Haematopoiesis

Dr Mahmoud Shbair

Consultant Pediatric Hematologist and Oncologist

Haematopoiesis

Haematopoiesis is the formation of blood cellular components which are derived from haematopoietic stem cells, it takes place in the bone marrow and lymphatic organs

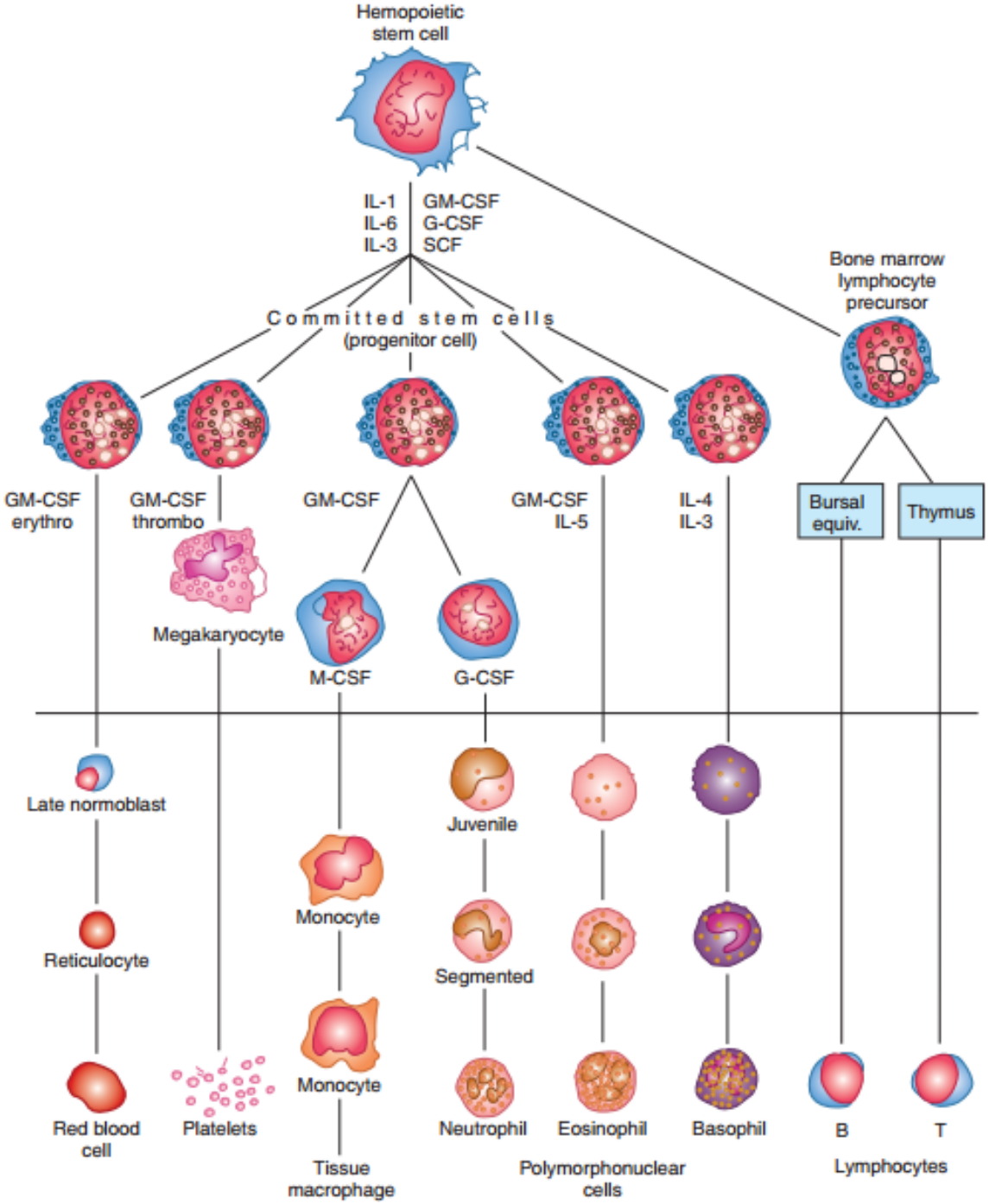
In early embryo these haematopoietic stem cells arise in the yolk sac mesoderm, in the second trimester, hematopoiesis occurs primarily in the developing liver, with the spleen playing a minor role

In the third trimester marrow in the medullary cavities of specific bones becomes the major hemopoietic organ.

Hemopoietic Stem Cells

All blood cells arise from a single type of **pluripotent** hemopoietic stem cell in the bone marrow, which are rare, proliferate slowly and give rise to two groups of multipotential progenitors:

- **lymphoid** progenitor cells migrate from the bone marrow to the thymus or the lymph nodes, spleen, and other lymphoid structures, where they proliferate and differentiate
- **Common myeloid** progenitor cells includes precursor cells (blasts) for erythropoiesis, thrombopoiesis, granulopoiesis, and monocytopoiesis, all in the bone marrow


Hemopoietic stem cell
IL-1
IL-6
IL-3
GM-CSF
G-CSF
SCF
Committed stem cells (progenitor cell)
Bone marrow lymphocyte precursor
GM-CSF erythro
GM-CSF thrombo
GM-CSF
GM-CSF IL-5
IL-4 IL-3
Bursal equiv.
Thymus
Megakaryocyte
M-CSF
G-CSF
Late normoblast
Juvenile
Monocyte
Reticulocyte
Segmented
Monocyte
Red blood cell
Platelets
Neutrophil
Eosinophil
Basophil
B
T
Tissue macrophage
Polymorphonuclear cells
Lymphocytes

BONE MARROW

Bone marrow is found in the medullary canals of long bones and in the small cavities of cancellous bone, it is of 2 types

- **red bone marrow** active blood-forming, abundance of blood and hemopoietic cells
- **yellow bone marrow**, which is filled with adipocytes that exclude most hemopoietic cells, present in the shaft of long bones in adults

In the newborn all bone marrow is red and active in blood cell production, but as the child grows, most of the marrow changes gradually to the yellow variety.

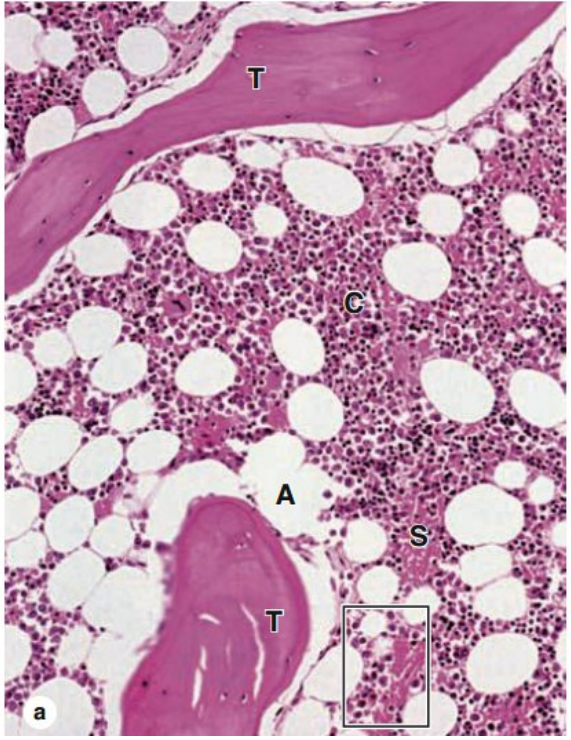
Red bone marrow

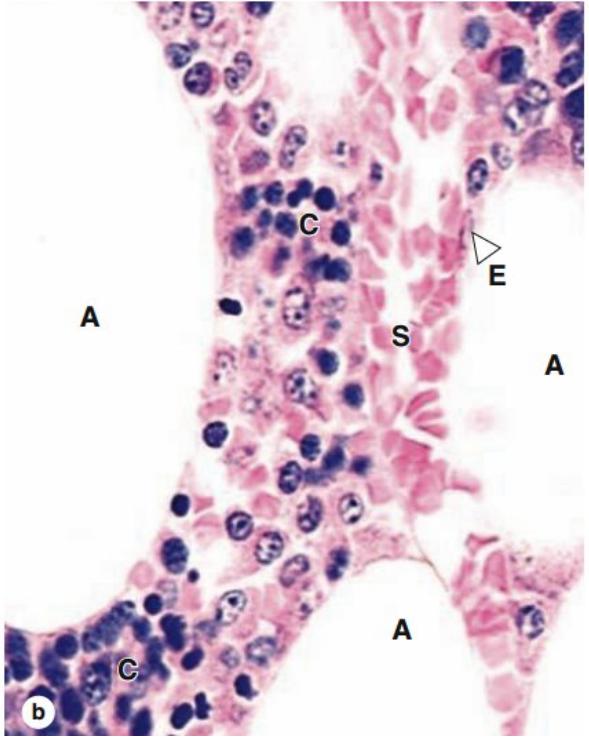
Stroma of reticular connective tissue

- Meshwork of specialized fibroblastic cells called **stromal cells** (also called **reticular** or **adventitial cells**) and a delicate web of reticular fibers supporting the hemopoietic cells and macrophages.
- The matrix of bone marrow also contains collagen type I, proteoglycans, fibronectin, and laminin.
- Play a role in hematopoietic growth factor production

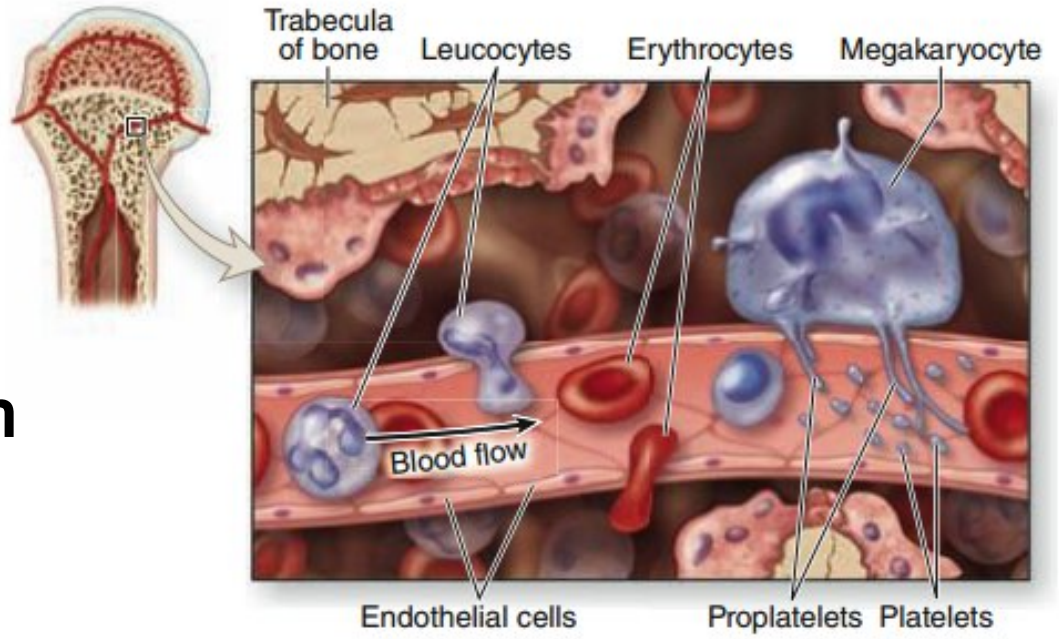
Hemopoietic cords or **islands** of cell which contains the immature stages of all blood cells

Sinusoidal capillaries run between the hematopoietic cords, have discontinuous endothelium, through which newly differentiated blood cells and platelets enter the circulation



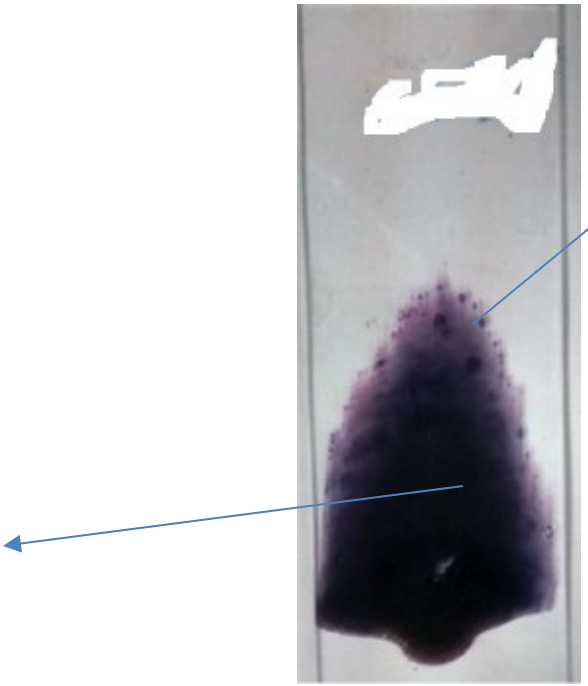


Red bone marrow



Sinusoidal endothelium in active marrow

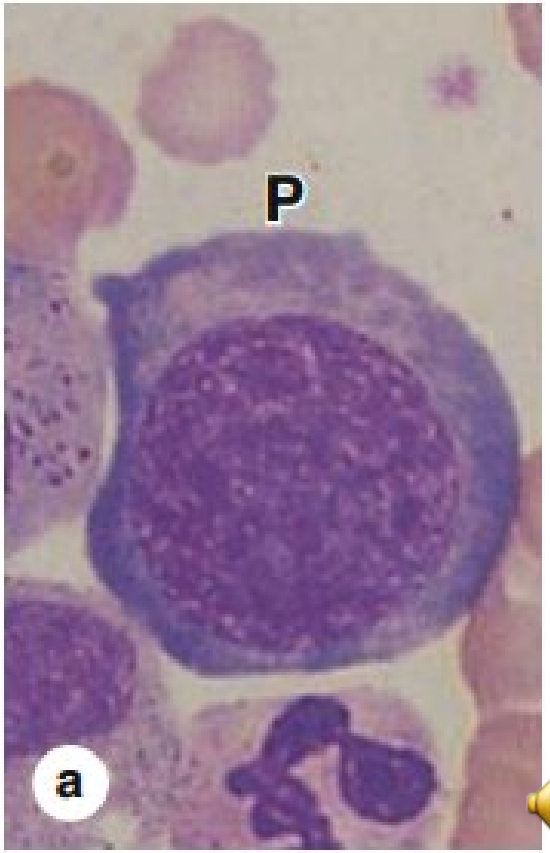
Representative
cellular trail



MATURATION OF ERYTHROCYTES (erythropoiesis)

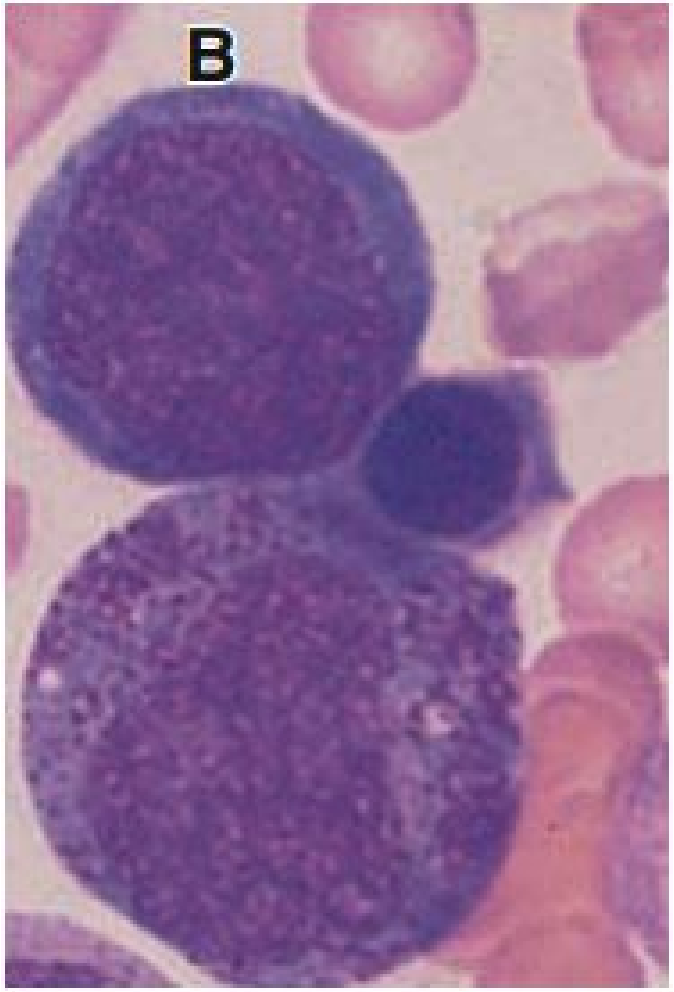
The distinct erythroid progenitor cell is the **proerythroblast** , it originates from the common myeloid erythroid progenitors under the influence of erythropoietin

proerythroblast is a large cell with loose, lacy chromatin, nucleoli, and



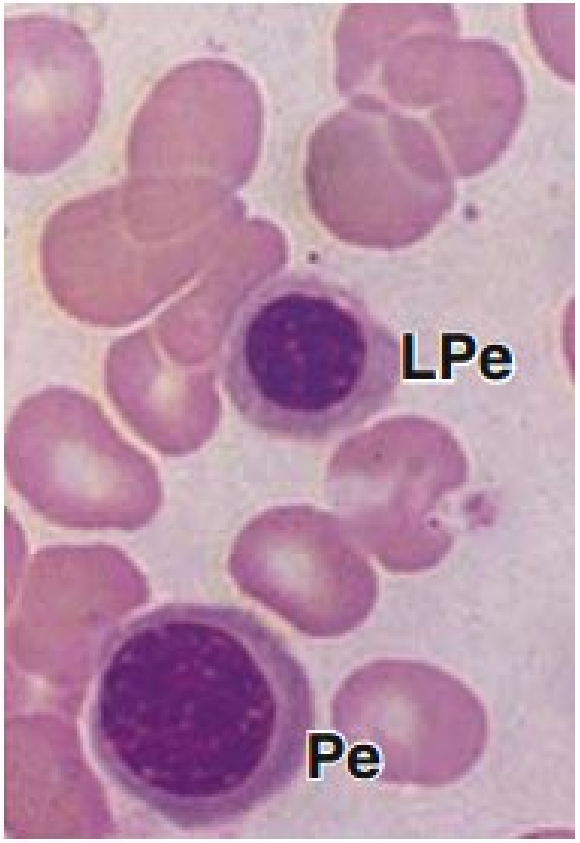
MATURATION OF ERYTHROCYTES (erythropoiesis)

Basophilic erythroblast is slightly smaller with cytoplasmic basophilia (from large number of free polysomes synthesizing hemoglobin) and a more condensed nucleus.



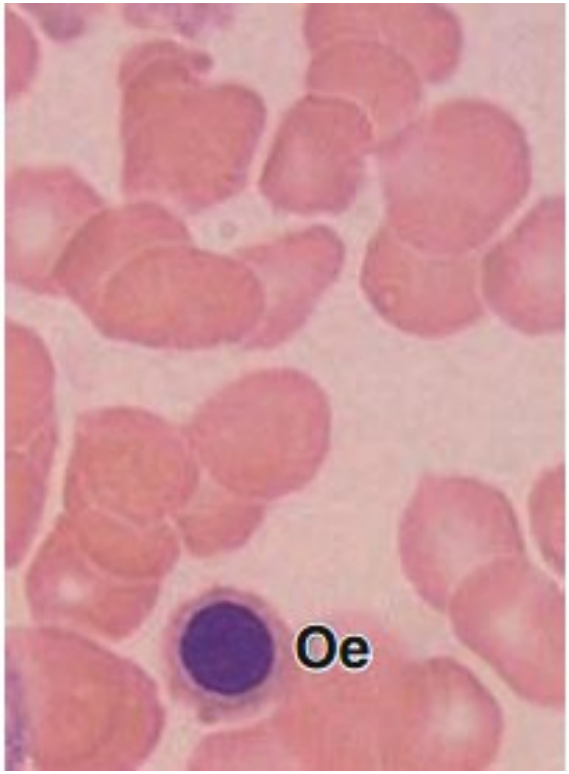
MATURATION OF ERYTHROCYTES (erythropoiesis)

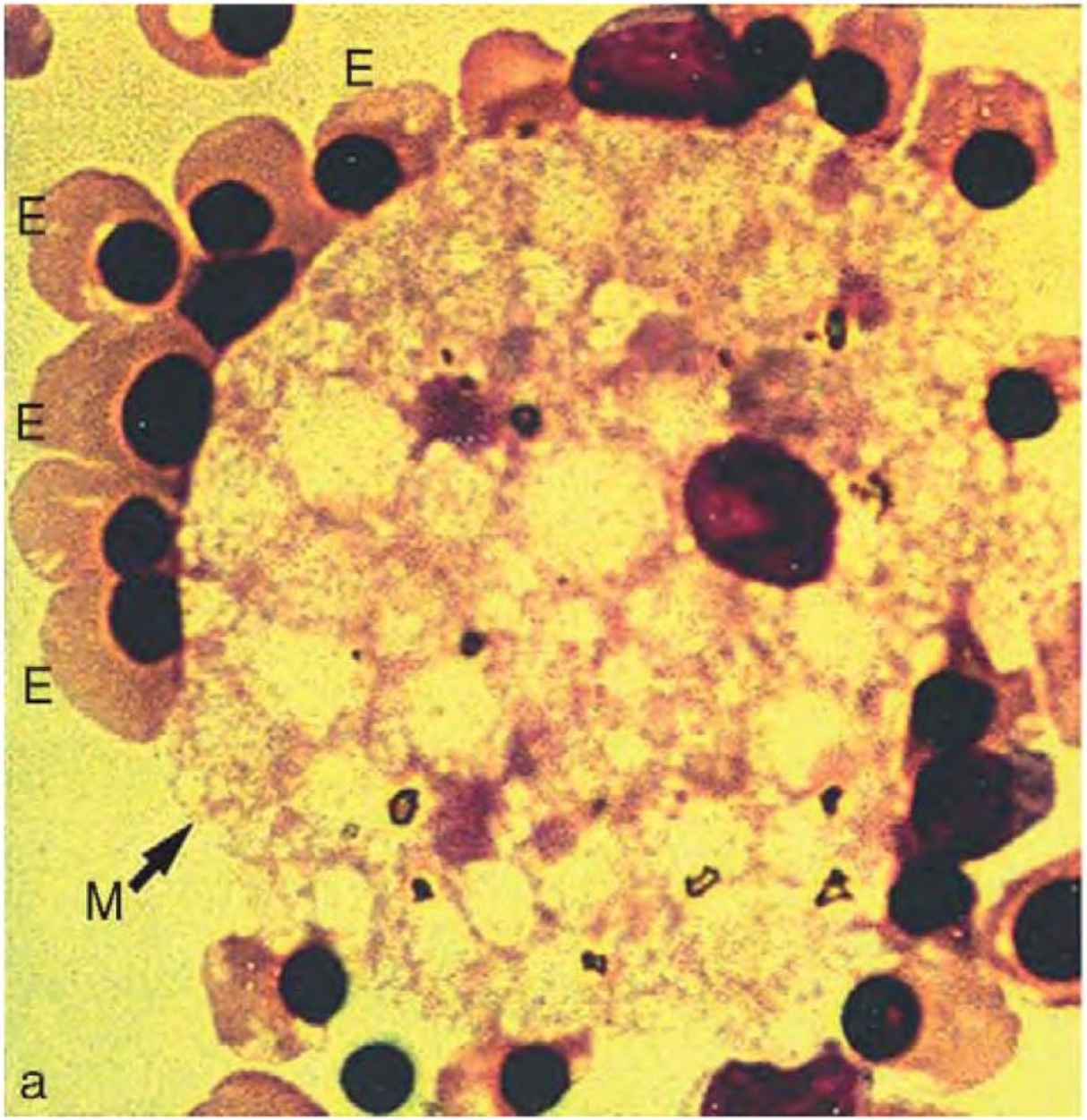
Polychromatophilic erythroblast, cell volume is reduced, polysomes decrease, and some cytoplasmic areas begin to be filled with hemoglobin, producing regions of both basophilia and acidophilia



MATURATION OF ERYTHROCYTES (erythropoiesis)

Orthochromatophilic erythroblasts (normoblasts): Cell and nuclear volumes continue to condense and basophilia is gradually lost, producing cells with uniformly acidophilic cytoplasm

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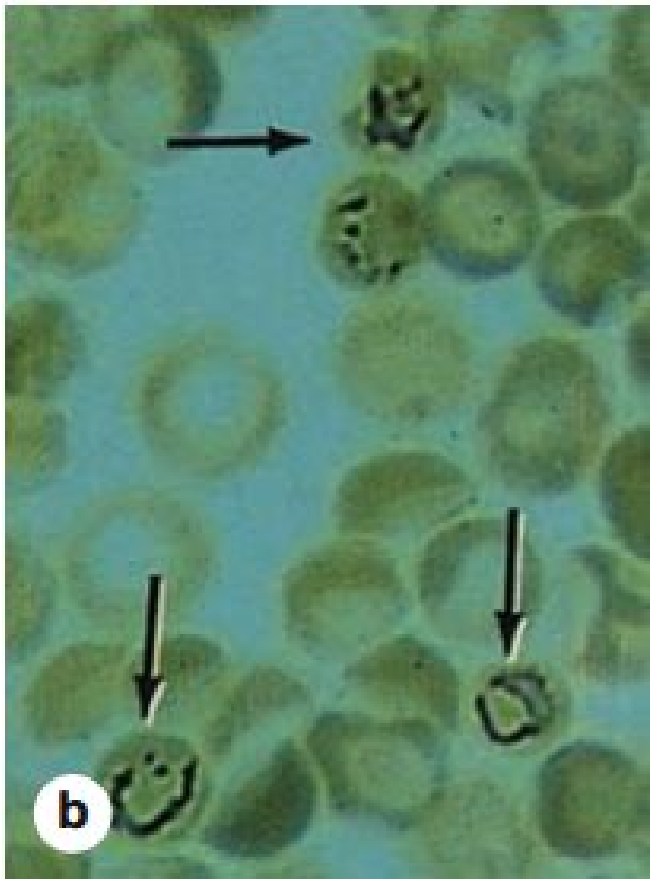
MATURATION OF ERYTHROCYTES (erythropoiesis)

Reticulocytes: the cell nucleus is ejected and undergoes phagocytosis by macrophages

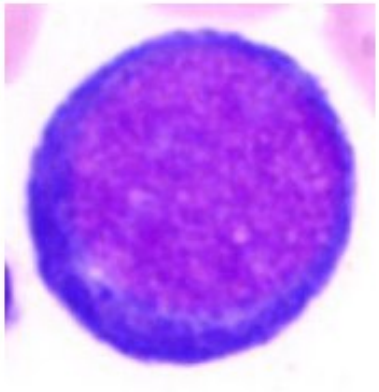
Cell still retains a few polyribosomes that look like faintly stained network when treated with supravital stains, hence the name

These cells enter the circulation, quickly loose there ribosomes and mature into erythrocyte

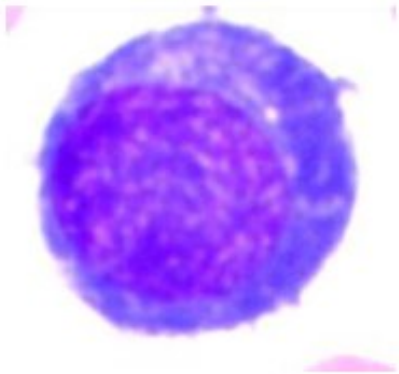


MATURATION OF ERYTHROCYTES (erythropoiesis)

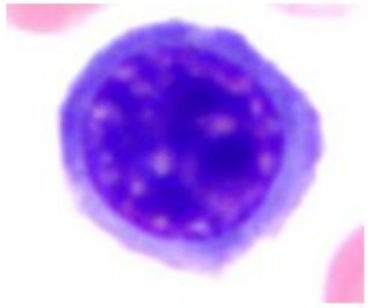
Several major changes take place during **erythropoiesis.** Cell and nuclear volumes decrease, while the nucleoli diminish in size and disappear. Chromatin density increases until the nucleus presents a pyknotic appearance and is finally extruded from the cell. There is a gradual decrease in the number of polyribosomes (basophilia), with a simultaneous increase in the amount of hemoglobin (a highly eosinophilic protein).



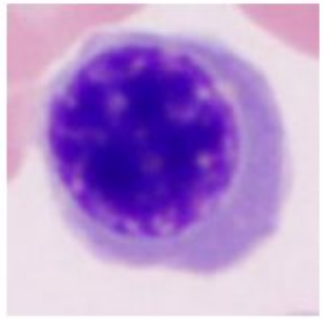
Proerythroblast



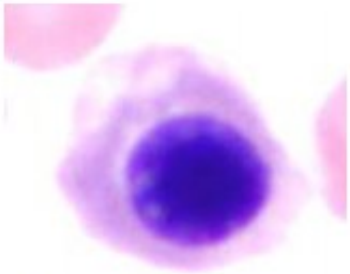
Basophilic Erythroblast



Polychromatic Erythroblast (early)



Polychromatic Erythroblast (late)

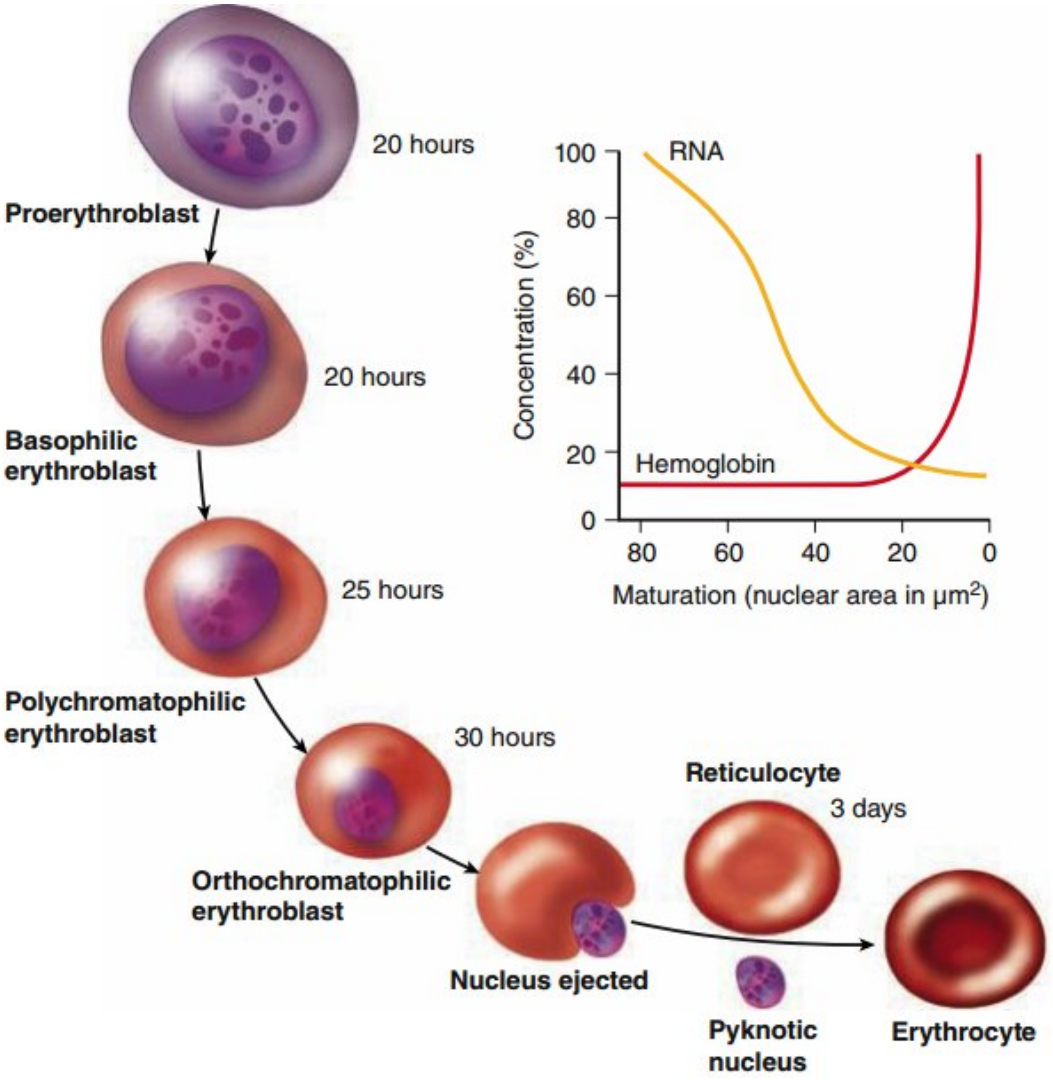


Orthochromatic Erythroblast

Reticulocyte

Mature Erythrocyte

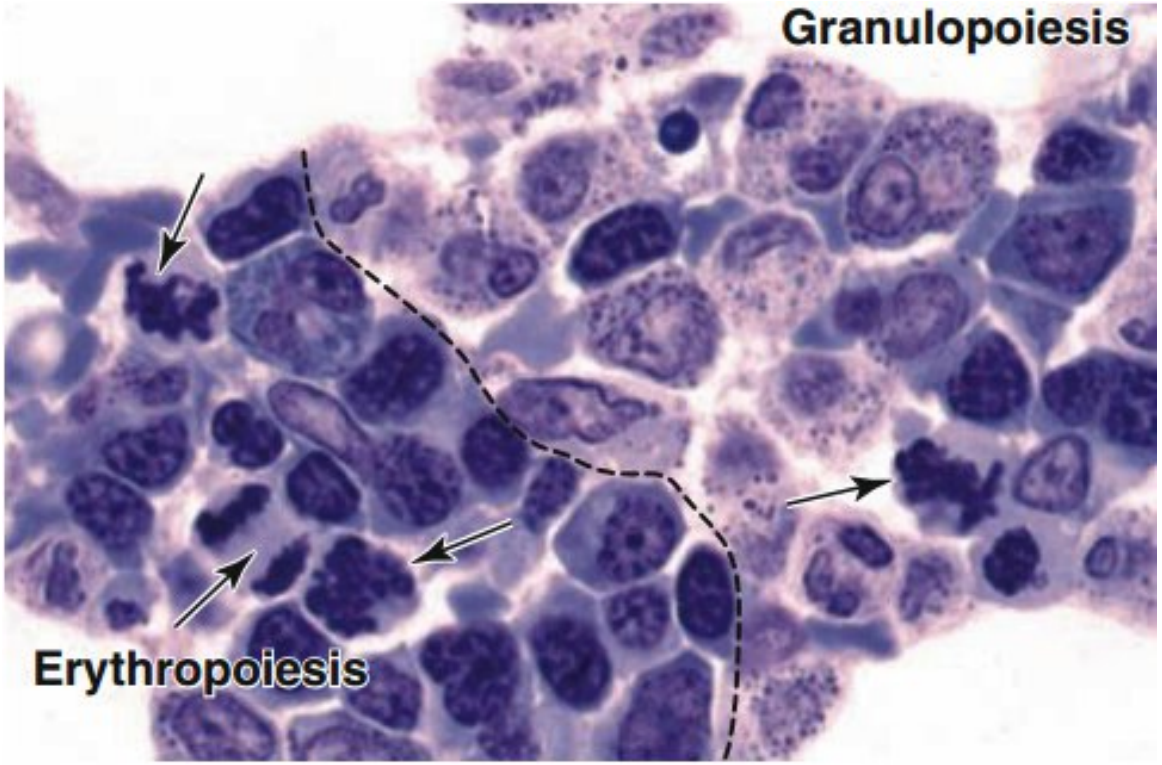
MATURATION OF ERYTHROCYTES (erythropoiesis)

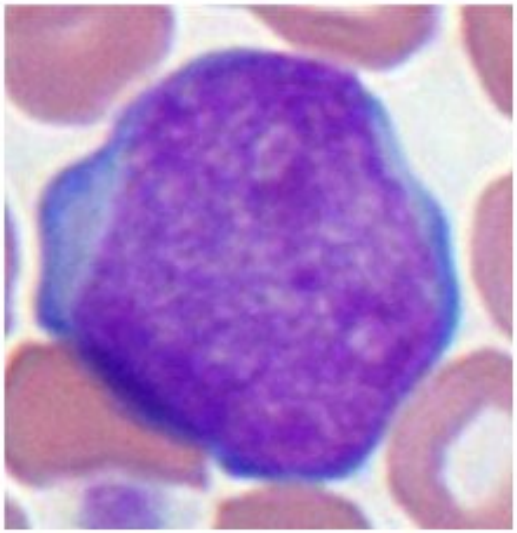


MATURATION OF GRANULOCYTES (Granulopoiesis)

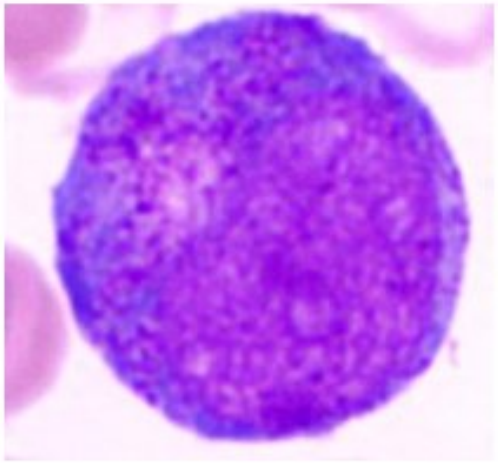
Characterized by cytoplasmic changes resulting from synthesis of proteins for the **azurophilic granules** and **specific granules**

These proteins are produced in the rough ER and the prominent Golgi apparatus in two successive stages , formed first are the azurophilic granules then specific granules, whose contents differ in each of the three types of granulocytes and endow each type with certain different properties

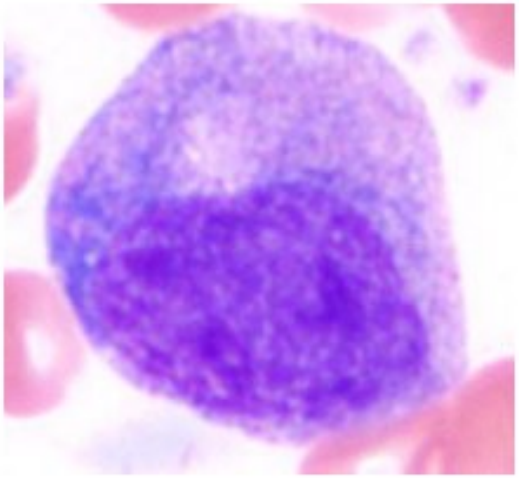

Granulopoiesis
Erythropoiesis



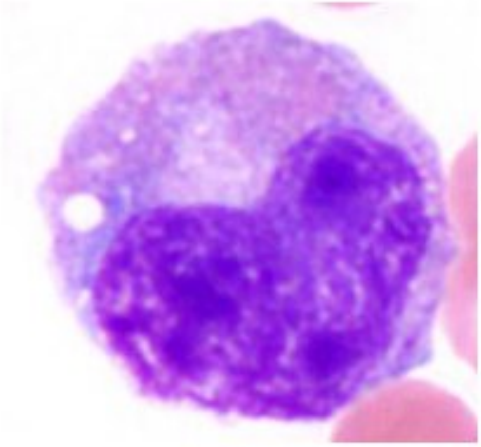
Myeloblast



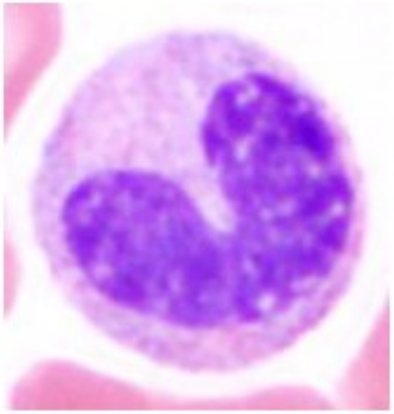
Promyelocyte



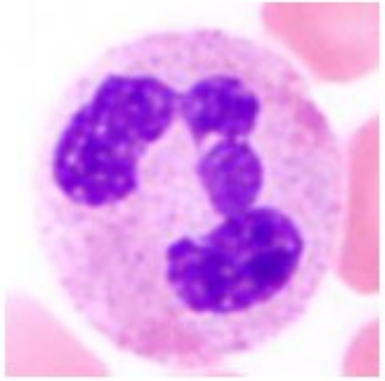
Myelocyte



Metamyelocyte



Band

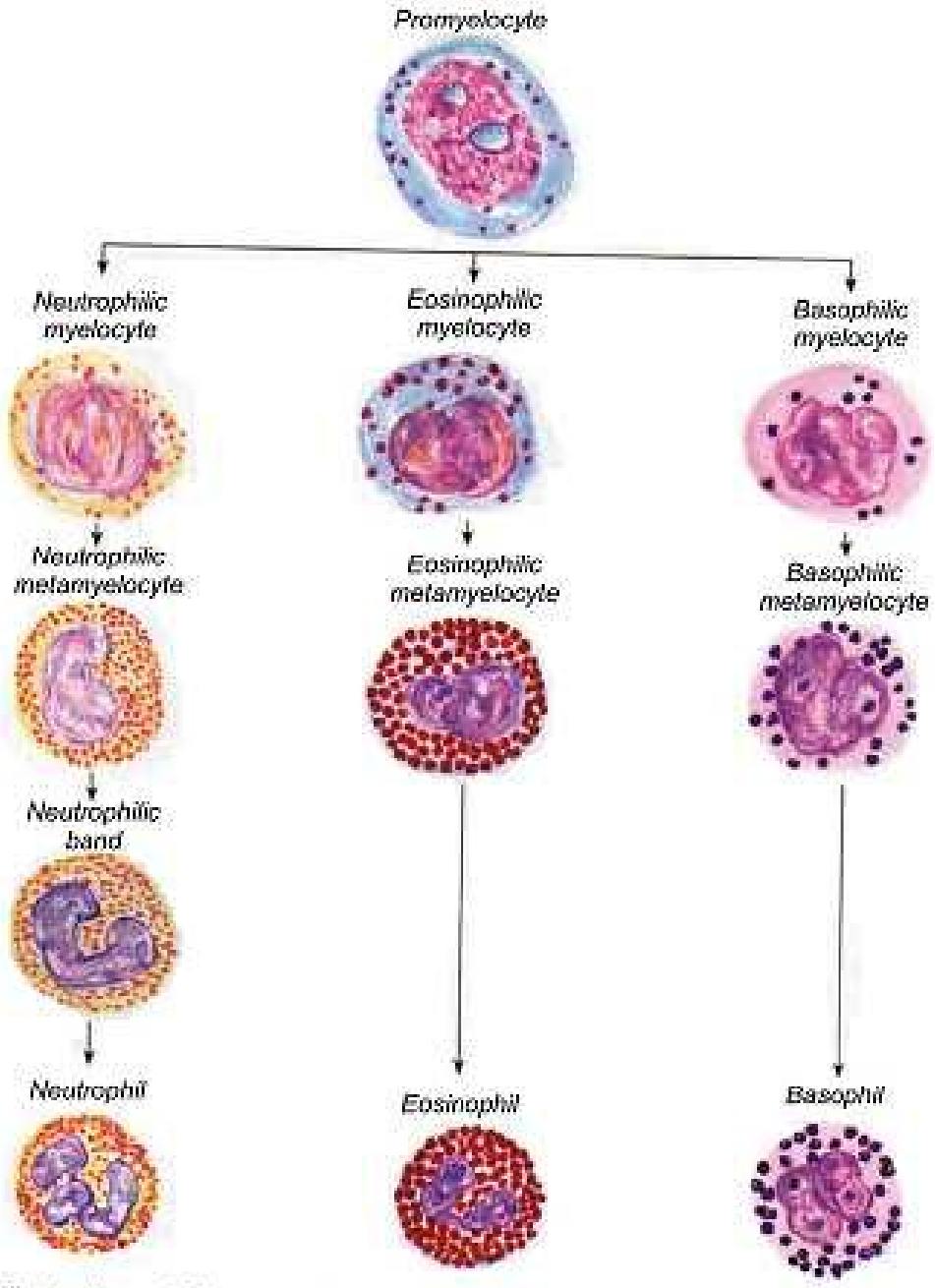


Mature
Neutrophil

MATURATION OF GRANULOCYTES (Granulopoiesis)

The **myeloblast** is the most immature recognizable cell in the myeloid series, these have finely dispersed chromatin, and faint nucleoli

Promyelocytes give rise to the three different types of granulocytes, with first visible sign of this differentiation appears in the **myelocyte** stage, specific granules gradually increase in number and eventually occupy most of the cytoplasm at the **metamyelocyte** stage


Promyelocyte
Neutrophilic myelocyte
Eosinophilic myelocyte
Basophilic myelocyte
Neutrophilic metamyelocyte
Eosinophilic metamyelocyte
Basophilic metamyelocyte
Neutrophilic band
Neutrophil
Eosinophil
Basophil

MATURATION OF GRANULOCYTES (Granulopoiesis)

neutrophilic, basophilic, and eosinophilic metamyelocytes mature with further condensation of their nuclei

neutrophilic granulocyte passes through an intermediate stage **band cell**

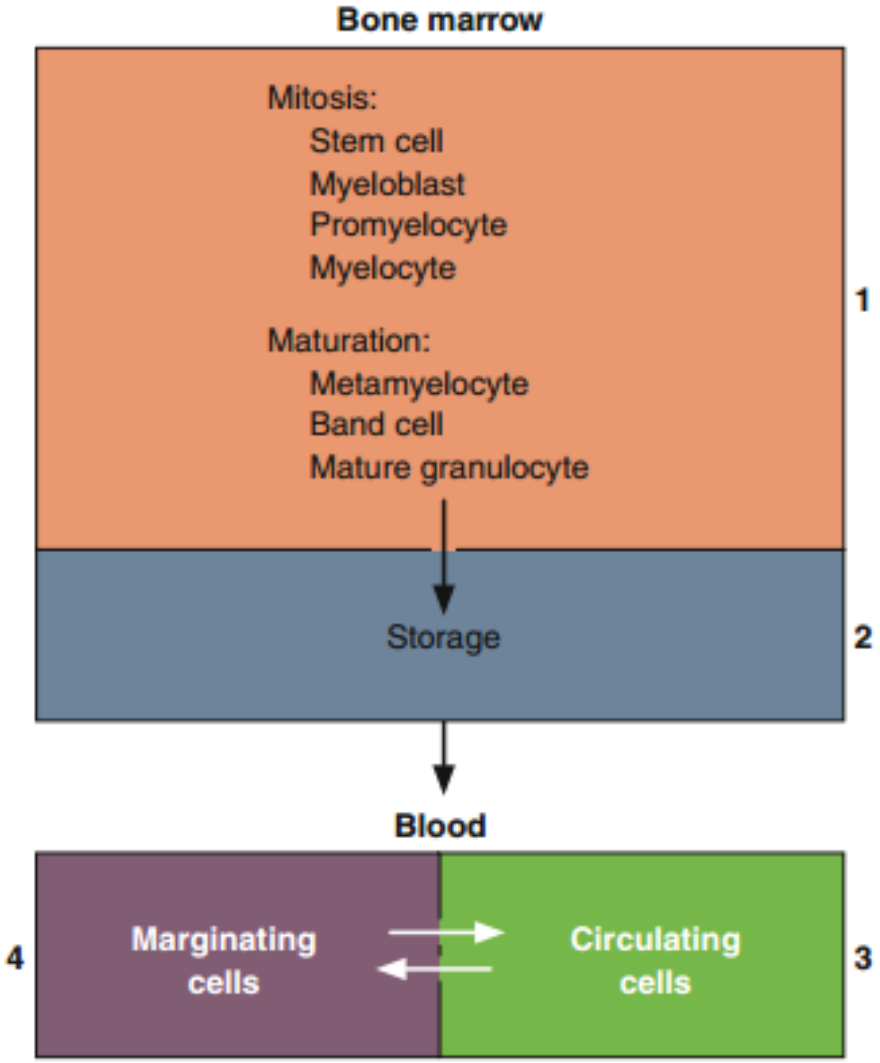
Band is characterized by elongated curved or coiled nucleus that is not polymorphic or separated in segments, it proceeds in maturation to mature neutrophilic granulocyte

MATURATION OF GRANULOCYTES (Granulopoiesis)

Neutrophils exist in at least four anatomically and functionally distinct compartments

- A **granulopoietic** compartment in bone marrow
- A **storage** (reserve) compartment, in bone marrow
- A **circulating** compartment throughout the blood
- A **marginating** compartment, temporarily at the surface endothelium in venules and small veins and constantly interchanging cells with the circulating compartment

Compartments of developing and mature neutrophils



MATURATION OF GRANULOCYTES (Granulopoiesis)

Neutrophils and other granulocytes may enter the connective tissues at the site of infection or injury by migrating through intercellular junctions between endothelial cells of postcapillary venules by **diapedesis**

Neutrophils reside in injured or infected connective tissues for a few days and then die by apoptosis, regardless of whether they have performed their major function of bacterial phagocytosis.

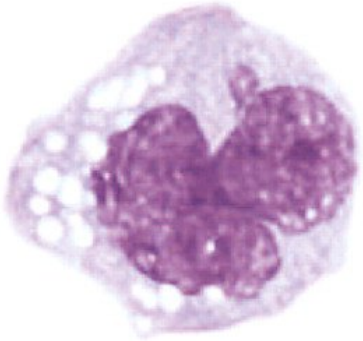
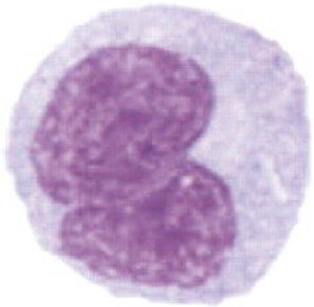
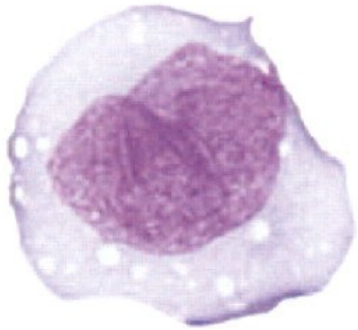
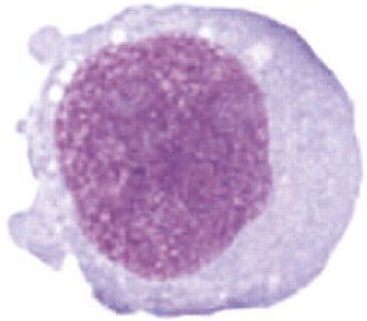
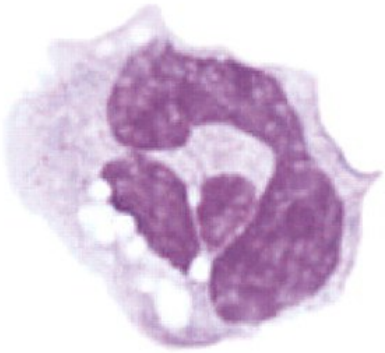
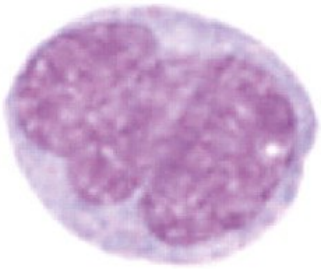
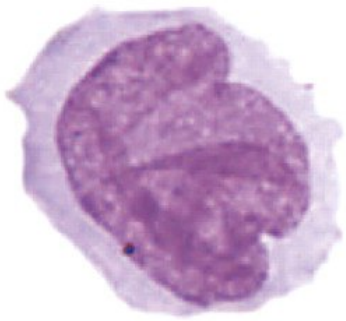
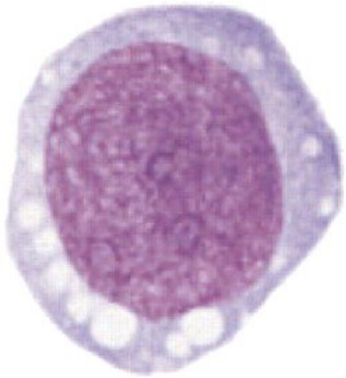
MATURATION OF AGRANULOCYTES

Monocytes

The **monoblast** is a committed progenitor cell that is virtually identical to the myeloblast morphologically.

Monoblast differentiate to **promonocyte,** a large cell (up to 18 μm in diameter) with basophilic cytoplasm and a large, slightly indented nucleus with lacy chromatin and evident nucleoli

Promonocytes divide twice as they develop into **monocytes**

Monocyte	Immature	Promonocyte	Monoblast
			
			

MATURATION OF AGRANULOCYTES

Monocytes

Monocytes contain extensive RER and large Golgi complexes forming lysosomes, which are observed as fine azurophilic granules at maturity

Monocytes circulate in blood for several hours and enter tissues where they mature as **macrophages** and function for up to several months.

Lymphocytes

Early lymphocyte progenitor cells originate in the bone marrow, then either

- Migrate to the thymus, where they acquire the properties of T that populate specific regions of peripheral lymphoid organs
- Differentiate into B lymphocytes in the bone marrow and then migrate to peripheral lymphoid organs

The first identifiable progenitor of lymphoid cells is the **lymphoblast**, that divides two or three times to form **lymphocytes**

Lymphocytes

As lymphocytes develop their nuclei become smaller, nucleoli disappear, and cell size decreases

Mature and functionally active B and T cells are generally larger than newly formed lymphocytes.

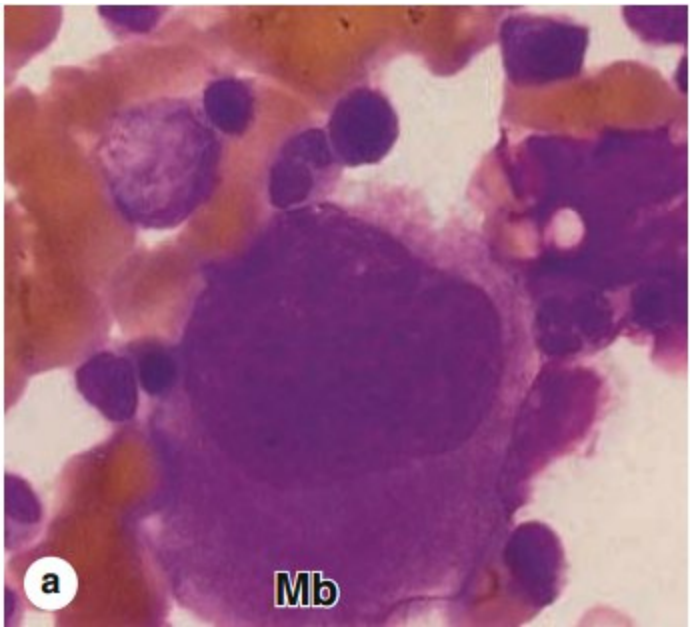
In the bone marrow and in the thymus, these cells synthesize the specific cell surface proteins that characterize B or T lymphocytes

Subsets of lymphocytes acquire distinctive cell surface proteins during differentiation that can be detected by immunocytochemical techniques and used to sort the specific lymphocytic types

ORIGIN OF PLATELETS

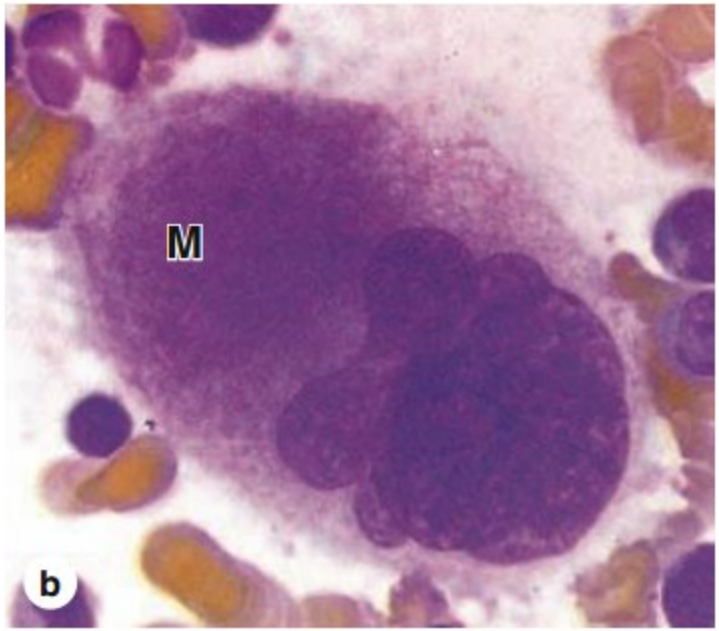
Platelets or thrombocytes originate in the red bone marrow from mature **megakaryocytes** which in turn differentiate from **megakaryoblasts**

Megakaryoblasts is 25-50 μm in diameter and has a large ovoid or kidney-sl
often with several small nucl



ORIGIN OF PLATELETS

Megakaryoblasts undergo endomitosis, with repeated rounds of DNA replication not separated by cell divisions, resulting in a nucleus that is highly polyploid

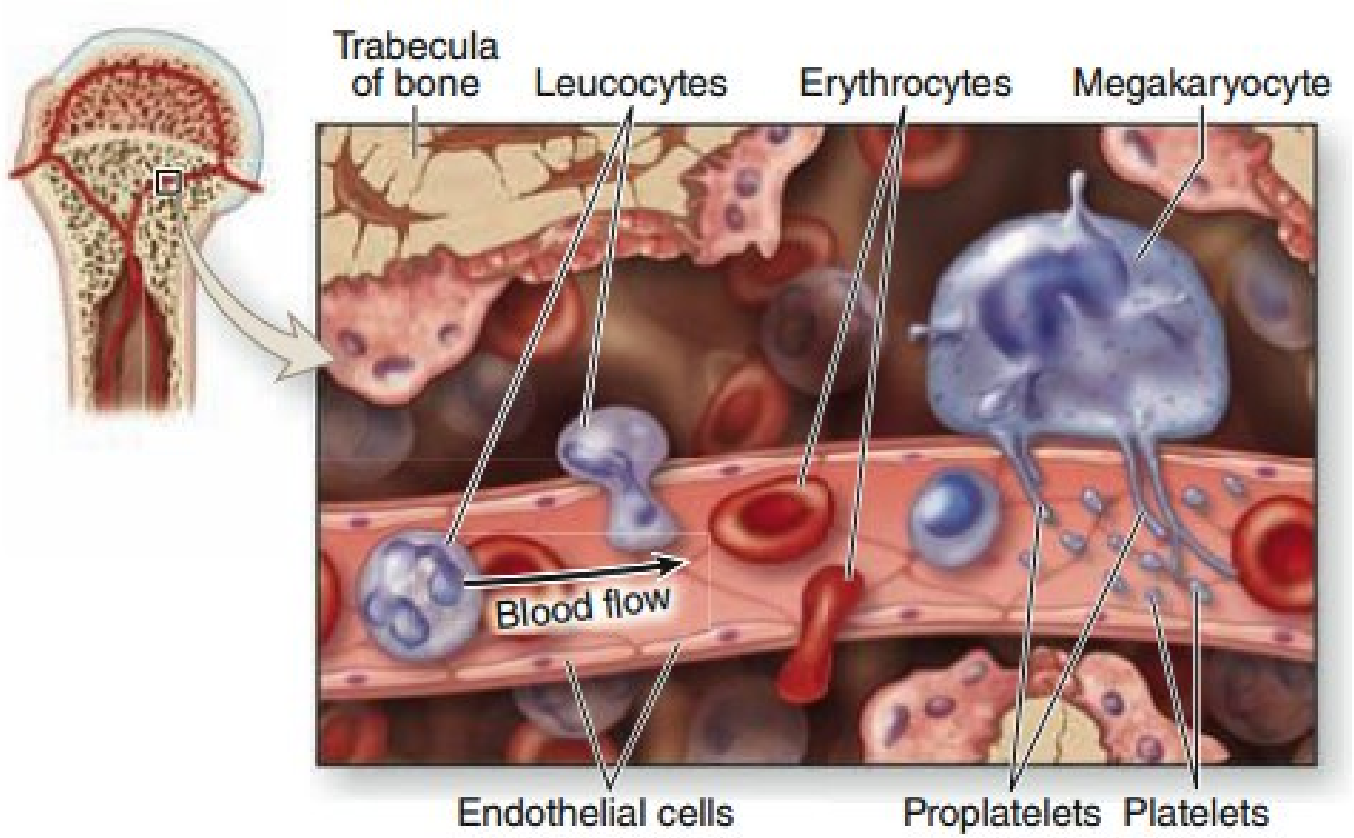


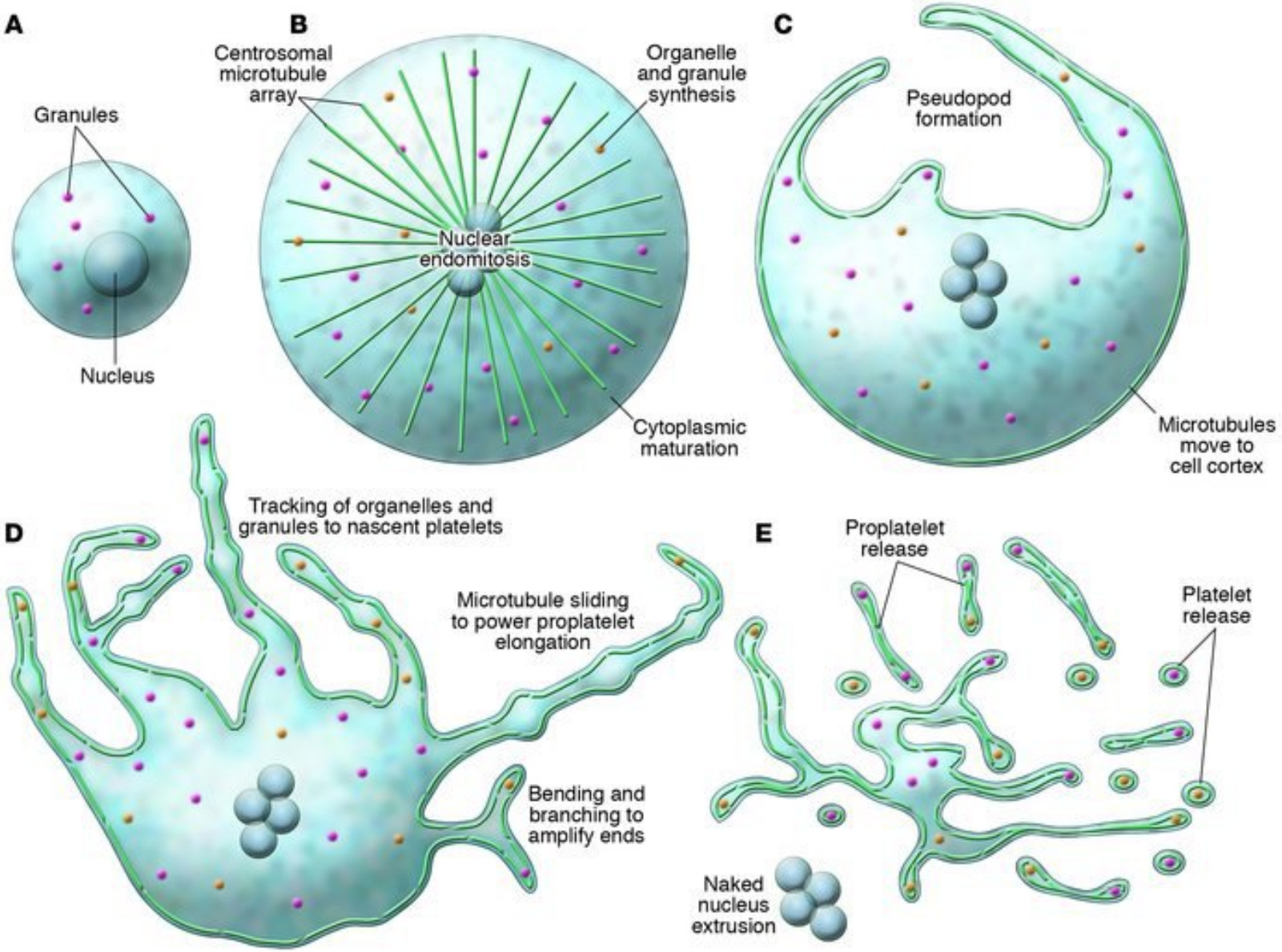
Megakaryocytes are giant cells, up to 150 μm in diameter, and the polyploid nuclei are large and irregularly lobulated with coarse chromatin.

Their cytoplasm contains numerous mitochondria, a well-developed RER, and an extensive Golgi apparatus from which arise the specific granules of platelets

Megakaryocytes are widely scattered in marrow, typically near sinusoidal capillaries

Proplatelets are megakaryocytes branching processes that penetrate the sinusoidal endothelium and are exposed in the circulating blood of the sinusoids


Trabecula of bone
Leucocytes
Erythrocytes
Megakaryocyte
Blood flow
Endothelial cells
Proplatelets
Platelets


A
Granules
Nucleus
B
Centrosomal microtubule array
Organelle and granule synthesis
Nuclear endomitosis
Cytoplasmic maturation
C
Pseudopod formation
Microtubules move to cell cortex
D
Tracking of organelles and granules to nascent platelets
Microtubule sliding to power proplatelet elongation
Bending and branching to amplify ends
E
Proplatelet release
Platelet release
Naked nucleus extrusion

ORIGIN OF PLATELETS

Proplatelets have a framework of actin filaments and microtubules along which membrane vesicles and specific granules are transported.

A loop of microtubules forms a teardrop-shaped enlargement at the distal end of the proplatelet, and cytoplasm within these loops is pinched of to form platelets

Each megakaryocyte produces a few thousand platelets, after which the remainder of the cell shows apoptotic changes and is removed by macrophages

Thank you and good Luck

Histology of Blood

Dr Mahmoud S Shbair

Consultant Pediatric Hematologist and Oncologist

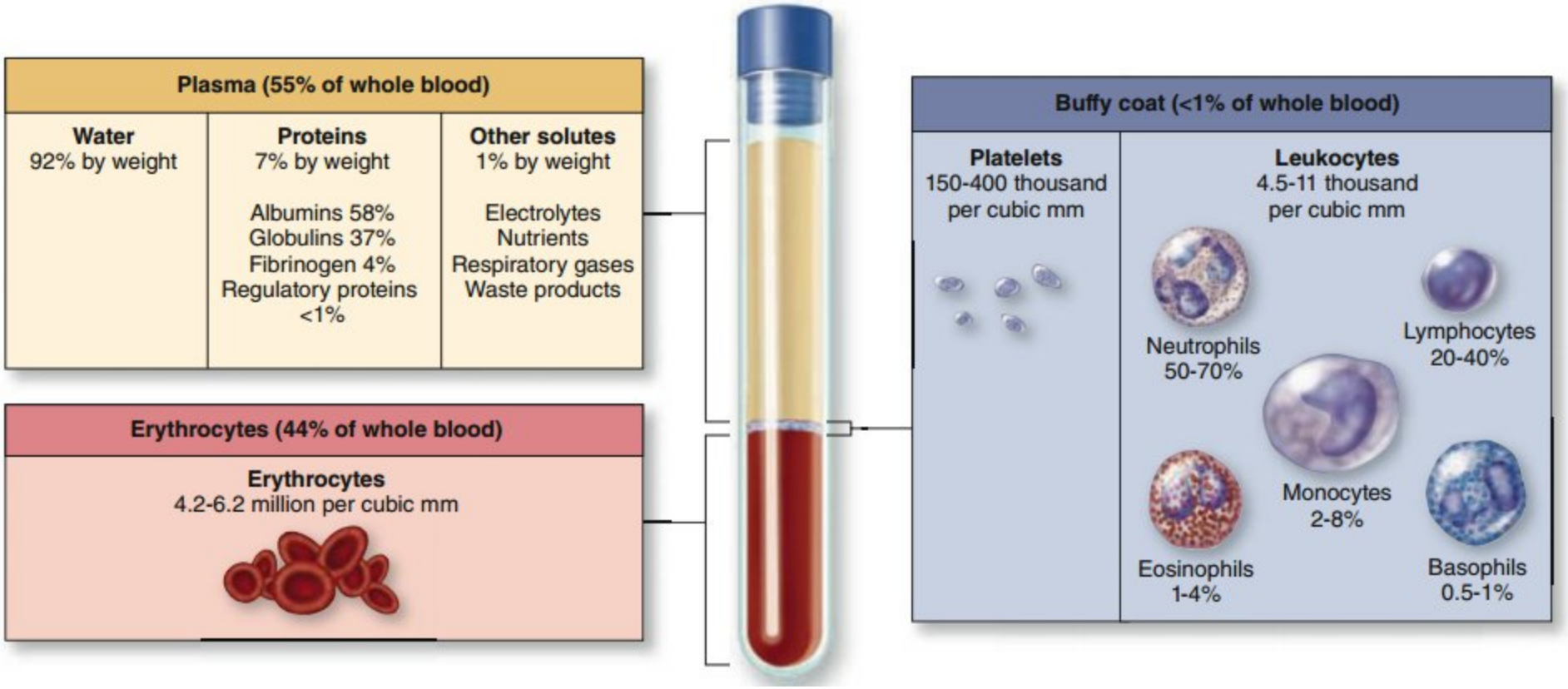
Blood

Blood is a modified type of connective tissue within the circulatory system and is mesodermal in origin

Cellular (formed) elements are erythrocytes, leukocytes and thrombocytes.

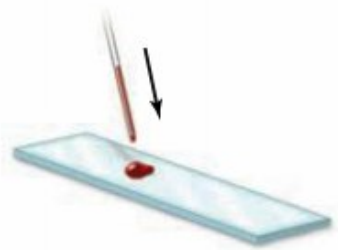
The extracellular matrix is the blood plasma

Plasma Composition

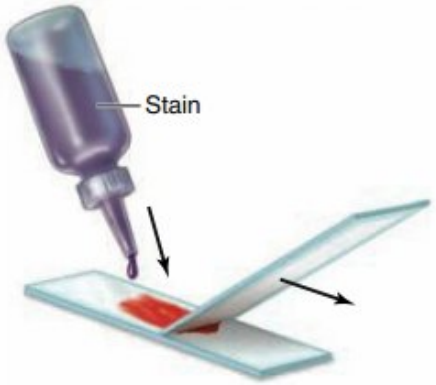


The Blood Smear

(1) Prick finger and collect a small amount of blood using a micropipette.

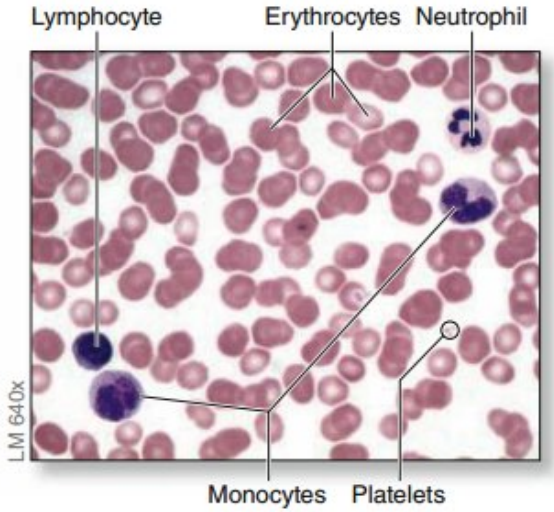


(2) Place a drop of blood on a slide.



(3a) Using a second slide, pull the drop of blood across the first slide's surface, leaving a thin layer of blood on the slide.

(3b) After the blood dries, apply a stain briefly and rinse. Place a coverslip on top.

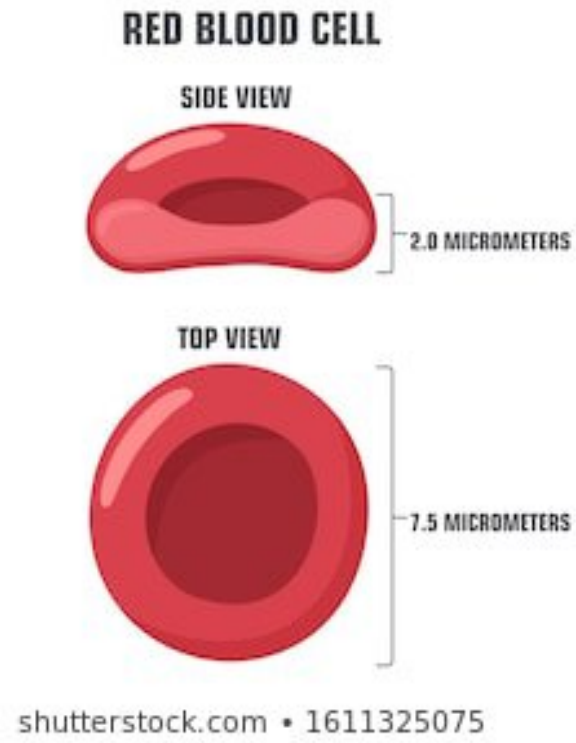


(4) When viewed under the microscope, blood smear reveals the components of the formed elements.

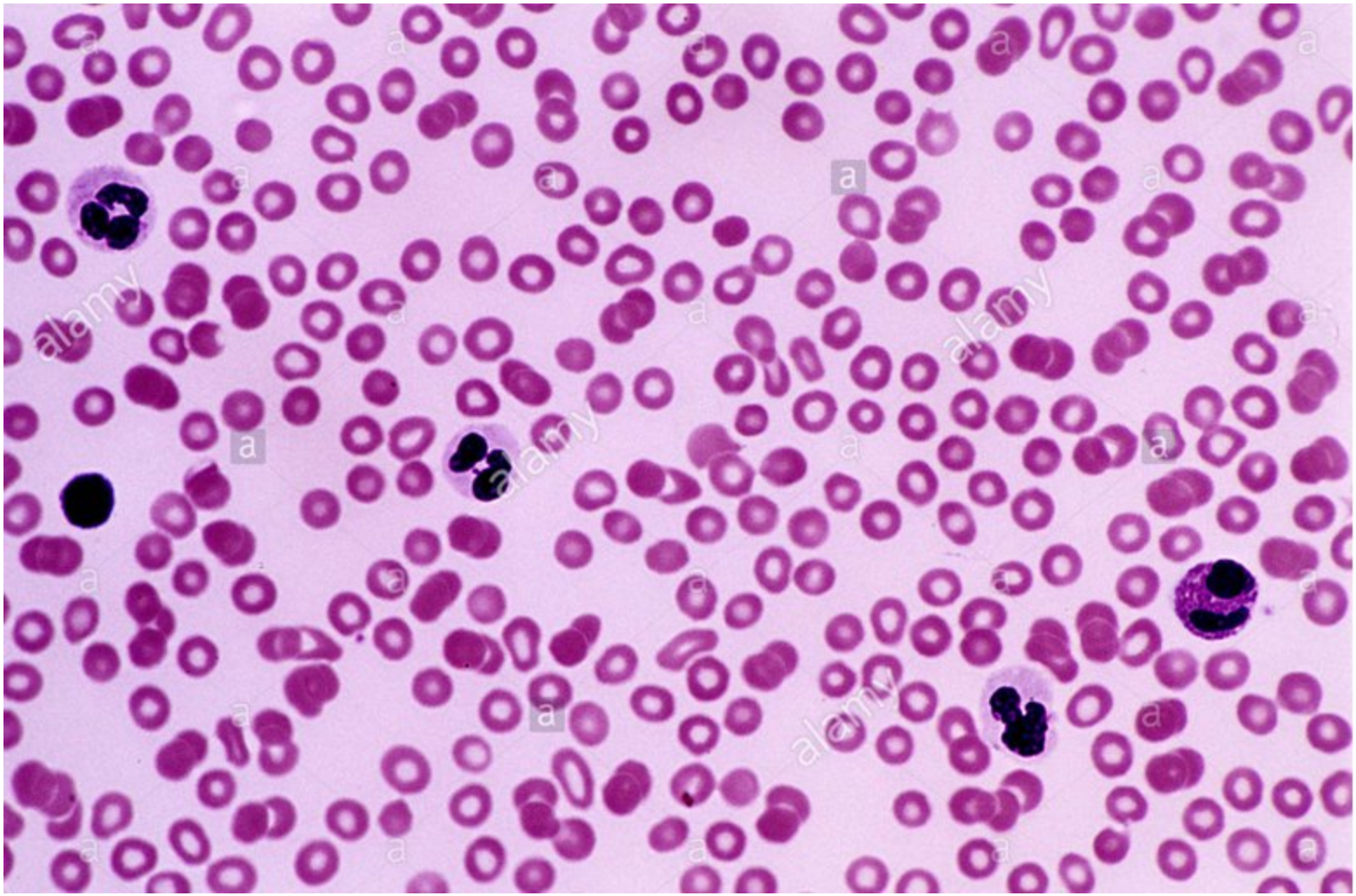
Formed Elements of the Blood
Red Blood Cells (RBCs)

Mature erythrocytes are denucleated biconcave disks with a diameter of 7-8 μm, edge thickness 2 μm, central thickness 0.8 μm

- Increase in surface area (SA) by 20-30%
- Huge SA/volume ration enabling more efficient gas transfer



Light Microscopy Of RBCs



Light Microscopy Of RBCs

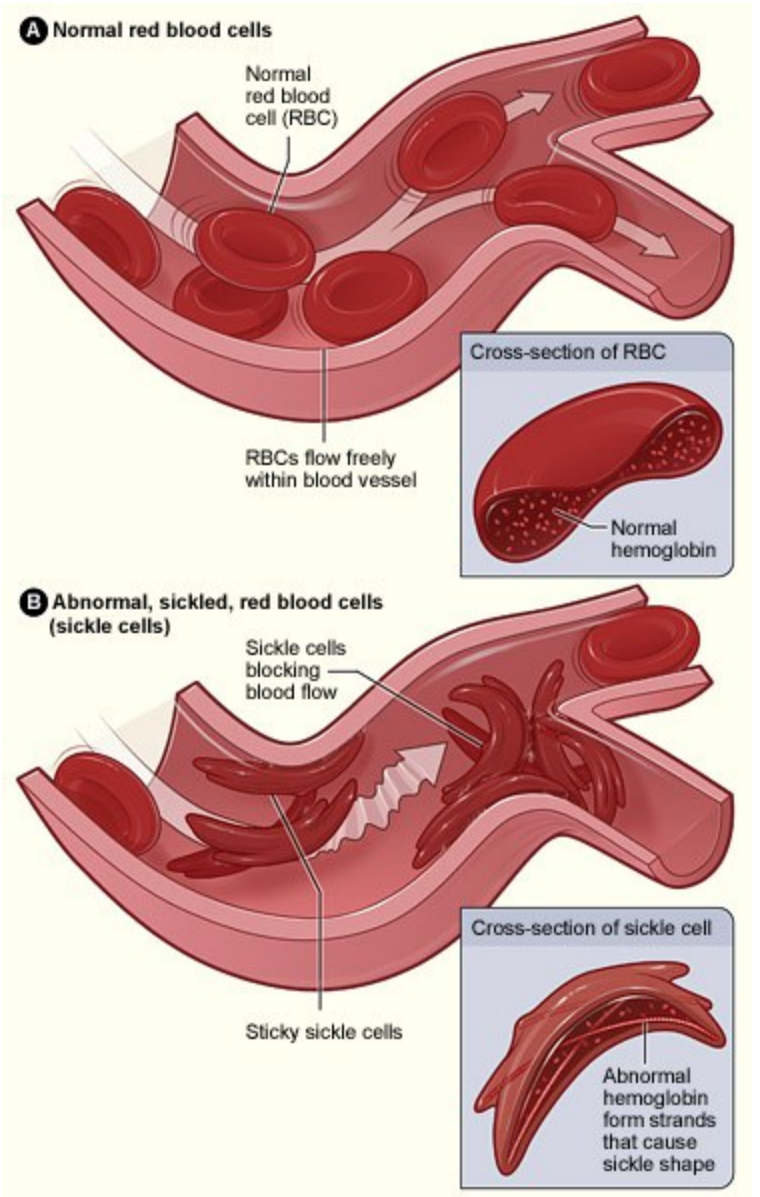
Round non nucleated acidophilic (pink color)

Dark periphery and pale centre, approximately 1/3 of the transverse diameter due to the biconcave shape

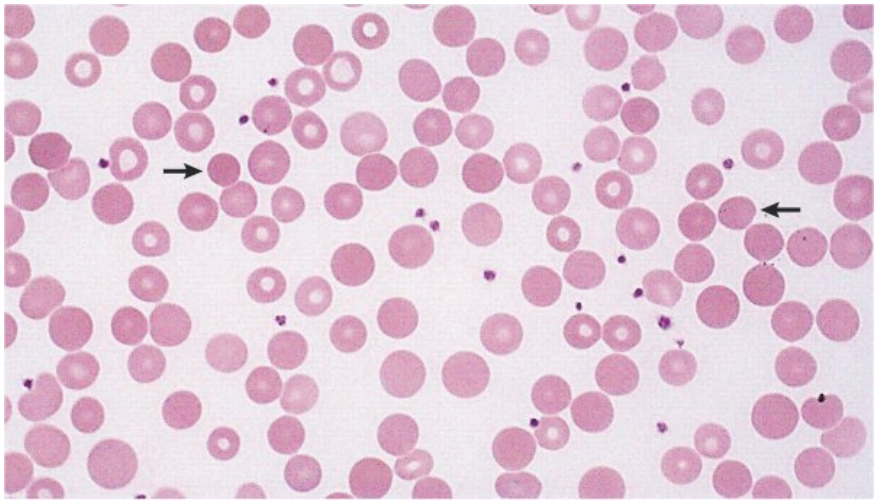
Pliability and shape of RBCs enables them to squeeze them self through narrow capillaries

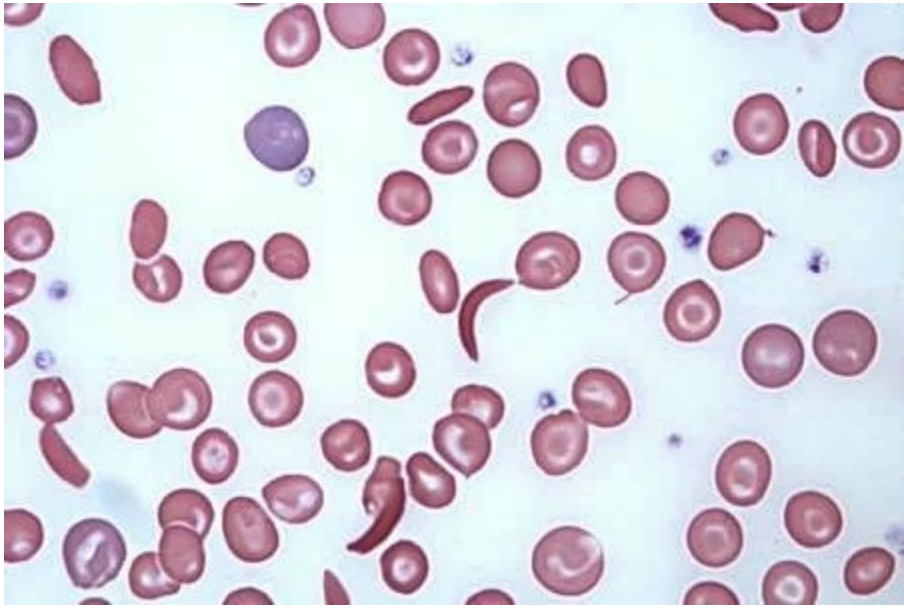
Changes may occur either in the membrane cytoskeletal proteins or hemoglobin content that deprive RBCs of

Examples of such changes include sickle cell disease (crescent shaped RBCs due to hemoglobin abnormality) and hereditary spherocytosis (spherical)



Light Microscopy





Electron Microscopy

Denucleated cell devoid of organelles, containing hemoglobin that appears as electron dense homogenous material

Cell membrane is very flexible, it is composed of 40% lipids, 50% proteins and 10% carbohydrates, proteins are integral and help in maintaining the shape

Immediately under the plasma membrane there is a network of filaments, the cytoskeletal membrane proteins, include spectrin, actin and membrane associated proteins

Counts

The normal concentration of RBCs in blood is 3.5-5.5 million/mm^3 in women and 4.3-5.9 million^3 in men

Higher counts in men are attributed to the erythrogenic effects of androgens and the regular menstrual losses of women

The packed volume of blood cells per total volume of blood is known as hematocrit, normally it is 36-46% in women, 41-53% in men

Fate of RBCs

Life span for RBCs is 120 days

When aging, RBCs develop subtle changes, macrophages in the bone marrow, spleen and liver engulf and digest them

The iron and hem are recycled, the iron is used to form ferritin stores till being reused for new RBCs, hem is catabolized to bilirubin which is secreted in bile.

White Blood Cells (WBCs)

Th number of leukocytes in the blood varies according
to age, sex, and physiologic conditions. Healthy adults have 4500-11,000 leukocytes per microliter of blood.

Leukocytes leave the blood and migrate to the tissues where they perform various activities related to immunity

They are spherical in blood, amoeboid in tissues

Divided into two major groups, granulocytes and agranulocytes

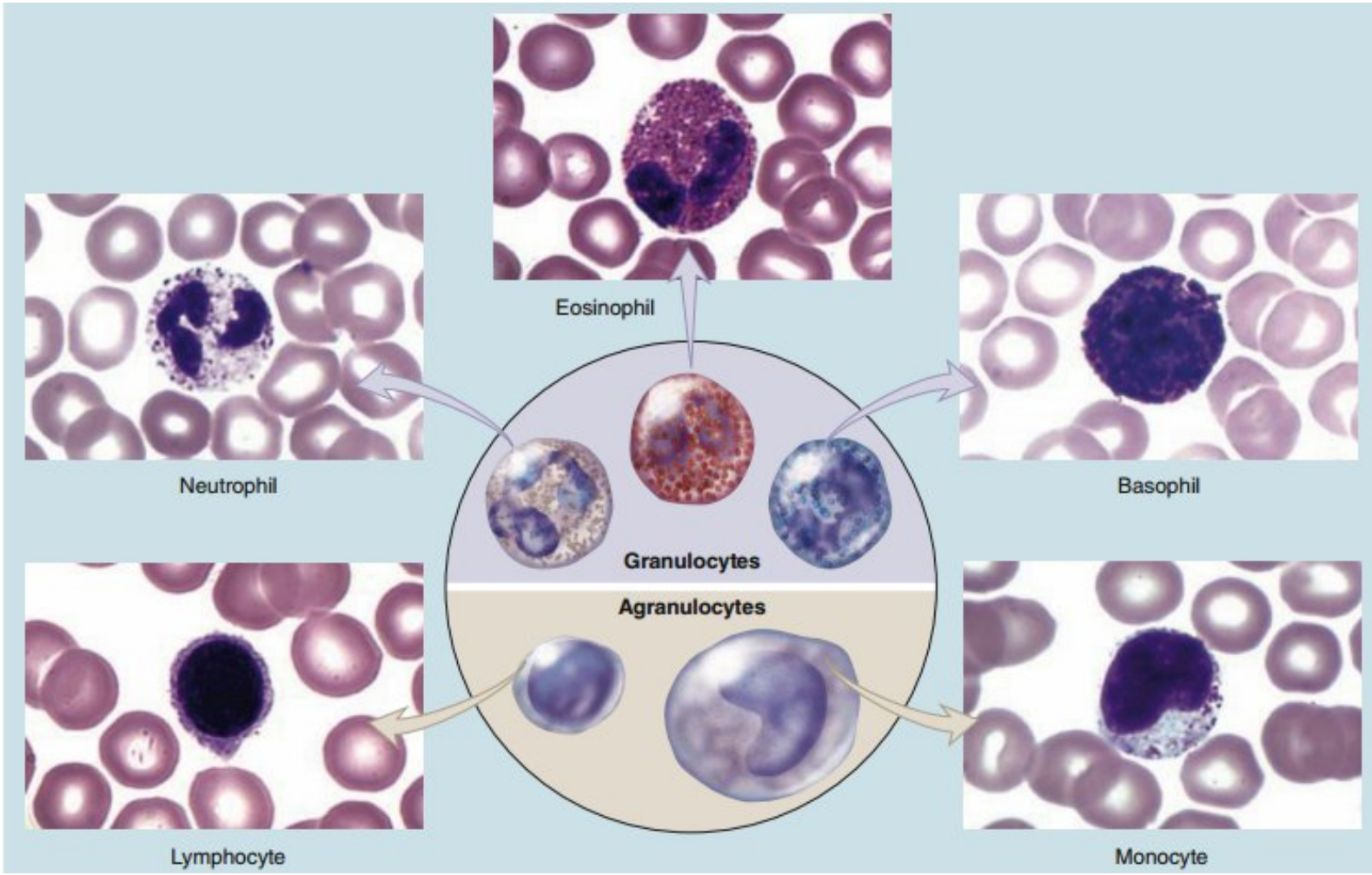
White Blood Cells (WBCs)

Granulocytes possess two types of cytoplasmic granules

- **Azurophilic** (non specific) granules with staining affinity to azure dyes
- **Specific granules** that bind neutral, basic, or acidic stains and have specific functions, based on these granulocytes are classified to **neutrophils, eosinophils** , and **basophils**

Granulocytes also have **polymorphic nuclei** with two or more distinct (almost separated) lobes

Agranulocytes lack specific granules, but do contain some azurophilic granules (lysosomes). The nucleus is spherical or indented but not lobulated. This group includes the **lymphocytes** and **monocytes**


Eosinophil
Neutrophil
Basophil
Granulocytes
Agranulocytes
Lymphocyte
Monocyte

Neutrophils (Polymorphonuclear Leukocytes)

Mature neutrophils constitute 50%-70% of circulating leukocytes

Neutrophils are 12-15 μm in diameter

Nuclei having two to five lobes linked by thin nuclear extensions, variability in shape and number of lobes gave the name of PMNs

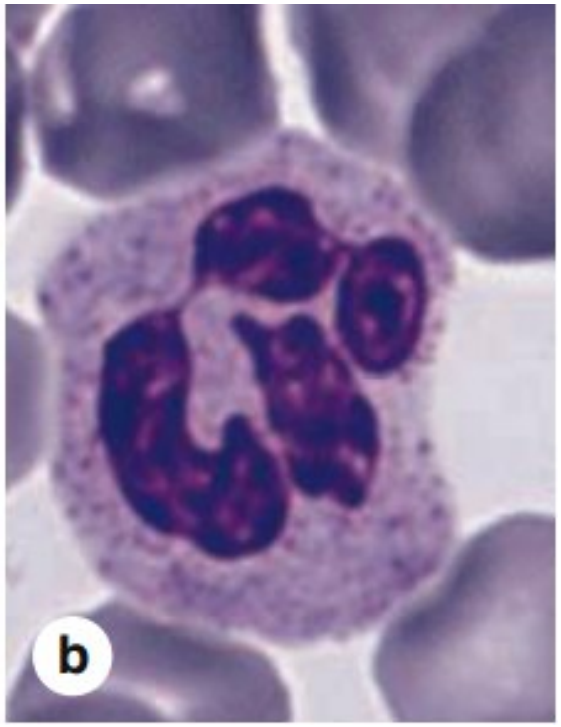
Half-life of 6-8 hours in blood and a life span of 1-4 days in connective tissues before dying by apoptosis.

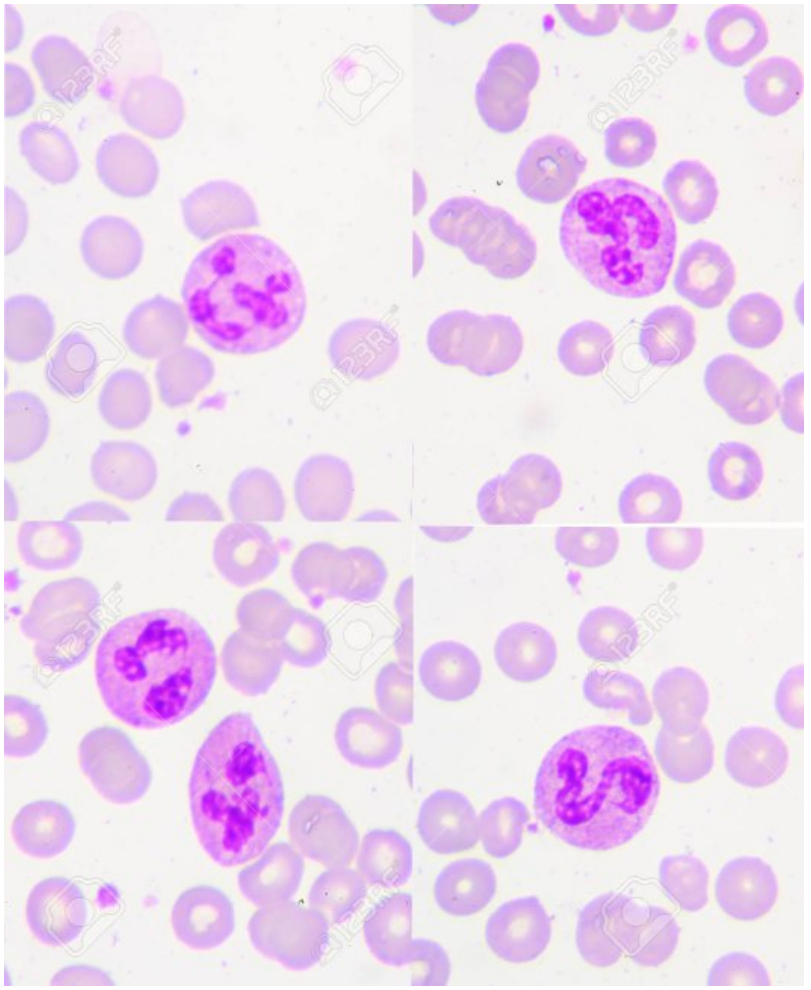
Neutrophils (Polymorphonuclear Leukocytes)

The cytoplasm is stippled, light pink and contains

- **Azurophilic primary granules**
  - Lysosomes, large, dense vesicles
  - Contain proteases and antibacterial proteins, including **Myeloperoxidase, Lysozyme and Defensins**
  - Major role in killing and degrading engulfed microorganisms,
- **Specific secondary granules**
  - Smaller and less dense, stain faintly pink,
  - Secret various ECM-degrading enzymes such as collagenases, deliver additional bactericidal

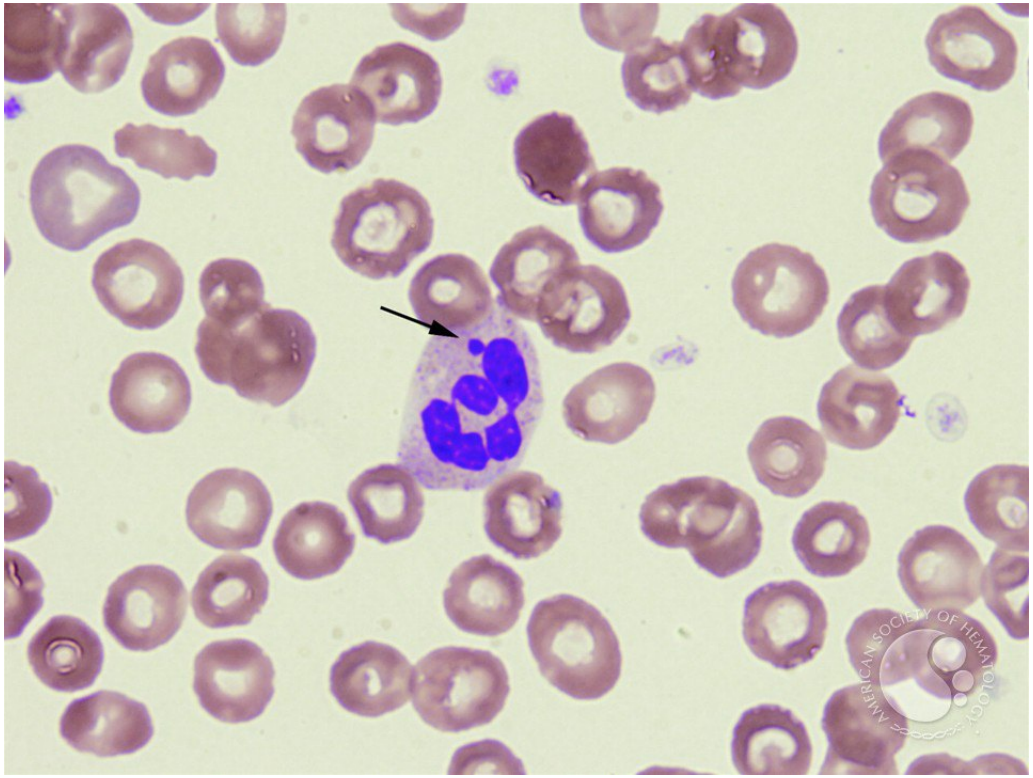
Neutrophils (Polymorphonuclear Leukocytes)





Neutrophils (Polymorphonuclear Leukocytes)

In females, the inactive X chromosome may appear as a drumstick-like appendage on one of the lobes of the nucleus called **Barr body**



Neutrophils (Polymorphonuclear Leukocytes)

Under electron microscopy

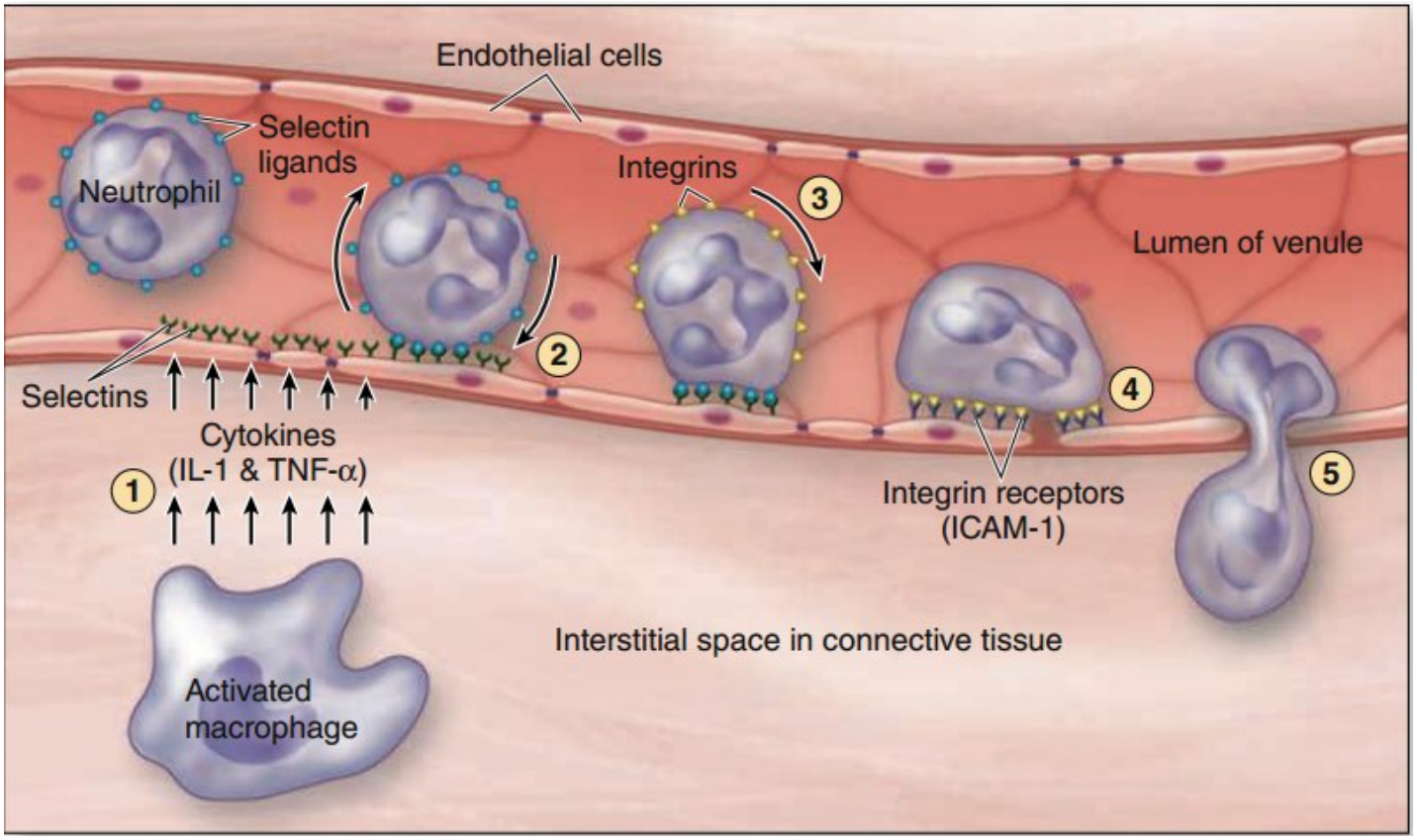
- Specific granules and azurophilic granules
- paucity of organelles
- abundant glycogen granules which is broken down to glucose to yield energy via gycolysis

Neutrophils are usually the first leukocytes to arrive at sites of infection

Chemotaxis

Chemical substances attract neutrophils and macrophages to a site of injury / inflammation

Associated with increased capillary membrane permeability to facilitate movement of the WBCs from the blood into tissue spaces



1. **proinflmmatory cytokines** signal endothelial cells to insert glycoprotein selectins
2. neutrophils cell surface glycoproteins bind the **selectins**
3. expression of new **integrins** on the rolling leukocytes and expression of the integrin ligand **ICAM-1** on the endothelial cells.
4. Integrins and their ligands provide firm endothelial adhesion of neutrophils to the endothelium
5. **diapedesis**

Phagocytosis

Cellular ingestion of an offending agent

Selection of offending agent:

- Rough surface
- Lack of protein coats
- Antibodies produced by lymphocytes coat the pathogens and make them susceptible to phagocytosis (Antibody + C3 protein = opsonization)

WBC encircles the organism by pseudopodia

The pseudopodia meet one another on the opposite side and fuse yielding a phagosome.

Intracellular killing

Neutrophils and macrophages contain an abundance of lysosomes filled with proteolytic enzymes

The lysosomes of macrophages (but not of neutrophils) contain large amounts of lipases, which digest the thick lipid membranes

Lysosomes and other granules fuse with and discharge their enzymes into vesicle, killing and digesting the engulfed microorganisms

Powerful oxidizing agents formed by peroxisomes

- Superoxide (O_2^-)
- Hydrogen peroxide(H_2O_2)
- Hydroxyl ions (OH^-)

Lactoferrin avidly binds iron crucial element in bacterial nutrition

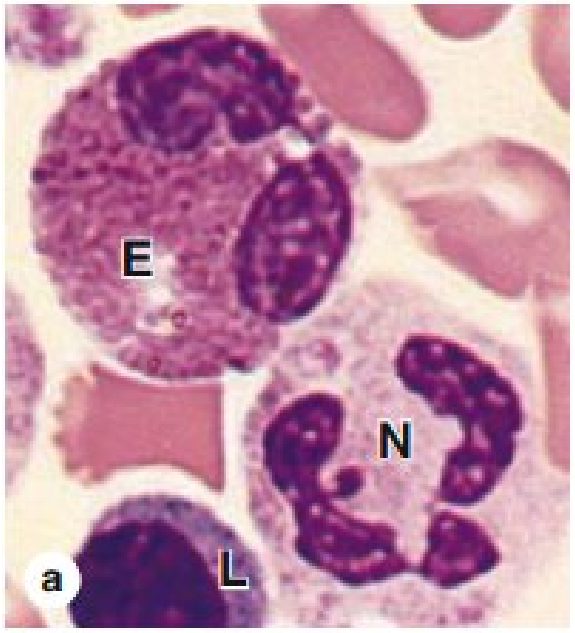
Eosinophils

Constitute 1-4% of leukocytes.

About the same size as a neutrophils

Characteristic bilobed nucleus

Abundant large, acidophilic specific granules typically staining pink or red.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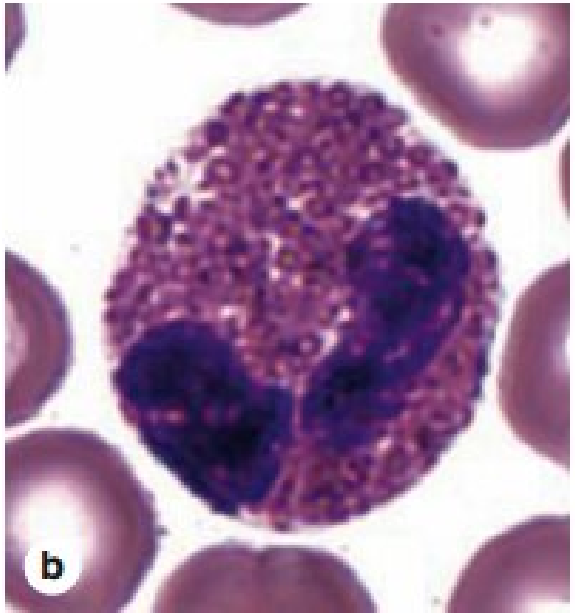
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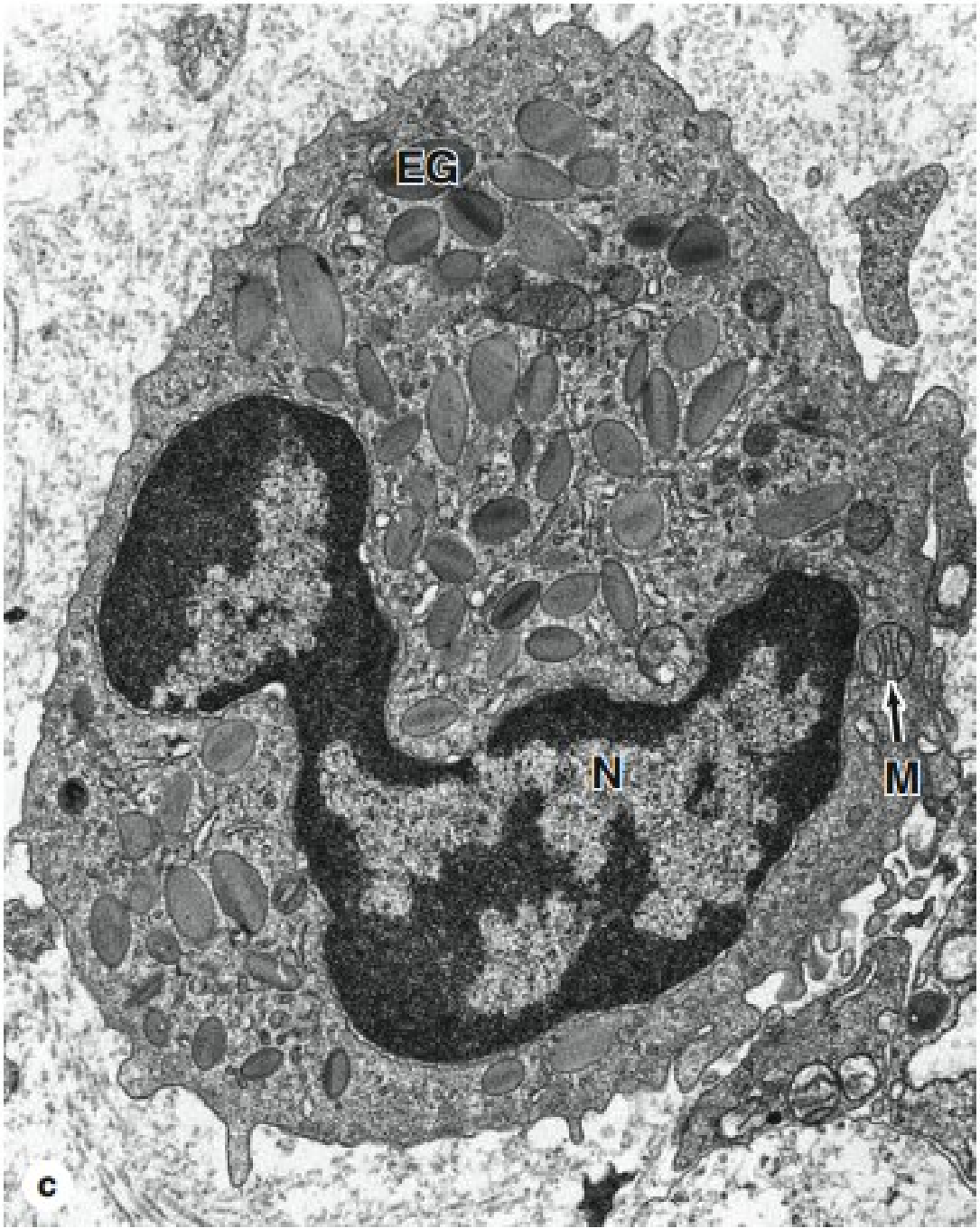
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Eosinophils

Electron microscopy

- Specific granules are oval in shape, with disc shaped crystalline cores
- Specific granules contain **major basic proteins (MBP)** that is responsible for the granule oesinophilia
- Few lysosomes and mitochondria filling the cytoplasm

Eosinophils

Defensive functions

- Weak phagocytes, exhibit chemotaxis
- Eosinophils attach themselves to parasites and kill them by
  - Hydrolytic enzymes
  - Highly reactive forms of oxygen
  - MBP which is highly larvacidal polypeptide
- Modulate and detoxify some of the inflammation inducing substances released by the mast cells and basophils
- Remove antigen-antibody complexes from interstitial fluid by phagocytosis.

The number of circulating eosinophils increases during helminthic infections and allergic reactions and conditions.

Eosinophils are particularly abundant in connective tissue of the intestinal lining and at sites of chronic inflammation, such as lung tissues of asthma patients.

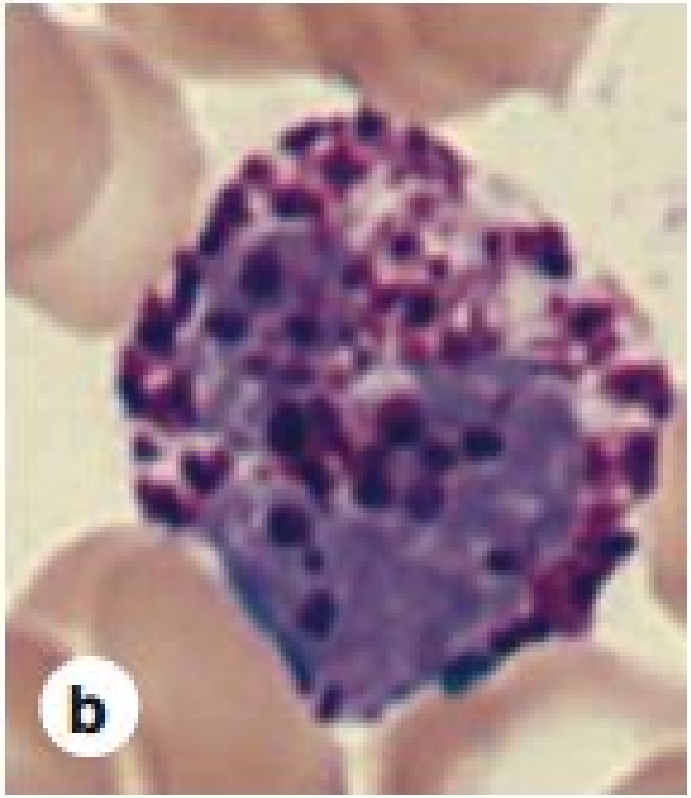
Basophils

Less than 1% of circulating leukocytes, 12-15 μm in diameter

The nucleus is divided into two irregular lobes

Large specific granules overlying the nucleus usually obscure its shape

The specific granules (0.5 μm in diameter) typically stain purple with the basic dye of blood smear stains and are fewer, larger, and more irregularly shaped than the granules of other granulocytes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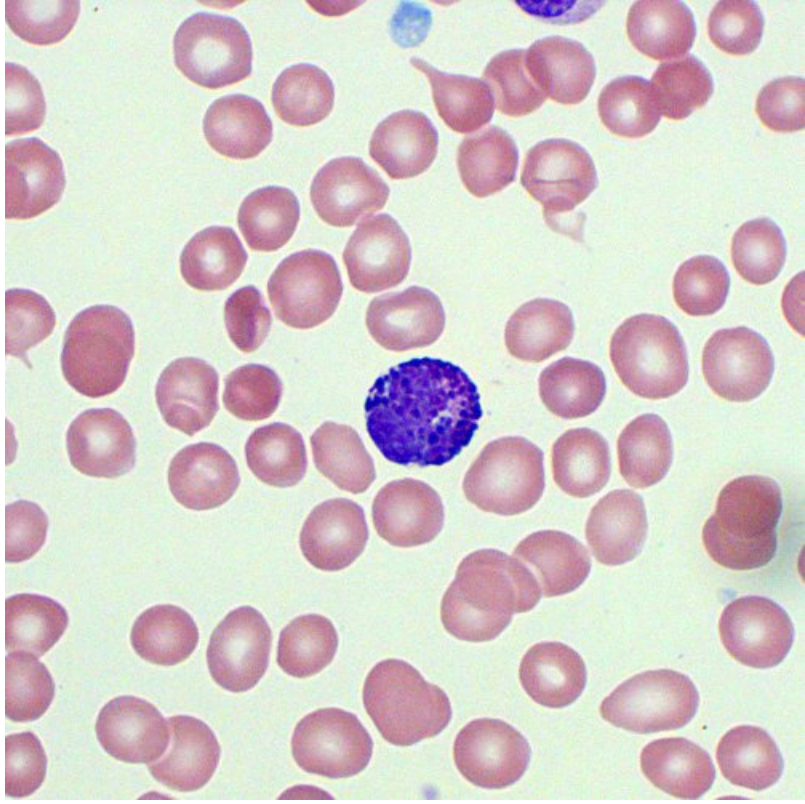

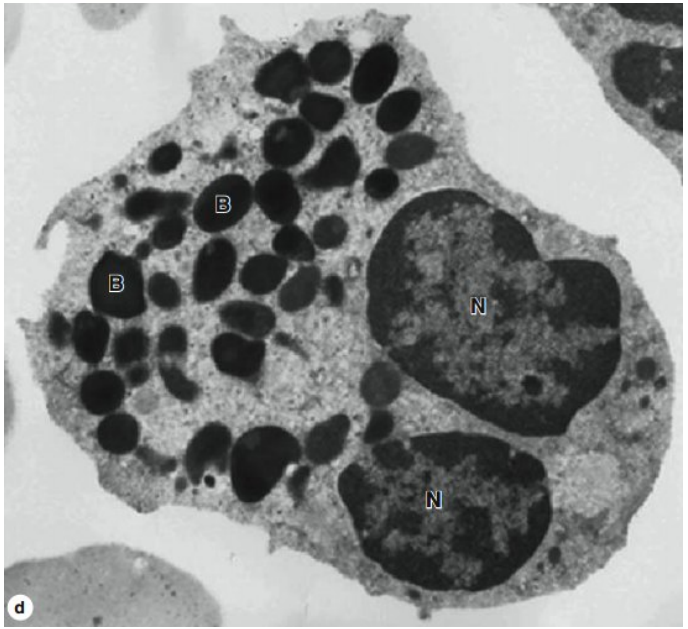
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Basophils

The strong basophilia of the granules is due to the presence of heparin and other sulfated GAGs.

Specific granules also contain histamine and other mediators of inflammation, including platelet activating factor, eosinophil chemotactic factor, and the enzyme phospholipase A that catalyzes an initial step in producing lipid-derived proinflmmatory factors leukotrienes.

Basophils have surface receptors for immunoglobulin E **(IgE)** and secrete their granular components in response to certain antigens and allergens that bind to the IgE on their surface.

Lymphocytes

Most numerous type of agranulocytes in normal blood smears constituting 20-30% of all leukocytes

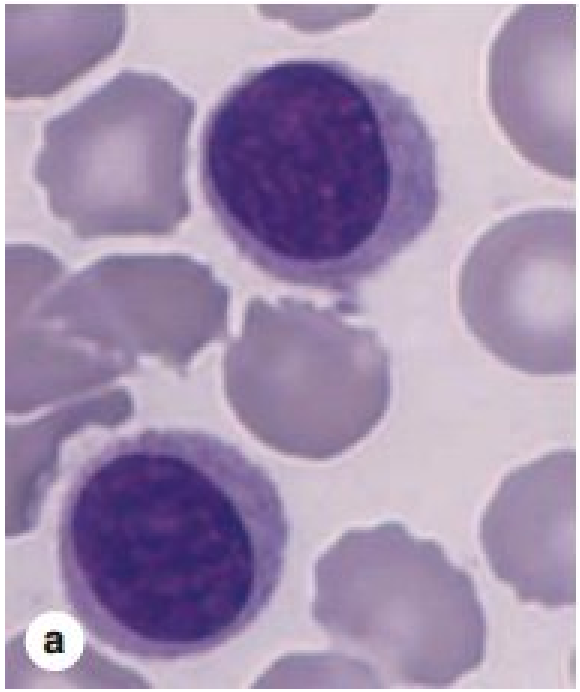
Circulating lymphocytes have a wider range of sizes

Small, newly released lymphocytes have diameters similar to those of RBCs while medium and large lymphocytes are 9-18 μm in diameter, with the latter representing activated lymphocytes or NK cells

Lymphocytes

Small lymphocytes

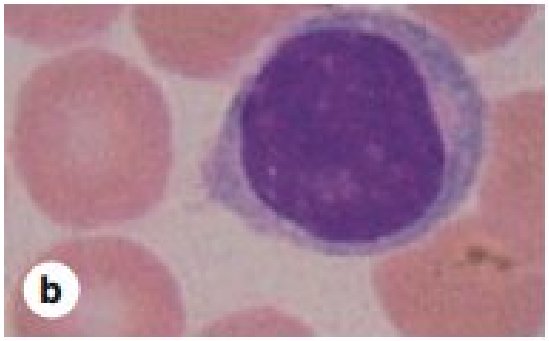
- The most numerous
- Slightly larger than the neighboring erythrocytes and have only a thin rim of cytoplasm surrounding the spherical nucleus.

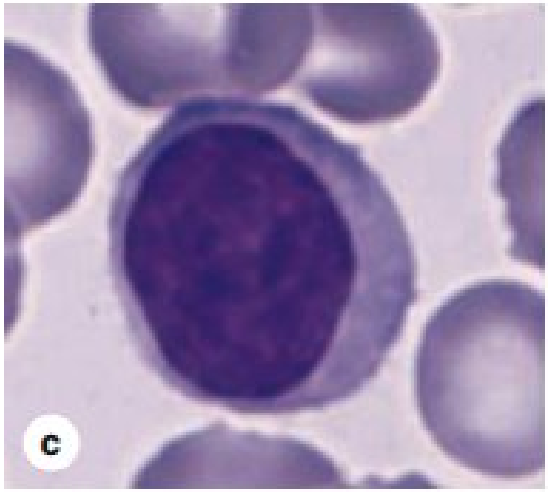


Lymphocytes

Medium and large lymphocytes are distinctly larger than erythrocytes

May represent activated cells that have returned to the circulation





Lymphocytes

They are morphologically similar and indistinguishable

Mature lymphocytes can be subdivided into functional groups by distinctive surface molecules called “cluster of differentiation” or CD markers, e.g. CD 4 CD 8 for T cells and CD 20 for B cells

Different types of lymphocytes have diverse roles in immune defenses against invading microorganisms and certain parasites or abnormal cells

T lymphocytes, unlike B cells and all other circulating leukocytes, differentiate outside the bone marrow in the thymus.

Monocytes

Monocytes are agranulocytes that are precursor cells of macrophages, osteoclasts, microglia, and other cells of the mononuclear phagocyte system in connective tissue

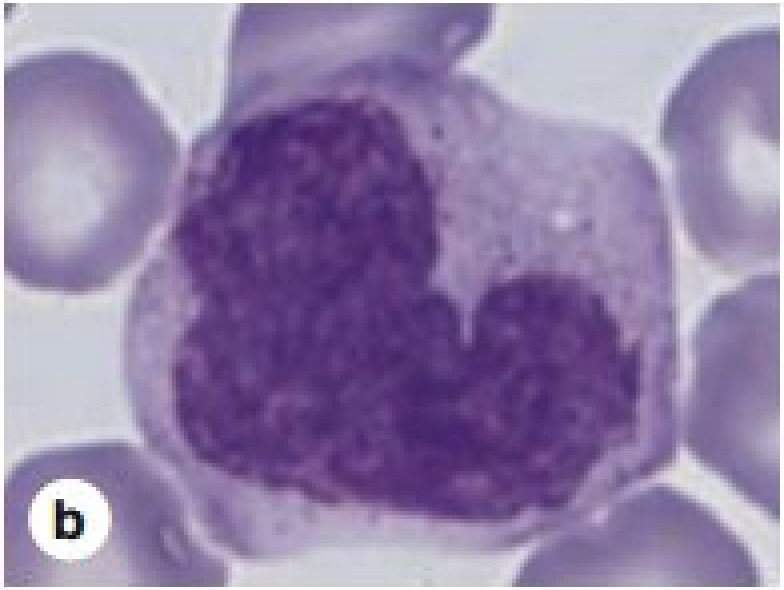
All monocyte-derived cells are antigen-presenting cells and have important roles in immune defense of tissues.

Monocytes

Circulating monocytes have diameters of 12-15 μm, macrophages are larger.

The monocyte nucleus is large and usually distinctly indented or C-shaped, the chromatin is less condensed than in lymphocytes and typically stains lighter than that of large lymphocytes.

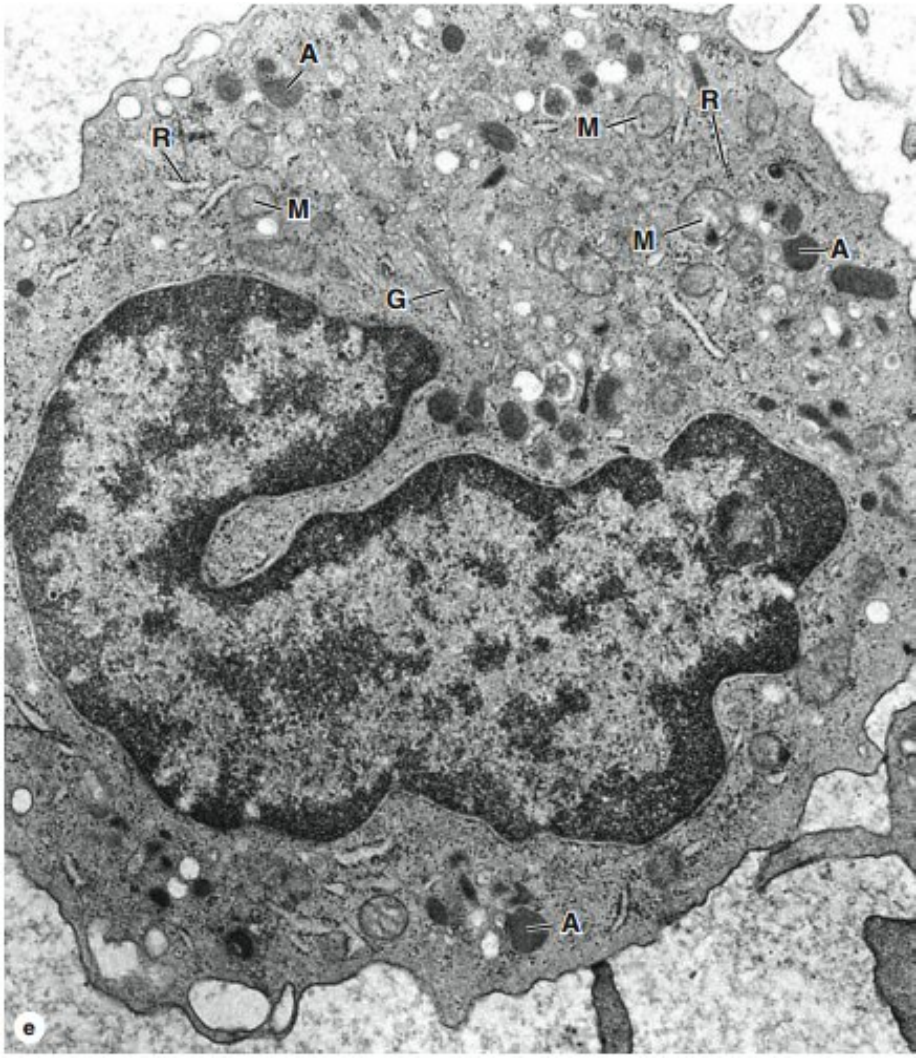
The cytoplasm of the monocyte is basophilic and contains
many small lysosomal azurophilic granules



Monocytes

The granules are distributed through the cytoplasm, giving it a bluish gray color in stained smears

Mitochondria and small areas of rough ER are present, along with a Golgi apparatus



Platelets

Very small non-nucleated, membrane-bound cell fragments, 2-4 μm in diameter

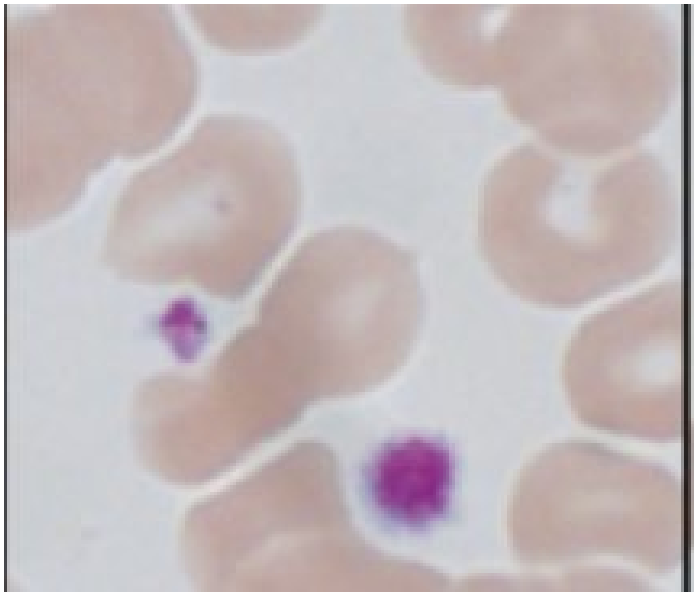
Normal platelet counts range from 150,000 to 400,000/μL (mm3) of blood. Circulating platelets have a life span of about 10 days.

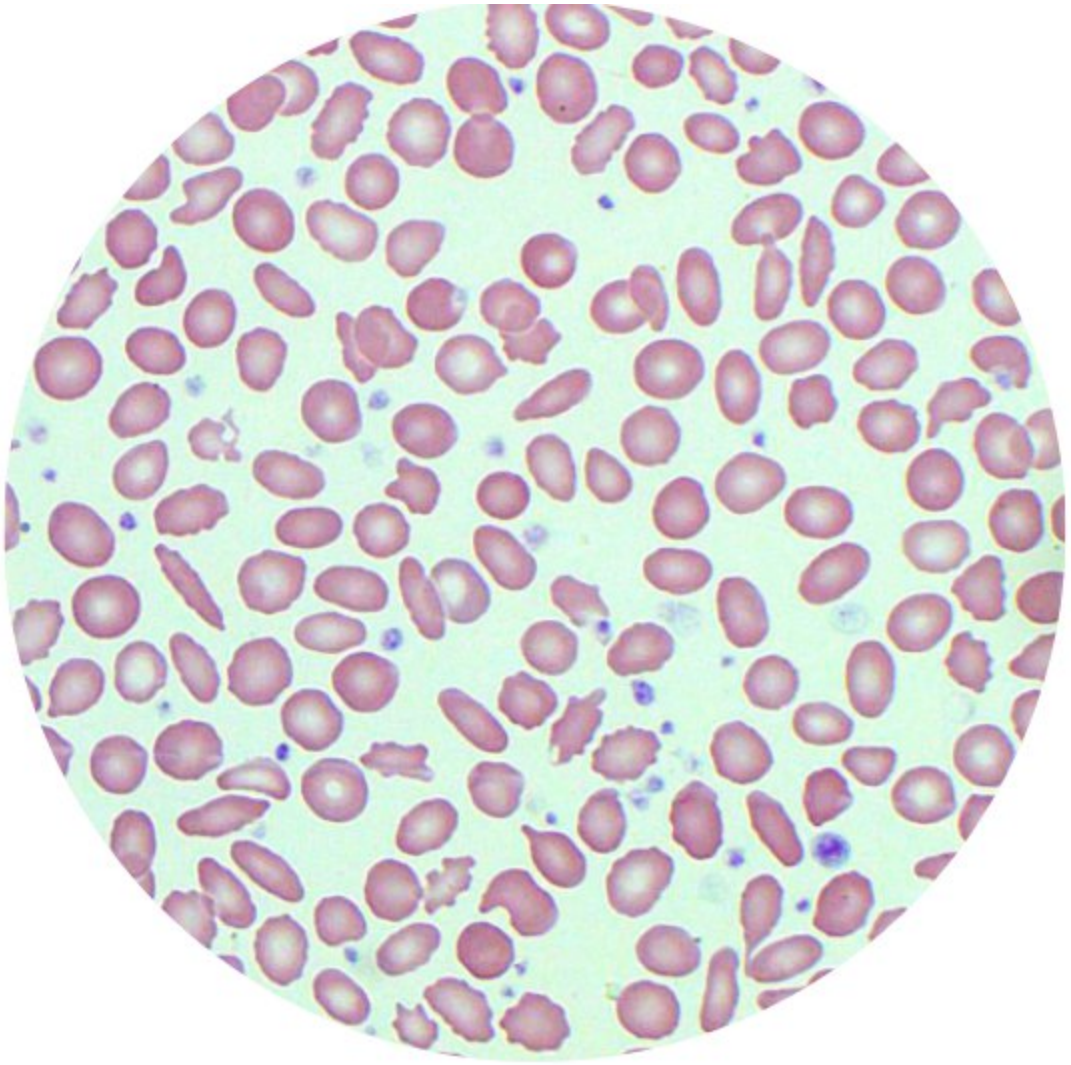
Originate by separation from the ends of cytoplasmic processes extending from giant polyploid bone marrow cells called megakaryocytes

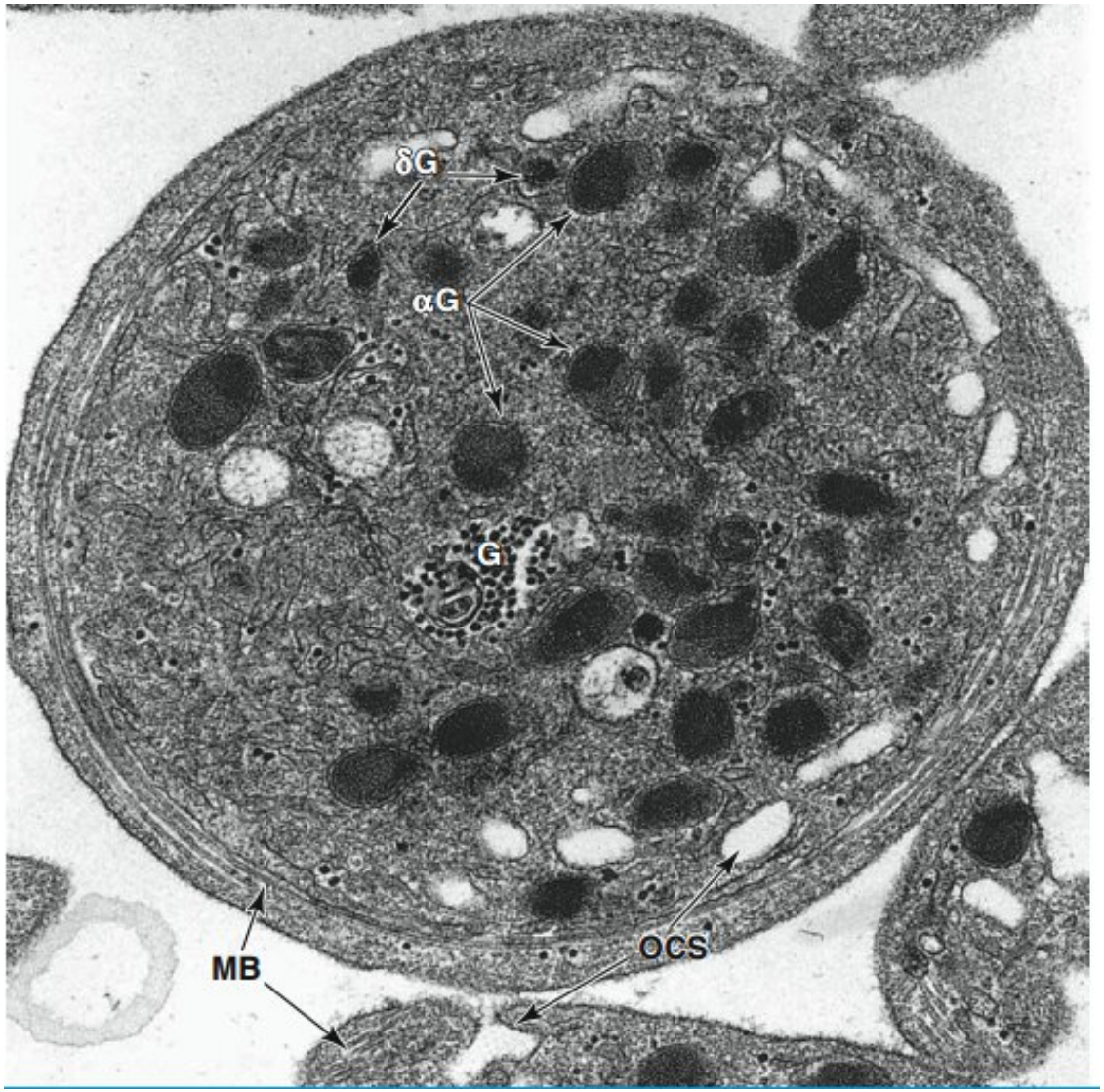
In stained blood smears, each individual platelet is generally discoid and has 2 zones

- Very lightly stained peripheral zone, the **hyalomere**
- darker-staining central zone rich in granules, called the **granulomere**

A sparse glycocalyx surrounds the platelet plasmalemma is involved in adhesion and activation during blood coagulation






δG
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Ultrastructural

- **Marginal bundle** of microtubules and microfilaments, which helps to maintain the platelet's shape
- **Hyalomere** contains 2 systems of membrane channels

  Open canalicular system of vesicles is connected to invaginations of the plasma membrane.

  Dense tubular system is derived from the ER and stores Ca2+ ions

  These two systems facilitate the extremely rapid exocytosis
  of proteins from platelets (degranulation) upon

- Central granulomere, contains
  - Sparse population of mitochondria and glycogen particles
  - **Delta granules (δG)**, 250-300 nm in diameter, contain ADP, ATP, and serotonin
  - **Alpha granules (αG)** are larger (300-500 nm in diameter) and contain platelet derived growth factor (PDGF), platelet factor 4, and several other platelet-specific proteins.
- Most of the stained granules seen in platelets with the light microscope are alpha granules.

Thank You