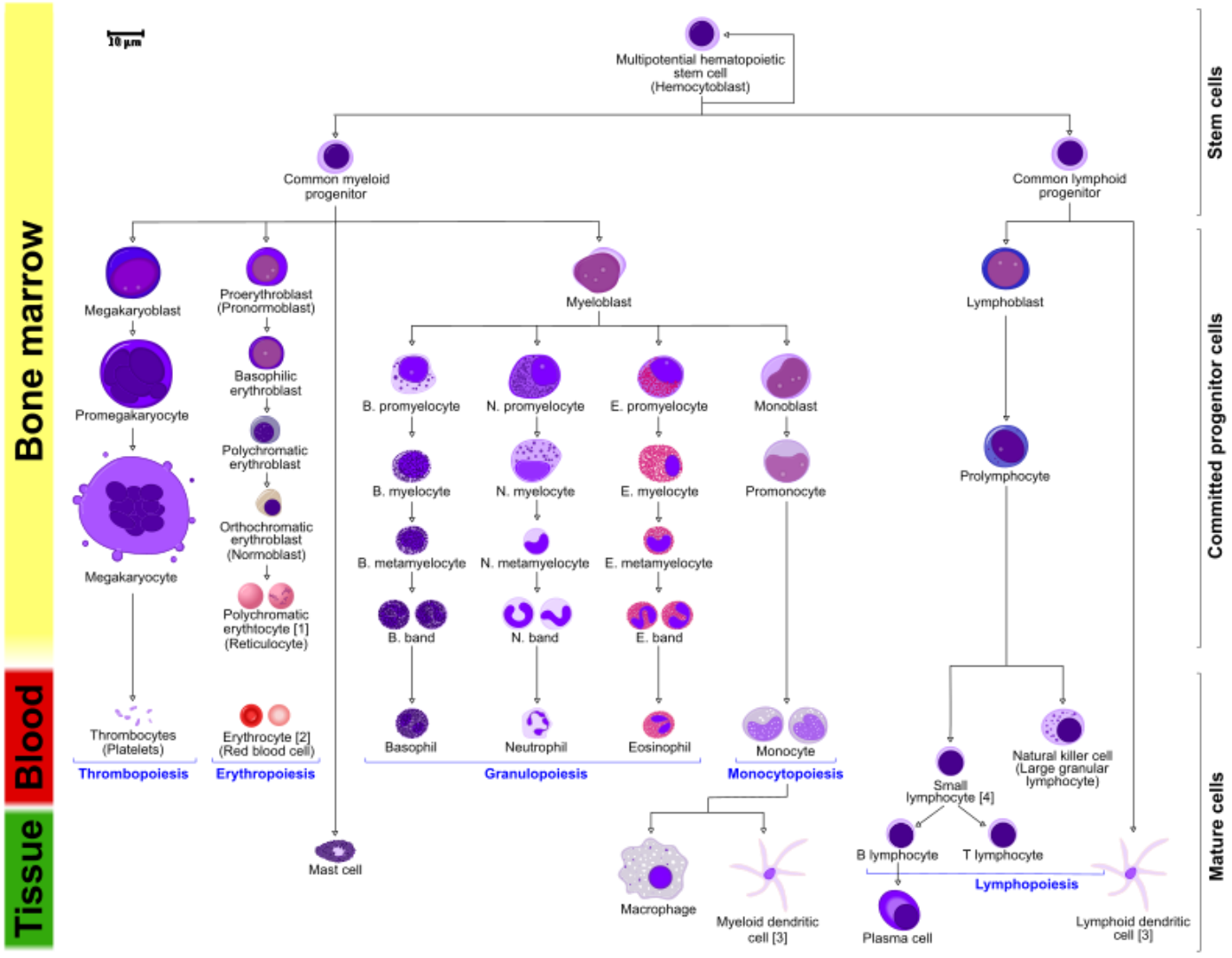
Haematopoiesis

علي موجد الدهموشي

Hematopoiesis is the process of forming blood cells, which occurs during embryogenesis and throughout normal life. Hematopoiesis occur in: embryonic yolk sac during early embryonic development, spleen, and liver, in adults the predominant site of hematopoiesis is the bone marrow (multipotent hematopoietic stem cells (HSCs)). The HSCs are able to differentiate into both myeloid or lymphoid cell lines. Hematopoiesis occurs in the flat bones, including the sternum, ribs, skull, pelvis, shoulders, vertebrae



Hematopoiesis is essential to the continued production of all blood cell lineages. Three major cell types exist; red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes).

Blood: -

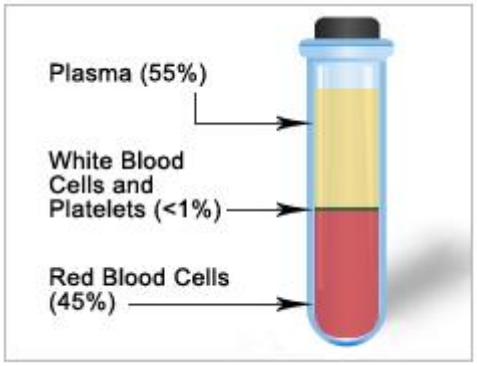
Is a body fluid in humans that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from the cells.

Components

Whole blood contains red cells , white cells, and platelets suspended in plasma

1-Plasma

Plasma is the pale yellowish liquid portion of blood – a protein- solution in which red and white blood cells and platelets are suspended. Plasma, which is 92 percent water, constitutes 55 percent of blood volume. Plasma contains albumin (the chief protein constituent), fibrinogen (responsible, in part, for the clotting of blood) and globulins (including antibodies). Plasma serves a variety of functions, from maintaining a satisfactory blood pressure and volume to supplying critical proteins for blood clotting and immunity



2-Serum

It is the blood plasma not including the fibrinogens. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms)

3-Blood cells

- Red blood cells (RBC) – Erythrocytes
- White blood cells (WBC) – Leukocytes
- Platelets – Thrombocytes.

Blood functions-:

- transporting oxygen **and** nutrients to the lungs and tissues
- carrying cells and antibodies that fight infection
- bringing waste products to the kidneys and liver, which filter and clean the blood
- regulating body temperature

Erythrocyte:

A cell that contains hemoglobin and can carry oxygen to the body. Also called a red blood cell (RBC). The reddish color is due to the hemoglobin. Erythrocytes are biconcave in shape, which increases the cell's surface area and facilitates the diffusion of oxygen and carbon dioxide. Erythrocytes are very flexible and change shape when flowing through capillaries. Immature erythrocytes, called reticulocytes, normally account for 1-2 percent of red cells in the blood

Normal RBC range is:

- Male: 4.7 to 6.1 million cells per microliter (cells/mcL)
- Female: 4.2 to 5.4 million cells/mcL.

Production of red blood cells is controlled by erythropoietin, a hormone produced primarily by the kidneys.

White blood cells:

(WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protection the body against both infectious disease and foreign invaders. All leukocytes are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell.

Five different and diverse types of leukocytes exist. These types are distinguished by their physical and functional characteristics.

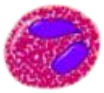
granulocytes

1-Neutrophil

Neutrophils are the most abundant white blood cell, constituting 60-70% of the circulating leukocytes. They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus.

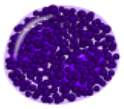
They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands. This gives the neutrophils the appearance of having multiple nuclei. The cytoplasm have fine granules. Neutrophils are the most common cell type seen in the early stages of acute inflammation. The life span of a circulating human neutrophil.6 hours–few days.

2-Eosinophil



Eosinophils compose about 2-4% of the total WBC. It rises in response to allergies, parasitic infections and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts. In general, their nucleus is bi-lobed. The lobes are connected by a thin strand.

3-Basophil



Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing vasodilation. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules that hide it.

A granulocytes

Lymphocyte

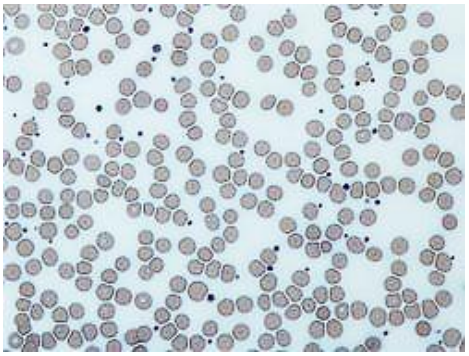
Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes include: T cell and B cell.

Monocyte

Monocytes are formed in the bone marrow and are released into peripheral blood, where they circulate for several days. They comprise about 5% to 10% of the circulating white blood cells in healthy individuals. monocytes are important in the immune system's ability to destroy invaders, but also in facilitating healing and repair.

Platelet

Also called "thrombocytes", Platelets circulate in the blood and are important following an injury, because they are involved in forming blood clots to prevent continued bleeding.



These unactivated platelets are biconvex discoid structures shaped like a lens, 2–3 µm in greatest diameter. Platelets are found only in mammals.

End Lecture Exercise:

1- What is hematopoiesis? Describe briefly.

2- serum is not used for detect clotting factor. Why?

3- They are usually first responders to microbial infection; their activity and death in large numbers forms pus.

- **Basophile**
- **Neutrophil**
- **Lymphocyte**

4- Normal RBC range is:……. ,……..

5- What are the type of leukocyte? Describe briefly.

6- numerate blood component's?

Production of Red Blood Cells: -

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Areas of the Body That Produce Red Blood Cells. In the early weeks of embryonic life, primitive, nucleated red blood cells are produced in the yolk sac. During the middle trimester of gestation, the liver is the main organ for production of red blood cells, but reasonable numbers are also produced in the spleen and lymph nodes. Then, during the last month of gestation and after birth, red blood cells are in bone marrow

Pluripotential Hematopoietic Stem Cells, Growth Inducers, and Differentiation Inducers.

The blood cells begin their lives in the bone marrow from a single type of cell called the pluripotential hematopoietic stem cell, from which all the cells of the circulating blood are derived. As these cells reproduce, a small portion of them remains exactly like the original pluripotential cells and is retained in the bone marrow to maintain a supply of these, although their numbers diminish with age. A committed stem cell that produces erythrocytes is called a colony-forming unit–erythrocyte, and the abbreviation CFU-E is used to designate this type of stem cell. Likewise, colony-forming units that form granulocytes and monocytes.

Growth and reproduction of the different stem cells are controlled by multiple proteins called *growth inducers.* Four major growth inducers. One of these, *interleukin-3,* promotes growth and reproduction of virtually all the different types of committed stem cells, the growth inducers promote growth but not differentiation of the cells. This is the function of another set of proteins called *differentiation inducers.*

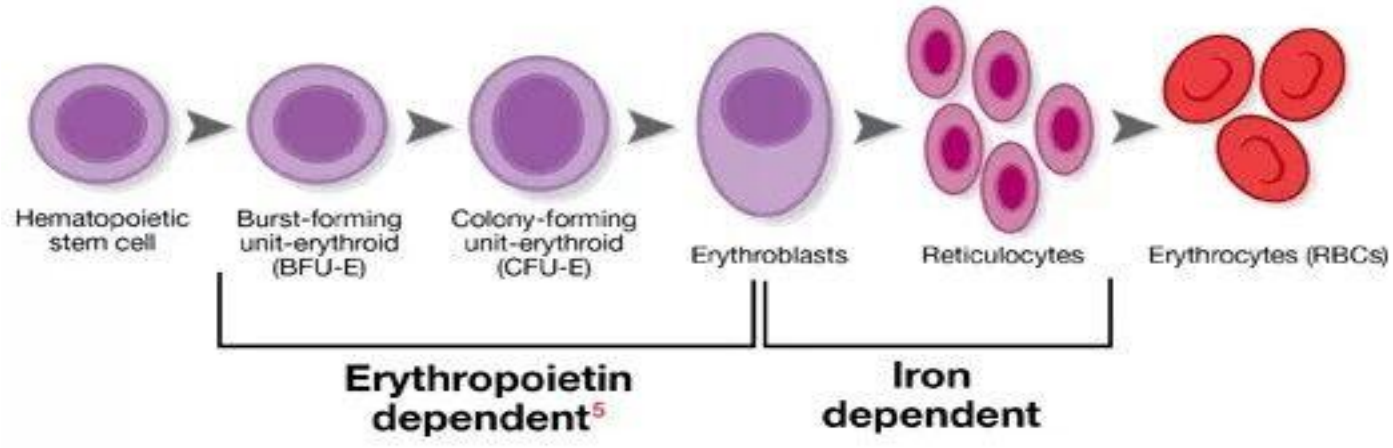
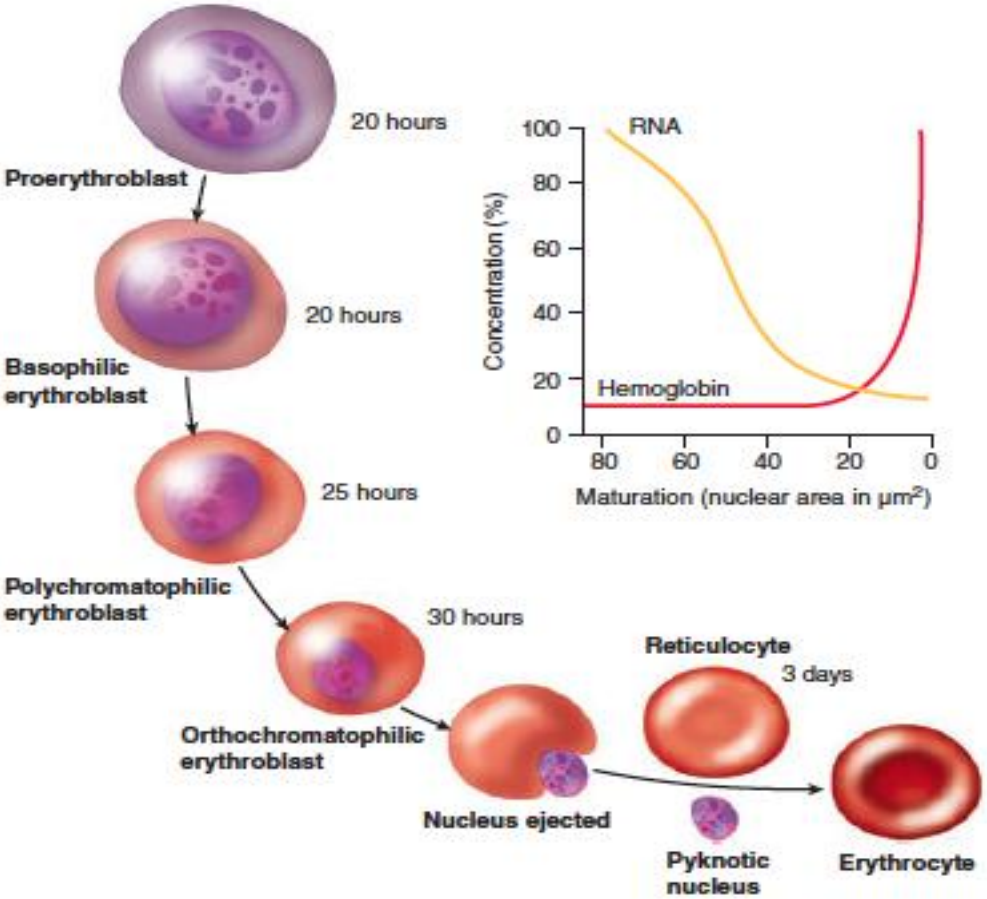


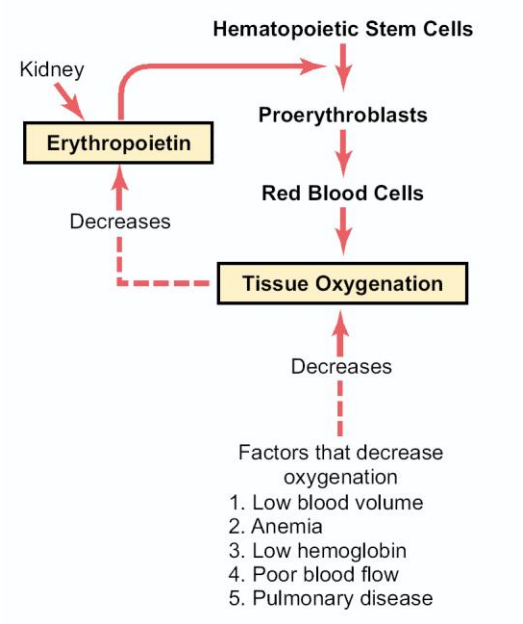
FIGURE **13–6** **Summary of erythrocyte maturation.**



Regulation of Red Blood Cell Production—Role of Erythropoietin

-Tissue Oxygenation Is the Most Essential Regulator of Red Blood Cell Production.

Any condition that causes the quantity of oxygen transported to the tissues to decrease ordinarily increases the rate of red blood cell production. Thus, when a person becomes extremely *anemic* as a result of hemorrhage or any other condition, the bone marrow immediately begins to produce large quantities of red blood cells. also, destruction of major portions of the bone marrow by any means, especially by x-ray therapy, causes hyperplasia of the remaining bone marrow, thereby attempting to supply the demand for red blood cells in the body. Various diseases of the circulation that cause decreased blood flow through the peripheral vessels, and particularly those that cause failure of oxygen absorption by the blood as it passes through the lungs, can also increase the rate of red cell production. This is especially apparent in prolonged *cardiac failure* and in many *lung diseases*, because the tissue hypoxia resulting from these conditions increases red cell production, with a resultant increase in hematocrit and usually total blood volume



Role of the Kidneys in Formation of Erythropoietin:

In the normal person, about 90 per cent of all erythropoietin is formed in the kidneys; the remainder is formed mainly in the liver. It is not known exactly where in the kidneys the erythropoietin is formed. One likely possibility is that the renal tubular epithelial cells secrete the erythropoietin, because anemic blood is unable to deliver enough oxygen from the peritubular capillaries to the highly oxygen-consuming tubular cells, thus stimulating erythropoietin production

Factors that decrease oxygenation

1. Low blood volume
2. Anemia
3. Low hemoglobin
4. Poor blood flow
5. Pulmonary disease

End lecture practice:

1- Talk about Red Blood Cell Production?

2- Growth inducers promotes growth and reproduction and differentiation of virtually all the different types of committed stem cells

-True

-False

3- describe Growth inducers. Briefly?

4- Erythropoietin Stimulates Red Cell Production, and Its Formation Increases in Response to Hypoxia. Draw diagram show the relationship between erythropoietin and hypoxia.

5- numerate factors that decrease oxygenation?

Polycythemia

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Polycythemia is an elevated red blood cell (RBC) count that is accompanied by an increased packed cell volume (PCV) and increased hemoglobin level. Polycythemia may be relative or absolute. In relative polycythemia, the RBC count is not truly increased, but the PCV is elevated because there is less fluid (plasma) in the blood, which makes the relative amount of RBCs appear to be high. Absolute polycythemia occurs when more RBCs are produced than normal and their count is truly elevated.

Causes:

- Increased fluid loss from sweating, vomiting or diarrhea.
- Increased urine output due to diuretics or osmotic diuresis.
- Severe burns
- Escape of fluid into the extravascular space.

Polycythemia types:

- Primary polycythemia (polycythemia Vera)
- Secondary polycythemia.

polycythemia Vera:

increasing of RBC production due to mutation HSCs or RBC progenitors (in bone marrow). RBC production occurred out of erythropoietin control (without erythropoietin stimulation). Some polycythaemia cases combined with leukocytosis (increasing WBC count).

Secondary polycythemia:

Secondary polycythaemia is due to increased erythropoietin effect due to over production of erythropoietin

Secondary polycythemia divided into:

- Physiological polycythaemia (chronic hypoxia)
- Non physiological polycythaemia.

Physiological polycythaemia (chronic hypoxia):

Increasing in erythropoietin production by kidney due to presence of chronic hypoxia (leak of oxygen in tissue).

Causes of Physiological polycythaemia

- Living at high altitude
- Chronic pulmonary disease
- Heart diseases.
- Hypoventilation syndromes.
- Abnormal haemoglobin with decreased oxygen delivery to tissues
- Carboxyhaemoglobin due to cigarette smoking .

Non physiological polycythaemia:

Increasing of erythropoietin production without hypoxia.

Causes of Non physiological polycythaemia:

- Kidney cancers
- Liver cancers
- Kidney diseases (cysts and renal artery stenosis).
- Androgen (injection of androgens hormones by athletes).
- Blood doping (injection of erythropoietin or hypoxia inducible factor by athletes).

Clinical signs:

Increasing in RBC levels lead to increase in blood viscosity make shortage oxygen delivery to tissue causes a symptoms including:

- Headaches
- Blurred vision (double vision).
- Red skin- particularly in the face, hands and feet.
- Weakness
- High blood pressure

Diagnosis:

- RBC count (high RBC level)
- Hemoglobin concentration (high Hb concentration)
- PCV (high level)
- Blood smear (to differentiate the high PCV with normal RBC (polycythemia) or macrocytic RBC).
- Blood erythropoietin levels (high in secondary polycythemia and low or absent in polycythemia Vera).
- Bone marrow smear (abnormal RBC progenitor in polycythemia Vera).

Normal red blood cells

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Erythrocytes, red blood cells (RBC), are the functional component of blood responsible for the transportation of gases and nutrients throughout the human body. Their unique shape and composition allow for these specialized cells to carry out their essential functions. The role of the erythrocyte is critical in investigating many disease processes in a variety of body systems.

Structure

- Human RBC is a flexible biconcave disk shape cells with approximately 6-8 micron in diameter.
- RBC biconcave disk shape offers the maximum surface area relative to volume for gas exchange.
- Absence of nucleus, mitochondria and normal cells organelles which give RBC flexibility to deformation its shape.
- The membrane of the RBC comprises a phospholipid bilayer and an underlying two – dimensional network of spectrin molecules (cytoskeleton).
- The bilayer has little resistance but contributes to bending resistance and helps to maintain cell surface area.
- The spectrin network or cytoskeleton is largely responsible for the elastic ability (flexibility of the RBC). this ability responsible for repeated large deformation in RBC shape.

Function

- RBC function is to transport O2 from lung to tissue and carry out Co2 from tissue to lung, RBC carry O2 but cannot use it in metabolism and get energy.
- Erythrocytes generate energy from glucose without oxygen via anaerobic glycolysis pathway (without oxygen), which turns glucose into lactate and generates adenosine triphosphate (ATP) for energy
- Haemoglobin molecules, essential for gas transport within the circulation, contained in the RBC cytosol.
- The cytosol is the intracellular RBC fluid and is regulated by the membrane and the heamoglobin makes up about 95% of RBC cytosol.
- .Because of RBC have no cellular organelles so it cannot synthesis proteins or other substance
- RBC lifespan is 120 days, the majority of erythrocytes (90%) are phagocytized and destroyed by spleen (extravascular hemolysis) and 10% are destroyed in the blood circulation (intravascular hemolysis).
- The hemoglobin is broken down into the heme ring and globin proteins. The iron is removed from the heme ring and either returns to the bone marrow to be inserted into new erythrocytes or enters the iron storage pool.

Abnormal red blood cells morphology

علي موجد الدهموشي

It is variation in appearance of red blood cells included variation in size (anisocytosis), color and shape (Poikilocytosis).

Size variation (Anisocytosis)

1- Macrocytes:

Macrocytosis is the enlargement of red blood cells and is defined by a mean corpuscular volume (MCV) of greater than 100 femtolitres. The enlarged erythrocytes are called **macrocytes** or **megalocytes** (both words have roots meaning "big cell").

Pathophysiology

macrocytosis is often associated with abnormalities of RBC nuclear maturation and cell development, and this process may be described as "megaloblastic change." This usually occurs as a result of impaired DNA synthesis. Vitamin B12 acts as an essential cofactor for the methylation of homocysteine to methionine, which is essential for the synthesis of thymidine, one of the nucleotides in DNA. Dietary folic acid is necessary to maintain the folate compounds used as substrates in these reactions. A deficit in either of these vitamins will limit thymidine available for DNA synthesis, resulting in impaired nuclear maturation.

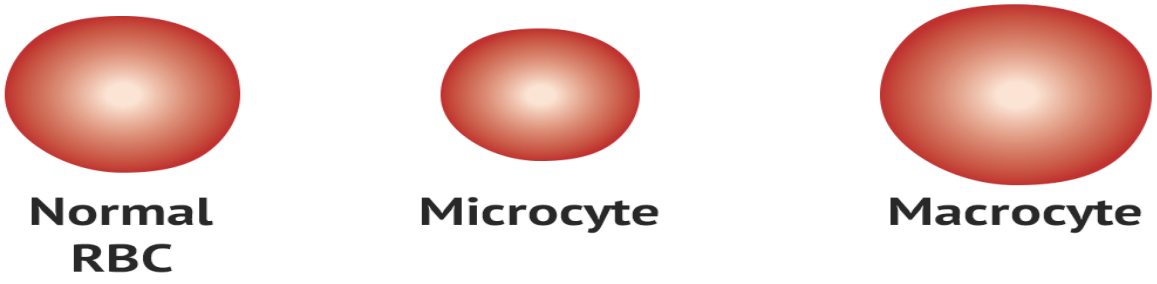
A maturation defect causes the developing cell to have an imbalance between the cytoplasm and the nucleus. The result will be an RBC precursor with more cytoplasm compared to the nucleus.

2- Microcytes:

Microcytosis or **microcythemia** is a condition in which red blood cells are unusually small as measured by their mean corpuscular volume. When associated with anemia, it is known as microcytic anemia.

Pathophysiology:

Microcytic anemia is not caused by reduced DNA synthesis. The most common cause of this type of anemia is decreased iron reserves of the body which may be due to multiple reasons. This may be due to decreased iron in the diet, poor absorption of iron from the gut, acute and chronic blood loss, increased demand of iron in certain situations like pregnancy or recovering from a major trauma or surgery.



Variation in color:

1- Hypochromic \ hypochromasia:

Hypochromic red cells contain less than the normal amount of hemoglobin and the central pale area is increased to more than one- third of the cell diameter.in severe hypochromatia the hemoglobin appears as a thin rim at the periphery of the cell. the cells are usually microcytic and assume target shape. it is appeared in iron deficiency anemia, thalassemia.

2- hyperchromia \ hyperchromatic:

the RBC is darker in color that normal; this may be due to dehydration.

3- Polychromatic:

blue-staining RBCs, indicating that they are immature due to early release from the bone marrow. it is appeared in sever blood lose and reticulocytosis.

End lecture exercise:

1- Explain the pathophysiology of macrocytosis

2- Cells with mean corpuscular volume (MCV) greater than 100 femtolitres, what is name? draw it.

3- Anemia is caused by reduced iron reserves of the body ? Define the cells that causes this anemia and explain pathophysiology of it.

4- mention the variation in color of RBC ?

Abnormal red blood cells morphology

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Shape variation (Poikilocytosis).

1- Elliptocytes, also known as ovalocytes, are abnormally shaped red blood cells that appear oval or elongated, from slightly egg-shaped to rod or pencil forms. They have normal central pallor with the hemoglobin appearing concentrated at the ends of the elongated cells when viewed through a light microscope.

Pathophysiology

is **caused** by mutations in structural components of the erythrocyte cell membrane. Mutations in genes coding for protein 4.1, alpha spectrin, beta spectrin, band 3, and Glycophorin C have all been implicated in hereditary **elliptocytosis**

The most common genetic defects (present in two-thirds of all cases of hereditary elliptocytosis) are in genes for the polypeptides α-spectrin or β-spectrin. These two polypeptides combine with one another *in vivo* to form an αβ heterodimer. These αβ heterodimers then combine to form spectrin tetramers. These spectrin tetramers are among the basic structural subunits of the cytoskeleton of all cells in the body. it is generally true that α-spectrin mutations result in an inability of α-spectrin to interact properly with β-spectrin to form a heterodimer. In contrast, it is generally true that β-spectrin mutations lead to αβ heterodimers being incapable of combining to form spectrin tetramers. In both cases the end result is a weakness in the cytoskeleton of the cell.

2- Acanthocytes (spiny cells)

Spheroidal cells with 3-12 spicules (projection) of uneven length irregularly distributed over the cell surface. It is seen in disorders of lipid metabolism, alcoholic liver cirrhosis.

3- Echinocytes (crenate cells \ Burr cells):

Red cells showing numerous, short, evenly distributed spicules (projections) of equal length. these are probably the most common artifacts in a blood film consistently found in blood samples that have been stored for some time room temperature and because of diffusion of alkaline substances from the slide into the cells resulting in an increase in PH and thus crenation of the cells. They are seen in uremia, pyruvate kinase deficiency and neonatal liver diseases. Burr cells are small bearing a few spines. They are found particularly in uremia.

4- Dacrocytes (Tear drop cells)

A dacrocyte (or dacryocyte) is a type of poikilocyte that is shaped like a teardrop (a "teardrop cell"). These tear drop cells are found primarily in diseases with bone marrow fibrosis. One theory regarding dacrocyte formation is that red blood cells containing various inclusions undergo "pitting" by the spleen to remove these inclusions, and in the process, they can be stretched too far to return to their original shape. It is also thought that this can similarly occur when red blood cells with large inclusions are obstructed from passing through the microcirculation, and the portion containing the inclusion thus gets pinched, leaving a tailed end.

5- drepanocytes (sickle cells)

Sickle cells, also referred to as drepanocytes, are formed as a result of the presence of hemoglobin S in the red cell. In the absence of oxygen, hemoglobin S polymerizes into rods, causing the sickle cell shape. Sickle cells (drepanocytes) are elongated red blood cells with pointed ends. They are seen in sickling hemoglobinopathies such as sickle cell anemia

6- Schistocytes (fragmented cells)

Two types can be distinguished in this circumstance. the first one is small fragments of cells of varying shape, sometimes with sharp angles or spines (e.g. sour cell) sometimes round in contour, usually staining deeply but occasionally palely as a result of loss of hemoglobin at the time of fragmentation. the other type is larger cells have split off, e.g. helmet cell. They are seen in certain genetically determined disorders (e.g. thalassemia and hereditary elliptocytosis), acquired disorders of red cell formation, megaloblastic and iron deficiency anemia.

7- Codocytes (target cells)

Codocytes, also known as target cells, are red blood cells that have the appearance of a shooting target with a bullseye. Target cells may appear in association with Liver disease: Lecithin—cholesterol acyltransferase (LCAT) activity may be decreased in obstructive liver disease. Decreased enzymatic activity increases the cholesterol to phospholipid ratio, producing an absolute increase in surface area of the red blood cell membranes or may be

increased red cell membrane fluidity. Target cells, or codocytes, have an excess of cell membrane relative to cell volume.

8- Stomatocytes:

These are cells with a narrow slit like area of central pallor. They are common findings in liver diseases.

9- Spherocytes \ Microspherocytes:

These are dense staining spherical cells with smaller diameter and greater thickness than normal. They are formed as a result of loss membrane due to chemicals, bacterial toxins, antibody – mediated hemolytic anemia. They are commonly seen in hereditary spherocytosis that is associated with abnormalities in membrane protein, lipid loss and excessive flux of Na+ across the membrane.

Red cell inclusions:

1- Basophilic stippling \ punctate basophilic

The red cells contain small irregularly shaped granules which stain blue in wright stain . The presence of **basophilic stippling** is attributed to aggregates of ribosomes or fragments of ribosomal RNA precipitated throughout the cytoplasm of circulating erythrocytes. This finding is associated with acquired and heritable hematologic disorders affecting erythropoiesis and erythrocyte maturation.

2- Heinz bodies:

The presence of Heinz bodies represents damage to hemoglobin and is classically observed in G6PD deficiency, a genetic disorder that causes hemolytic anemia.

Heinz bodies are indicative of oxidative injury to the erythrocyte. They are clumps of irreversibly denatured hemoglobin attached to the erythrocyte cell membrane. Although the precise mechanism is not entirely understood, the presumption is that amino acid substitutions in the beta-chains of the hemoglobin polypeptides allow them to precipitate within the red blood cell and form Heinz bodies.

3- Howell – Jolly bodies:

Howell-Jolly bodies are nuclear remnants found in red blood cells (erythrocytes) under various pathological states. They most commonly present in patients with absent or impaired function of the spleen; this is because one of the spleen's functions is to filter deranged blood cells and remove the intracellular inclusions left by the erythrocyte precursors.

4- Cabot's rings:

These are incomplete or complete rings , even figures of "8" that appear as reddish – violet fine filamentous configuration in Wright – stained films. They are remnants of the microtubules of the mitotic spindle. Cabot ring may be found in megaloblastic anemia.

5- Blood parasites: Malaria, Babesia.

Hemoglobin:

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Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs.

Hemoglobin is made up of four protein molecules (globulin chains) that are connected together. The normal adult hemoglobin (abbreviated Hgb or Hb) molecule contains two alpha-globulin chains and two beta-globulin chains. In fetuses and infants, beta chains are not common and the hemoglobin molecule is made up of two alpha chains and two gamma chains. As the infant grows, the gamma chains are gradually replaced by beta chains, forming the adult hemoglobin structure.

Each globulin chain contains an important iron-containing porphyrin compound termed heme. Embedded within the heme compound is an iron atom that is vital in transporting oxygen and carbon dioxide in our blood. The iron contained in hemoglobin is also responsible for the red color of blood.

Hemoglobin also plays an important role in maintaining the shape of the red blood cells. In their natural shape, red blood cells are round with narrow centers resembling a donut without a hole in the middle. Abnormal hemoglobin structure can, therefore, disrupt the shape of red blood cells and impede their function and flow through blood vessels.

Types of Haemoglobin:

There are seven types of haemoglobin molecules throughout a human's life.

Embryonic Haemoglobin:

The form of haemoglobin most common and in highest proportion in an embryo is Haemoglobin Gower I ($\zeta_2\varepsilon_2$) The four polypeptide chains that compose this type of haemoglobin are two zeta and two epsilon chains.

The other three forms of haemoglobin are present at much lower levels and are:

- Haemoglobin Gower II ($\alpha_2\varepsilon_2$) – Composed of two alpha and two epsilon chains.
- Haemoglobin Portland I ($\zeta_2\gamma_2$) – Comprised of two zeta and two gamma polypeptides.
- Haemoglobin Portland II ($\zeta_2\beta_2$) – Made of two zeta and two beta polypeptide chains.

Fetal Haemoglobin:

Once an embryo develops into a fetus and the four types of embryonic haemoglobin molecules disappear they are replaced by Haemoglobin F ($\alpha_2\gamma_2$)

This type of haemoglobin is used due to it having a greater affinity for oxygen than adult haemoglobin. Therefore, the growing fetus is able to take its mother's oxygen which is in her bloodstream.

Adult haemoglobin:

Haemoglobin F remains in the child's blood until it is around six months old and then almost all of it is replaced with adult haemoglobin.

The two types of adult Haemoglobin are:

- Haemoglobin A ($\alpha_2\beta_2$) – Has two alpha chains and two beta chains
- Haemoglobin A_2 ($\alpha_2\delta_2$) – Has two alpha polypeptides and two delta polypeptides.

There is also a small amount of Haemoglobin F remaining.

Haemoglobin A is the most prevalent as it makes up about 97% of adult haemoglobin

Variant Forms:

As with all biological substances mutations can occur and these mutations cause a change in the genes coding for haemoglobin and so variant forms of haemoglobin are formed. There are

Several hundred abnormal forms of hemoglobin (variants) have been identified, but only a few are common and clinically significant.

Common hemoglobin variants

- Hemoglobin S: this is the primary hemoglobin in people with sickle cell disease (also known as sickle cell anemia). it is combined with another hemoglobin mutation, such as that causing Hb C or beta thalassemia.
- Hemoglobin C: It usually causes a minor amount of hemolytic anemia and a mild to moderate enlargement of the spleen.
- Hemoglobin E: Hemoglobin E is one of the most common beta chain hemoglobin variants in the world. generally, have a mild hemolytic anemia, microcytic red blood cells, and a mild enlargement of the spleen.

Less common variant:

There are many other variants. Some are silent – causing no signs or symptoms – while others affect the functionality and/or stability of the hemoglobin molecule. Examples of other variants include: Hemoglobin D, Hemoglobin G, Hemoglobin J, Hemoglobin M.

Anemia:

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Anemia (also spelled **anaemia**) is a decrease in the total amount of red blood cells (RBCs) or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen.

When anemia comes on slowly, the symptoms are including:

feeling tired, **weakness**, **shortness of breath**, and a **poor ability to exercise**.

When the anemia comes on quickly, symptoms may include:

confusion, **loss of consciousness**, **and increased thirst**. Additional symptoms may occur depending on the underlying cause. For people who require surgery, pre-operative anemia can increase the risk of requiring a blood transfusion following surgery.

Anemia can be caused by **blood loss**, **decreased red blood cell production**, **and increased red blood cell breakdown**.

Causes of **blood loss** include :

-trauma and gastrointestinal bleeding.

Causes of **decreased production** include :

-iron deficiency, vitamin B12 deficiency, thalassemia, and a number of neoplasms of the bone marrow.

Causes of **increased breakdown** include

-genetic conditions such as sickle cell anemia, infections such as malaria, and certain autoimmune diseases.

Anemia can also be classified based on the size of the red blood cells and amount of hemoglobin in each cell. If the cells are small, it is called microcytic anemia; if they are large, it is called macrocytic anemia; and if they are normal sized, it is called normocytic anemia.

Basic laboratory examination for anemia:

1`-complete blood count (CBC): include Hb, PCV.

2- Reticulocytes count: if increased its main that anemia with increased R.B.Cs loss or destruction and reverse is a true.

3- red blood cells indices: MCV, MCH, MCHC, RDW.

4- Blood film: to observe size, shape, and inclusion bodies

5- serum iron & TIBC: to confirm the diagnosis of IDA from other.

6- bone marrow biopsy: it is useful in case of unexplained anemia.

7- serum B.12 & folic acid: to exclude megaloblastic anemia.

8- Certain hemolytic anemia:

a) Direct coombs test

b) Osmotic fragility (hereditary spherocytosis)

c) Sickle cell test (sickle cell anemia)

Megaloblastic anemia:

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Megaloblastic anemia is a type of macrocytic anemia that results from inhibition of DNA synthesis during red blood cell production. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to continuing cell growth without division, which presents as macrocytosis. The defect in red cell DNA synthesis is most often due to hypovitaminosis, specifically vitamin B12 deficiency or folate deficiency. Loss of micronutrients may also be a cause.

Megaloblastic anemia not due to hypovitaminosis may be caused by antimetabolites that poison DNA production directly, such as some chemotherapeutic or antimicrobial agents (for example azathioprine or trimethoprim).

The pathological state of megaloblastosis is characterized by many large immature and dysfunctional red blood cells (megaloblasts) in the bone marrow and also by hypersegmented neutrophils (defined as the presence of neutrophils with six or more lobes). These hypersegmented neutrophils can be detected in the peripheral blood (using a diagnostic smear of a blood sample).

Causes:

- Vitamin B12 deficiency
- Folate deficiency
- Combined Deficiency: vitamin B12 & folate.
- Inherited Pyrimidine Synthesis Disorders
- Inherited DNA Synthesis Disorders
- Toxins and Drugs

Diagnosis

- low blood level of Vitamin B_{12}
- A measurement of methylmalonic acid (methylmalonate) can provide an indirect method for partially differentiating Vitamin B_{12} and folate deficiencies. The level of methylmalonic acid is not elevated in folic acid deficiency.
- Direct measurement of blood cobalamin

Blood findings

The blood film can point towards vitamin deficiency:

- Decreased red blood cell (RBC) count and hemoglobin levels.
- Increased mean corpuscular volume (MCV, >100 fL) and mean corpuscular hemoglobin (MCH)

- Normal mean corpuscular hemoglobin concentration (MCHC, 32–36 g/dL)
- Neutrophil granulocytes may show multisegmented nuclei ("senile neutrophil").
- Anisocytosis (increased variation in RBC size) and poikilocytosis (abnormally shaped RBCs).
- Macrocytes (larger than normal RBCs) are present.
- Ovalocytes (oval-shaped RBCs) are present.
- Howell-Jolly bodies (chromosomal remnant) also present.

Blood chemistries will also show:

- An increased lactic acid dehydrogenase (LDH) level. The isozyme is LDH-2 which is typical of the serum and hematopoietic cells.
- Increased homocysteine and methylmalonic acid in Vitamin B_{12} deficiency
- Increased homocysteine in folate deficiency

Bone marrow (not normally checked in a patient suspected of megaloblastic anemia) shows megaloblastic hyperplasia

Pernicious Anemia

Pernicious anemia is a type of anemia.In pernicious anemia, the body can't make enough healthy red blood cells because it doesn't have enough vitamin B12.

Pernicious anemia also can cause other problems, such as nerve damage, neurological problems (such as memory loss), and digestive tract problems. People who have pernicious anemia also may be at higher risk for weakened bone strength and stomach cancer.

Causes

Pernicious anemia is caused by a lack of intrinsic factor or other causes, such as infections, surgery, medicines, or diet.

Diagnosis

- Medical and Family Histories
- Physical Exam
- Diagnostic Tests : (Complete Blood Count)
- Bone Marrow Tests

Hemostasis:

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Hemostasis or haemostasis is a process to prevent and stop bleeding, meaning to keep blood within a damaged blood vessel.

Stages of hemostasis:

1) Vessel Spasm

When arteries are damaged the first thing that happens is that the smooth muscles surrounding the vessels immediately constrict, to reducing blood flow. This occur by endothelin-1 that released from damaged endothelium. ***Serotonin and Thromboxane A2 are activated.**

2) Formation of Platelet Plug

If a vessel is damaged, the collagen is exposed and platelets bind to it, vWF allows this binding to occur. The platelets become activated through this adhesion and degranulation in process called platelet release reaction. These granules contain ADP, Serotonin & ThromboxaneA2. Net result is the formation of an aggregate layer of activated platelets at the site of the injury, eventually forming a platelet plug full of activated platelets.

3) Blood Coagulation

Fibrin formation begins through either intrinsic\ extrinsic pathway, which converges on Factor X. This begins the common pathway which is when Factor X combines with Factor V

(together they are prothrombinase) and convert prothrombin into thrombin. Thrombin then turns fibrinogen into a loose Fibrin network. Factor XII stabalizes the fibrin by making it resistant to proteolytic cleavage.***VITAMIN K plays important role.**

4) Clot Retraction

After clot formation actin and myosin (cytoskeletal proteins) contract to pull the clot together and squeeze out any serum/fluid from injured site.

5) Clot Dissolution (Lysis)

Plasmin is produced from plasminogen (proteolytic enzyme) and this enzyme breaks down the clot once healing has begun.

Serotonin:

Serotonin is a monoamine neurotransmitter. Its biological function is complex and multifaceted, modulating mood, cognition, reward, learning, memory, and numerous physiological processes such as vomiting and vasoconstriction. Transported by platelets and released upon activation. This induces constriction of injured blood vessels and enhances platelet aggregation to minimize blood loss.

Thromboxane A2:

Thromboxane A2 (TXA2) is a type of thromboxane that is produced by activated platelets during hemostasis and has prothrombotic properties: it stimulates activation of new platelets as well as increases platelet aggregation and serves as a vasocontrictor.

Collagen:

Collagens, located in the matrix underlying vascular endothelial cells, are not exposed to flowing blood. After injury, blood will flow directly over subendothelial structures including connective tissue that contains a high percentage of collagen. Thus collagen is ideally situated to initiate hemostasis.

vWF:

Von Willebrand factor (vWF, or VWF), glycoprotein that plays an important role in stopping the escape of blood from vessels (hemostasis) following vascular injury. Von Willebrand factor (VWF) works by mediating the adherence, activation and aggregation of platelets and the interaction of platelets with components such as collagen in the damaged vessel lining

ADP:

Adenosine diphosphate (ADP) is a platelet agonist that causes platelet shape change and aggregation as well as generation of thromboxane A_2.

Coagulation factors:

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Number and/or name
I (fibrinogen)
II (prothrombin)
III (tissue factor or tissue thromboplastin)
IV (calcium)
V (proaccelerin, labile factor)
VI
VII (stable factor, proconvertin)
VIII (Antihemophilic factor A)
IX (Antihemophilic factor B or Christmas factor)
X (Stuart-Prower factor)
XI (plasma thromboplastin antecedent)
XII (Hageman factor)
XIII (fibrin-stabilizing factor)

Coagulation cascade

The coagulation cascade of secondary hemostasis has two initial pathways which lead to *fibrin* formation. These are the *contact activation pathway* (also known as the intrinsic pathway), and the *tissue factor pathway* (also known as the extrinsic pathway), which both lead to the same fundamental reactions that produce fibrin

Tissue factor pathway (extrinsic)

The main role of the tissue factor pathway is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps:

1. Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
2. TF-FVIIa activates FIX and FX.
3. The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).
4. FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.
5. Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which forms a complex with FIX), and activates and releases FVIII from being bound to vWF.

6. FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.)

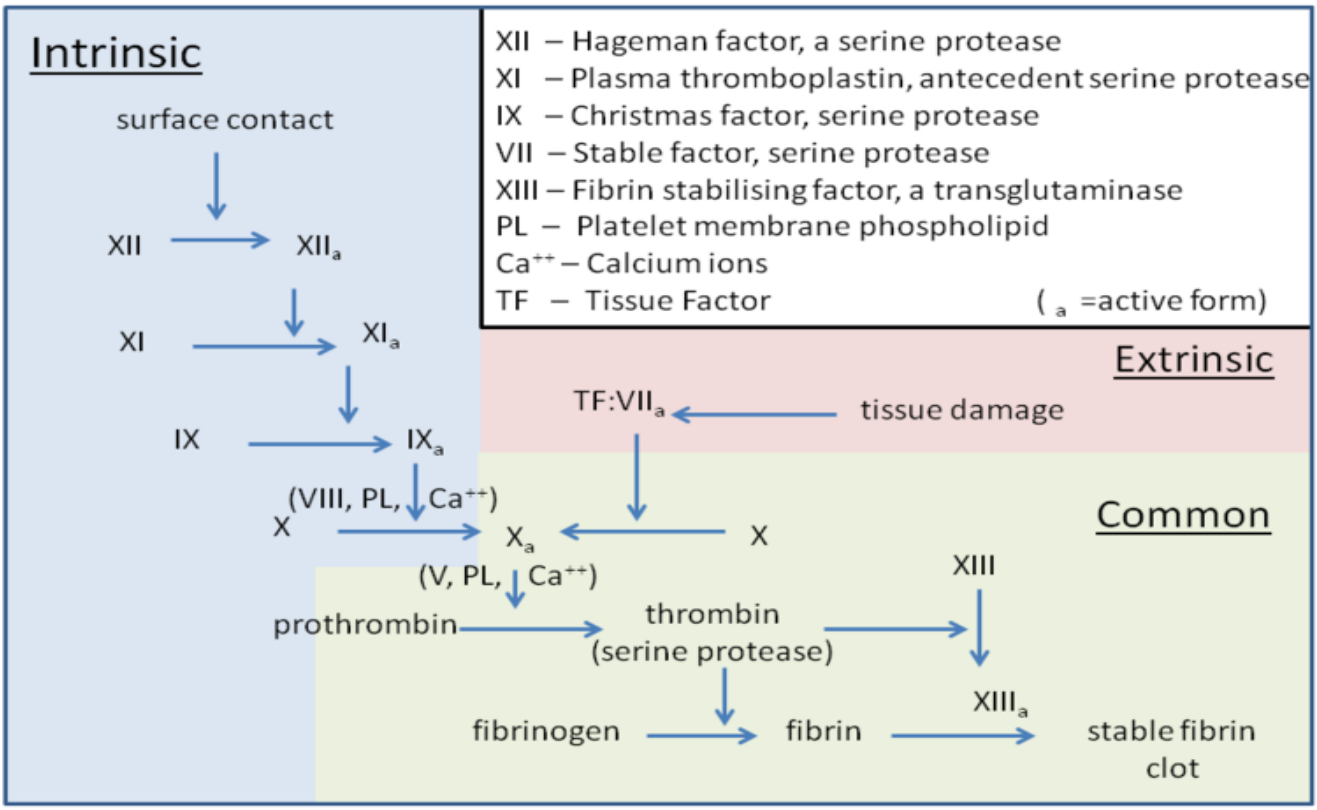
Contact activation pathway (intrinsic)

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa.

Final common pathway

The coagulation cascade is therefore classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

The three pathways that makeup the classical blood coagulation pathway



Cofactors

Various substances are required for the proper functioning of the coagulation cascade:

Calcium and phospholipid

Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function.

Vitamin K

Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X.

Bleeding Disorders:

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Bleeding disorders are a group of conditions that result when the blood cannot clot properly. In normal clotting, platelets, a type of blood cell, stick together and form a plug at the site of an injured blood vessel. Proteins in the blood called clotting factors then interact to form a fibrin clot, essentially a gel plug, which holds the platelets in place and allows healing to occur at the site of the injury while preventing blood from escaping the blood vessel. While too much clotting can lead to conditions such as heart attacks and strokes, the inability to form clots can be very dangerous as well, as it can result in excessive bleeding. Bleeding can result from either too few or abnormal platelets, abnormal or low amounts of clotting proteins, or abnormal blood vessels.

Types - Bleeding Disorders

Bleeding disorders can be inherited, or they can be acquired. Acquired bleeding disorders are more common than inherited bleeding disorders.

Acquired bleeding disorders

A bleeding disorder may develop if disease or a medicine, causes body to stop making blood clotting factors or causes the blood clotting factors to stop working correctly. In addition, problems with blood vessels can lead to bleeding.

Acquired bleeding disorders include:

- Disseminated intravascular coagulation (DIC)
- Liver disease-associated bleeding
- Vitamin K deficiency bleeding

Von Willebrand disease and hemophilia, two conditions that are most often inherited, may also develop as a result of a medical condition.

Inherited bleeding disorders

hemophilia and Von Willebrand disease are two conditions that are most often inherited bleeding disorders .

Hemophilia:

Hemophilia is a rare, inherited bleeding disorder that can range from mild to severe, depending on how much clotting factor is present in the blood. Hemophilia is classified as type A or type B, based on which type of clotting factor is lacking (factor VIII in type A and factor IX in type B). Hemophilia results from a genetic defect found on the X chromosome. Women have two X chromosomes. Women who have one X chromosome with the defective gene are termed carriers and they can pass the disease onto their sons. Due to random chromosome activation, some women carriers may range from asymptomatic to symptomatic

depending on how much of their factor VIII or IX is inactivated. In fact, some women may have “mild hemophilia,” though this is less common. Men have one X and one Y chromosome, so if their X chromosome has the defective gene, they will have hemophilia.

Because blood does not clot properly without enough clotting factor, any cut or injury carries the risk of excessive bleeding. In addition, people with hemophilia may suffer from internal bleeding that can damage joints, organs, and tissues over time.

Von Willebrand disease :

is an inherited condition that results when the blood lacks functioning von Willebrand factor, a protein that helps the blood to clot and also carries another clotting protein, factor VIII. It is usually milder than hemophilia and can affect both males and females. Women are especially affected by von WIllebrand disease during menses. Von Willebrand disease is classified into three different types (Types 1, 2, and 3), based on the levels of von Willebrand factor and factor VIII activity in the blood. Type 1 is the mildest and most common form; Type 3 is the most severe and least common form.

Other inherited bleeding disorders include other factor deficiencies, such as I, II, V, V + VIII, VII, X, XI, or XIII deficiencies. These rare bleeding disorders are named by the clotting factor causing the problem.

Signs and symptoms:

Signs and symptoms of bleeding disorders may include:

Easy bruising

Bleeding gums

Heavy bleeding from small cuts or dental work

Unexplained nosebleeds

Heavy menstrual bleeding

Bleeding into joints

Excessive bleeding following surgery

White blood cells (WBCs):

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White blood cells (**WBCs**), also called **leukocytes** or **leucocytes**, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system. All white blood cells have nuclei, which distinguishes them from the other blood cells, the anucleated red blood cells (RBCs) and platelets.

The number of leukocytes in the blood is often an indicator of disease, The normal white cell count is usually between 4,000 to 11,000 white blood cells per microliter of blood. White blood cells make up approximately 1% of the total blood volume in a healthy adult, making them substantially less numerous than the red blood cells at 40% to 45%. However, this 1% of the blood makes a large difference to health, because immunity depends on it.

The name "white blood cell" derives from the physical appearance of a blood sample after centrifugation. White cells are found in the *buffy coat*, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. The scientific term *leukocyte* directly reflects its description. It is derived from the Greek roots *leuk-* meaning "white" and *cyt-* meaning "cell".

The buffy coat may sometimes be green if there are large amounts of neutrophils in the sample, due to the heme-containing enzyme myeloperoxidase that they produce.

Types:

All white blood cells are nucleated, which distinguishes them from the anucleated red blood cells and platelets. Types of leukocytes can be classified in standard ways. Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or by cell lineage (myeloid cells or lymphoid cells). These broadest categories can be further divided into the five main types: neutrophils, eosinophils, basophils, lymphocytes, and monocytes. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. Further subtypes can be classified, for example, among lymphocytes, there are B cells (named from bursa or bone marrow cells), T cells (named from thymus cells), and natural killer cells.

- **Neutrophil**

Neutrophils are the most abundant white blood cell, constituting 60-70% of the circulating leukocytes, and including two functionally unequal subpopulations: neutrophil-killers and neutrophil-cagers. They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers form pus.

They are commonly referred to as polymorphonuclear (PMN) leukocytes, although, in the technical sense, PMN refers to all granulocytes. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands. This gives the neutrophils the appearance of having multiple nuclei, hence the name polymorphonuclear leukocyte. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytosed a few pathogens. Neutrophils are the most common cell type seen in the early stages of acute inflammation. The average lifespan of inactivated human neutrophils in the circulation has been reported by different approaches to be between 5 and 135 hours.

- **Eosinophil**

Eosinophils compose about 2-4% of the WBC total. This count fluctuates throughout the day, seasonally, and during menstruation. It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts.

- **Basophil**

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study.

They excrete two chemicals that aid in the body's defenses: histamine and heparin. Histamine is responsible for widening blood vessels and increasing the flow of blood to injured tissue. It also makes blood vessels more permeable so neutrophils and clotting proteins can get into connective tissue more easily. Heparin is an anticoagulant that inhibits blood clotting and promotes the movement of white blood cells into an area. Basophils can also release chemical signals that attract eosinophils and neutrophils to an infection site.

- **Lymphocyte**

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes include:

- B cells make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction. T cells &Natural killer cells

- **Monocyte**

Monocytes, the largest type of WBCs, share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an extra role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and become tissue macrophages, which remove dead cell debris as well as attack microorganisms. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney-shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

- ✓ **Fixed leucocytes**

Some leucocytes migrate into the tissues of the body to take up a permanent residence at that location rather than remaining in the blood. Often these cells have specific names depending upon which tissue they settle in, such as fixed macrophages in the liver, which become known as Kupffer cells. These cells still serve a role in the immune system.

- Histiocytes
- Dendritic cells (Although these will often migrate to local lymph nodes upon ingesting antigens)
- Mast cells
- Microglia

Morphology of normal leukocytes:

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1. ***Polymorphonuclear neutrophil:***

- Neutrophil measures 14-15 μm in size.
- Its cytoplasm is colorless and contains multiple, small, fine, mauve granules.
- Nucleus has 2-5 lobes that are connected by fine chromatin strands.
- A segmented neutrophil has at least 2 lobes connected by a chromatin strand.
- A band neutrophil shows non-segmented U-shaped nucleus of even width.
- Normally band neutrophils comprise less than 3% of all leukocytes. Majority of neutrophils have 3 lobes, while less than 5% have 5 lobes. In females, 2-3% of neutrophils show a small projection (called drumstick) on the nuclear lobe. It represents one inactivated X chromosome.

2. ***Eosinophil:***

- Eosinophils are slightly larger than neutrophils (15-16 μm).
- The nucleus is often bilobed and the cytoplasm is packed with numerous, large, bright orange-red granules.

3. ***Basophils:***

- Basophils are seen rarely on normal smears.
- They are small (9-12 μm), round to oval cells, which contain very large, coarse, deep purple granules.

4. ***Monocytes:***

- Monocyte is the largest of the leukocytes (15-20 μm).
- It is irregular in shape, with oval or clefted (kidney-shaped) nucleus and fine, delicate chromatin.
- Cytoplasm is abundant, bluegray with ground glass appearance and often contains fine azurophil granules and vacuoles.
- After migration to the tissues from blood, they are called as macrophages.

5. ***Lymphocytes:***

- On peripheral blood smear, two types of lymphocytes are distinguished: small and large. The majority of lymphocytes are small (7-8 μm).
- These cells have a high nuclearcytoplasmic ratio with a thin rim of deep blue cytoplasm.
- The nucleus is round or slightly clefted with coarsely clumped chromatin.

- Large lymphocytes (10-15 μm) have a more abundant, pale blue cytoplasm, which may contain a few azurophil granules. Nucleus is oval or round and often placed on one side of the cell.

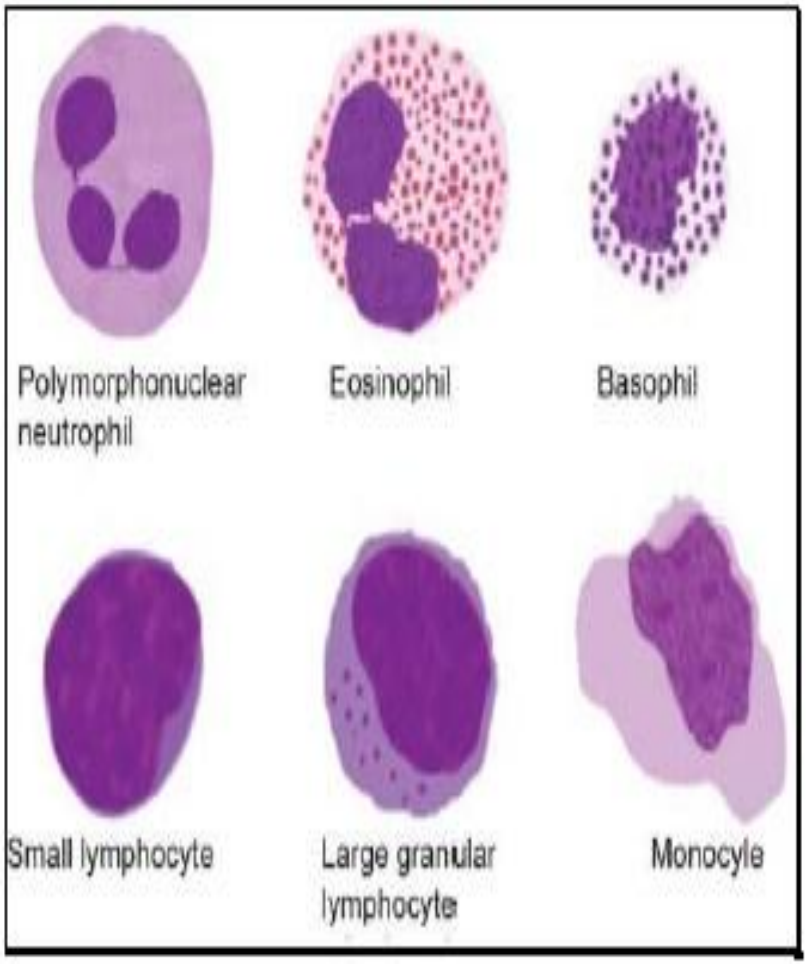

Figure (1). Normal mature white blood cells in peripheral blood

Morphology of abnormal leukocytes:

1. ***Toxic granules:*** These are darkly staining, bluepurple, coarse granules in the cytoplasm of neutrophils. They are commonly seen in severe bacterial infections.

2. ***Döhle inclusion bodies:*** These are small, oval, pale blue cytoplasmic inclusions in the periphery of neutrophils. They represent remnants of ribosomes and rough endoplasmic reticulum. They are often associated with toxic granules and are seen in bacterial infections.

3. ***Cytoplasmic vacuoles:*** Vacuoles in neutrophils are indicative of phagocytosis and are seen in bacterial infections.

4. ***Shift to left of neutrophils:*** This refers to presence of immature cells of neutrophil series (band forms and metamyelocytes) in peripheral blood and occurs in infections and inflammatory disorders.

5. ***Hypersegmented neutrophils:*** Hypersegmentation of neutrophils is said to be present when >5% of neutrophils have 5 or more lobes. They are large in size and are also called as macropolycytes. They are seen in folate or vitamin B_{12} deficiency

6. ***Pelger-Huet cells:*** In Pelger-Huet anomaly (a benign autosomal dominant condition), there is failure of nuclear segmentation of granulocytes so that nuclei are rod-like, round, or have two segments. Such granulocytes are also observed in myeloproliferative disorders (pseudo-Pelger-Huet cells).

7. ***Atypical lymphocytes:*** These are seen in viral infections, especially infectious mononucleosis. Atypical lymphocytes are large, irregularly shaped lymphocytes with abundant cytoplasm and irregular nuclei. Cytoplasm shows deep basophilia at the edges and scalloping of borders. Nuclear chromatin is less dense and occasional nucleolus may be present.

8. ***Blast cells:*** These are most premature of the leukocytes. They are large (15-25 μm), round to oval cells, with high nuclear cytoplasmic ratio. Nucleus shows one or more nucleoli and nuclear chromatin is immature. These cells are seen in severe infections, infiltrative disorders, and leukemia. In leukemia and lymphoma, blood smear suggests the diagnosis or differential diagnosis and helps in ordering further tests (see Figur).

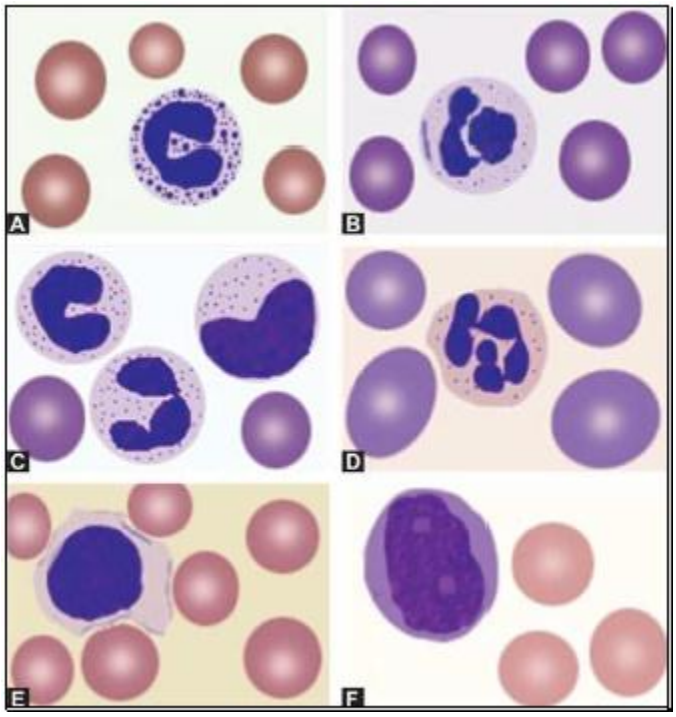

Figure (2) Morphological abnormalities of white blood cells: (A) Toxic granules; (B) Döhle inclusion body; (C) Shift to left in neutrophil series; (D) Hypersegmented neutrophil in megaloblastic anemia; (E) Atypical lymphocyte in infectious mononucleosis; (F) Blast cell in acute leukemia