1

Clinical Haematology

T. Rashad Saleh M. Alkhwlany

MSc in medical microbiology

COMPOSITION AND FUNCTIONS OF BLOOD

Hematology: is the study of the blood-forming tissues and circulating blood components.

- **Physical properties of blood:**
  - Your total blood volume is: 5-6 liters (8% of body weight or 80 ml/kg body weight).
  - Specific gravity: 1050-1060
  - Viscosity: 4-5 times that of water
  - PH: 7.4 + - 0.05; alkaline

Composition of Blood

If anticoagulant is add to blood sample and allowed to stand in a narrow tube, it separates out into cells and plasma.

- **Cells of blood**

  The cellular elements of blood represent 45% of the total blood volume, called Packed Cell Volume (PCV) or Haematocrit. It includes:

  1. Erythrocytes or Red Blood Cells (RBC's):
     Normal count 5 million/ mm^3 (5 x 10^6 per mm^3).
  2. Leucocytes or White Blood Cells (WBC's):
     Normal count 4,000 -11,000/ mm^3 (4-11 x 10^3 per mm^3).
  3. Platelets or Thrombocytes:
     Normal count: 150000-400000 PLTs/ mm^3 (150 - 400 x 10^3 per mm3).

- **Plasma**

  Plasma is a clear, straw colored fluid portion of the blood and represents 55% of the total blood volume. It composed of serum and fibrinogen. The serum contains:

  1. 91% water.
  2. 9% solids. The solids comprises of:
     a. 1% inorganic molecules and
     b. 8% organic molecules.
     - The major inorganic molecules are:
       - Na^+, Ca^{2+}, Cl^-, HCO_3^- (mainly extracellular).

- K^+, Mg^{2+}, Cu^{2+}, PO_4 (mainly intracellular).
- Fe^{2+}, Fe^{3+}.

- Of 8% total organic molecules:
  - 7% are plasma proteins.
  - 1% is other substances like NON protein Nitrogenous (NPN) substances, sugar, fats, enzymes and hormones.

Plasma Proteins

- Normal value: 6.4-8.3 gm%
- Components;

1. 55% Albumin: 3-5 gm% (Average: 4.8 gm %)
2. 38% Globulin: 2-3 gm% (Average: 2.3 gm %)
   i. 13% α - Globulin: 0.79-0.84 gm%
   ii. 14% β - Globulin : 0.78-0.81 gm%
   iii. 11% γ - Globulin : 0.66-0.70 gm%

   A/G ratio; Albumin: Globulin: : 1.7
3. 7% Fibrinogen: 0.3 gm%
4. Prothrombin: 40 mg%

Non-Protein Nitrogenous (NPN) Substances

- Normal: 28-40 mg%
- These are derivatives of food and in parts are the waste products of tissue catabolism. These include:

1. Urea : 20-40 mg%
2. Uric acid : 2-4 mg%
3. Creatine : 1-2 mg%
4. Creatinine : 0.6-1.2 mg%
5. Xanthine : Traces
6. Hypoxanthine : Traces

Other Substances

These include:

1. Neutral ‘fats (triglycerides) : 30-150 mg%
2. Phospholipids e.g. Lecithin, sphingomyelin, cephalin etc. : 150-300 mg%

3. Glucose (fasting) : 70-90 mg%
4. Cholesterol : 150-240 mg%

Functions of Blood

1. Respiratory: Transport of oxygen from lungs to the tissues and of carbon-dioxide (CO_2) from the tissues to the lungs.
2. Nutritive: Transport absorbed food materials, glucose, amino acids, fatty acids, vitamins, electrolytes and trace metals from the alimentary canal to the tissues for utilization and storage.
3. Excretory: Transports the metabolic wastes e.g. urea, uric acid, creatinine etc. to the kidney, skin and intestine for their removal.
4. 'Homeostatic' for water, pH and electrolyte concentration: Blood forms internal environment of the cell. Buffering power of haemoglobin helps to maintain constancy of blood pH.
5. Regulation of body temperature: Blood preserves the very narrow range in body temperature. How?

   Blood whose major constituent is water has:

   i. High specific heat - This buffer sudden change (rise or fall) in body temperature.
   ii. High conductivity - This helps to take out heat from an organ for uniform distribution throughout the body.
   iii. High latent heat of evaporation.
6. Chemical for communication and protection:
   i. Concentration of hormones and various substances in the blood is regulated through feedback mechanisms.
   ii. Within blood circulates the entire complex of humoral antibodies important in defence against infection, initiation of inflammation and regulation of Haemostasis (clotting mechanism).
7. Plasma proteins functions:
   i. Regulation of the osmotic pressure which influences the exchange of fluid between blood and tissues.

ii. Act as a reservoir of proteins.

iii. Combine with many substances e.g. iron, thyroxine and steroid hormones to form transportable complexes from which the active components are released at the appropriate sites.

Serum

If the blood is allowed to clot in a test tube, then the clot retracts and gives out serum. Therefore, serum is plasma minus fibrinogen and clotting factors (II, V and VIII), because these factors get consumed during clotting (remaining do not).

Serum has higher serotonin (5 hydroxy-tryptamine - 5HT) content because of the breakdown of platelets during clotting.

Haematopoiesis

(From Ancient Greek: haima: blood; poiesis to make) (Or hematopoiesis in the United States; sometimes also haemopoiesis or hemopoiesis).

Is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells. In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are produced daily in order to maintain blood levels in the peripheral circulation.

○ Site of haematopoiesis:

1. Fetus: 0-2 months: yolk sac.
   2-7 months: liver and spleen.
   5-9 months: bone marrow.
2. Infants: Bone marrow (practically all bones).
3. Adults: Vertebrae, ribs, sternum, skull, sacrum and pelvis. Proximal ends of femur and humeri.

- In the first few weeks of gestation the yolk sac is the main site of haernopoiesis. However, definitive haernopoiesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoi¬etic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow.

○ Extramedullary

In some cases, such as some disease, the liver, thymus, and spleen may resume their haematopoietic function, if necessary. This is called *extramedullary haematopoiesis*. It may cause these organs to increase in size substantially.

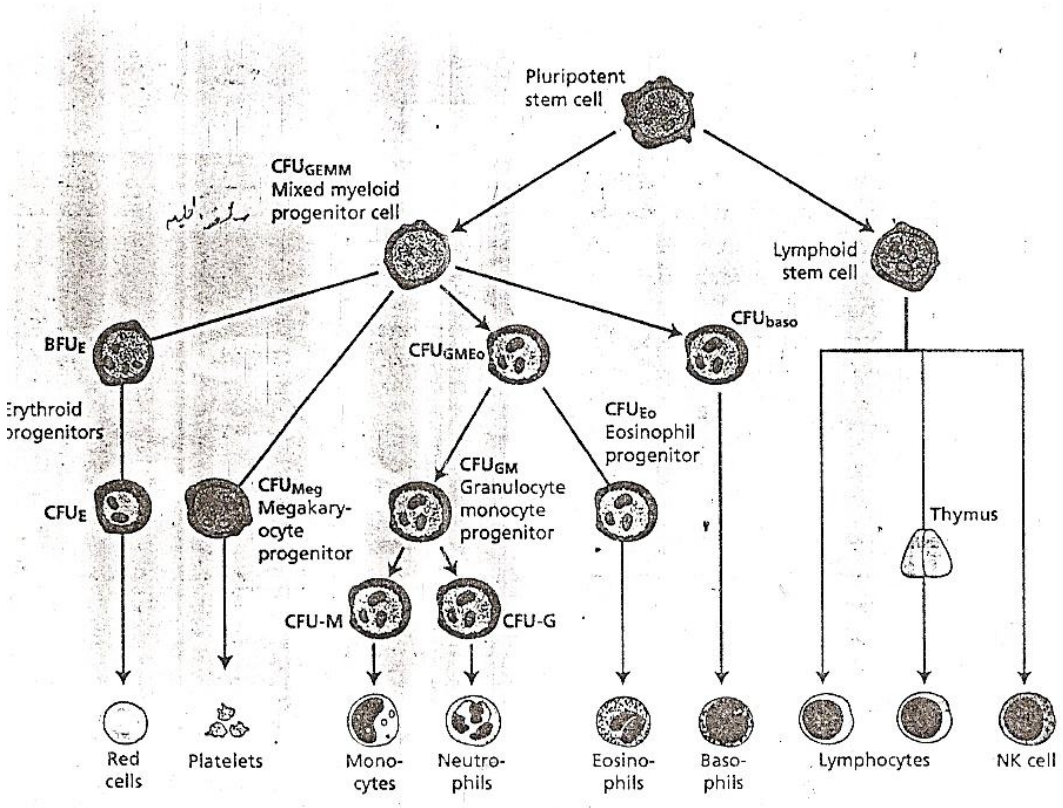
○ Stages of haematopoiesis:

➢ Stem cells:

All cellular blood components are derived from haematopoietic stem cells. The stem cells found within the bone marrow are the origin of all blood cells. Blood cells are formed by a process of differentiation from stem cell. The stem cell has the capability of self-renewal (so that marrow cellularity remains constant in a

normal healthy steady state). One stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions. The precursor cells are, however, capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises.

1. Pluripotential stem cells: are the earliest (least developed) stem cells. They are distinguished by their ability to generate pure and mixed of progenitor cells on the spleens of irradiated mice; thus, they were first called colony-forming units-spleen (CFU-S).
   a. CFU-S isolated from the lymphoid node of human have certain identifying characteristics.
      1) They are morphologically identical to small lymphocytes.
      2) They do not form rosettes on exposure to sheep red blood cells, indicating that they lack a typical antibody response to antigen.
   b. Colony-forming units-blast (CFU-blast): a cell with properties very similar to those of the CFU-S has been identified in human. These cells are thought to be the human pluripotential stem cells.
2. Multipotential stem cells develop from the differentiation of pluripotential stem cells. Two cell lines can develop:
   a. Lymphoid multi potential stem cells: Which give rise to cells from which develop B and T cells.
   b. Myeloid multipotential stem cells: These give rise to erythroid, granulocytic, monocytic, and megakaryocytic progenitor cells, from which develop mature red blood cells, platelets, monocytes, neutrophils, basophils, and eosinophils.



- **Nomenclature of haematopoietic cells:**

Between 1948 and 1950, the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs issued reports on the nomenclature of blood cells. An overview of the terminology is shown below, from earliest to final stage of development:

- [root]**blast**
- **pro**[root]**cyte**
- [root]**cyte**
- **meta**[root]**cyte**
- **mature cell name**

The root for CFU-E is "**rubri**", for CFU-GM is "**granulo**" or "**myelo**" and "**mono**", for CFU-L is "**lympho**" and for CFU-Meg is "**megakaryo**". According to this terminology, the stages of red blood cell formation would be: rubriblast,

prorubricyte, rubricyte, metarubricyte, and erythrocyte. However, the following nomenclature seems to be, at present, the most prevalent:

Committee	**"lympho"**	**"rubri"**	**"granulo" or "myelo"**	**"mono"**	**"megakaryo"**
Lineage	Lymphoid	Myeloid	Myeloid	Myeloid	Myeloid
CFU	CFU-L	CFU-GEMM→CFU-E	CFU-GEMM→CFU-GM→CFU-G	CFU-GEMM→CFU-GM→CFU-M	CFU-GEMM→CFU-Meg
Process	lymphocytopoiesis	erythropoiesis	granulocytopoiesis	monocytopoiesis	thrombocytopoiesis
[root]blast	Lymphoblast	Proerythroblast	Myeloblast	Monoblast	Megakaryoblast
pro[root]cyte	Prolymphocyte	Polychromatophilic erythrocyte	Promyelocyte	Promonocyte	Promegakaryocyte
[root]cyte	-	Normoblast	Eosino/neutro/basophilic myelocyte		Megakaryocyte
meta[root]cyte	Large lymphocyte	Reticulocyte	Eosinophilic/neutrophilic/basophilic metamyelocyte, Eosinophilic/neutrophilic/basophilic band cell	Early monocyte	-
mature cell name	Small lymphocyte	Erythrocyte	granulocytes (Eosino/neutro/basophil)	Monocyte	thrombocytes (Platelets)

Osteoclasts also arise from haemopoietic cells of the monocyte/neutrophil lineage, specifically CFU-GM.

○ Colony-forming units

There are various kinds of colony-forming units:

- Colony-forming unit lymphocyte (CFU-L)
- Colony-forming unit erythrocyte (CFU-E)

- Colony-forming unit granulo-monocyte (CFU-GM)
- Colony-forming unit megakaryocyte (CFU-Me)
- Colony-forming unit Basophil (CFU-B)
- Colony-forming unit Eosinophil (CFU-Eo)

○ The regulation of haemopoiesis

Haemopoiesis starts with stem cell division in which one cell replaces the stem cell (self-renewal) and the other is committed to differentiation. These early committed progenitors express low levels of transcription factors that may commit them to discrete cell lineages. Which cell lineage is selected for differentiation may depend both on chance and on the external signals received by progenitor cells.

Several transcription factors have been isolated that regulate differentiation along the major cell lineages. For instance, PU.l commits cells to the myeloid lineage whereas GATA-l has an essential role in erythropoietic and megakaryocytic differentiation.

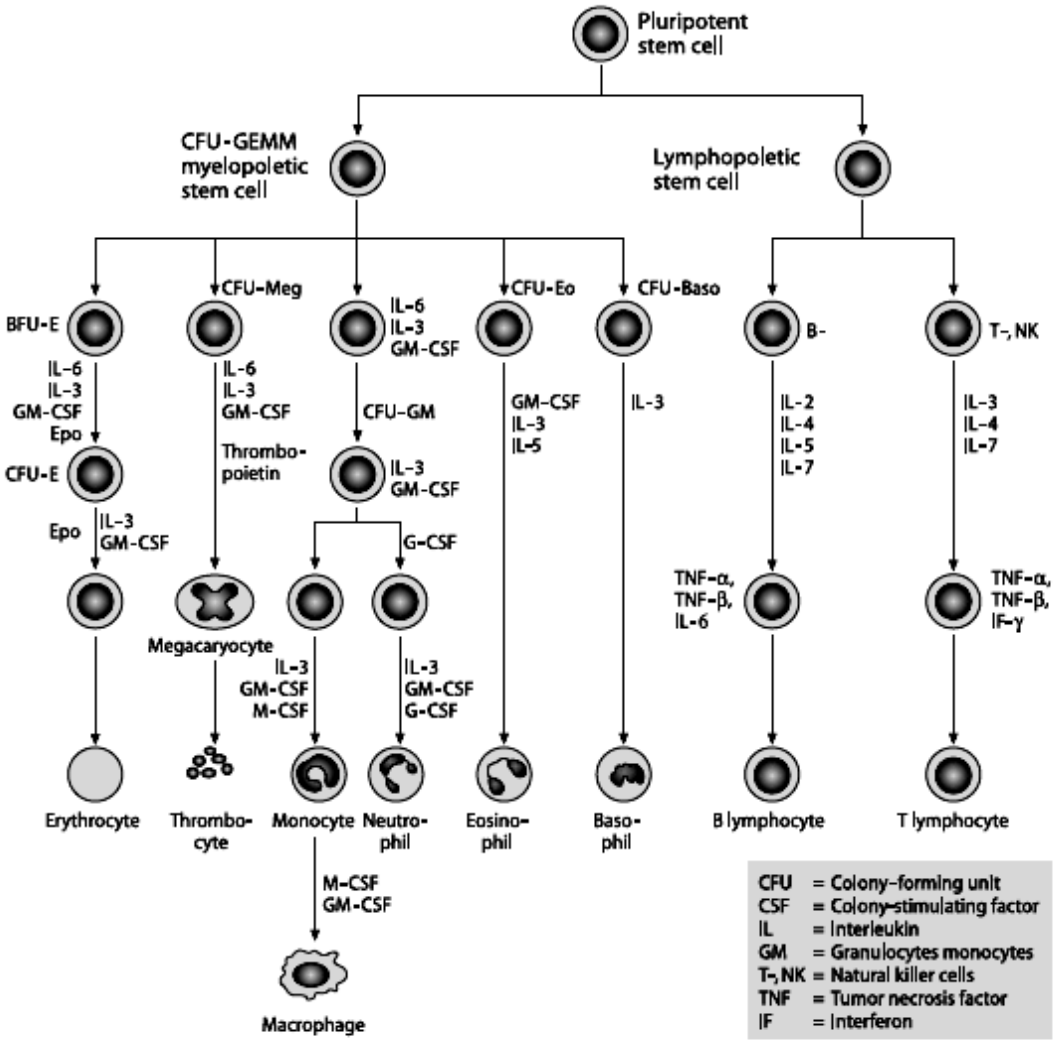
○ Haematopoietic growth factors

Red and white blood cell production is regulated in healthy humans, and the production of granulocytes is rapidly increased during infection. The proliferation and self-renewal of these cells depend on stem cell growth factor. Growth factors are glycoprotein that regulates the proliferation, also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells that enter the blood from the marrow. The more factors are:

Human hematopoietic growth factors

Growth Factor	Source	Major Function
GM-CSF	T-Lymphocyte, endothelial cells, Fibroblasts	Stimulates production of neutrophils, eosinophils, monocytes, red cells and platelets.
G-CSF	Monocytes, Fibroblasts	Stimulates production of neutrophils.
M-CSF	Macrophages, endothelia cells	Stimulates production of Monocytes
ERYTHROPOIETIN	Peritubular cells, Liver, Macrophages	Stimulates production of red cells.
IL-1	Macrophages, activated lymphes, endothelial cells.	Cofactor for IL-3 and IL-6. Activated T cells
IL-2	Activated T cells	T cell growth factor. Stimulates IL-1 synthesis. Activated B cells and NK cells

IL-3	T cells	Stimulates production of all non-lymphoid cells.
IL-4	Activated T cells	Growth factor for activated B cells, resting T cells and mast cells.
IL-5	T cells	Induces differentiation of activated B cells and eosinophils.
IL-6	T cells	Stimulates CFU-GEMM. Stimulates Ig synthesis
IL-7	T cells, Fibroblasts, Endothelial cells	Growth factor for pre B cells



Erythropoiesis

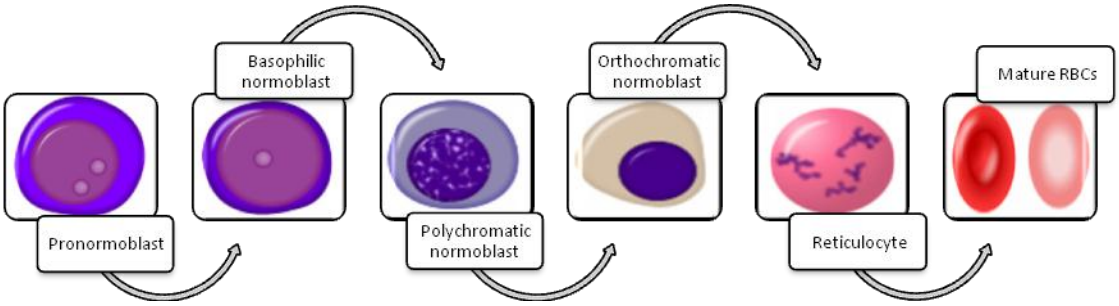
- The term erythropoiesis (erythro = RBC, and poiesis = to make) used to describe the process of RBC formation or production. In humans, erythropoiesis occurs almost exclusively in the red bone marrow.
- There are approximately 10^{12} new erythrocytes (red cells) each day by the complex and finely regulated process of erythropoiesis.
- Erythropoiesis passes from the stem cell through the progenitor cells colony-forming unit granulocyte, erythroid، monocyte and megakaryocyte (CFU_{GEMM}), burst-forming unit erythroid (BFU_E) and erythroid CFU (CFU_E) to the first recognizable erythrocyte precursor in the bone marrow، the pronormoblast.

Erythrocyte differentiation

1. Pluripotent hematopoietic stem cell
2. Multipotent stem cell
3. Myeloid stem cell
4. Pronormoblast
5. Basophilic normoblast/early normoblast
6. Polychromatophilic normoblast/intermediate normoblast
7. Orthochromatic normoblast/late normoblast
8. Reticulocyte
9. Mature RBCs → In blood circulation

In bone marrow (5 days)

Spends 1-2 days in the marrow and also circulates in the peripheral blood for 1-2 days before maturing، mainly in the spleen.



College of American Pathologists (CAP) vs. American Society for Clinical Pathology (ASCP) Terminology for Red Cells

CAP	ASCP
Pronormoblast	Rubriblast
Basophilic normoblast	Prorubricyte
Polychromatophilic normoblast	Rubricyte
Orthochromic normoblast	Metarubricyte
Reticulocyte	Reticulocyte
Erythrocyte	Erythrocyte

☒ Pronormoblast

- **Size:** 18 to 20 μm, the largest immature, the "mother cell".
- **N:C ratio**: 4:1.
- **Nuclear chromatin:** Round nucleus with condensation chromatin, evenly distributed, fine texture with deep violet color, nucleoli may be present but are difficult to visualize.
- **Cytoplasm:** Dark marine blue.

☒ Basophilic Normoblast

- **Size:** 16 μm.
- **N:C ratio:** 4:1.
- **Nuclear chromatin:** Round nucleus with crystalline chromatin, red-purple color to chromatin.
- **Cytoplasm:** Blue.

☒ Polychromatophilic Normoblast

- **Size:** 13 μm.
- **N:C ratio:** 2:1.
- **Nuclear chromatin:** Chromatin is condensed.
- **Cytoplasm:** A color mixture, blue layered with tinges of orange red, the hemoglobin begins to be synthesized.

☒ Orthochromic Normoblast-Nucleated (nRBC)

- **Size:** 8 μm.
- **N:C ratio:** 1:1.
- **Nuclear chromatin:** Dense, velvet-appearing homogeneous chromatin.
- **Cytoplasm:** Increased volume, with orange-red color with slight blue.

☒ Reticulocyte

- **Size:** 8 μm.
- **Appearance:** Remnant of RNA visualized as reticulum, filamentous structure in chains or as a single dotted structure in new methylene blue stain.
- **Nucleus:** The cell has extruded its nucleus, but is still capable of producing hemoglobin.

☒ Mature Red Cell

- **Size:** 6 to 8 μm.
- **Appearance:** Disk-shaped cell filled with hemoglobin, having an area of central pallor of 1 to 3 μm.

Major changes and features of erythropoiesis

- Decrease the total size of the cell.
- Increase amount of cytoplasm.
- Decrease basophilic staining.
- Increase haemoglobin synthesis.
- Condensation of chromatin.
- The N:C ratio decreases as the cell matures.

Note:

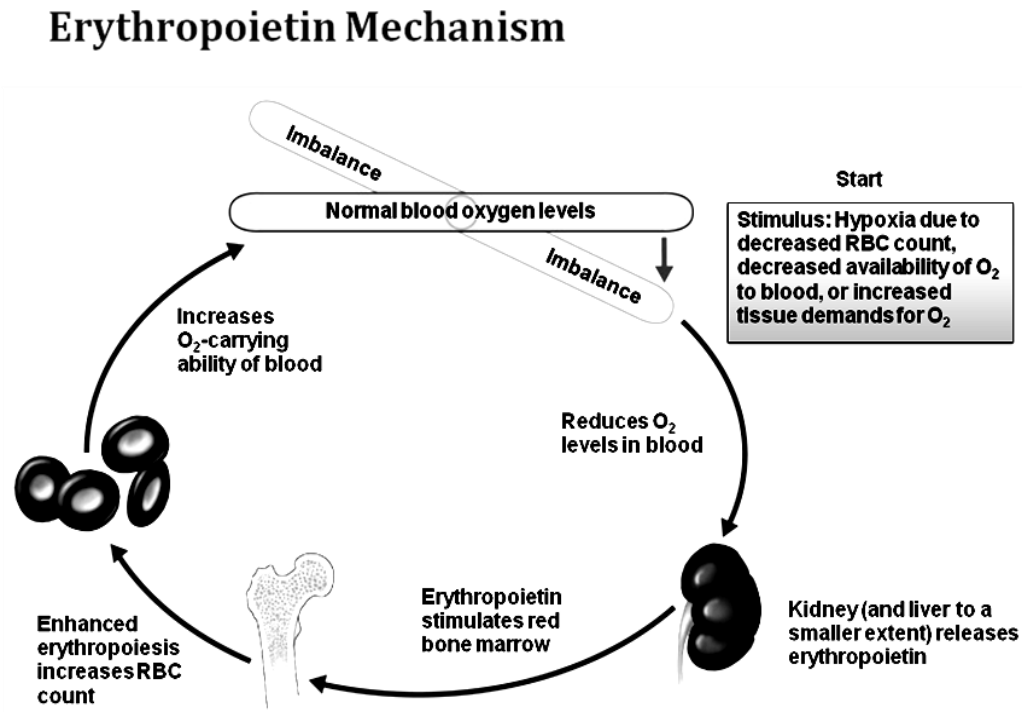
Previous cell are studied from where:

- Cytoplasm (amount, color, texture).
- Nucleus (Size, color, position, texture).
- Nucleoli (presence and number).
- Ratio of cytoplasm to nucleus.

Control of erythropoiesis:

By erythropoietin (formed in kidneys) is released in response to lowered tissue oxygen. Erythropoietin is glycoprotein and stimulates erythropoiesis.

It act on the BFU-E (burst-forming units), CFU-E (colony-forming units) and on pronormoblast.



Hemoglobin

Hemoglobin (also spelled haemoglobin and abbreviated Hb or Hgb) is the iron-containing oxygen-transport metalloprotein in the red blood cells. The hemoglobin was discovered by Hünefeld in 1840.

إن الوظيفة الأساسية لكريات الدم الحمراء هي نقل الأوكسجين من الرئتين إلى الأنسجة وحمل ثاني أكسيد الكربون من الأنسجة إلى الرئتين. وهذه العملية تتم بواسطة الهيموجلوبين..

كل جزيء من الهيموجلوبين يتكون من أربع ذرات من الهيم وأربع ذرات من الجلوبين، أما الهيم فيتكون من الحديد الثنائي F^{++} والبورفرين Porphyrine وأما الجلوبين فهو بروتين يتكون من سلاسل من عديدات الببتيد Polypeptide والتي تتكون من تجمع من الأحماض الأمينية amino acid.

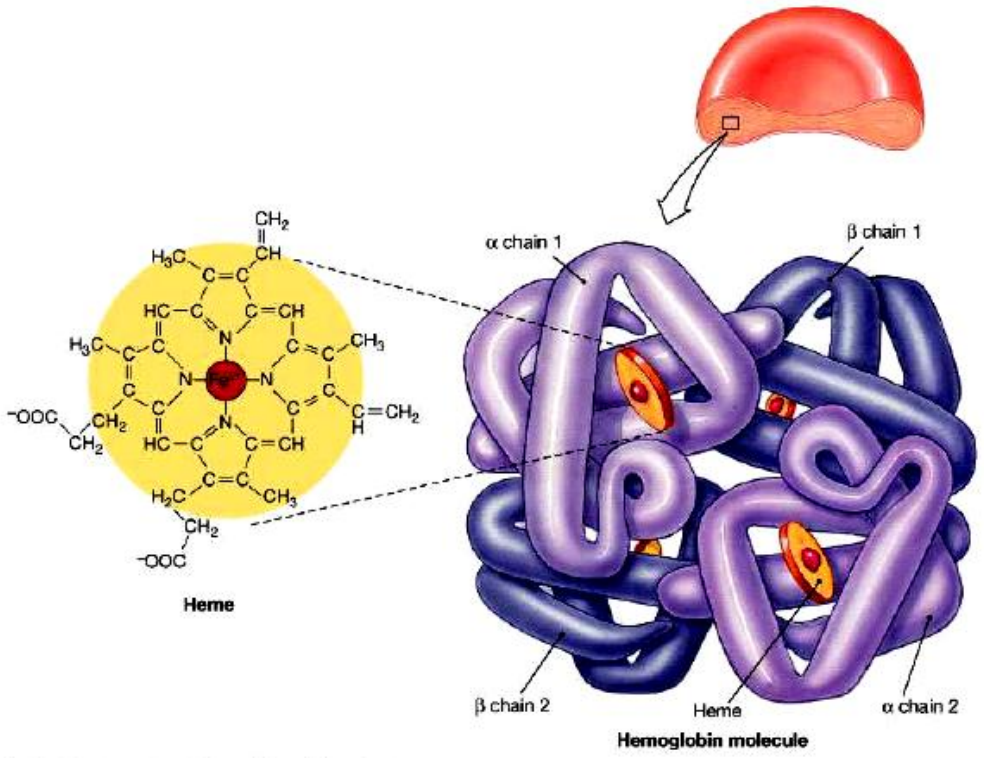
Each molecule of haemoglobin contains four polypeptide (globin) chains and four molecules of haem.

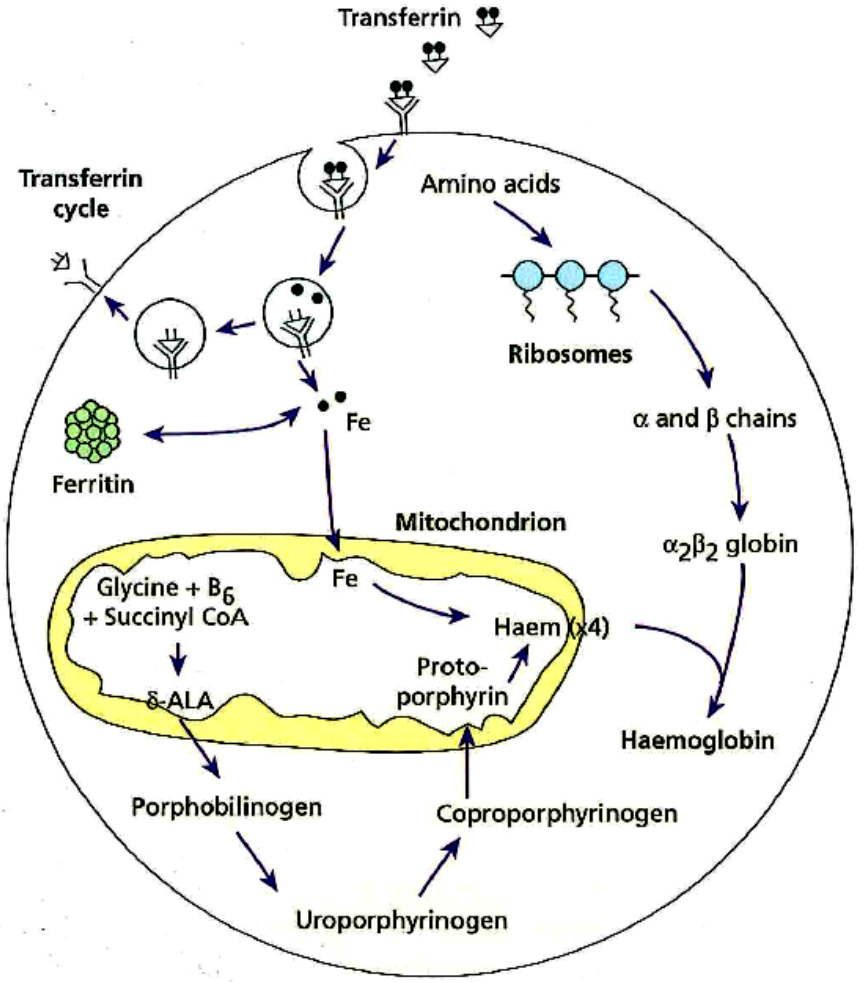
Globin in adult consist of two alpha (α) chain containing 141 amino acid and two beta (β) chains containing 146 amino acids.

الجلوبين يتكون في نخاع العظم وبالذات في ريبوسومات الخلايا الحمراء الغير ناضجة Immature RBCs.

أما الهيم فيتكون بشكل أساسي في الميتوكوندريا Mitochondria.

هناك العديد من العناصر التي تساعد في تكوين الهيموجلوبين مثل $Vitamin\ B_{12}$ و Folic acid و Vitamin C و النحاس و Vitamine 6 والحديد Fe^{++}.





Types in humans

- **In the embryo:**
  - ✓ Gower 1 ($\zeta_2\varepsilon_2$)
  - ✓ Gower 2 ($\alpha_2\varepsilon_2$)
  - ✓ Hemoglobin Portland ($\zeta_2\gamma_2$)
- **In the fetus:**
  - ✓ Hemoglobin F ($\alpha_2\gamma_2$)
- **In adults:**
  - ✓ Hemoglobin A ($\alpha_2\beta_2$) - a normal amount 96-98%
  - ✓ Hemoglobin A2 ($\alpha_2\delta_2$) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.2%
  - ✓ Hemoglobin F ($\alpha_2\gamma_2$) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells, it has a normal range of 0.5-0.8%

However, the level of Hb F can be elevated in persons with sickle-cell disease and thalassemia.

Hb A هو الشائع في جسم الإنسان بعد الشهر الثالث، أما في الأطفال يكون Hb F بنسبة 60-80% لكن هذه النسبة تتراجع بعد الشهر الثالث وإذا لم تتراجع فأنها تؤدي للإصابة بالأمراض.

Genetics

Subunit Name	Gene	Chromosomal Locus
Hb α_1	HBA_1	Chromosome 16 p13.3
Hb α_2	HBA_2	Chromosome 16 p13.3
Hb β	HBB	Chromosome 11 p15.5

Hb derivatives:

- Oxyhemoglobin
- Deoxyhemoglobin
- Methaemoglobin
- Carboxyhaemoglobin
- Sulfhaemoglobin

Abnormal Hb:

- Thalassemia (β_4)
- HbS ($\alpha_2\beta^S_2$)
- HbC ($\alpha_2\beta^C_2$)
- HbSC

Red Blood Cells (RBCs)

- Red blood cells are also known as RBCs, **red blood corpuscles** (an archaic term), erythrocytes (from Greek erythros for "red" and cyte translated as "cell". The term Red Blood Cells is the proper name.
- The first person to describe red blood cells or erythrocytes was a Dutch biologist Jan Swammerdam, in 1658. Anton van Leeuwenhoek provided further descriptions of the RBCs in 1674.
- The color of erythrocytes is due to the heme group of hemoglobin. The blood plasma alone is straw-colored, but the red blood cells change color depending on the state of the hemoglobin:
  - when combined with oxygen (oxyhemoglobin) is scarlet.
  - when oxygen has been released (deoxyhemoglobin) is darker.

RBCs function:

RBCS transport the respiratory gases O_2 and Co_2

Life Span:

120 days, then destroy at RE (reticuloendothelial system) system. The important breakdown products are heam and globin that recirculated in the body. The heme are broken down into Fe and biliverdin. The biliverdin is reduced to bilirubin, which is released into the plasma and recirculated to the liver bound to albumin. The iron is released into the plasma to be recirculated by a carrier protein called transferrin. Almost all erythrocytes are removed in this manner from the circulation before they are old enough to hemolyze. Hemolyzed hemoglobin is bound to a protein in plasma called haptoglobin which is not excreted by the kidney.

RBCs structure:

Red cell is structure from cell membrane and cytoplasm filled completely with Hb. The RBCs lack the others organelles (nucleus, ribosome, Golgi apparatus and mitochondria).

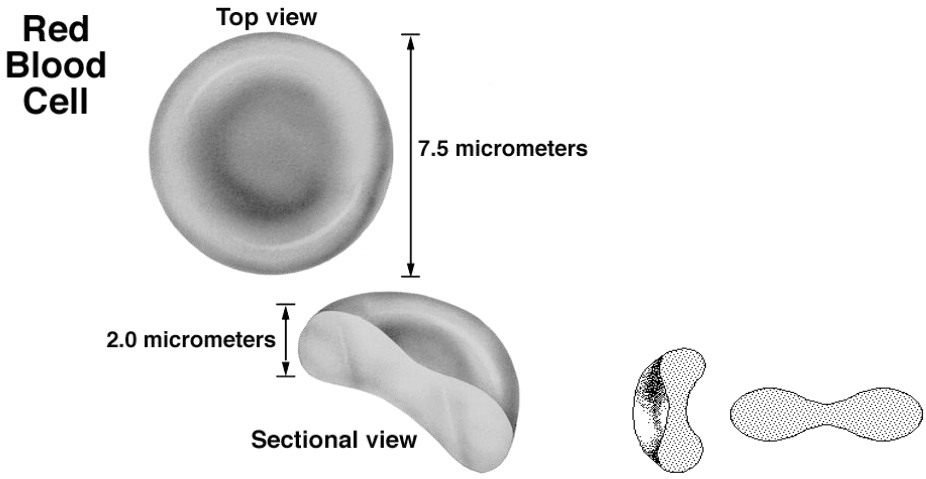
- Prior to discharge from B.M (bone marrow) into P.B. (peripheral blood) ⟶ RBCs shed their nuclei. This gives the advantages:

  1. Reduced weight.

2. Transformation into biconcave shape with increased flexibility compared with rigid nucleated cell.

RBCs is biconcave shape (from side) and spherical (disc) with central pallor from top (⅓ diameter of cell) with 7.5-8 µm in diameter, must be able to pass repeatedly through the microcirculation whose minimum diameter is 3.5-5 µm, in order to:

1. Maintain Hb in reduced 9ferrous Fe^{++}) form.
2. Maintain osmotic equilibrium inside the cell.

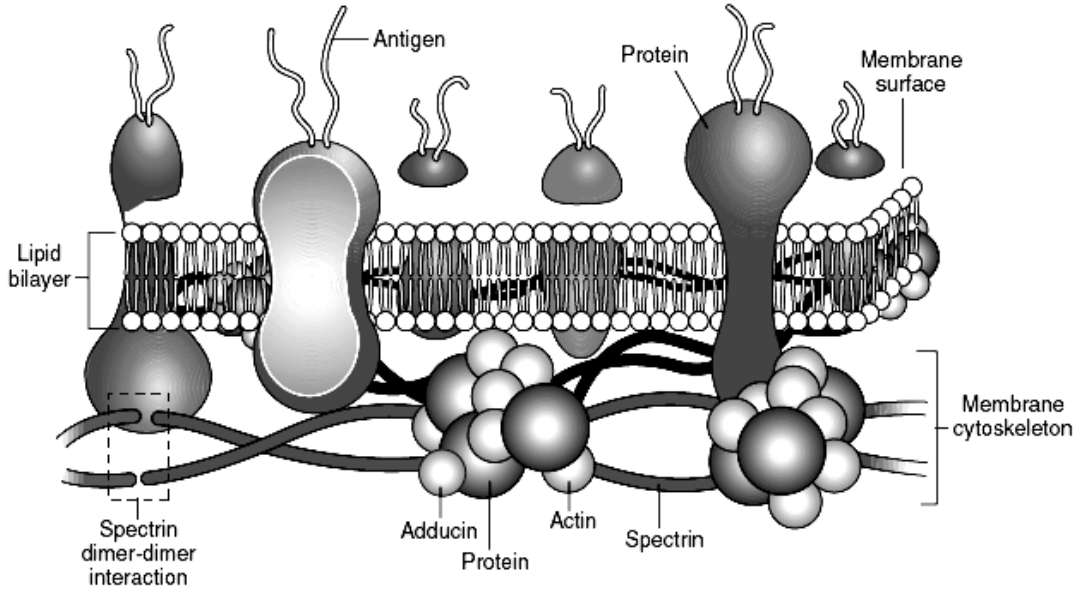


- **RBCs membrane:**

The red cell membrane comprises a lipid bilayer, integral membrane proteins with surface carbohydrate.

- **10% carbohydrate** → On the surface
  → Ags of blood group.
- **50% protein**
  - Which include → α and β spectrin
    → Ankyrin
    → Protein band 4.1 and 4.2
    → Actin
    → Band 3
  - These protein formed the RBCs skeleton.
  - These protein are important in maintain biconcave shape and transmembrane.
  - Any change or defect in these protein may cases some alteration in RBCs shape eg: hereditary spherocytosis and elliptocytosis.
- **40% lipid:**

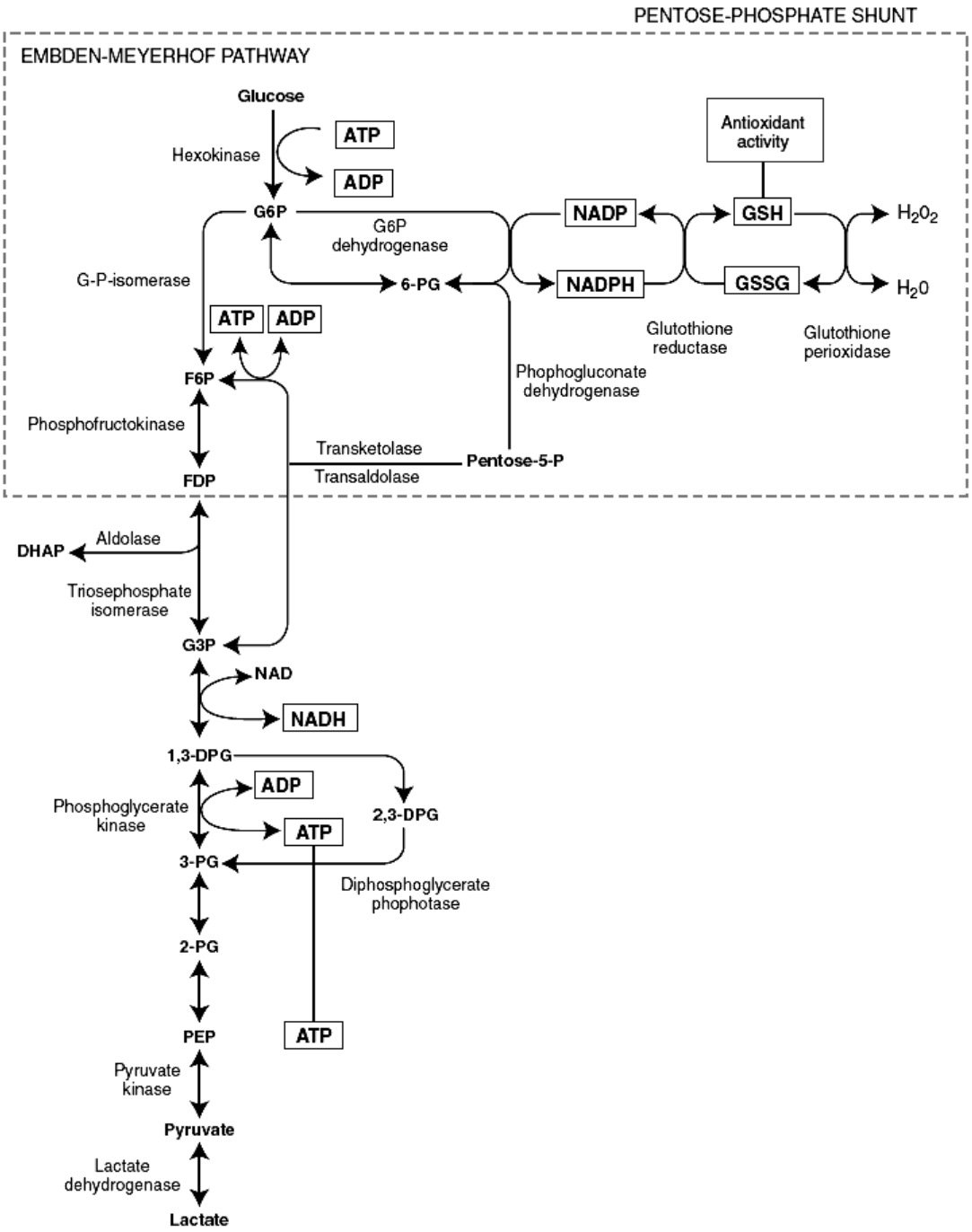
- Which include cholesterol and phospholipid.
- Alterations in lipid composition may be associated with others shape abnormalitiis eg:
  - ✓ Increase in cholesterol and phospholipid causes target cells formation.
  - ✓ Large increase in cholesterol cause acanthocyte formation.



▪ RBCs metabolism:

RBCs able to produce energy that required it as:

- **ATP:**
  - ✓ From anaerobic glycolysis (Embden Meyerhof pathway).
  - ✓ To maintain RBCs shape, volume, flexibility.
- **NADH:**
  - ✓ Also from anaerobic glycolysis
  - ✓ Reducing power, to maintain Hb in reduced form (reduced met Hb ⟶ normal Hb)
- **NADPH:**
  - ✓ 5% from glycolysis enter HMP (Hexose monophosphate pathway) .
  - ✓ As NADH also to remove the toxicity from RBCs (antioxidant).
- **2,3 DPG (2,3 diphosphoglycerate):**
  - ✓ From Luebering-Rapoport (part from glycolysis)
  - ✓ Regulation of Hb-oxygen affinity.



Embden Meyerhof pathway

- **The red blood cells diseases include:**
  - **Anemia** are diseases characterized by low oxygen transport capacity of the blood, because of low red cell count or some abnormality of the red blood cells or the hemoglobin.
  - **Polycythemia** are diseases characterized by increase of red blood cells.

LEUKOCYTES (WHITE BLOOD CELLS)

Several types of leukocytes, or white blood cells (WBCs), are found in the blood. The normal WBC count is ~4,000 to 11,000/µL. Leukocytes are usually divided into **granulocytes**, which have specific granules, and **agranulocytes**, which lack specific granules.

Granulocytes are divided into **neutrophils** (with faintly staining granules), **eosinophils** (with large reddish or eosinophilic granules), and **basophils** (with large dark blue or basophilic granules). Agranulocytes are divided into **lymphocytes** and **monocytes**.

Although they are called white blood cells, leukocytes predominantly function in tissues. They are only in the blood transiently, while they travel to their site of action.

- **Functions of neutrophils**
  - About half the neutrophils circulating in the blood (included in WBC count). The other half, marginate along the walls of blood vessels and in capillaries, (not included in WBC count).
  - Neutrophils have a short life-span. After circulating in the blood for 6–10 hours they pass into the tissues where they become phagocytes, they are important in defending the body from infection.
  - They mobilize and migrate to sites of infection or inflammation, they attracted by chemical substances released by bacteria, complement components, damaged tissue, and other leukocytes (process called chemotaxis).
  - Neutrophils have receptors for IgG antibody and complement (C_3b) and are therefore able to recognize, phagocytose, and kill bacteria coated with IgG or C_3b. Bacteria are ingested and destroyed by chemicals released from neutrophil granules.
  - Following phagocytosis, neutrophils die at the site of infection, forming pus cells. Neutrophils not involved in the inflammatory process are removed by the spleen after 1–2 days.
- **Functions of eosinophils**
  - Eosinophils circulate in blood for about 8 hours after which they enter the tissues. They are found mainly in the skin, gastrointestinal tract and lungs where

they are involved in hypersensitivity reactions, e.g. asthma, hay fever, eczema. Eosinophils contain substances that are able to inactivate histamine and factors released during anaphylaxis.

- Eosinophils are important in parasitic helminth immune responses in which IgG and IgE antibodies are produced. Lymphokines released from T lymphocytes stimulate the production of IgE which binds to mast cells at the site of parasitic infection.
- Parasitic antigens cause chemotactic substances to be released from mast cells which attract eosinophils to the site. Eosinophils bind to the antibody-coated parasite and release cytotoxic substances which damage the surface of the parasite, leading to its destruction.
- Antibody dependent cell-mediated cytotoxicity by eosinophils is effective against parasites which are located in the tissues, or have a tissue invading stage, e.g. schistosomes, filarial worms, hookworms, *Trichinella*, *Strongyloides*, *Ascaris*, *Fasciola*, *Fasciolopsis*, *Paragonimus*, and *Toxocara*.

Functions of basophils

- Basophils circulate only in the blood. They are not found in tissues (mast cells are tissue equivalents to basophils). Basophils bind IgE on their surface and are involved in anaphylactic, hypersensitivity, and inflammatory reactions.
- Basophils interact with eosinophils and macrophages in allergic reactions. When specific antigen reacts with IgE bound to basophils, degranulation of the cells occurs with the release of inflammatory substances including heparin, histamine and platelet activating factor.

Functions of monocytes and macrophages

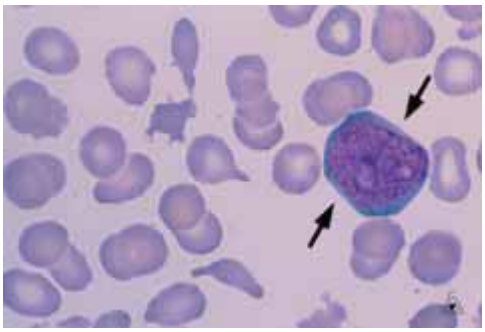
- Monocytes pass from the bone marrow into the blood circulation. Within 2–3 days they reach the tissues where they develop into macrophages, becoming fixed tissue macrophages in the spleen, liver (Küpffer cells), lymph nodes, connective tissues, and central nervous system, and free macrophages in lung alveoli, peritoneum and inflammatory granulomas.

- Macrophages form the mononuclear phagocytic system, i.e. reticuloendothelial (RE) system. Unlike neutrophils, mononuclear phagocytes do not die following phagocytosis. Macrophages live in the tissues for several months or longer.
- As phagocytic cells, monocytes ingest microorganisms and cellular debris, including malaria pigment. The RE system is involved in the destruction of bacteria, viruses, fungi, protozoal parasites, and malignant cells.
- Macrophages process and present antigens to T lymphocytes, and regulate many T and B cell activities.
- When activated, macrophages synthesize and secrete cytokines (e.g. interleukins, tumour necrosis factor, GM-CSF) which are involved in the activation of lymphocytes, the inflammatory process (particularly chronic inflammation), cell-mediated immune responses and haematopoiesis.
- An increase in circulating monocytes (monocytosis) is found in protozoal parasitic infections, and also in tuberculosis and other chronic bacterial infections and rickettsial infections.

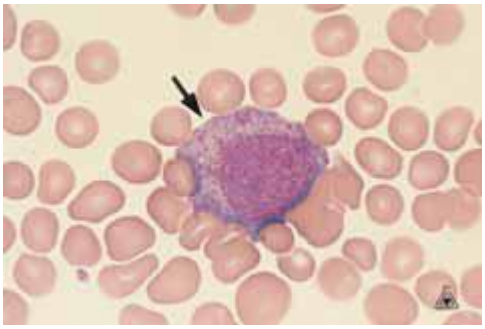
Leukocyte differentiation

Myeloblast

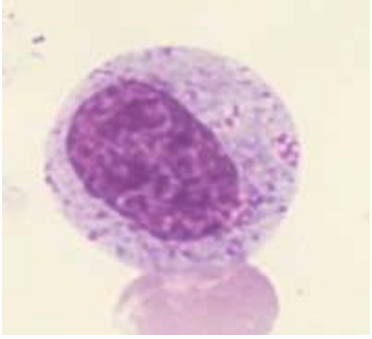
- **Size:** 12 to 20 µm
- **N:C:** 4:1 with round, oval
- **Cytoplasm:** Moderate blue and usually non-granular
- **Differentiating characteristic:** Nucleus has thin chromatin strands that are distributed throughout the nucleus uniformly; chromatin appears smooth and velvety.
- **Cluster designation:** (CD)45, CD38, CD34, CD33, CD13, human leukocyte antigen (HLA)-DR



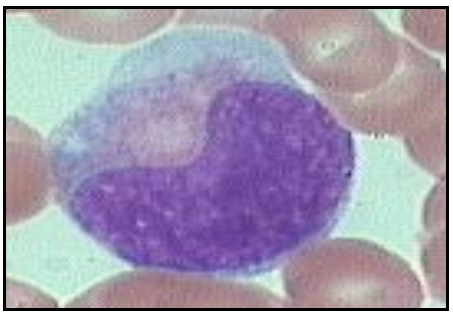
- **Promyelocyte**
  - **Size**: 15 to 21 µm
  - **N:C:** 3:1, oval, round
  - **Cytoplasm**: Moderate blue color but difficult to observe because fine to large blue-red azurophilic granules are scattered; granules are NONSPECIFIC
  - **Differentiating characteristic***:* Cell is larger than the blast with large prominent nucleoli, nuclear chromatin is slightly coarse.
  - **Cluster designation:** CD45, CD33, CD13, CD15



- **Myelocyte**
  - **Size**: 10 to 18 µm
  - **N:C:** 2:1
  - **Cytoplasm**: Specific granules present, neutrophilic granules are dusty, fine, and red-blue; eosinophilic granules are large red-orange, and singular; basophil granules are large, deep blue purple
  - **Differentiating characteristic***:* Small pink-purple granules for the neutrophilic myelocyte, nucleus stains deeper color, granular pattern to the chromatin.
  - **Cluster designation:** CD45, CD33, CD13, CD15, CD11b/11c



- **Metamyelocyte**
  - **Size**: 10 to 15 µm
  - **N:C:** 1:1
  - **Cytoplasm**: Pale blue to pinkish tan with moderate specific granules
  - **Differentiating characteristics:** Nuclear indentation and condensed chromatin with no nuclei.
  - **Cluster designation:** CD markers are the same as for the myelocyte



- **Band**
  - **Size**: 9 to 15 µm
  - **Chromatin**: Band shaped like a cigar band, C or S shaped, unable to see filament, coarselyclumped almost like leopard spot coarseness
  - **Cytoplasm**: Brown-pink, with many fine secondary granules
  - **Differentiating characteristics***:* No filament, may resemble a metamyelocyte but indentation is more severe and chromatin is more clumped.
  - **Cluster designation:** CD45, CD13, CD15, CD11b/11c