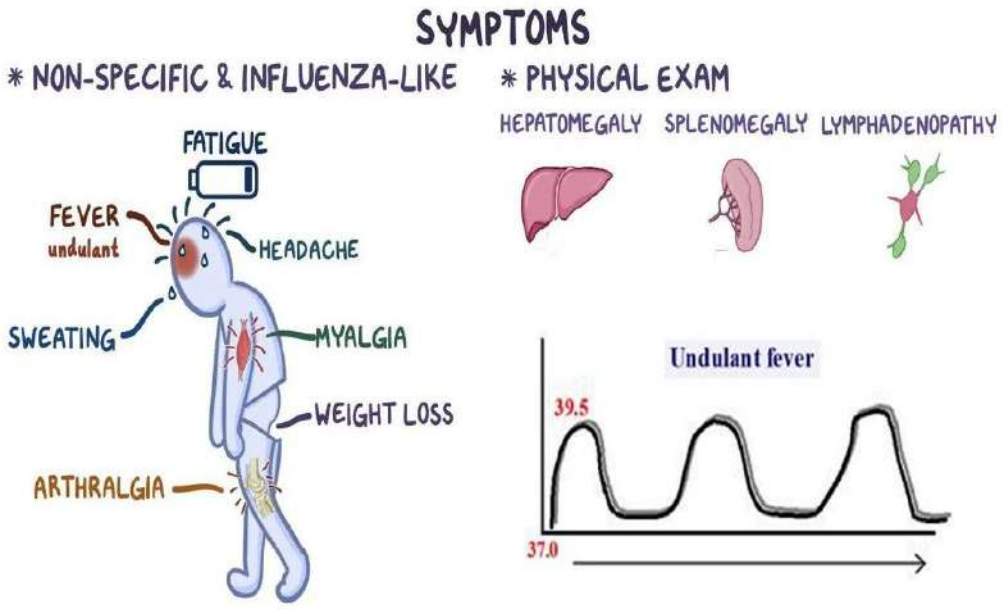


Pathogenesis

- Endotoxin
- Facultative intracellular parasite: the organism causes septicemia then localizes in macrophages of the reticuloendothelial system (RES).
- Causes Granulomatous response with central necrosis.
- Spread in the body: Brucella-infected phagocytic cells move to the bone marrow, lymph nodes, spleen, and liver.

Disease: Brucellosis (Undulant Fever)

- Acute septicemia.
- Undulant fever (Temperature rises gradually and falls like a wave over days to weeks).
- Influenza-like symptoms, including arthralgia, anorexia, myalgia, and back pain.
- Profuse sweating.
- Hepatomegaly, splenomegaly, and lymphadenopathy.



Diagnosis and Prevention

- **Diagnosis:** Blood culture is diagnostic but hazardous. Serum agglutination test (fourfold increase in titer of antibodies >1:160) is considered positive.
- **Prevention:** Vaccinate cattle and pasteurize milk, especially goat milk.

YERSINIA PESTIS

Features

- Small gram-negative rods with bipolar staining.
- Facultative intracellular parasite.
- Coagulase positive.
- Reservoir: Zoonosis; rodents (e.g., rats, chipmunks, squirrels).
- Potential bio-warfare agent.

Transmission

- Wild rodents, flea bite (rat to man) → sylvatic plague.
- Human-to-human transmission by respiratory droplets.

Pathogenesis

- Coagulase via contaminated mouth parts of flea.

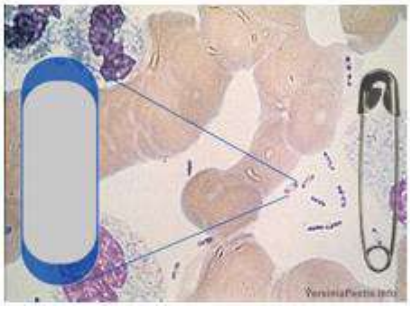
- Endotoxin and exotoxin production.
- Envelope antigen (F-1) inhibits phagocytosis.
- Suppression of cytokine production and resistance to phagocytic killing.

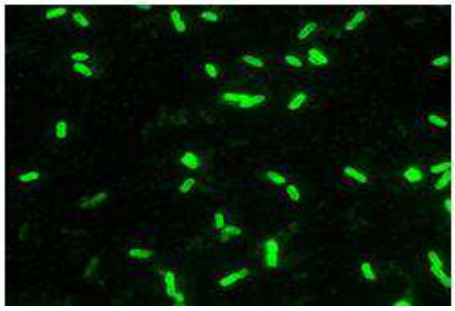
Diseases

- **Bubonic plague:** Flea bites an infected animal then a human. Symptoms include rapidly increasing fever and regional buboes (swollen inflamed lymph nodes). Leads to septicemia and death if untreated.
- **Pneumonic plague:** Rapidly progressing pneumonia. Arises from septic pulmonary emboli in bubonic plague or inhalation of organisms. Highly contagious.

Diagnosis and Prevention

- **Diagnosis:** Clinical specimens (lymph node aspirate, sputum, or blood) and cultures are hazardous. Serodiagnosis or direct immunofluorescence used. Bipolar staining appears as a "closed safety pin".
- **Prevention:** Animal control; avoid sick and dead animals. Killed vaccine available for military use.





BARTONELLA HENSELAE

- **Characteristics:** Gram-negative rods.
- **Reservoir/Transmission:** Cats and dogs; bites, scratches, and fleas.
- **Disease:** Cat scratch fever (lymphadenopathy with stellate microabscesses) and Bacillary angiomatosis in AIDS patients (red-purple papules that bleed easily).

FRANCISELLA TULARENSIS

- **Distinguishing Features:** Small gram-negative rod, facultative intracellular pathogen, potential bio-warfare agent, and zoonosis.
- **Reservoir:** Many species of wild animals, especially rabbits, deer, and rodents.
- **Transmission and Diseases:**
    - Tick bite → ulceroglandular disease (fever, ulcer at bite site, regional lymph node enlargement and necrosis).
    - Traumatic implantation (skinning rabbits) → ulceroglandular disease.
    - Aerosols (skinning rabbits) → pneumonia.
    - Ingestion (undercooked meat or contaminated water) → typhoidal tularemia (hepatosplenomegaly).
- **Diagnosis:** Culture is diagnostic but hazardous; Immunofluorescence assay for Francisella antibodies.
- **Prevention:** Protection against tick bites, glove use while butchering rabbits, and live attenuated vaccine for those at high risk.

BLOODSTREAM INFECTION (BSI)

- **Definitions:**
    - **Bacteremia:** Simple presence of bacteria in bloodstream with no symptoms or mild fever.
    - **Septicemia:** Presence and multiplication of bacteria in bloodstream with clinical symptoms; a life-threatening condition.
- **General Risk Factors:** Factors impairing the immune system (malignancies, diabetes) and factors facilitating pathogen entry (catheters, IV drug use).
- **Common BSI Pathogens and Risk Factors:**
    - *Staphylococcus aureus:* Skin & soft tissue infections, osteomyelitis.
    - *Coagulase negative Staphylococci:* Prosthetic implants, central venous catheters.
    - *Enterobacteriaceae (E. coli):* Urinary tract infection, intra-abdominal pathology.
    - *Pseudomonas aeruginosa:* Prolonged hospitalization.
    - *Enterococci:* Colorectal cancer, urinary tract infections.

Blood Borne Pathogens

Definition: Pathogens carried in the bloodstream and spread through contact with blood or fluids.

- **Primary concern:** HIV, Hepatitis B and C.
- **Others:** CMV, EBV, HTLV, Treponema pallidum.

Fever of Unknown Origin (FUO)

Definition: Temperature >38.3°C recorded on multiple occasions lasting >3 weeks with no clear etiology.

Common infectious causes:

1. Tuberculosis
2. Brucellosis
3. Q fever
4. Sub-acute bacterial endocarditis
5. Complicated urinary tract infection
6. Abscess

References:

- Kaplan Medical USMLE Step 1 Lecture Notes (2021) Immunology and Microbiology.

Viral Lymphadenitis

Objectives By the end of this lecture, the student will be able to:

- List the major viruses causing lymphadenitis.
- Describe the taxonomy and the structure of some important viruses causing lymphadenitis.
- Mention the mode of transmission of some important viruses causing lymphadenitis.

Epstein-Barr Virus (EBV)

Family: Herpesviridae (Icosahedral, enveloped, double-stranded DNA).

Clinical Manifestation: Causes painful localized bilateral cervical or generalized lymphadenitis.

Transmission:

- Body secretions, especially infected saliva ("kissing disease").
- Others include blood transfusion or bone marrow transplantation.

Pathogenesis:

I. EBV uses the CD21 receptor on B lymphocytes and epithelial cells of the oropharynx.

II. Infected B lymphocytes trigger:

- **Humoral immune response:** Production of heterophile antibodies (non-specific antibodies recognizing Paul-Bunnell antigen).
- **Cellular immune response:** High concentration of atypical CD8+ cytotoxic T lymphocytes (Downey cells).
- **Malignant transformation:** Arises from immortal B cells.

III. EBV can cause latent infection of B cells in the presence of competent T cells.

Diseases Associated with EBV

A. **Infectious Mononucleosis (Heterophile-positive):** A "civil war" between infected B cells and protective T cells. Symptoms include fever, sore throat, enlarged lymph nodes, and splenomegaly.

B. **Lymphoproliferative Disease:** Occurs in immunocompromised patients when T cells cannot control B-cell growth.

C. **Hairy Oral Leukoplakia:** White plaques on the tongue of AIDS patients due to hyperproliferation of infected lingual epithelial cells.

D. **Malignancies:** African or Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin and non-Hodgkin lymphoma.

Laboratory Diagnosis of EBV

- **Sample:** Blood.
- **Hematologic:** Lymphocytosis with atypical lymphocytes (Downey cells).
- **Serology:**
  1. **Monospot/Paul Bunnell Test:** Screening test to detect heterophile IgM antibodies.
  2. **Specific Serologic Tests:** Detect antibodies to viral capsid antigen (VCA) and EBV nuclear antigen (EBNA).
     - *Acute Infection:* VCA-IgM (+), VCA-IgG (+).
     - *Past Infection:* VCA-IgG (+), EBNA-IgG (+).

Note: EBNA = Never Acute.

Cytomegalovirus (CMV)

- **Family:** Herpesviridae (Icosahedral, enveloped, double-stranded DNA).
- **Clinical Manifestation:** Causes painful generalized lymphadenitis.
- **Transmission:** Blood transfusion, sexual contact, transplacental, perinatal, body fluids (saliva), and organ transplants.
- **Pathogenesis:** Infects epithelial cells and becomes latent in mononuclear cells.
- **Diseases:**
  1. **Immunocompetent:** Mostly asymptomatic; <10% develop CMV mononucleosis (heterophile-negative).
  2. **Immunocompromised:** Pneumonia, retinitis, esophagitis, colitis, or encephalitis.
  3. **Congenital:** Cytomegalic inclusion disease.
- **Diagnosis:**
  - "Owl-eye" inclusion bodies in biopsy or urine.
  - PCR (primary method).
  - Serology (IgM for current, IgG for past infection).

Human Immunodeficiency Virus (HIV)

Family: Retroviridae (Icosahedral).

Structure:

- Envelope
- Two copies of ss (+) RNA
- Reverse transcriptase
- Integrase
- Protease

Transmission:

- Sexual contact
- Bloodborne (needles, transfusions)
- Vertical transmission.

Pathogenesis:

Surface glycoprotein gp120 binds to CD4 on T-helper cells and macrophages, leading to the destruction of CD4+ T cells and loss of immunity.

HIV Clinical Course

- **Stage 1 (Acute):** 2–4 weeks after infection; mononucleosis-like symptoms; high viral load.
- **Stage 2 (Chronic):** Clinical latency; virus multiplies at low levels; can last 10+ years without treatment.
- **Stage 3 (AIDS):** Final stage; severe immune damage; opportunistic infections and malignancies; wasting syndrome.

AIDS-Associated Conditions

- **Infections:** Pneumocystis jirovecii, Candida, CMV retinitis, Mycobacterium tuberculosis, and Toxoplasma.
- **Malignancies:** Kaposi sarcoma, Invasive cervical carcinoma, Burkitt lymphoma, and Primary CNS lymphoma.

Laboratory Diagnosis of HIV

- **Initial Screening:** Detection of HIV p24 antigen and IgM/IgG antibodies.
- **Confirmation:** HIV-1/HIV-2 differentiation immunoassay, PCR, or Western blot.
- **Progression Monitoring:** CD4 count and CD4:CD8 ratio.

Window Period:

- The 3–8 weeks period post-infection before antibodies are detectable.
- Tests may be false-negative, but the virus can still be transmitted.

References:

- Kaplan Medical USMLE Step 1 Lecture Notes (2021) Immunology and Microbiology.

Parasites of lymphatic and reticule-endothelial system

By the end of the lecture the student will be able to know:

1. Visceral leishmanisis types, life cycle, epidemiology, pathophysiology and clinical presentation, diagnosis, treatment and prevention.

2. Types of lymphatic filariasis causative parasites, life cycle, epidemiology, pathophysiology and clinical presentation, diagnosis, treatment and prevention.

- **Parasites of lymphatic and reticulo-endothelial system**

A. Parasites of Lymphatic system

Helminthes (Tissue Nematoda)

- Filarial worms 1. *Wuchereria bancrofti*. 2. *Brugia malyai*.

B. Parasites of reticulo-endothelial system

- **Protozoa**

1. *Toxoplasma gondii* *2- Leishmania* *3- Trypanosoma*

Filarial Nematodes

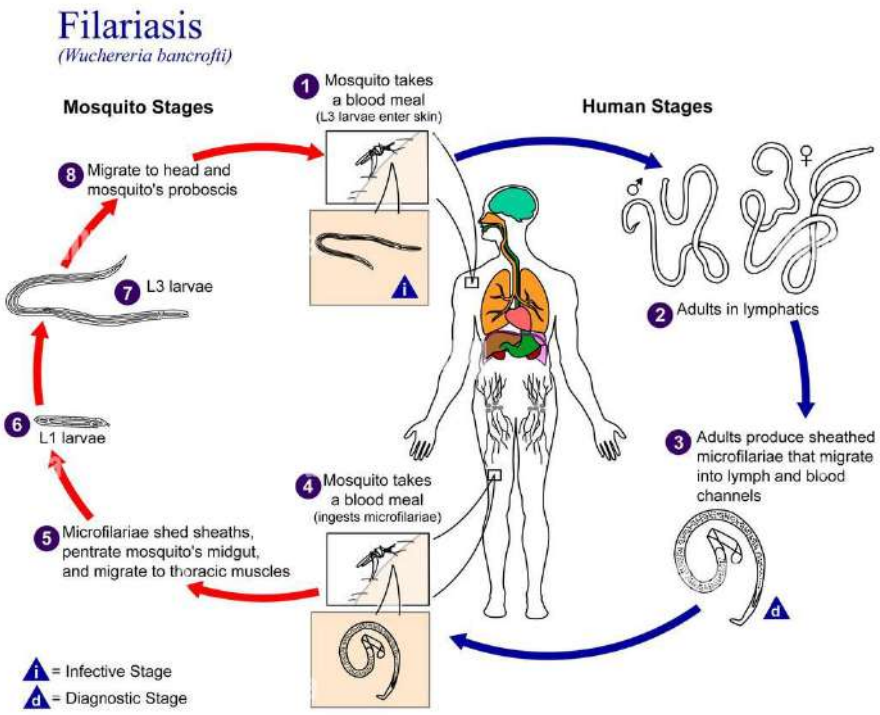
1-*Wuchereria bancrofti*:

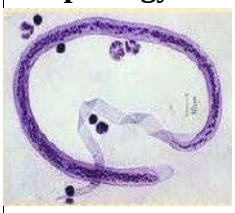
Disease:	**Bancroftian or Lymphatic filariasis, elephantiasis.**
Geog. distribution:	✓ Tropics and subtropics in Africa, Asia and South America ✓ In Egypt it is present in Qalubiya, Dakahlia, Sharkia, Cairo, Giza and Assuit.
Habitat:	Adult worms found in lymphatics of lower limbs, groin and epididymis in males and labial glands in females.
D.H. :	Man
Vector (I. H.)	Female Mosquitoes (*Culex, Anopheles and Aedes*). In Egypt, it is mainly transmitted by *Culex pipiens*.
Infective stage:	Infective filariform larva (L3)
Diagnosic stage	Microfilaria in peripheral blood by night, between 10 pm and 2 am

Mode of infection	When mosquito bites man ,the infective larvae pierce the l abium attracted by the warmth of skin,penetrate the skin o r enter through the bite wound or any abrasion

Life cycle:

- ✓ Adults live in lymph vessel and glands of man which is the only definitive host
- ✓ Microfilaria reaches the circulation via the lymphatics.
- ✓ They appear in peripheral blood by night maximum between 10 pm to 2 am and disappear by daytime (nocturnal periodicity)
- ✓ The vector (I.H) is a female mosquito sucks blood containing microfilaria.
- ✓ Microfilariae lose their sheath in the stomach of the mosquito, penetrate gut wall to the thoracic muscles where they moult . On the 10^{th} day they become infective filariform larvae, measuring 1.4 to 2mm in length migrate to the labium. They don't multiply in mosquitoes but only grow and develop (Cyclodevelopmental transmission) cycle in mosquitoes takes 1-3 weeks.
- ✓ When mosquito bites man, the infective larvae pierce the labium attracted by the warmth of skin, penetrate the skin or enter through the bite wound or any abrasion
- ✓ larvae pass to lymph vessels and nodes, mature to adults and mate, microfilariae produced by female worms appear in peripheral blood in 8-12 months


Filariasis
(Wuchereria bancrofti)
Mosquito Stages
Human Stages
1 Mosquito takes a blood meal (L3 larvae enter skin)
2 Adults in lymphatics
3 Adults produce sheathed microfilariae that migrate into lymph and blood channels
4 Mosquito takes a blood meal (ingests microfilariae)
5 Microfilariae shed sheaths, pentrate mosquito's midgut, and migrate to thoracic muscles
6 L1 larvae
7 L3 larvae
8 Migrate to head and mosquito's proboscis
i = Infective Stage
d = Diagnostic Stage

Morphology 	• Adult: Creamy white thread like worm with filariform esophagus. Male: 2-4 cm, its tail is sharply curved with two spicules. Female: 7-10 cm, viviparous. • Microfilaria (Diagnostic stage): Found in the blood at night (10pm-2 am). It is about 300× 8 µm, the anterior end is rounded and the posterior end is pointed. It has a loose sheath and the body form graceful curves. Body contains nuclei. The anterior end and the tail are free from nuclei. • Infective filariform larvae (Infective stage)1.4 to 2 mm in length

Pathogenesis and clinical pictures:

Incubation period: From the entry of the third stage infective larvae into the skin until the microfilariae appear in the blood. It may last for one year.

A symptomatic stage: detected only by blood examination especially in endemic areas

B. Inflammatory (acute) stage:

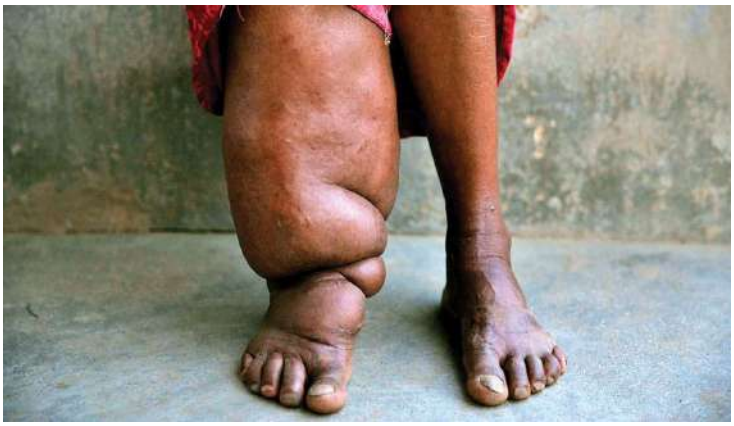
-Recurrent attacks of lymphangitis and Lymphadenitis:

- ✓ **Lymphangitis**: The affected lymph vessels of lower limb and genitalia appear raised, red, hot, swollen, and tender streaks .
- ✓ **Lymphadenitis**: Regional lymph nodes are enlarged and tender with temporary oedema of the affected limb. Abscesses may occur due to secondary bacterial infection.

C. Chronic stage:

- ✓ Repeated inflammatory attacks lead to fibrosis and obstruction of lymphatics, distally lymphedema occurs with hard, brawny edema, thickening and verrucous changes in the skin (elephantiasis)

D. Tropical Eosinophilia: characterized by pulmonary infiltrates, peripheral eosinophilia, cough and asthmatic like attacks (especially at night). These patients have high IgE levels, high antifilarial antibody titers, and no microfilariae in peripheral blood (amicrofilaremic filariasis). It is considered a hyperimmune reaction against lymphatic filariasis. The patients improve dramatically with anti-filarial treatment.



Diagnosis

I. Clinical diagnosis: History and clinical picture.

II. Laboratory diagnosis:

1- Direct method :

1. Detection of microfilariae in peripheral blood (taken at night between 10 pm & 2 am while the patient is sleeping.
2. Wet drop: Examination of a drop of fresh blood to detect living moving microfilariae.
3. Thick and thin blood film stained with Giemsa to identify the species according to the morphology.
4. Provocative test: If it is difficult to obtain blood by night, give the individual 50-100 mg Diethyl Carbamazine (DEC) orally and examine the blood within 30- 50 min.

Indirect methods (immunodiagnosis): Serological tests :Detection of antibody by ELISA, IFAT and CFT (antigen prepared from the dog filarial worm, Dirofilaria immitis).

Blood examination: High eosinophilia.

Radiological examination : Ultrasonography: For detection of adult. Viable adults may be seen moving in lymphatics (filarial dance sign).

X-ray: Shows calcified worms .

Lymphangiography: May show characteristic lymphatic changes specially dilatation of vessels.

Treatment

1. DEC (Hetrazan): (affect mainly microfilariae & to a less extent the adult worms) 2 mg /kg body weight 3 times daily given orally for 3 weeks to be repeated once after 6 months.
2. Ivermectin: Is effective for microfilariae but does not kill the adult worm, thus microfilariae reappear in the circulation. Treatment should be repeated half yearly or yearly.
3. Combination of DEC and ivermectin gives better result
4. Symptomatic treatment: Antibiotics, anti-fungal therapy, physiotherapy and bandaging.
5. Elephantoid tissues and chronic hydrocele: May be corrected surgically.

Prevention and control

Control of mosquito , Treatment of patients

2- *Brugia malayi*

Similar to W. bancrofti and differs as regards

Disease: Malayan filariasis.

Geographical distribution: Far East.

D.H.: Man**.**

Habitat: adult worms found in lymphatics of legs below knee and arms below elbow. Genital involvement and chyluria are rare**.**

I.H: Mainly female *Mansonia* and some species of *Anopheles* and *Aedes.*

Diagnostic stage: Microfilaria in peripheral blood shows non periodicity and sometimes nocturnal periodicity.

Infective stage: Infective filariform larvae.

Microfilaria: Sheathed with kinky curves with 2 deeply stained nuclei in the tail end one infront of the other. Sometimes it shows non periodicity and sometimes nocturnal periodicity.

Pathogenesis and clinical picture:

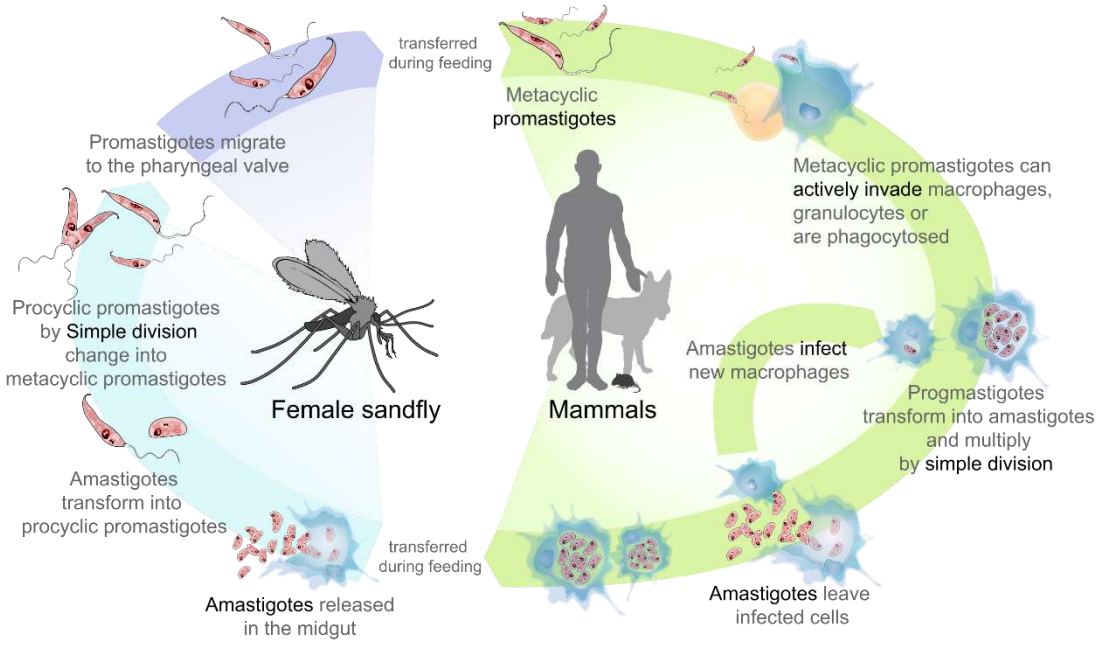
1. Milder disease.
2. Elephantiasis affects legs below knees and arms below elbow. Genital involvement and chyluria are rare.
3. Allergic manifestations are common).

This parasite has a similar life cycle, pathogenesis, diagnosis, treatment and control like that of *W. bancrofti*

3-Leishmania spp.

Disease	**Visceral leishmaniasis**
Definitive host: vector	Man and other mammalian hosts *Phlebotomus papatasii* (sandfly)
Habitat:	Intracellular found in reticuloendothelial cells of the spleen, bone marrow, leucocytes, liver cells and lymph nodes
Infective stage: **Diagnostic stage:**	➢ promastigotes) ➢ Amastigote forms
Mode of infection:	1. Through the bite of Phlebotomus papatasii. 2. blood transfusion 3. Congenital transmission.

Life cycle:



Pathogenesis and clinical picture

- Incubation period: Long (about 4 months).
- Promastigotes are engulfed by skin macrophages and transformed to amastigotes that start multiplication.
- The phagocytosed parasites are present in small numbers in blood since they are taken by the reticuloendothelial cells of the spleen, liver, lymph nodes, bone marrow, intestinal mucosa and various other organs which will show marked hyperplasia .
- The onset of the disease is gradual with initial fever (usually intermittent with double daily rise), accompanied with chills and sweating.
- Diarrhoea and dysentery are common.(amastigotes appear in stool).
- Splenomegaly may be the first sign (hard, non-tender, huge spleen with no ascites).
- Hepatomegaly (the parasite affects the Kupffer cells not hepatocytes).
- Lymphadenopathy.
- Invasion of bone marrow results in anaemia, leukopenia and thrombocytopenia. Also, there is hypergammaglobulinemia (reversal of albumin / globulin ratio) due to elevation of gamma globulin.
- Glomerulonephritis (amastigotes appear in urine).

- Skin changes may occur in the form of dark depigmented macules over the body.Abutterfly distribution over the nose is common .
- Post- kala-azar dermal leishmanoid may develop due to spontaneous arrest of the disease or incomplete treatment .
- Toxemia, anorexia and loss of weight.
- Death may occur due to secondary bacterial infection as pneumonia and septicaemia.
- **Clinical Diagnosis:**

  Laboratory Diagnosis:

  A. Direct methods: Detection of the parasite in blood (rare) or material from spleen (risk of splenic rupture), liver, bone marrow or lymph nodes. *Specimens are examined by three methods:

  1. Stained smear: Giemsa stain of amastigote intracellularly in macrophage and extracellularly due to rupture of parasitized cells.

  2. Culture: it is cultured on NNN (Novy McNeal Nicolle) medium and examine microscopy for motile promastigotes.

  3. Animal Inoculation: Intra - peritoneal inoculations of material in golden hamsters or mice then, examine amastigote in animal viscera after 2-3 months.
- **Indirect Methods:**

  1. **Serological tests:** They are used to detect anti- *Leishmania* antibodies in serum (CFT, IFAT, IHAT, ELISA).

  2. Leishmanin skin test (Montenegro test):

  It is a delayed hypersensitivity skin test. gives a delayed reaction (after 3 days). In kala-azar, the skin test becomes positive usually only 6 to 8 weeks after cure from the disease and it is negative in active cases due to the absence of DTH (suppressed cell mediated immunity).
- **Molecular diagnosis:** PCR

  Treatment

  Pentavalent antimonial compounds IV or IM for 6-10 days (sodium stibogluconate or Pentostam 600 mg/day) or Diamidine compound (Pentamidine) IM for 15 days (2-4 mg/kg body weight /day).

  Amphotericin is used with or after an antimony compound for visceral leishmaniasis unresponsive to the antimonial alone.

Recently, a new drug was developed, **Miltefosine**. This is a membrane signaling pathway inhibitor. This can be taken orally and is very effective against visceral leishmaniasis.

Allopurinol 20 mg/kg 3 times daily (for AIDS patients).

Antibiotics for secondary bacterial infections.

Bed rest, good nutrition.

Blood transfusion in severe anaemia, splenectomy in resistant cases.

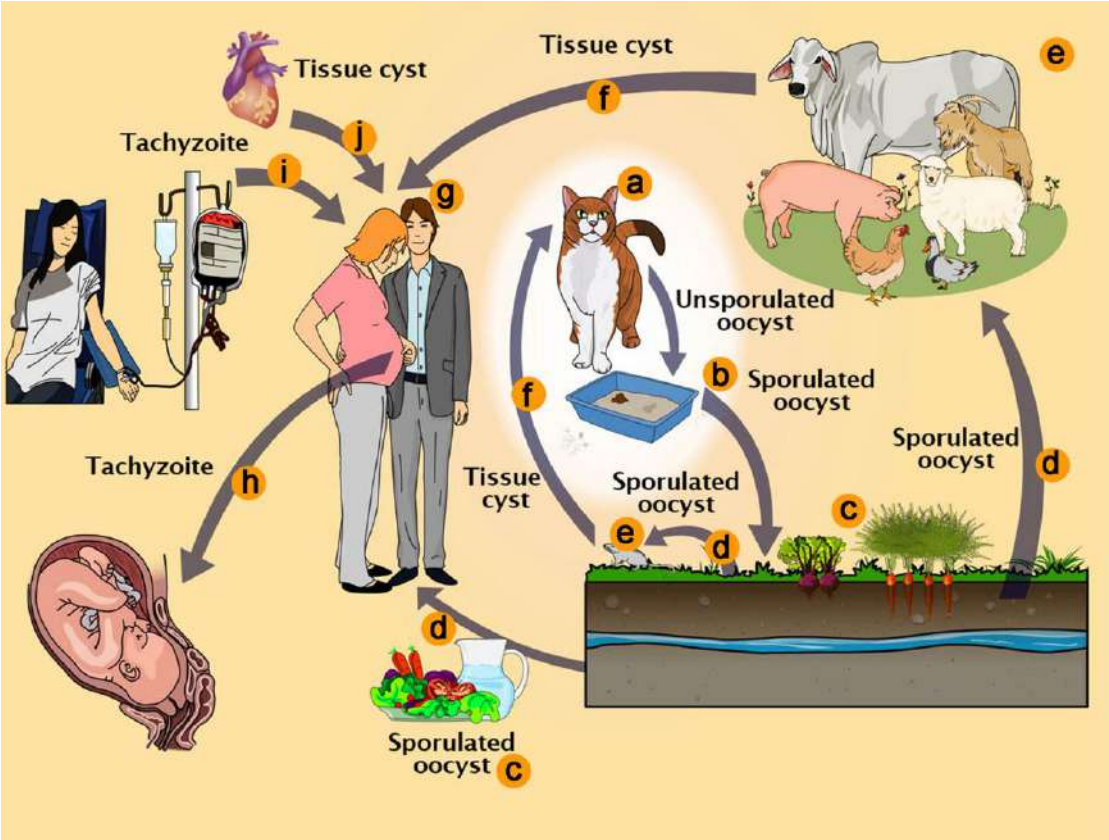
Prevention and control

- Treatment of patients.
- Destruction of animal reservoir hosts.
- Vector control by using wire screens, mosquito nets or repellents.
- Vaccination in endemic areas using suspension of living promastigotes resulting in lasting immunity.

Toxoplasma gondii

Causative parasite:	Toxoplasma gondii
Geog. Dist.	cosmopolitan distribution
Definitive host: Intermediate host:	The domestic cat (predator) All vertebrate hosts including humans serve as prey hosts.
Habitat	obligate intracellular parasites and are found in all nucleated cells
Mode of infection	1. Ingestion of sporulated oocysts in contaminated vegetables or water or during handling of litter trays or by aids of flies 2. Ingestion of tachyzoites or bradyzoites in cysts in undercooked meat or during handling infected raw meat. 3. Blood transfusion and organ transplant. 4. Congenital transmission
Infectious stages	the tachyzoites, the bradyzoites (in tissue cysts), and the sporozoites (in oocysts in cat feces).

The life cycle of *Toxoplasma gondii*



The organisms are obligate intracellular parasites and are found in two forms:

1. The actively proliferating trophozoites or tachyzoites are usually seen in the early, more acute phases of the infection, they invade any tissue, proliferating in the macrophages

2. The resting forms or cysts are found primarily in muscle and brain, probably as a result of the host immune response.

Pathogenesis and clinical picture:

A. **Congenital infection from mother to fetus** results from primary acquired maternal infection during prgnancy.

1. Fetal abnormalities could occur depending on the stage of pregnancy. The infection is more severe in the first trimester of pregnancy causing abortion or stillbirth.

2. Neurological affection includes hydrocephalus, microcephaly, intracranial calcifications with epilepsy, psychomotor or mental retardation.
3. Chorioretinitis, strabismus, or even blindness.
4. Hepatic enlargement, ascites, cardiomegaly, thrombocytopenia, and anemia.
5. Low birth weight and preterm labor

B. Acquired toxoplasmosis

1. Usually asymptomatic or mononucleosis-like syndrome (fever, rash, lymphadenopathy, hepatosplenomegly and pneumonia) in immune- competent patients.
2. Infection remains latent for life unless reactivation due to immunosuppression.

C. Immunocompromised (most commonly AIDS)

1. Encephalitis with focal CNS lesions seen as single or multiple ring- enhancing masses on CT (and focal neurological signs)
2. Lymph node, liver and spleen enlargement and pneumonitis, may proceed to heart failure and death.
3. Chorioretinitis.

Diagnosis:

1. History and clinical picture
2. Serological tests: ELISA and Indirect immunofluorescence tests can be used for the detection of IgM (acute infection) and IgG antibodies (chronic infection).
3. PCR technique.
4. Biopsy from liver or lymph node aspirates stained with Giemsa to detect the parasite.
5. Ultrasonography for detection of congenital infection and intracranial calcification.

Treatment and Prevention:

1. There is no completely satisfactory treatment. The damage caused by transplacental infection is irreversible.

2. Spiramycin could be used to prevent transplacental transmission pyrimethamine + sulfadiazine (add folinic acid).
3. Prvention is perfoemed through proper hand hygiene and cooking meat thoroughly to the proper temperature

Parasitic infections of blood

Human Malarial Parasites (*Plasmodium* spp.)

***Plasmodium* species affecting man:**

- ***P. vivax*** (benign tertian malaria) is the most common cause of malaria and is found in subtropical and temperate areas of the world.
- ***P. falciparum*** (malignant subtertian malaria) is found in tropical regions and causes the most severe and fatal disease.
- ***P. malariae*** (quartan malaria) is limited to subtropical areas.
- ***P. ovale*** (ovale tertian malaria) is the least common malarial species and is endemic in Africa.

Causative protozoa :	***Plasmodium vivax, P. ovale, P. malariae, P. falciparum.***
Geog. Dist.:	Tropics and subtropics.
Definitive host:	Female Anopheles mosquitoes
Habitat:	➢ Liver cells and R.B.Cs
Infective stage:	➢ Sporozoites
Diagnostic stage:	➢ All blood stages
Mode of infection:	➢ Through the bite of female *Anopheles* mosquitoes. ➢ Blood transfusion. ➢ Congenital transmission.

Clinical Presentation:

- Flu-like symptoms. (till establishment of cycles).
- Malaria Paroxysms includes 3 stages:
    i. Cold stage: The patient complains of sudden chill, extreme cold

ii. Hot stage: there is headache, high fever.

iii. Sweating stage: there is profuse sweating, temperature falls the patient is weak and exhausted.

1. ***P. vivax* and *P. ovale*:** chills and fever every 48 h (**benign tertian malaria**) and(ovale **tertian malaria)** respectively.
2. ***P. malariae***: chills and fever every 72 h (**Quartan malaria**).
3. ***P. falciparum***: can be every 36-48h or irregular (**malignant subtertian**)

- Anemia due to destruction of RBCs.
- Hepatosplenomegaly.
- Relapse: recurrence of malarial attacks after many months due to the reactivation (entering the erythrocytic cycle) of dormant liver hypnozoites of either *P. ovale* or *P. vivax.*

Complications:

1. ***P. malariae*** can cause nephrotic syndrome.
2. ***P. falciparum* (most lethal and called malignant malaria):**

- Cerebral malaria - with progressive headache followed by coma, uncontrollable rise in temperature and convulsions.
- Algid malaria, which is a rapid development of shock, with circulatory failure. The skin is cold, and the peripheral veins are constricted.
- Septicemia with toxemia and massive gastrointestinal hemorrhage.
- Black water fever: It is an acute, massive lysis of RBCs, which lead to high levels of free hemoglobin and breakdown products of hemoglobin in the blood and urine. The urine is quite dark, hence the name of the condition. There is also jaundice, fever and vomiting.

Sever hemolytic anemia: due to the massive lysis of RBCs occurs as a result of destruction of both parasitized and non-parasitized erythrocytes due to autoimmune mechanism.

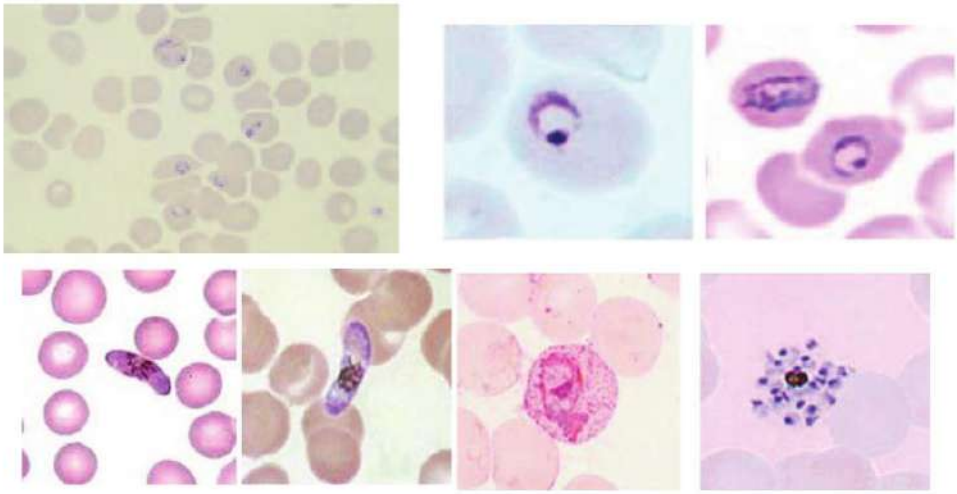
Diagnosis

A. Clinical picture: The characteristic paroxysms of cold stage (a shaking chills), followed by a fever stage (40–41°C), and finally a wet stage (profuse sweating). The

patient is exhausted but well until the next cycle of fever begins. Other symptoms include tender **hepatosplenomegaly and anemia**

B. Detection of the parasite in blood smear:

1. Thick blood film (Giemsa stain) for presence of organisms.
2. Thin blood film (Giemsa stain) for species, RBCs morphology and quantification of parasites
3. **Serological tests:** Fluorescent antibody test and ELISA.
4. Sternal puncture in case of malignant malaria when the parasite does not appear in blood films.



Treatment:

1. **Prophylaxis:** proguanil, daraprim, resochin.
2. **Clinical cure:** chloroquine.
3. **Radical cure:** Primaquine.

If drug resistance occurred: a combination of pyrimethamine and sulphadoxin

Prevention:

1. Treatment of patients.
2. Destruction of breeding places by draining ponds or by filling them with earth or by using larvicidal oil.
3. The use of small fish (*Gambusia*) which eat mosquito larvae.
4. The use of insecticides on the inner walls of houses to destroy adult mosquitoes.

5. Screening houses and the use of bed nets.
6. Application of skin repellents over exposed areas of skin.
7. prevention with antimalarial prophylaxis,

Trypanosomes

Human African trypanosomiasis

(Sleeping sickness)

Causative parasite:	✓ ***Trypanosoma brucei gambiense*** **(West African trypanosomiasis)** ✓ ***Trypanosoma brucei rhodesiense*** **(East African trypanosomiasis)**
Habitat:	Blood, lymph channel throughout the body, CSF, connective tissue, intracellular space, brain
Infective stage:	Metacyclic trypomastigotes
Diagnostic stage:	Trypomastigotes form in blood film
Mode of infection:	1. By the vector which is tsetse fly. 2. Transplacental (Mother-to-child). 3. Blood transfusion 4. contaminated needle

Pathogenesis:

- **During early "Haemolymphatic " stage** of infection the parasites multiply and spread throughout the bloodstream, lymphatic system, and lymph nodes.
- **Late "Encephalitic " stage** of the disease, the trypanosomes traverse the (blood brain barrier) BBB to the CNS where it shows picture of meningoencephalitis.

Clinical picture:

Stage 1:

- Chancre occurs especially 3-7 days after infection.
- followed by fever and parasitemia for few days.
- followed by enlargement of lymph nodes. A characteristic sign is enlarged posterior cervical nodes (Winterbottom's sign).

- followed by remittent fever, anemia, leukocytopenia, high IgM.
- Heart involvement, jaundice, pneumonia are frequent in T.b. rhodesiense which may be fatal.

Stage 2:

1. Involvement of the CNS within 3-4 weeks (rapid) in ***T. b. rhodesiense***, whereas it takes many months or years (slow) in ***T. b. gambiense.***
2. Associated with changes of behavior, confusion, sensory disturbances, and poor coordination.
3. Characteristic disruption of the normal sleep-wake cycle gives the disease its name, with the presence of nocturnal insomnia and daytime somnolence
4. ends in deep coma and death.

Diagnosis

1. Clinical picture
2. Laboratory diagnosis:

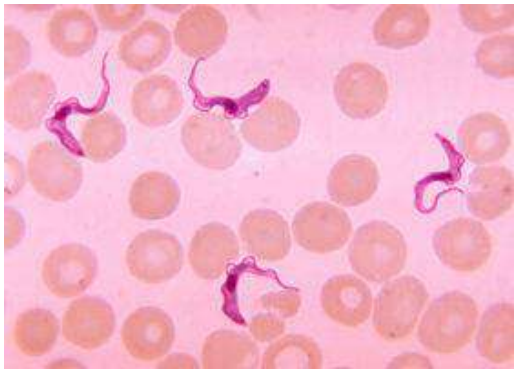
A. Direct by demonstration of trypomastigotes. Stained thin blood smears showed polymorphic flagellates.

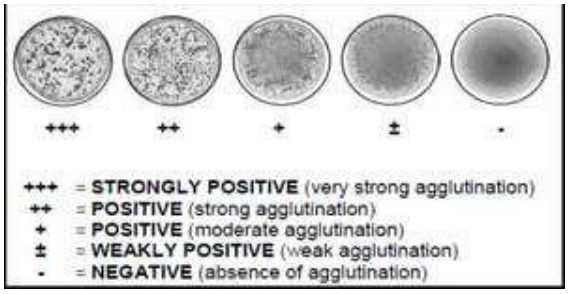
B. Indirect diagnosis

I. Serology: antigen or antibody detection: The Card Agglutination Test for Trypanosomiasis (CATT) has been used extensively for disease screening.

II. PCR for detection of the parasite DNA.

III. Culture on NNN media in case of low parasitemia for better demonstrate of the parasite.





Treatment:

During blood and lymphatic stages:

1. Pentamidine isothionate.
2. Suramin sodium.

In late stage with C.N.S. invasion:

1. Melarsoprol.
2. Tryparsamid.

Control:

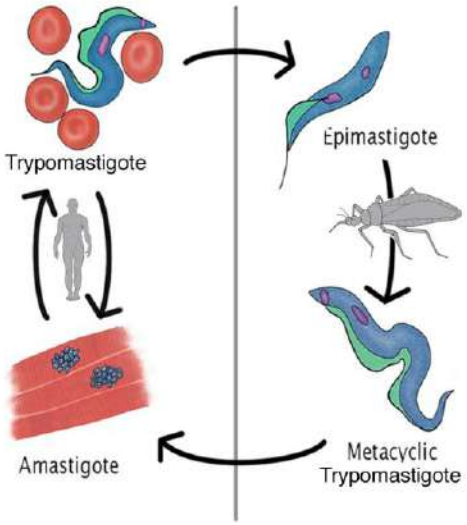
1. Treatment of carriers.
2. Prophylactic treatment with pentamidine isothionate.
3. Destruction of insect vector.

Trypanosoma cruzi "Chagas disease"

- Disease: American trypanosomiasis "Chagas disease"
- Geographical distribution: Central and South America.
- Host: Man, especially infants and young children.
- Reservoir host: The most important reservoir hosts are armadillos.
- Vector: Triatoma megista (kissing or cone-nosed or winged-bug).
- Habitat: Cells of the reticulo-endothelial system (liver, spleen, bone marrow, and lymph nodes), myocardium, smooth muscles, and nervous system.
- Infective stage: metacyclic trypomastigotes.

Mode of infection:

1. Biological transmission by Triatomine bugs (posterior station transmission).
2. Blood transfusion.
3. Congenital transmission (via the placenta from the mother to the fetus).
4. Organ transplants by using organs from infected donors.



Clinical picture:

Chagas disease (acute and chronic)

Acute form: common in infants and young children.

1. **Chagoma:** primary cutaneous indurated lesion develops at the site of vectors bite due parasite multiplication inside macrophages.
2. **Romana's sign:** It is a unilateral swelling of the patient's eyelids associated with inflammation of the lacrimal gland and conjunctivitis. **It is a marker of acute Chagas disease.**
3. Invasion of the reticulo-endothelial cells causes generalized lymphadenopathy, splenomegaly and hepatomegaly.
4. Presence of anemia, continuous fever and severe headache.
5. In rare cases, infected individuals may develop acute myocarditis or acute meningoencephalitis which is life-threatening

Chronic form: common in adults

- The parasites are hidden in organs (the heart, digestive smooth muscles and nervous system), no parasites found in blood.
- Destruction of autonomic nerve ganglion in muscles of the heart and hollow organs resulting in mega organ disease

✓ **Cardiomegaly.**

- ✓ **Megaesophagus:** dilatation of the esophagus leading to dysphagia and chronic achalasia.
- ✓ **Megacolon:** dilatation of the colon leading to constipation and patients with advanced disease can go for weeks between bowel movements

Diagnosis:

I. Clinical picture.

II. Laboratory diagnosis:

1. **In acute cases**:

- Monomorphic trypomastigotes can be found by microscopic examination of thin blood smears and aspirates from chagoma, lymph node, bone marrow, and CSF
- Tissue sections from lymph nodes or heart for definitive diagnosis of acute stage Chagas disease (Amastigote form).
- Cultivation of the suspected blood on N.N.N. medium (Novy-MacNeal-Nicolle Medium) and examined after (1-4 weeks) to show epimastigotes and trypomastigotes

References:

- USMLE®Step 1 -Lecture Notes- 2020-Immunology and Microbiology
- Introduction To Medical Parasitology
- Websites : for Microbiology, immunology and parasitology
- Garcia, Lynne Shore, and David A. Bruckner. Diagnostic Medical Parasitology. New York: Elsevier, 2016.

Hemoglobin

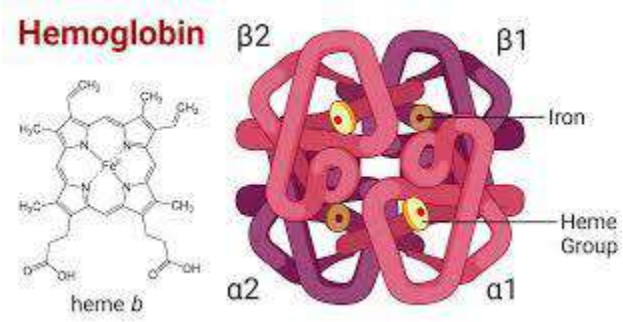
ILOs

1. **Describe the biochemical structure of hemoglobin, List the different types and derivatives of hemoglobin.**
2. **List the steps of heme synthesis, determine the sites where they occur, describe the regulation of heme synthesis and disorders of heme synthesis.**
3. **Define HMP shunt, describe its regulation, List the products**
4. **Define hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency (Favism).**

It is an oxygen/CO_2 carrier protein present in the red blood corpuscles of blood. Hemoglobin is a conjugated chromo-protein having heme as its prosthetic group. Heme is the prosthetic group, not only of hemoglobin but also of myoglobin, cytochromes etc.

Hemoglobin is formed by the combination of heme with globin (protein). Globin is made up of four polypeptide chains (an oligomeric protein). Two of these polypeptides are known as alpha (α) and the other two are known as beta (β). Each alpha chain has 141 amino acids and each beta chain has 146 amino acids.

Each polypeptide forms a cup like structure with a pocket like area where the prosthetic group, heme is buried. Heme has iron, which is linked to the imidazole nitrogen of the histidine in positions 58 and 87 of the alpha chains. In the beta chain the heme iron is linked with histidine in positions 92 and 63. Altogether there are four heme groups in one hemoglobin molecule.



Different types and derivatives of hemoglobin

Normal Hemoglobin Types

- **Hemoglobin A (HbA) (α2β2):** The primary adult hemoglobin, making up most of your blood.
- **Hemoglobin A2 (HbA2) (α2δ2):** A minor component in adults, with two delta chains replacing beta chains.
- **Fetal Hemoglobin (HbF) (α2γ2):** Dominant in fetuses, with gamma chains; switches to HbA after birth

Each chain is synthesized by the information obtained from the gene for hemoglobin, α chain is synthesized from **α** genes of hemoglobin, β chain from β genes of hemoglobin likewise y and **δ** from their respective genes. There are 2 pairs of **α** genes but only one pair each of β, γ and δ genes.

Abormal Hemoglobin Types

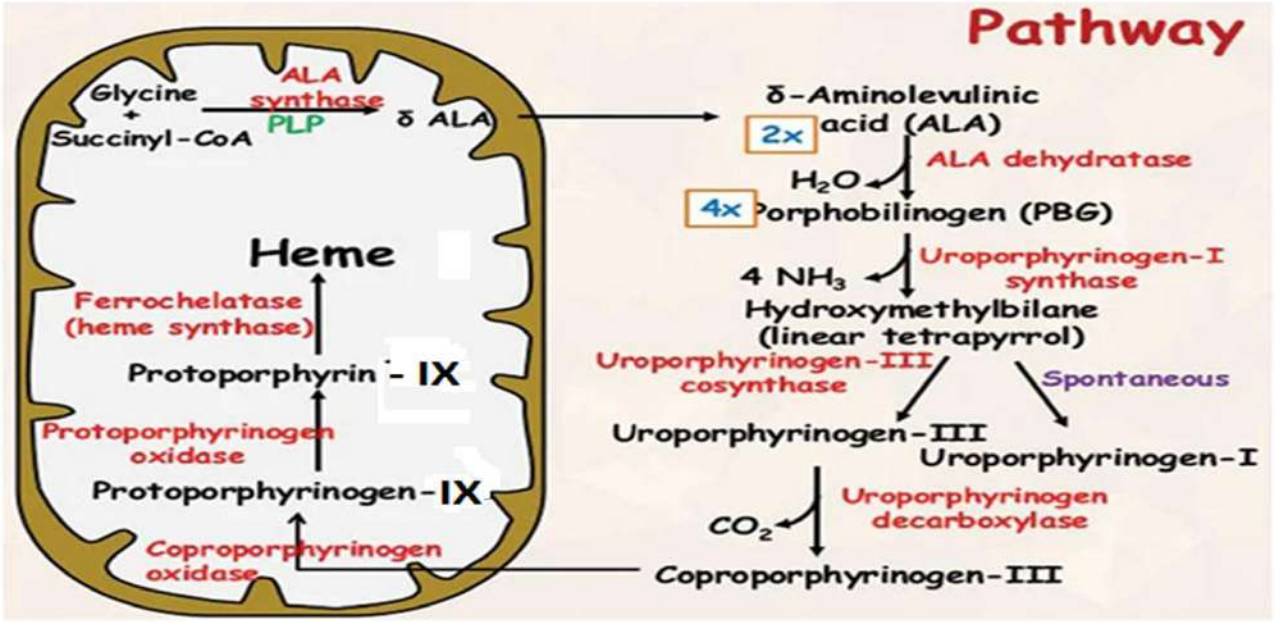
Abnormal hemoglobin's arise due to mutation in the gene for the hemoglobin synthesis. There are about 300 abnormal hemoglobin's. Some of them are those which have defect in α genes and some are with defective β chains.

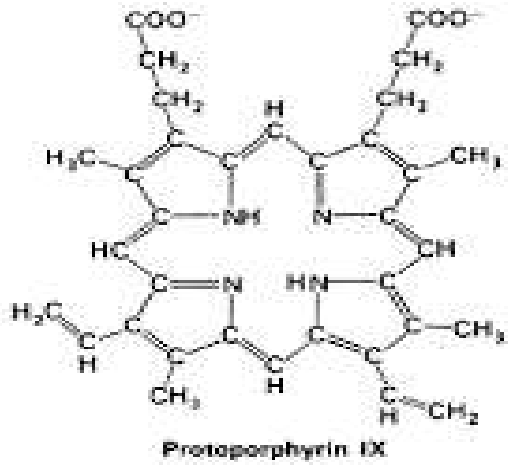
Name of Hb	Abnormality present at position	Actual amino acid present in normal Hb	Replaced amino acids in abnormal Hb
Hemoglobin (Hb) with abnormal α chain			
HbI	16	Lysine	Glutamic acid
HbG	23	Glutamic acid	Valine
HbM_B	58	Histidine	Tyrosine
Hemoglobin (Hb) with abnormal β chain			
HbS	6	Glutamic acid	Valine
HbC	6	Glutamic acid	Lysine
HbM_S	63	Histidine	Tyrosine

Biosynthesis of heme

Heme synthesis starts in mitochondria with the condensation of succinyl-CoA with the amino acid glycine, activated by pyridoxal phosphate. ALA synthase catalyzes this irreversible reaction forming an intermediate amino-ketoadipic acid. ALA synthase is the rate

limiting enzyme of heme synthesis. Two forms of ALA synthase are found: erythroid (ALAS2) and hepatic (ALAS1). ALA molecules enter the cytoplasm where their union in the presence of ALA dehydratase yields porphobilinogen (PBG) and water molecules. ALAD is inhibited by lead, and heme synthesis is inhibited leading to anemia. Four PBG molecules are joined by uroporphyrinogen I synthase (PBG deaminase) as a linear tetrapyrrole called hydroxymethylbilane (HMB). Linear tetrapyrrole cyclizes to form a ring known as uroporphyrinogen III (UPG) with participation of uroporphyrinogen III synthase. Uroporphyrinogen III has one asymmetric side chain.All acetyl groups of UPG are coverted to groups by decarboxylation and coproporphyrinogen III (CPG) is generated. CPG is acted upon in mitochondria by CPG oxidase, which decarboxylates and oxidizes two propionic side chains to vinyl groups. Protoporphyrinogen thus formed is further oxidized to protoporphyrins. Molecular oxygen is required for conversion of CPG to protoporphyrins. Finally, iron is incorporated to generate heme.The heme synthesis pathway is carried out by bone marrow (major contribution) and the liver. Heme, the product of this pathway, regulates its synthesis by decreasing synthesis of ALAS1





Regulation of Heme Synthesis Rate-Limiting Step: The first committed step, catalyzed by ALA synthase (ALAS), is the primary control point.

1. **Cell-Specific ALAS**:

1. **ALAS1 (Liver)**: Inhibited by heme (negative feedback). Drugs increasing heme demand (like for P450 enzymes) upregulate ALAS1 activity.

2. **ALAS2 (Erythroid)**: Regulated by iron availability. Iron deficiency increases ALAS2 synthesis, promoting heme production for hemoglobin.

2. **Iron Availability**: Essential for the final step (ferrochelatase), iron levels influence overall pathway activity, especially in red blood cells.

3. **Oxygen Levels**: Low oxygen stimulates erythropoietin (EPO) release, boosting red blood cell production and heme synthesis.

4. **Heme as a Regulator**: High heme levels inhibit ALAS1 and signal cessation of synthesis, preventing toxic accumulation

Porphyrias (Heme Synthesis Disorders)

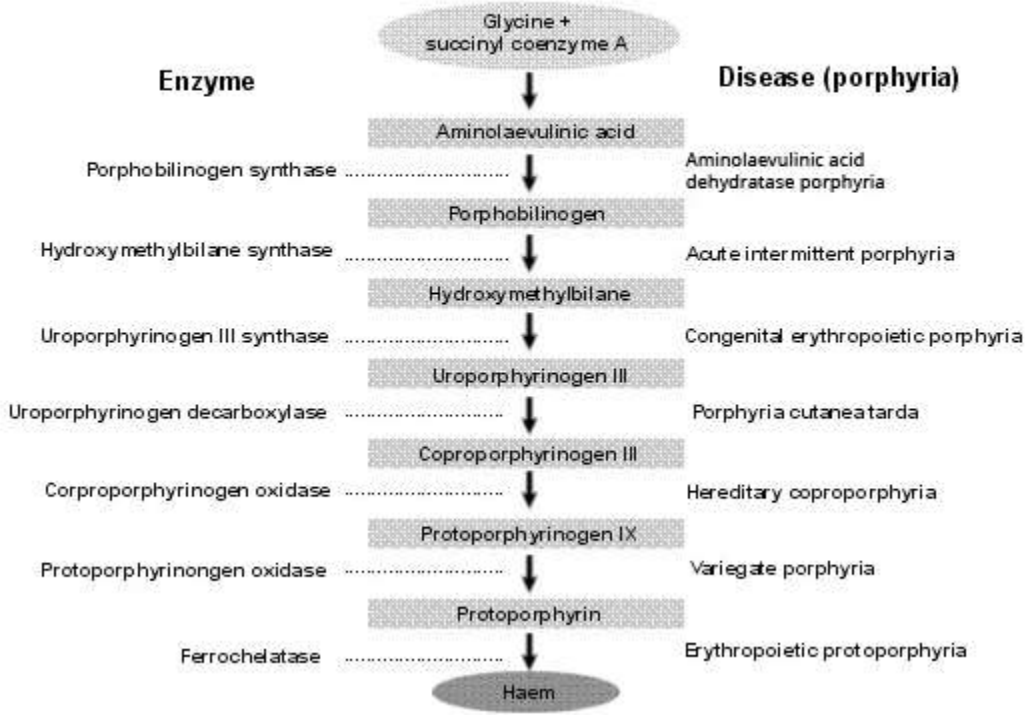
These disorders involve deficiencies in one of the enzymes in the heme pathway, leading to precursor accumulation.

Porphyrias are classified based on the **primary site of accumulation of heme precursors**:

1- The liver (hepatic)

2-The bone marrow/red blood cells (erythropoietic).

This difference in origin results in distinct clinical manifestations, though some types may involve both organs



- **Acute Hepatic Porphyrias** Defects in early enzymes (like PBG deaminase) cause accumulation of neurotoxic ALA and PBG, leading to attacks with abdominal pain, neurological, and psychiatric symptoms.
- **Erythropoietic Protoporphyria (EPP)**: Deficiency in Ferrochelatase (FECH) leads to protoporphyrin accumulation, causing severe skin photosensitivity.
- **Porphyria Cutanea Tarda (PCT)**: Porphyria Cutanea Tarda (PCT) is the most common of the Porphyrias and results from a deficiency of the enzyme uroporphyrinogen decarboxylase. Symptoms often triggered by alcohol or iron, causes skin fragility and blisters.

Sideroblastic Anemia (ALAS Deficiency)

A problem with the first enzyme, 5-aminolevulinic acid synthase (ALAS), specifically ALAS2 in red blood cells.

- **Cause**: X-linked genetic mutation or acquired, often from Vitamin B6 deficiency (due to alcohol, isoniazid), causing iron to accumulate in mitochondria (ring sideroblasts).

- **Symptoms**: Anemia, fatigue, iron overload in organs.

Other Related Conditions

- **Lead Poisoning**: Lead inhibits ALA dehydratase and Ferrochelatase, mimicking porphyria symptoms and causing basophilic stippling in red blood cells.

The Pentose Phosphate Pathway in red blood cells (RBCs)

The Pentose Phosphate Pathway (PPP) in red blood cells (RBCs) is crucial for producing **NADPH**, their *sole* source, which protects them from oxidative damage by regenerating reduced glutathione, neutralizing harmful free radicals, and preventing hemolytic anemia, also generating ribose-5-phosphate for nucleotide synthesis. The rate-limiting enzyme, Glucose-6-Phosphate Dehydrogenase (G6PD), starts the oxidative phase, generating NADPH, making RBCs highly dependent on this pathway for survival against oxidative stress.

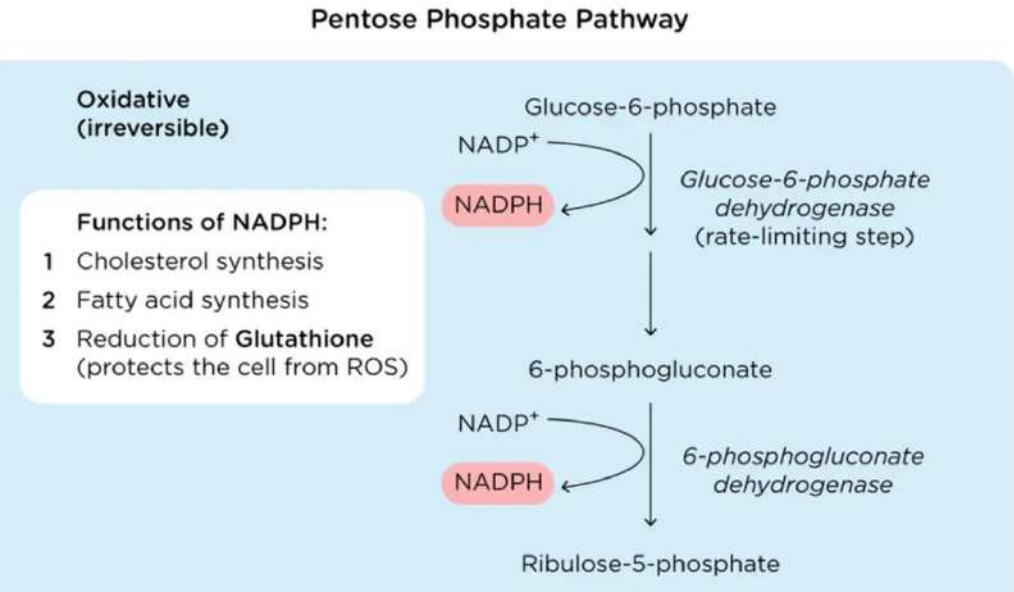
Key Functions in Red Blood Cells:

- **NADPH Production:** The primary role is to generate NADPH, which keeps glutathione in its reduced (active) form.
- **Oxidative Stress Protection:** Reduced glutathione detoxifies reactive oxygen species (ROS) generated during normal metabolism, preventing cell damage.
- **Red Blood Cell Integrity:** Without sufficient NADPH, RBCs become vulnerable to oxidative damage, leading to Heinz body formation and premature destruction (hemolysis).
- **Nucleotide Synthesis:** Produces ribose-5-phosphate, a precursor for DNA and RNA, though this is less critical in mature RBCs than NADPH production.

Steps:

1. **Glucose-6-Phosphate (G6P)** enters the pathway from glycolysis.
2. **G6PD** (Glucose-6-Phosphate Dehydrogenase) converts G6P to 6-phosphogluconolactone, reducing NADP+ to **NADPH**.
3. **6-phosphogluconolactone** is converted to **6-phosphogluconate**.

4. **6-phosphogluconate dehydrogenase** converts 6-phosphogluconate to **ribulose-5-phosphate**, producing another molecule of **NADPH**.



Glucose-6-phosphate dehydrogenase (G6PD) deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic disorder where red blood cells lack enough G6PD enzyme, making them vulnerable to breakdown (hemolysis) when exposed to triggers like certain drugs, infections, or fava beans, leading to jaundice, fatigue, and dark urine, especially in newborns. While most people are asymptomatic, the condition can cause hemolytic anemia, and management focuses on avoiding triggers, with treatment for severe jaundice in infant.

Causes & Inheritance

- **Genetic:** An X-linked recessive disorder, meaning it's more common in males.
- **Enzyme Role:** G6PD produces NADPH, which protects red blood cells from oxidative damage; deficiency reduces this protection.

Symptoms (During a Hemolytic Episode)

- Jaundice (yellow skin/eyes) and dark urine.
- Fatigue, weakness, pallor (pale skin).

- Shortness of breath, rapid heartbeat, dizziness.
- Abdominal pain, enlarged spleen (splenomegaly).

Common Triggers

- **Medications:** Certain antibiotics (sulfa drugs), antimalarials, aspirin.
- **Foods:** Fava beans (favism).
- **Infections:** Viral or bacterial infection

References

1. Lippincott Illustrated Reviews: Biochemistry. 8th ed. Philadelphia, PA: Wolters Kluwer; 2022:277-290
2. First Aid for the basic sciences P.281, 304, 785