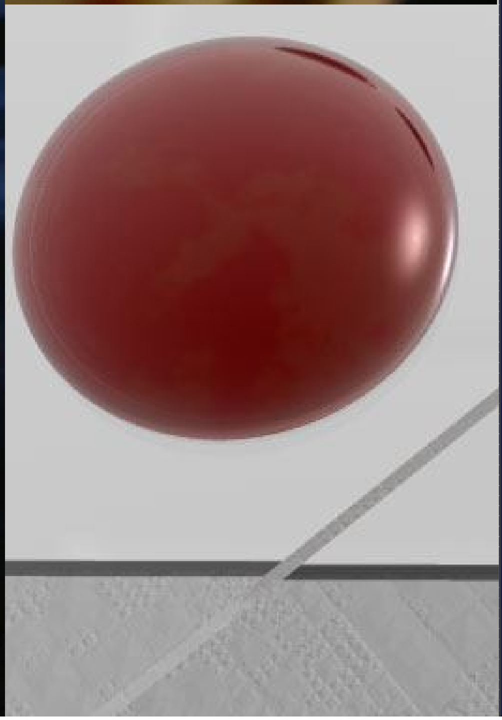


Hematology lecture

Danil Hammoudi.MD

PFC stands for perflourocarbons.
Perflourocarbons are a type of synthetic blood that helps carry dissolved gases in the blood. They are mixed with an emulsifier to create a liquid suspension that can be mixed with blood. PFCs can carry about 20 percent more gas than blood plasma.
PFCs may be especially helpful in these areas:

-Restoring the delivery of oxygen in the body
Treating traumatic brain injury
Treating anemia
Increasing the effectiveness of chemotherapy
Preventing the need for surgical blood transfusion

HEMOPOIESIS

- **Hemo: Referring to blood cells**
- **Poiesis: "The development or production of"**
- **The word Hemopoiesis refers to the production & development of all the blood cells:**
  - **Erythrocytes: Erythropoiesis**
  - **Leucocytes: Leucopoiesis**
  - **Thrombocytes: Thrombopoiesis.**
- **Begins in the 20th week of life in the fetal liver & spleen, continues in the bone marrow till young adulthood & beyond!**

SITES OF HEMOPOIESIS

- Active Hemopoietic marrow is found, in children throughout the:
  - Axial skeleton:
    - Cranium
    - Ribs.
    - Sternum
    - Vertebrae
    - Pelvis
  - Appendicular skeleton:
    - Bones of the Upper & Lower limbs
- In Adults active hemopoietic marrow is found only in:
  - The axial skeleton
  - The proximal ends of the appendicular skeleton.

Developmental Aspects

- Before birth, blood cell formation takes place in
  - **The fetal yolk sac,**
  - **Liver,**
  - **Spleen**
- By the **seventh month, red bone marrow is the primary hematopoietic area**
- Blood cells develop from mesenchymal cells called blood islands
- **The fetus forms HbF**, which has a higher affinity for oxygen than adult hemoglobin

Hemopoietic cells (those which produce blood) first appear in the yolk sac of the 2-week embryo.

By 8 weeks, blood making has become established in the liver of the embryo,

By 12-16 weeks the liver has become the major site of blood cell formation. It remains an active hemopoietic site until a few weeks before birth.

The spleen is also active during this period, particularly in the production of lymphoid cells, and the fetal thymus is a transient site for some lymphocytes.

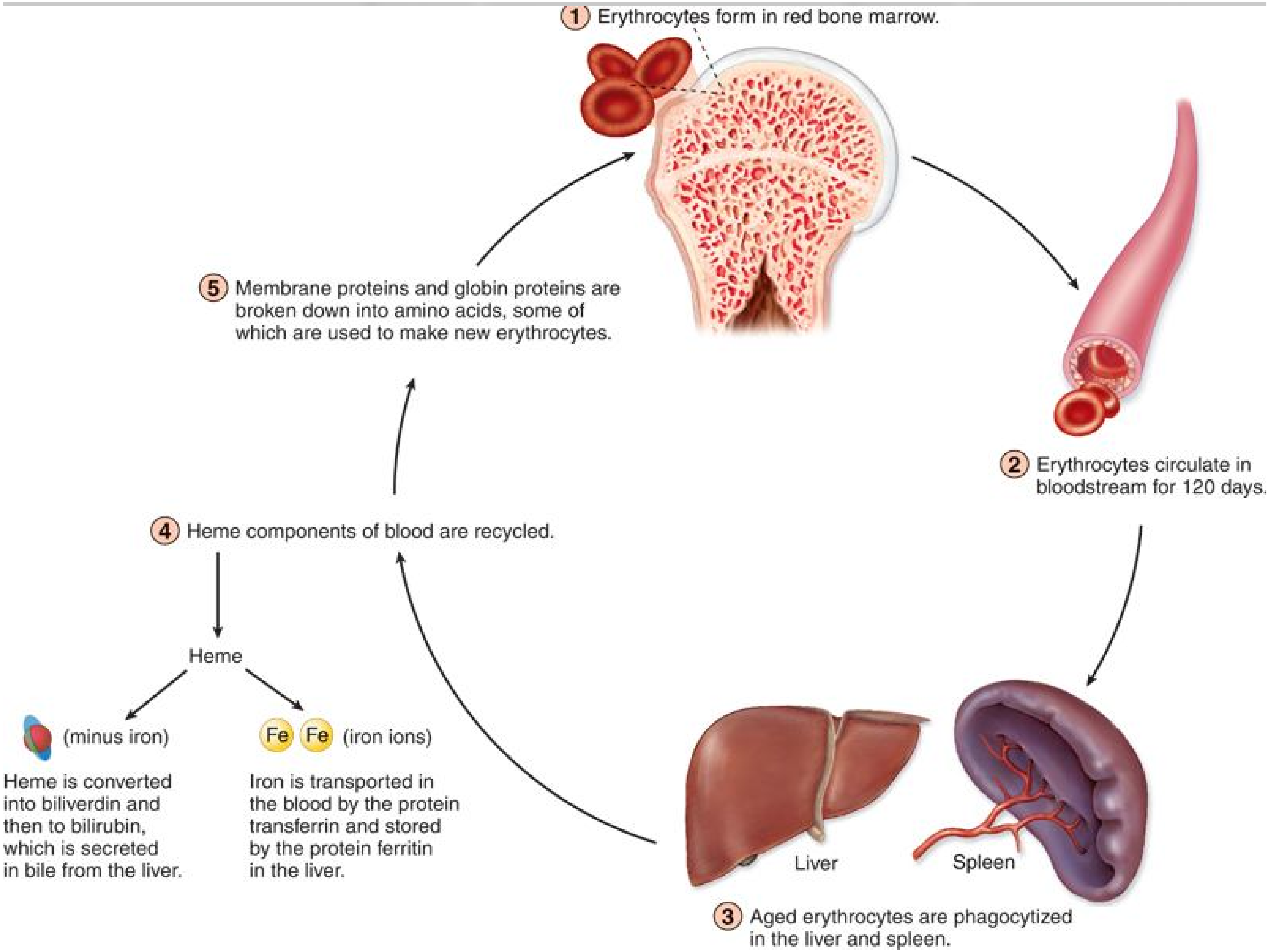
At birth, active blood making red marrow occupies the entire capacity of the bones and continues to do so for the first 2-3 years after birth.

The red marrow is then very gradually replaced by inactive, fatty, yellow, lymphoid marrow.
The latter begins to develop in the shafts of the long bones and continues until, by 20-22 years, red marrow is present only in the upper ends of the femur and humerus and in the flat bones of the sternum, ribs, cranium, pelvis and vertebra.

However, because of the growth in body and bone size that has occurred during this period, the total amount of active red marrow (approximately 1000-1500 g) is nearly identical in the child and the adult.

Developmental Aspects

- Age-related blood problems result from disorders of the heart, blood vessels, and the immune system
- Increased leukemias are thought to be due to the waning deficiency of the immune system
- Abnormal thrombus and embolus formation reflects the progress of atherosclerosis


1 Erythrocytes form in red bone marrow.
2 Erythrocytes circulate in bloodstream for 120 days.
3 Aged erythrocytes are phagocytized in the liver and spleen.
Liver
Spleen
4 Heme components of blood are recycled.
Heme
(minus iron)
Heme is converted into biliverdin and then to bilirubin, which is secreted in bile from the liver.
Fe
Fe
(iron ions)
Iron is transported in the blood by the protein transferrin and stored by the protein ferritin in the liver.
5 Membrane proteins and globin proteins are broken down into amino acids, some of which are used to make new erythrocytes.

•<u>**Pluripotent stem cells:**</u> **are mesenchymal cells representing less than .1% of Red Bone Marrow cells having the ability to develop into several other kinds of cells.**

•**These further lead to 2 other stem cells known as *Myeloid* and *lymphoid* stem cells.**

<u>**Myeloid Stem Cells lead to development of:**</u>

•***RBCs (erythrocytes)*** **(CFU-E)**
•***Platelets (thrombocytes)***
•***Eosinophils***
•***Neutrophils*** **(CFU-GM)**
•***Basophils***

Of the cell types listed above, all <u>**except eosinophils and basophils are produced by intermediate or Progenitor cells before fully developing**</u>

•Lymphoid Stem Cells lead to the development of T and B lymphocytes (T/B cells).

•HGFs (*Hemopoietic Growth factors*) control the production of progenitor cells. Examples include

- ***erythropoietin*** **(EPO)**
- ***Thrombopoietin*** **(TPO).**

•EPO cells are produced in the kidneys, therefore, renal failure contributes to insufficient production of RBCs.

• TPO originates in the liver to promote the synthesis of platelets from Megakaryocytes

•Cytokines - Typically acting as autocrines/paracrines,

- cytokines are glycoproteins synthesized by RBM cells, leukocytes, macrophages, fibroblasts and endothelial cells.
- They promote the spread of progenitor cells.

•2 Cytokine Families that stimulate production of WBCs:

- *CSFs (Colony-Stimulating Factors)*
- *Interleukins*

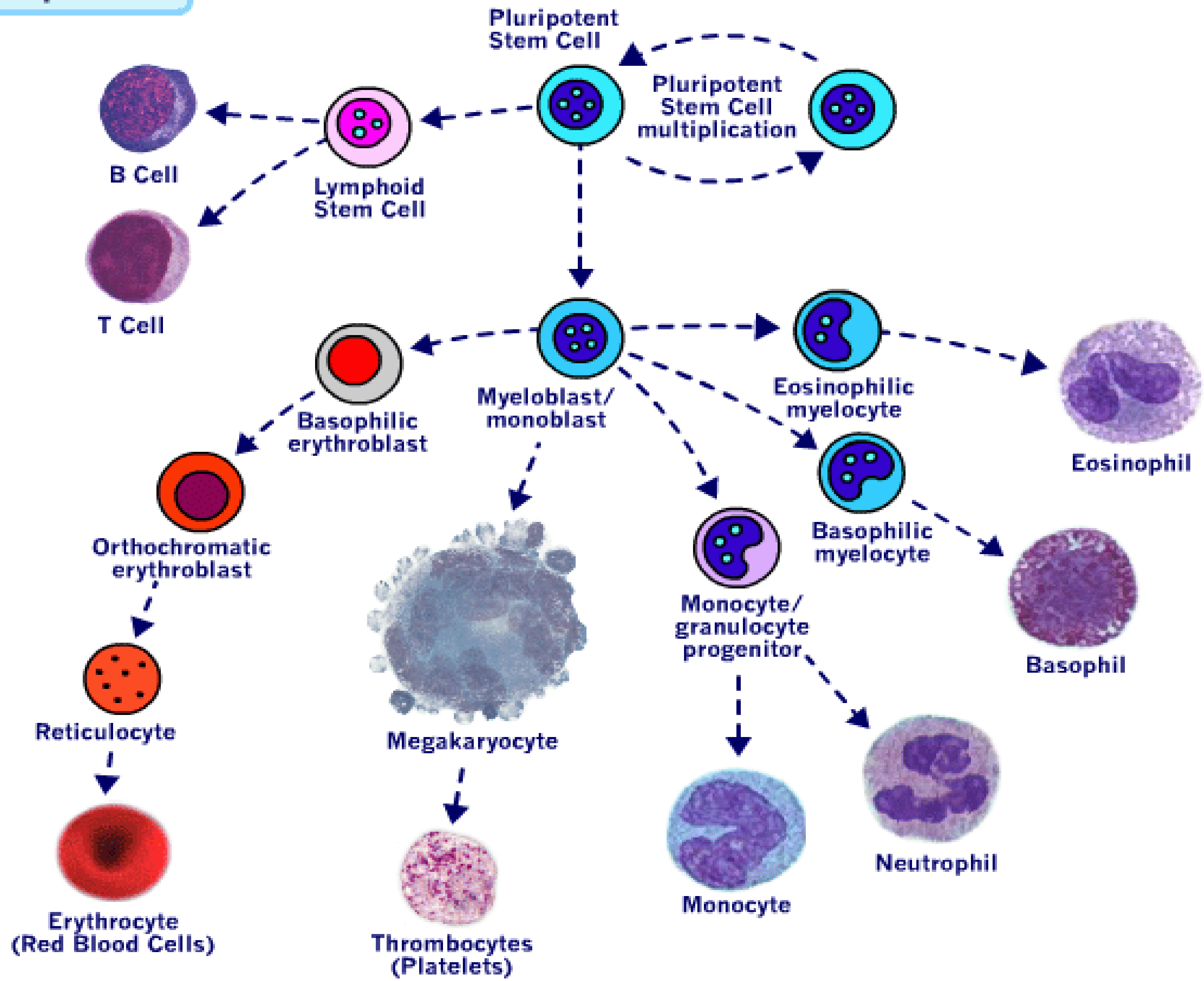
Erythropoietin , also known as *Epoetin alfa*, to treat

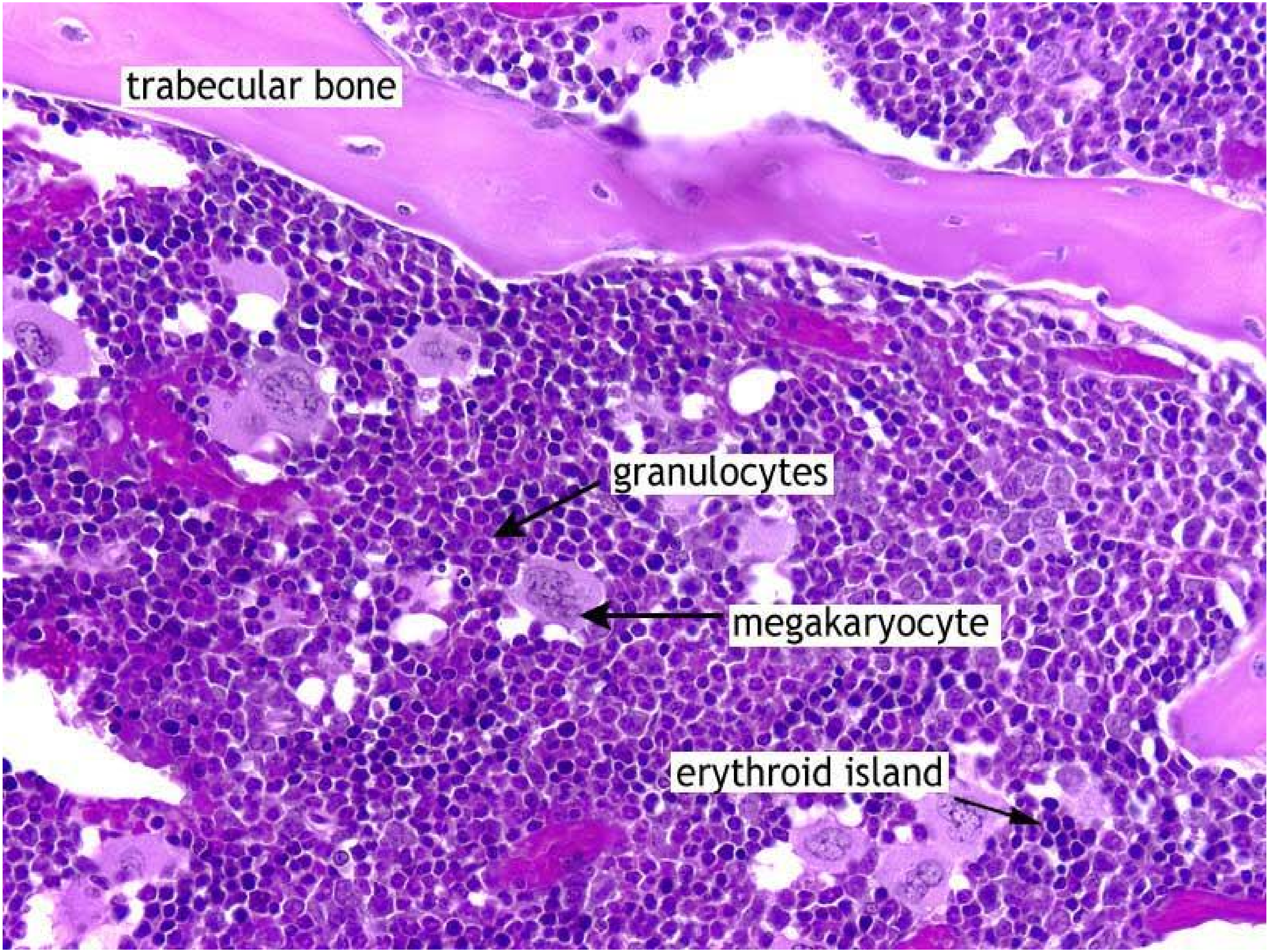
- **Low RBC concentration during end-stage kidney disease.**

- **Patients going through chemotherapy are treated with Granulocytic-Machrophage CSF and Granulocyte CSFs to treat low WBC concentration.**

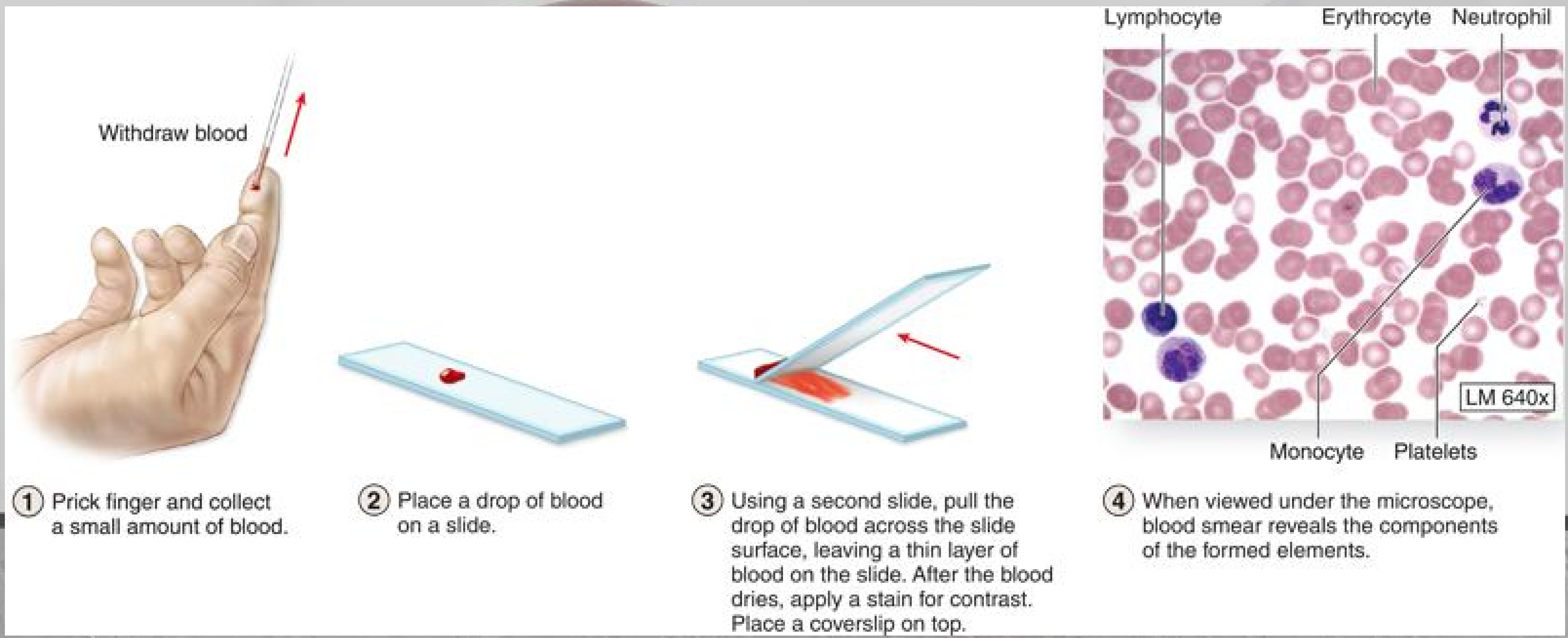
- **Thrombopoietin is used to treat platelet depletion.**

Hematopoiesis



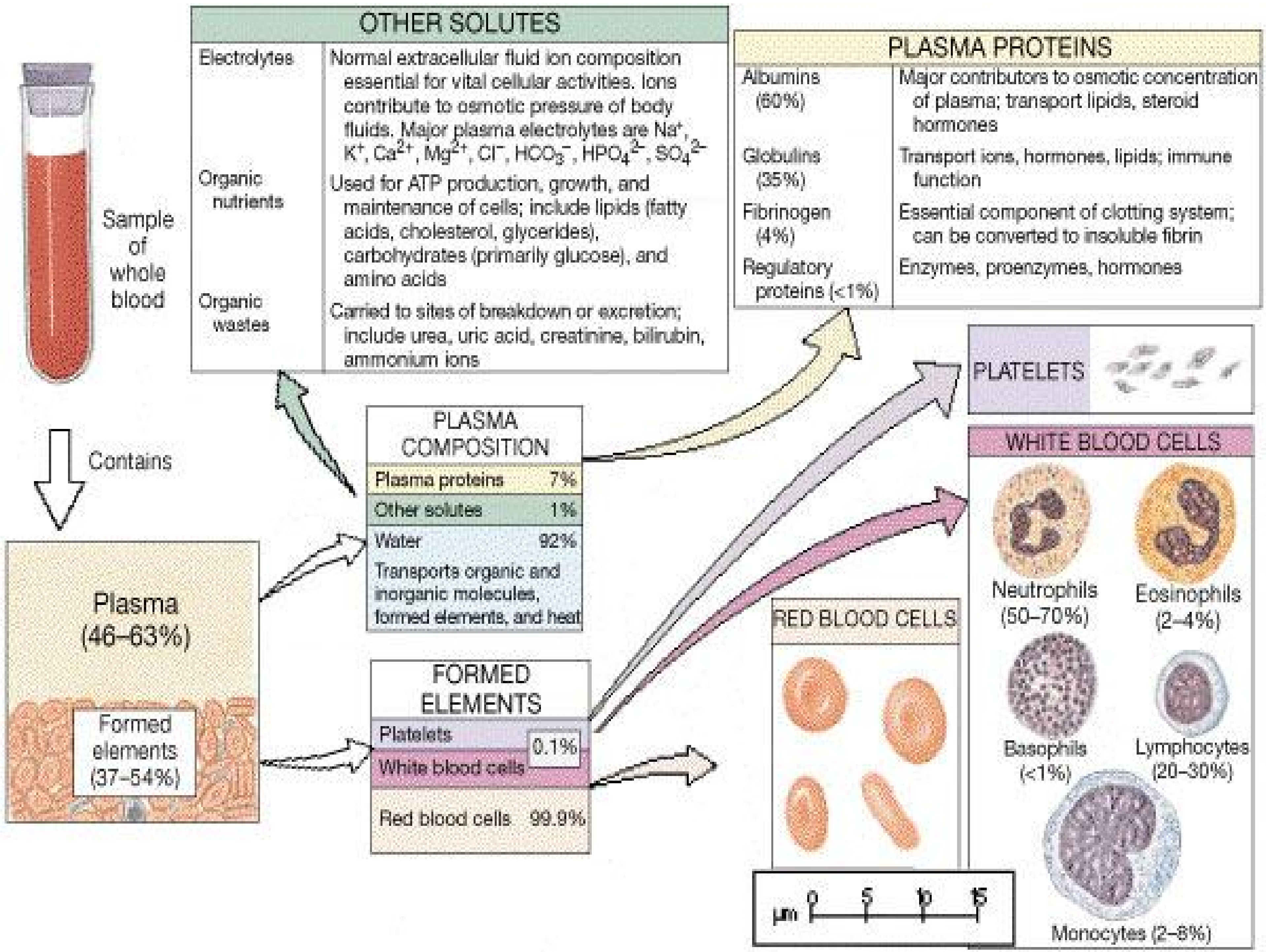

trabecular bone
granulocytes
megakaryocyte
erythroid island

Def and Generality



- **Blood is a connective tissue whose matrix is fluid.**
- **It is composed of:**
  - **red corpuscles,**
  - **white cells,**
  - **platelets,**
  - **blood plasma.**
- **It is transported throughout the body within blood vessels.**

- In human **adults about 5 liter of blood contribute 7-8 % to the body weight of the individual**.
- The contribution of red blood cells (erythrocytes) to the total volume of the blood **(hematocrit) is about 43%.**
- **Erythrocytes are the dominant (99%)** but not the only type of cells in the blood.
- **Erythrocytes, leukocytes and blood platelets = formed elements of the blood**.
- Erythrocytes and blood platelets perform their functions exclusively in the blood stream.
- In contrast, **leukocytes reside only temporarily in the blood.**
- Leukocytes can leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.

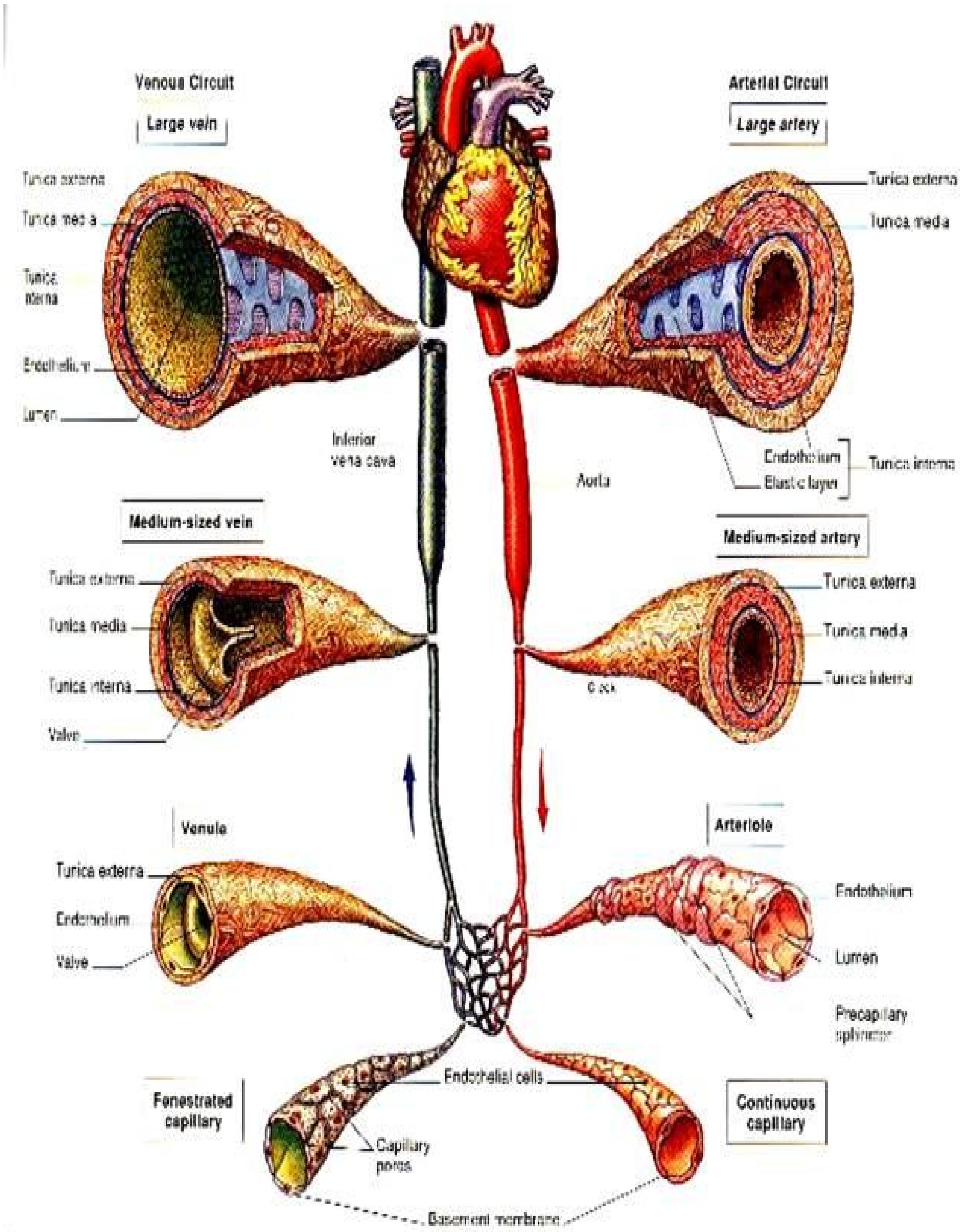


OTHER SOLUTES
Electrolytes
Normal extracellular fluid ion composition essential for vital cellular activities. Ions contribute to osmotic pressure of body fluids. Major plasma electrolytes are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}, SO_4^{2-}
Organic nutrients
Used for ATP production, growth, and maintenance of cells; include lipids (fatty acids, cholesterol, glycerides), carbohydrates (primarily glucose), and amino acids
Organic wastes
Carried to sites of breakdown or excretion; include urea, uric acid, creatinine, bilirubin, ammonium ions
PLASMA PROTEINS
Albumins (60%)
Major contributors to osmotic concentration of plasma; transport lipids, steroid hormones
Globulins (35%)
Transport ions, hormones, lipids; immune function
Fibrinogen (4%)
Essential component of clotting system; can be converted to insoluble fibrin
Regulatory proteins (<1%)
Enzymes, proenzymes, hormones
Sample of whole blood
Contains
Plasma (46–63%)
Formed elements (37–54%)
PLASMA COMPOSITION
Plasma proteins 7%
Other solutes 1%
Water 92%
Transports organic and inorganic molecules, formed elements, and heat
FORMED ELEMENTS
Platelets
0.1%
White blood cells
Red blood cells 99.9%
PLATELETS
WHITE BLOOD CELLS
Neutrophils (50–70%)
Eosinophils (2–4%)
Basophils (<1%)
Lymphocytes (20–30%)
Monocytes (2–8%)
RED BLOOD CELLS
µm 0 5 10 15

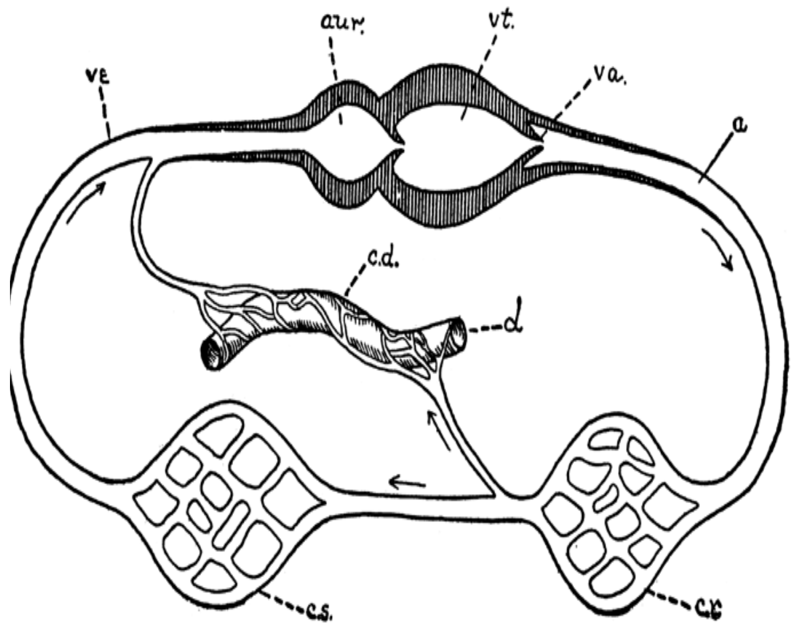
Overview of Blood Circulation

- Blood leaves the heart via arteries that branch repeatedly until they become capillaries
- Oxygen (O_2) and nutrients diffuse across capillary walls and enter tissues
- Carbon dioxide (CO_2) and wastes move from tissues into the blood

- Oxygen-deficient blood leaves the capillaries and flows in veins to the heart
- This blood flows to the lungs where it releases CO_2 and picks up O_2
- The oxygen-rich blood returns to the heart



Venous Circuit
Large vein
Tunica externa
Tunica media
Tunica interna
Endothelium
Lumen
Arterial Circuit
Large artery
Tunica externa
Tunica media
Endothelium
Elastic layer
Tunica interna
Inferior vena cava
Aorta
Medium-sized vein
Tunica externa
Tunica media
Tunica interna
Valve
Medium-sized artery
Tunica externa
Tunica media
Tunica interna
Venule
Tunica externa
Endothelium
Valve
Arteriole
Endothelium
Lumen
Precapillary sphincter
Fenestrated capillary
Endothelial cells
Capillary pores
Continuous capillary
Basement membrane



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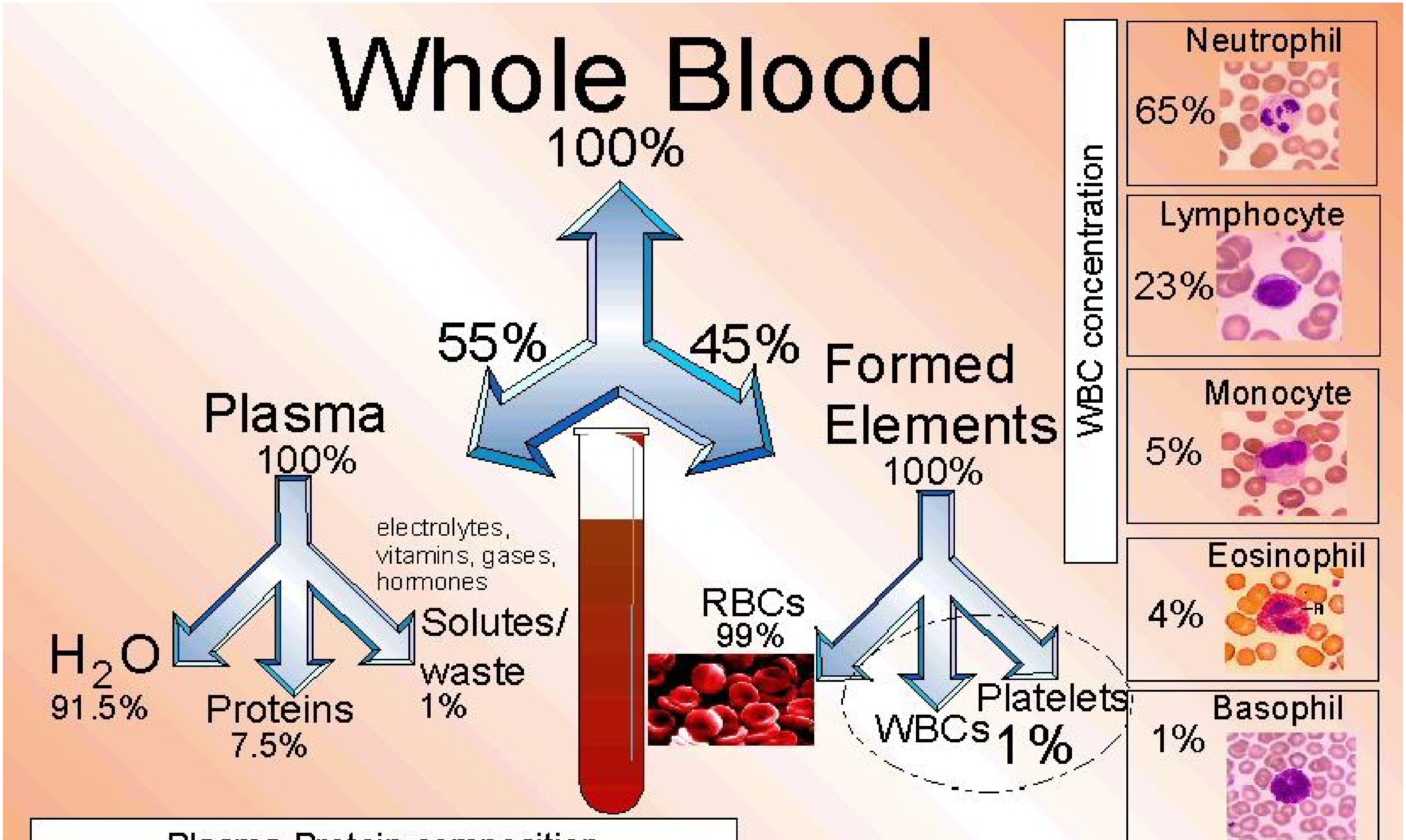
c.s.

Composition of Blood

- Blood is the **body's only fluid** tissue
- It is **composed of liquid plasma and formed elements**
- Formed elements include:
  - Erythrocytes, or red blood cells (RBCs)
  - Leukocytes, or white blood cells (WBCs)
  - Platelets
- Hematocrit – the percentage of RBCs out of the total blood volume



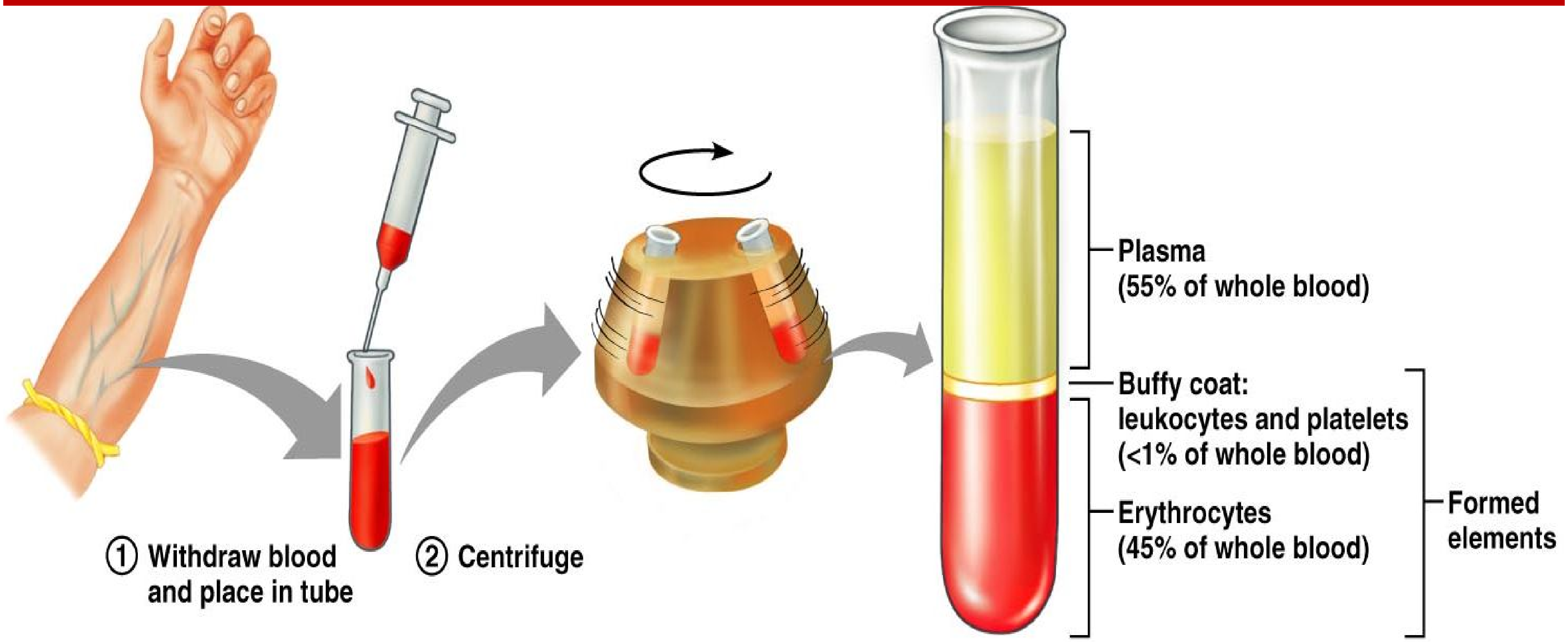
Plasma Protein composition		
Albumins 54% smallest, most abundant. transport fatty acids	**Globulins 38%** from plasma & liver cells; 38% Gamma Globulins Immunoglobulins	Fibrinogen **7%** + trace substances

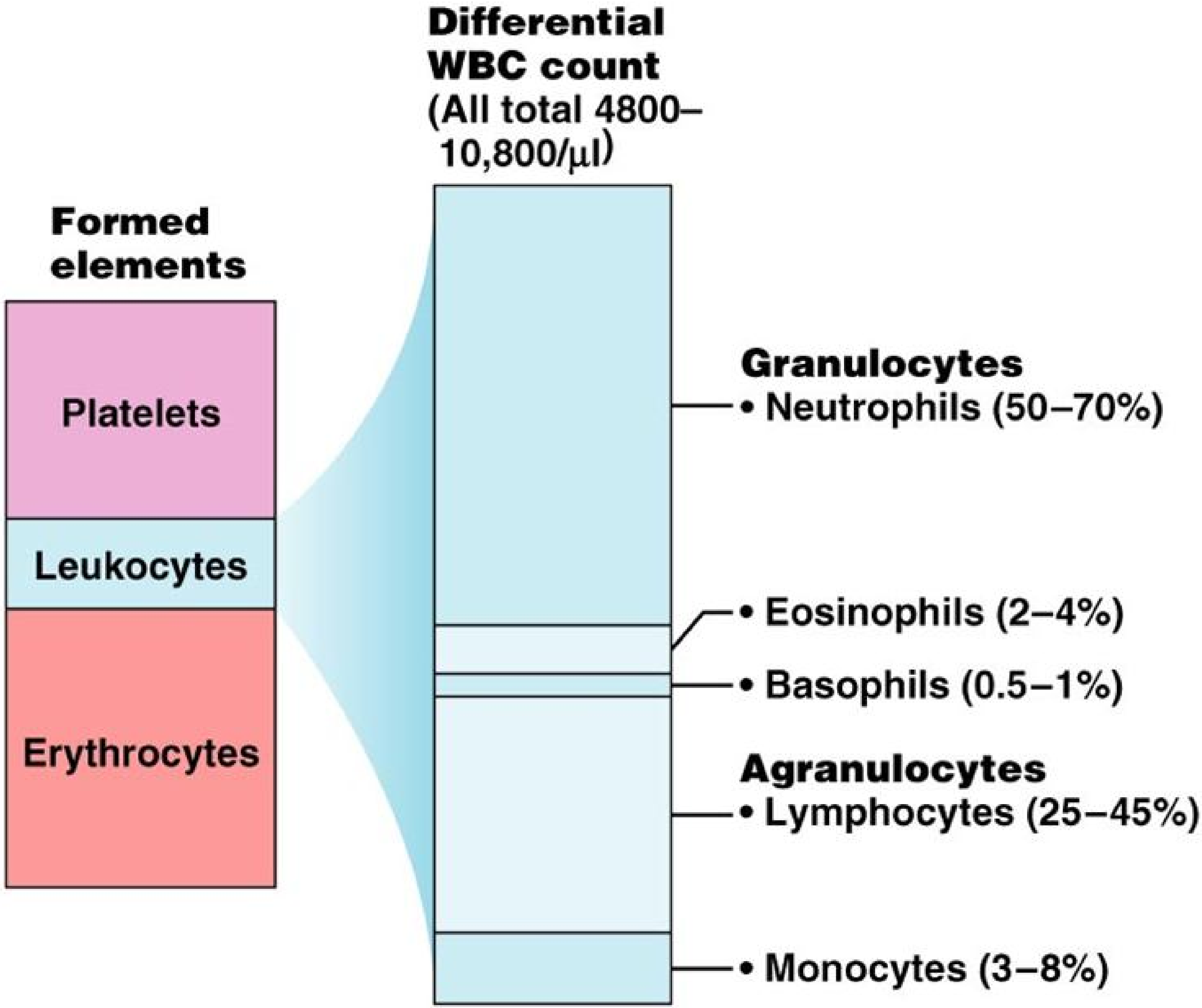
fibriniogen- 7%, made by liver

Components of Whole Blood

Whole blood is a living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues.

Whole blood contains red blood cells, white blood cells, and platelets suspended in a fluid called plasma.




Differential WBC count (All total 4800–10,800/μl)
Formed elements
Platelets
Leukocytes
Erythrocytes
Granulocytes
• Neutrophils (50–70%)
• Eosinophils (2–4%)
• Basophils (0.5–1%)
Agranulocytes
• Lymphocytes (25–45%)
• Monocytes (3–8%)

The volume of blood in an average-sized person (70 kg; 154 lbs) is approximately 5.5 L.

If we take the hematocrit to be 45 percent, then

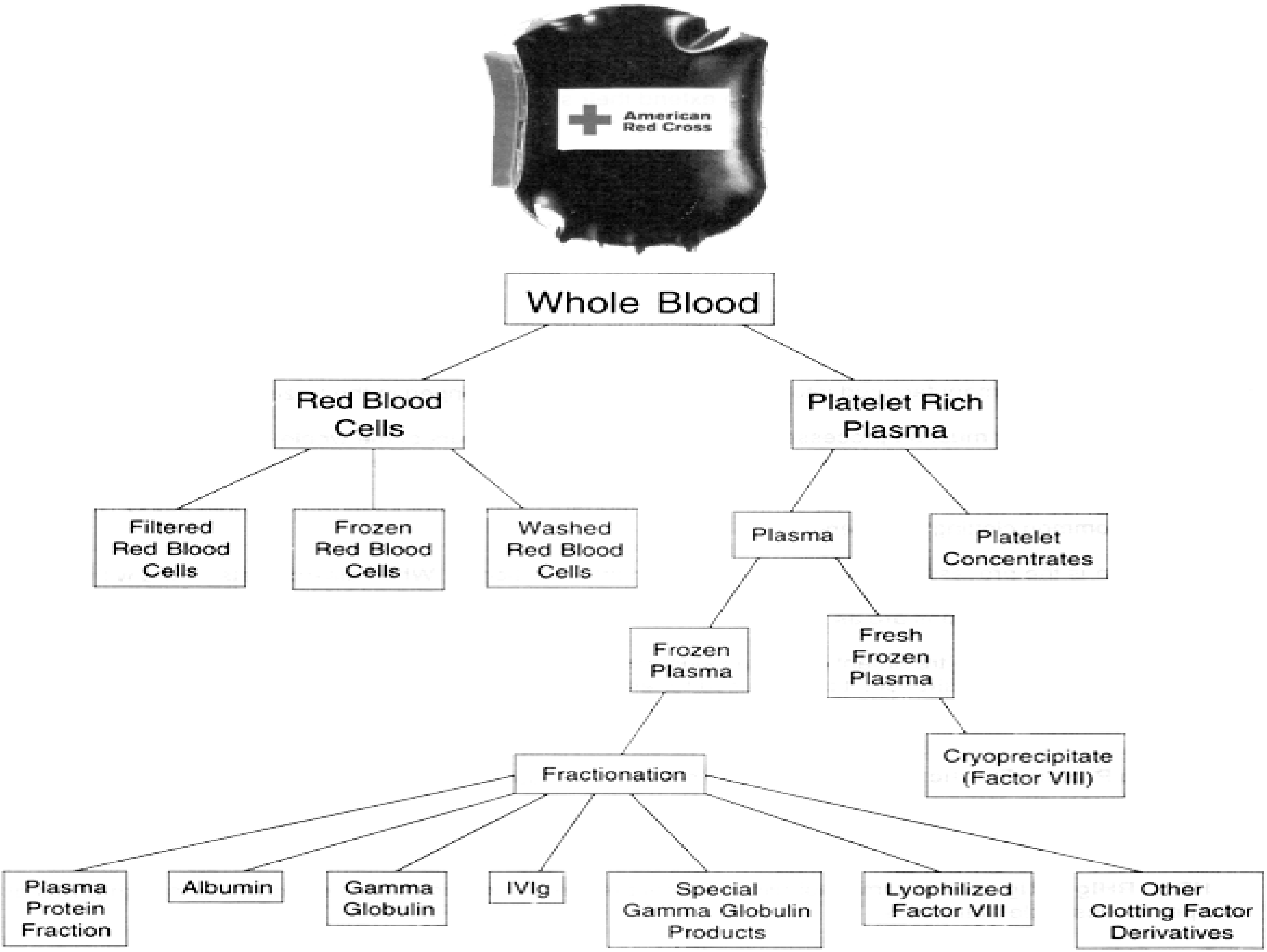
Erythrocyte volume = 0.45 x5.5 L =2.5 L

Since the volume occupied by the leukocytes and platelets is normally negligible, the plasma volume equals the difference between blood volume and erythrocyte volume; therefore, in our average person

Plasma volume = 5.5 L - 2.5 L = 3.0 L

Blood Volume

- Blood volume is about 8% of body weight.
- 1 kg of blood ≈ 1 L of blood
- 70 kg X 0.08 = 5.6 Kg = 5.6 L
- 45 % is formed elements
- 55% plasma


American Red Cross
Whole Blood
Red Blood Cells
Platelet Rich Plasma
Filtered Red Blood Cells
Frozen Red Blood Cells
Washed Red Blood Cells
Plasma
Platelet Concentrates
Frozen Plasma
Fresh Frozen Plasma
Cryoprecipitate (Factor VIII)
Fractionation
Plasma Protein Fraction
Albumin
Gamma Globulin
IVIg
Special Gamma Globulin Products
Lyophilized Factor VIII
Other Clotting Factor Derivatives

BLOOD PRODUCTS

- Blood-cells products
  - whole blood
  - packed red blood cells
  - leukocyte-poor (reduced) red cells
  - washed red blood cells
  - random-donor platelets concentrates
  - single-donor platelets concentrates [human leukocyte antigens(HLA)-matched platelets]
  - irradiated blood products (red blood cells and platelets concentrates)- after exposure 20 to 40 Gy
  - leukocyte (granulocyte) concentrates

- Plasma products
  - fresh-frozen plasma (FFP)
  - cryoprecipitate
  - factor concentrates (VIII, IX)
  - albumin
  - immune globulins

Physical characteristics

- **Viscosity** 4.5 - 5.5 (where H_2O = 1.0)
- **Temperature** 100.4F [38 degree Celsius]
- **pH** 7.35 - 7.45
- **Salinity** . 85% - .90%
- **Volume** 5 - 6 liters in males
  4 - 5 liters in females
- **Blood accounts for approximately 7-8% of body weight**

Blood is a sticky, opaque fluid with a <u>metallic taste</u>
Color varies from scarlet to dark red

- **Blood volume and osmotic pressure are regulated by several negative feedback mechanisms.**

- **Those mechanisms of specific interest involve**
  - **aldosterone,**
  - **ADH**
  - ***atrial natriuretic peptide***

Hematocrit

- The hematocrit (Ht or HCT) or packed cell volume (PCV) or erythrocyte volume fraction (EVF) is the proportion of blood volume that is occupied by red blood cells.

- The volume of RBCs refers to the amount of space that the RBCs occupy within the blood.
- **males the average is slightly higher at 47% (40-54%) due to higher levels of testosterone in males.**
- **Testosterone promotes synthesis of EPO (*erythropoietin*), which contributes to a higher RBC count.**

Hematocrit

- **At sea level, the hematocrit of a normal adult male averages about 47, which means that 47% of the blood volume is RBCs, while that of a normal adult female is 42.**
- **Ht=Hbx3**

- The normal ranges for hematocrit are dependent on age and, after adolescence, the sex of the individual.
- The normal ranges are:

- Newborns: 55%-68%
- One (1) week of age: 47%-65%
- One (1) month of age: 37%-49%
- Three (3) months of age: 30%-36%
- One (1) year of age: 29%-41%
- Ten (10) years of age: 36%-40%
- Adult males: 42%-54%
- Adult women: 38%-46%

Functions of Blood

- Blood performs a number of functions dealing with:
  - Substance distribution
  - Regulation of blood levels of particular substances
  - Body protection

Functions of Blood

- **Primary**
  - Transportation
  - Exchange
- **Secondary**
  - Immunity
  - Thermoregulation
  - Fluid volume balance
  - pH balance

Distribution

- Blood transports:
  - – Oxygen from the lungs and nutrients from the digestive tract
  - – Metabolic wastes from cells to the lungs and kidneys for elimination
  - – Hormones from endocrine glands to target organs

Regulation

- Blood maintains:
  - Appropriate body temperature by absorbing and distributing heat
  - Normal pH in body tissues using buffer systems
  - Adequate fluid volume in the circulatory system

Protection

- **Blood prevents blood loss by:**
  – Activating plasma proteins and platelets
  – Initiating clot formation when a vessel is broken
- **Blood prevents infection by:**
  – Synthesizing and utilizing antibodies
  – Activating complement proteins
  – Activating WBCs to defend the body against foreign invaders

Blood Plasma

- **Blood Plasma - Representing 55% of whole blood**
- **Of plasma, water makes up 91.5%, while 7.5% makes up proteins.**

•1% is a mix of other solutes including electrolytes, enzymes, hormones and waste products.

Blood Plasma

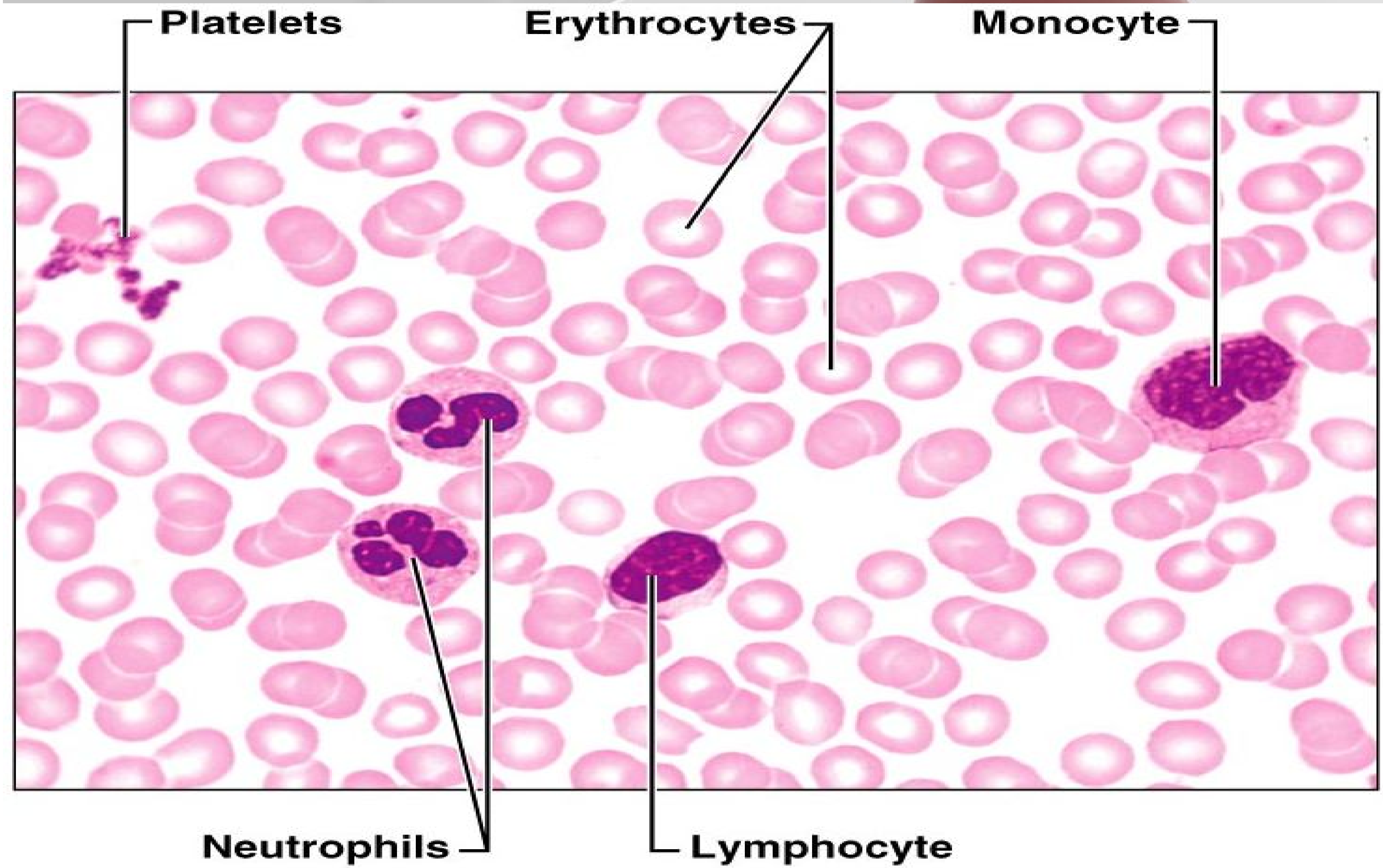
- **Blood plasma contains over 100 solutes, including:**
  - Proteins – albumin, globulins, clotting proteins, and others
  - Lactic acid, urea, creatinine
  - Organic nutrients – glucose, carbohydrates, amino acids
  - Electrolytes – sodium, potassium, calcium, chloride, bicarbonate
  - Respiratory gases – oxygen and carbon dioxide

- **There are 3 basic types of plasma proteins synthesized by hepatocytes (liver cells):**
  - ***Albumins* (54%)**
  - ***Globulins* (38%)**
  - ***Fibrinogen* (7%)**
- **Gamma globulins are also known as *immunoglobulins* or *antibodies*. bacterial or viral invasion prompts the production of millions of antibodies, which bind to an *antigen* (invader).**

TABLE 17.1	Composition of Plasma
CONSTITUENT	**DESCRIPTION AND IMPORTANCE**
Water	90% of plasma volume; dissolving and suspending medium for solutes of blood; absorbs heat
Solutes	
Plasma proteins	8% (by weight) of plasma volume; all contribute to osmotic pressure, maintaining water balance in blood and tissues; all have other functions (transport, enzymatic, etc.)
▪ Albumin	60% of plasma proteins; produced by liver; main contributor to osmotic pressure
▪ Globulins	36% of plasma proteins
alpha, beta	Produced by liver; mostly transport proteins that bind to lipids, metal ions, and fat-soluble vitamins
gamma	Antibodies released by plasma cells during immune response
▪ Fibrinogen	4% of plasma proteins; produced by liver; forms fibrin threads of blood clot
Nonprotein nitrogenous substances	By-products of cellular metabolism, such as urea, uric acid, creatinine, and ammonium salts
Nutrients (organic)	Materials absorbed from digestive tract and transported for use throughout body; include glucose and other simple carbohydrates, amino acids (digestion products of proteins), fatty acids, glycerol and triglycerides (fat products), cholesterol, and vitamins
Electrolytes	Cations include sodium, potassium, calcium, magnesium; anions include chloride, phosphate, sulfate, and bicarbonate; help to maintain plasma osmotic pressure and normal blood pH
Respiratory gases	Oxygen and carbon dioxide; oxygen mostly bound to hemoglobin inside RBCs; carbon dioxide transported bound to hemoglobin in RBCs or dissolved in plasma as bicarbonate ion or CO_2
Hormones	Steroid and thyroid hormones carried by plasma proteins

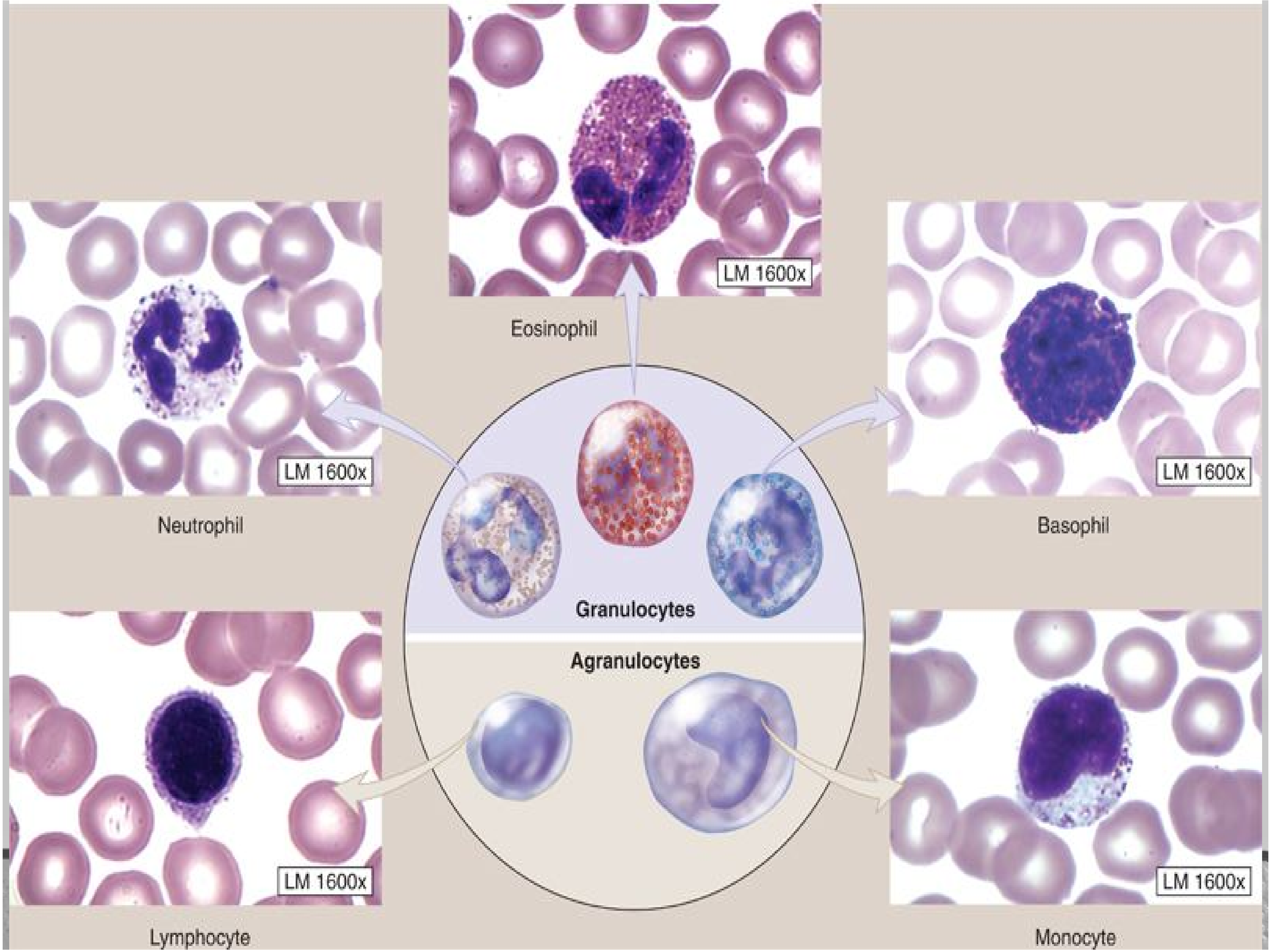
Table 17.1

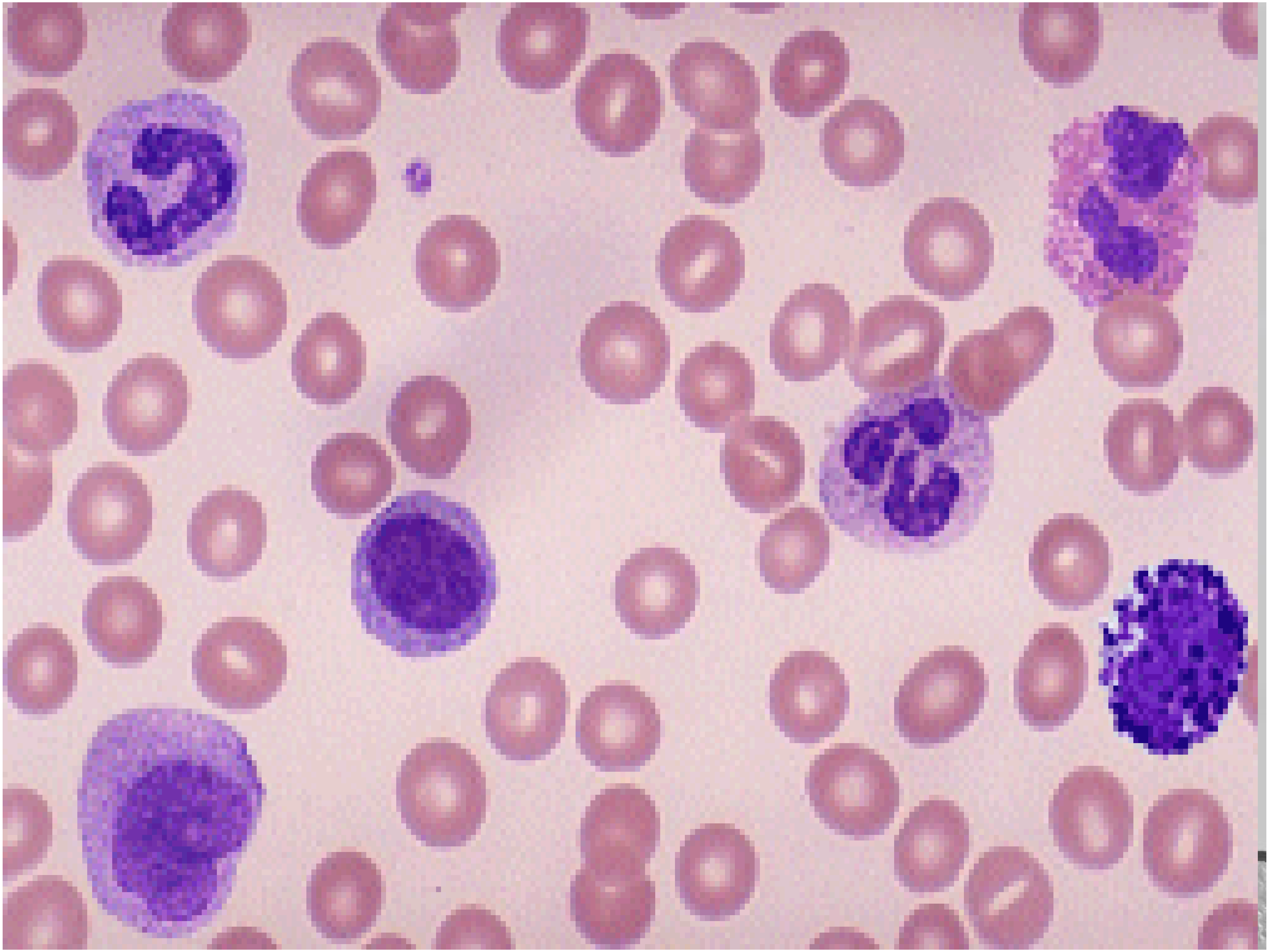
Components of Whole Blood



Formed Elements

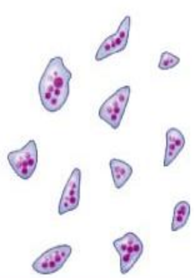
- Erythrocytes, leukocytes, and platelets make up the formed elements
  - Only WBCs are complete cells
  - RBCs have no nuclei or organelles, and platelets are just cell fragments
- Most formed elements survive in the bloodstream for only a few days
- Most blood cells do not divide but are renewed by cells in bone marrow


LM 1600x
Eosinophil
LM 1600x
Neutrophil
LM 1600x
Basophil
Granulocytes
Agranulocytes
LM 1600x
Lymphocyte
LM 1600x
Monocyte

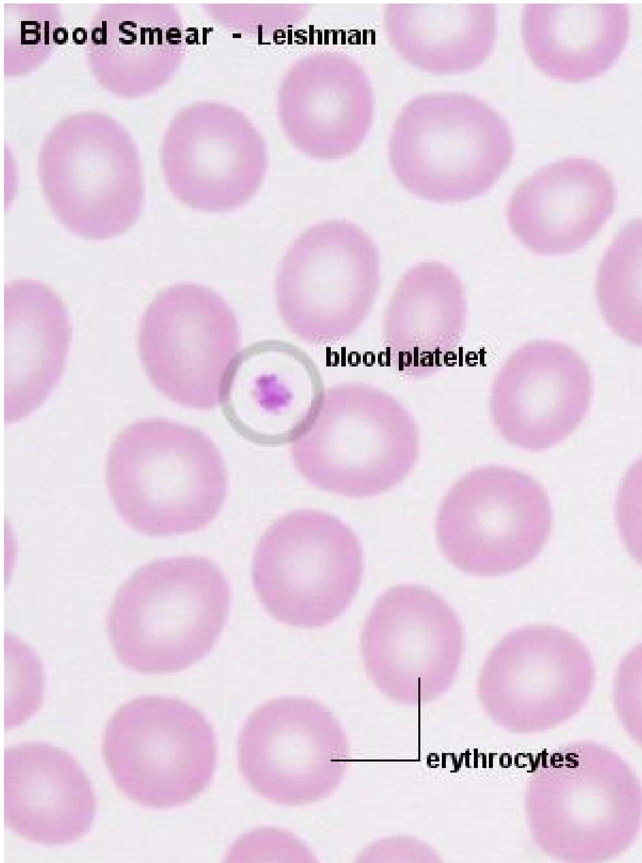


Name	Concentration	Characteristics	Functions
Erythrocytes (RBCs)	4.8 Million/microliter in females 5.4 Million/microliter in males	Biconcave disc no nucleus 120 day lifespan	Transports oxygen and carbon dioxide
Neutrophils	60% - 70% WBC concentration	Has 2-5 lobes joined by chromatin with a fine, pale granular cytoplasm	Phagocytosis. Uses lysozyme, super oxides and peroxides to destroy antigens.
Eosinophils	2% - 4% WBC concentration	has 2 - 3 lobes with large red/orange granules.	Responds to histamine in allergic reactions. Phagocytosis. destroys parasitic worms.
Basophils	0.5% - 1% WBC concentration	2 lobes with large, deep blue/purple granules	Intensify inflammatory response by liberating heparin, histamine and seratonin
Lymphocytes	20% - 25% WBC concentration	T/B cells. Cytoplasm forms ring around nucleus	Mediates immune response. B cells become plasma cells that form antibodies. T cells attack viruses, cancer cells and transplanted tissue cells.
Monocytes	3% - 8% WBC concentration	kidney-shaped nucleus with blue/gray cytoplasm	Becomes machrophage before phagocytosis
Thrombocytes (Platelets)	150,000 - 400,000/microliter	cells fragments, no nucleus	Forms platelet plug in hemostasis, and releases chemicals to promote vascular spasms and blood clotting.

TABLE 17.2 Summary of Formed Elements of the Blood *(continued)*

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Agranulocytes					
▪ Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 μm	1500–3000	D: days to weeks LS: hours to years	Mount immune response by direct cell attack or via antibodies
▪ Monocyte		Nucleus U or kidney shaped; gray-blue cytoplasm; diameter 14–24 μm	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 μm	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

*Appearance when stained with Wright's stain.



Erythrocytes

Life cycle

1. erythropoiesis
   a. negative - feedback regulation
      1. erythropoietin
2. 120 days ---> destruction
3. recycling bilirubin; jaundice

Erythropoesis-Brief

- Bone marrow
  - Pluripotent stem cells
  - Chemical regulation
    - Cytokines
    - Erythroid specific growth factor
    - Erythropoietin (EPO)
  - Life span
    - Reticulocyte- 4 days
    - RBC –120 days

Production of Erythrocytes

- **Hematopoiesis** – blood cell formation
- Hematopoiesis occurs in the red bone marrow of the:
  - Axial skeleton and girdles
  - Epiphyses of the humerus and femur
- **Hemocytoblasts** give rise to all formed elements

ERYTHROPOIESIS: SITES / PHASES

- **INTRAUTERINE LIFE:**
  - **INTRAVASCULAR PHASE: Upto 3rd month of Intra Uterine Life.**
    - **Endothelial cells = = = RBCs**
  - **HEPATIC PHASE: 3rd to 5th month IUL**
    - **Liver & Spleen**
    - **nRBCs from Mesenchymal cells.**
  - **MYELOID PHASE: From 5th month of IUL onwards.**

ERYTHROPOIESIS: SITES / PHASES contd.

- **POST NATAL LIFE:**
  - **CHILDREN:**
    - **Predominantly Red Bone Marrow of skeleton:**
      - **Axial &**
      - **Appendicular.**
  - **ADULTS:**
    - **Red Bone Marrow of Axial Skeleton.**

Production of Erythrocytes: Erythropoiesis

- A **hemocytoblast is transformed into a proerythroblast**
- Proerythroblasts develop into early erythroblasts
- The developmental pathway consists of three phases
  - ***1 – ribosome synthesis in early erythroblasts***
  - ***2 – Hb accumulation in late erythroblasts and normoblasts***
  - ***3 – ejection of the nucleus from normoblasts and formation of reticulocytes***
- Reticulocytes then become mature erythrocytes

ERYTHROPOIESIS

PROERYTHROBLAST

BASOPHILIC ERYTHROBLAST

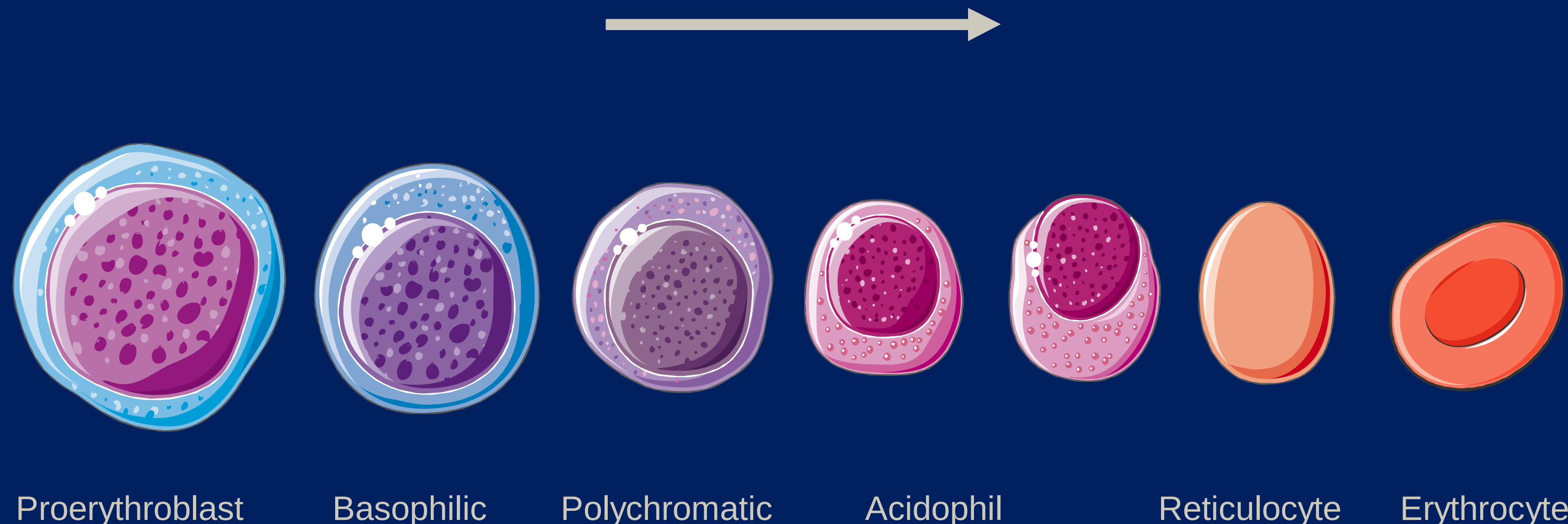
POLYCHROMATOPHILIC ERYTHROBLAST

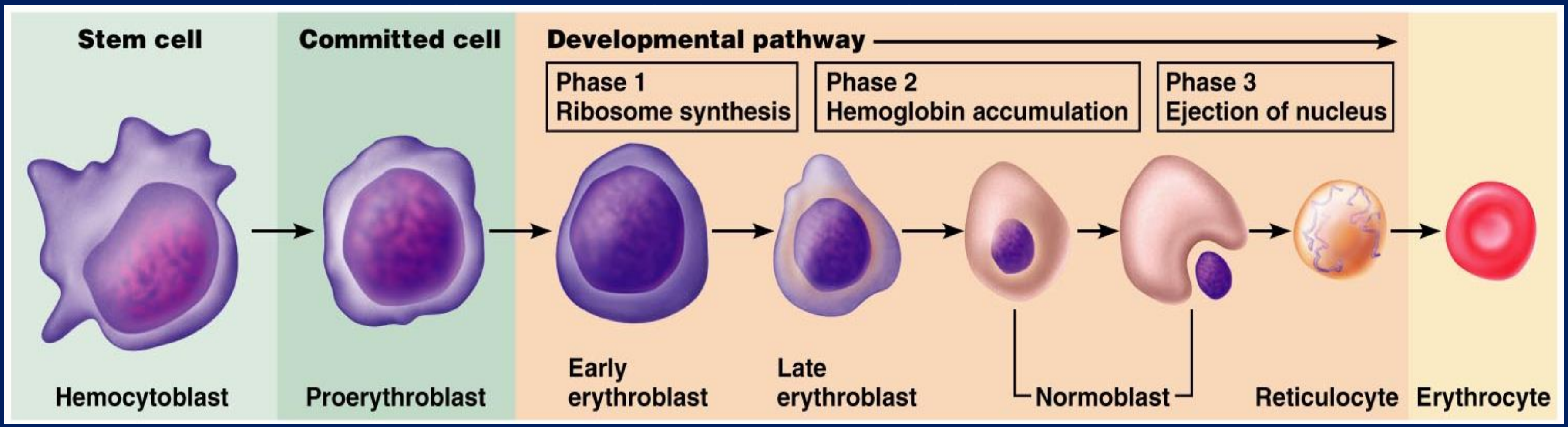
ORTHOCHROMATIC ERYTHROBLAST

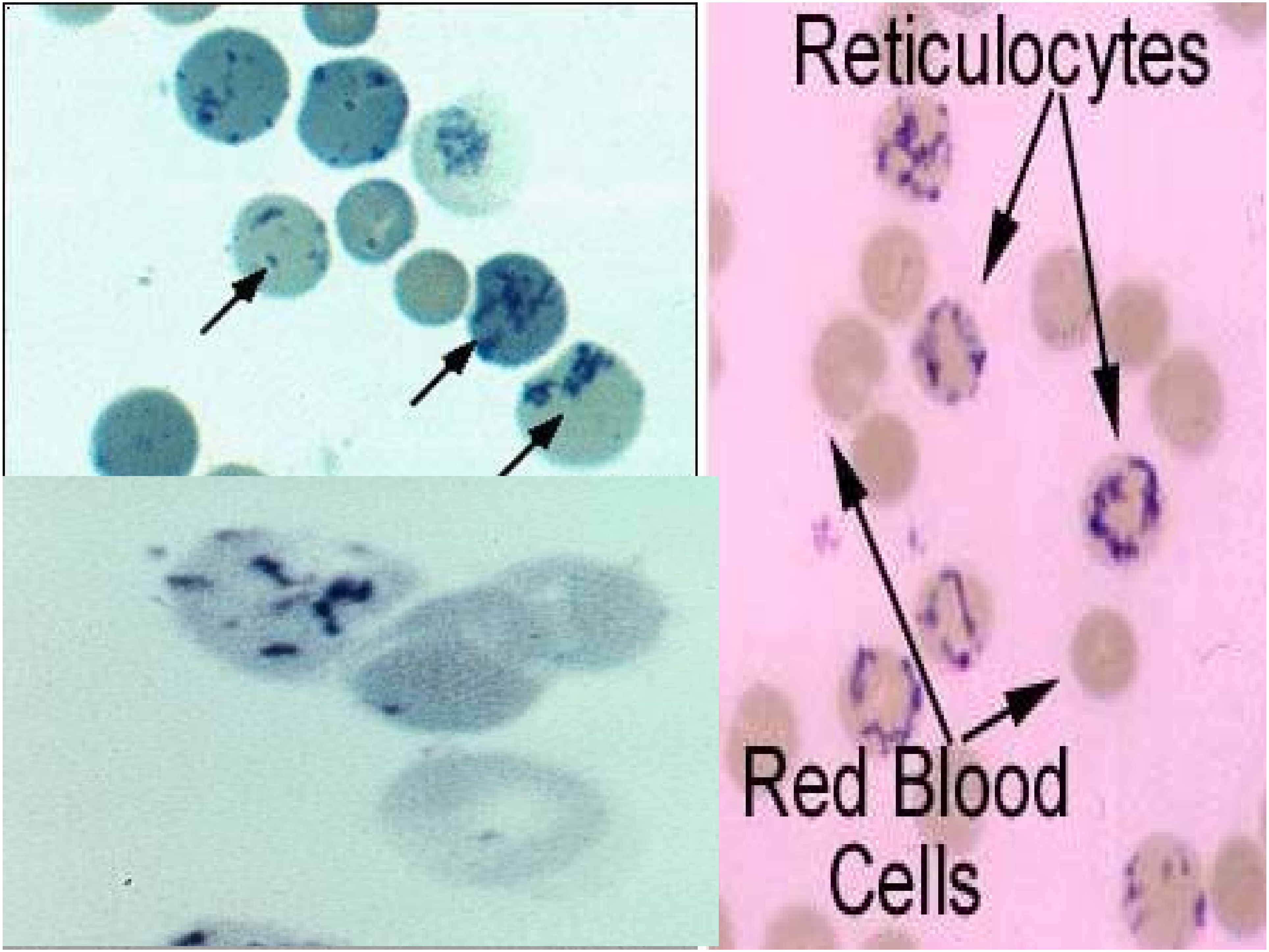
RETICULOCYTE

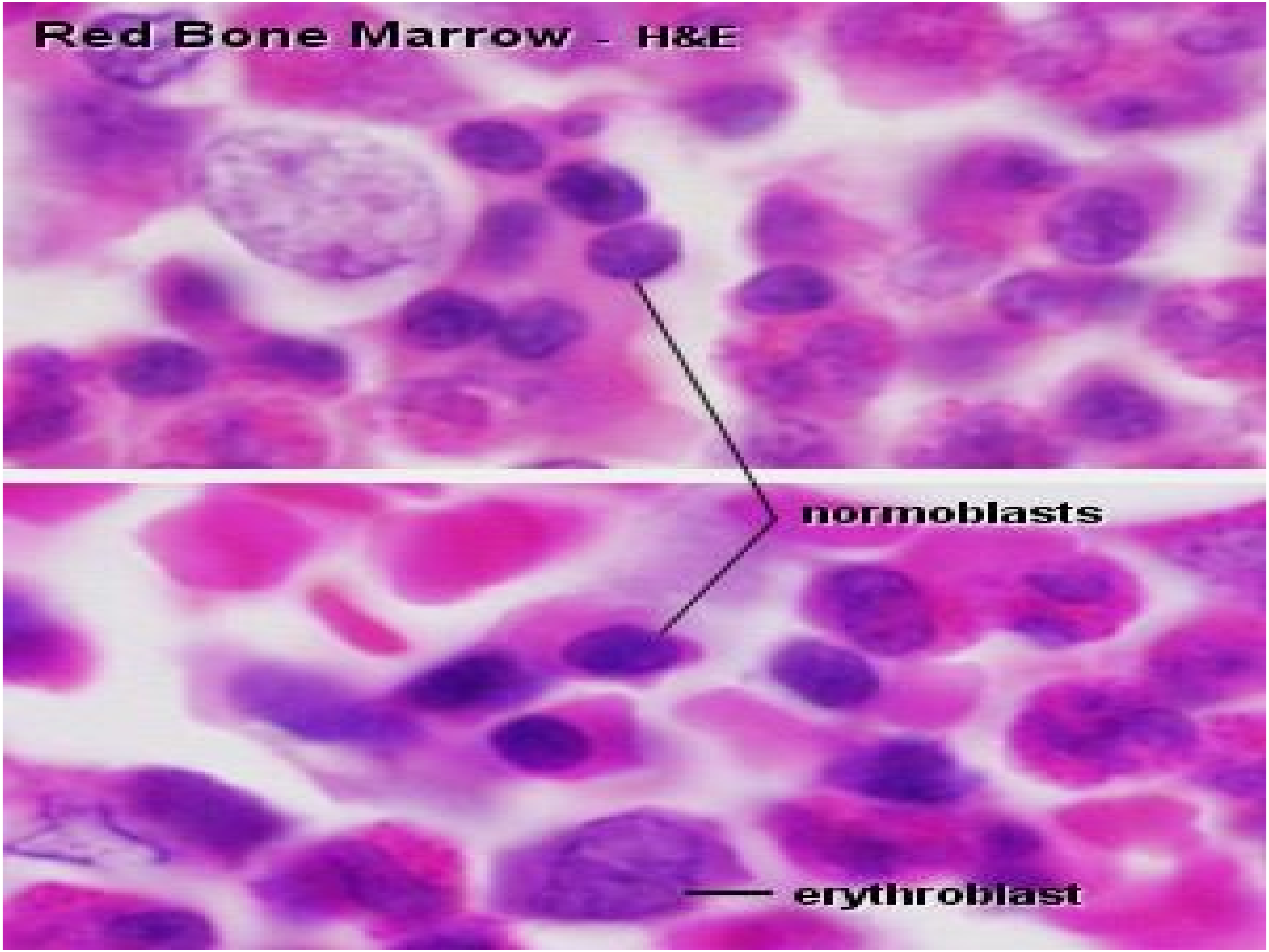
MATURE ERYTHROCYTES

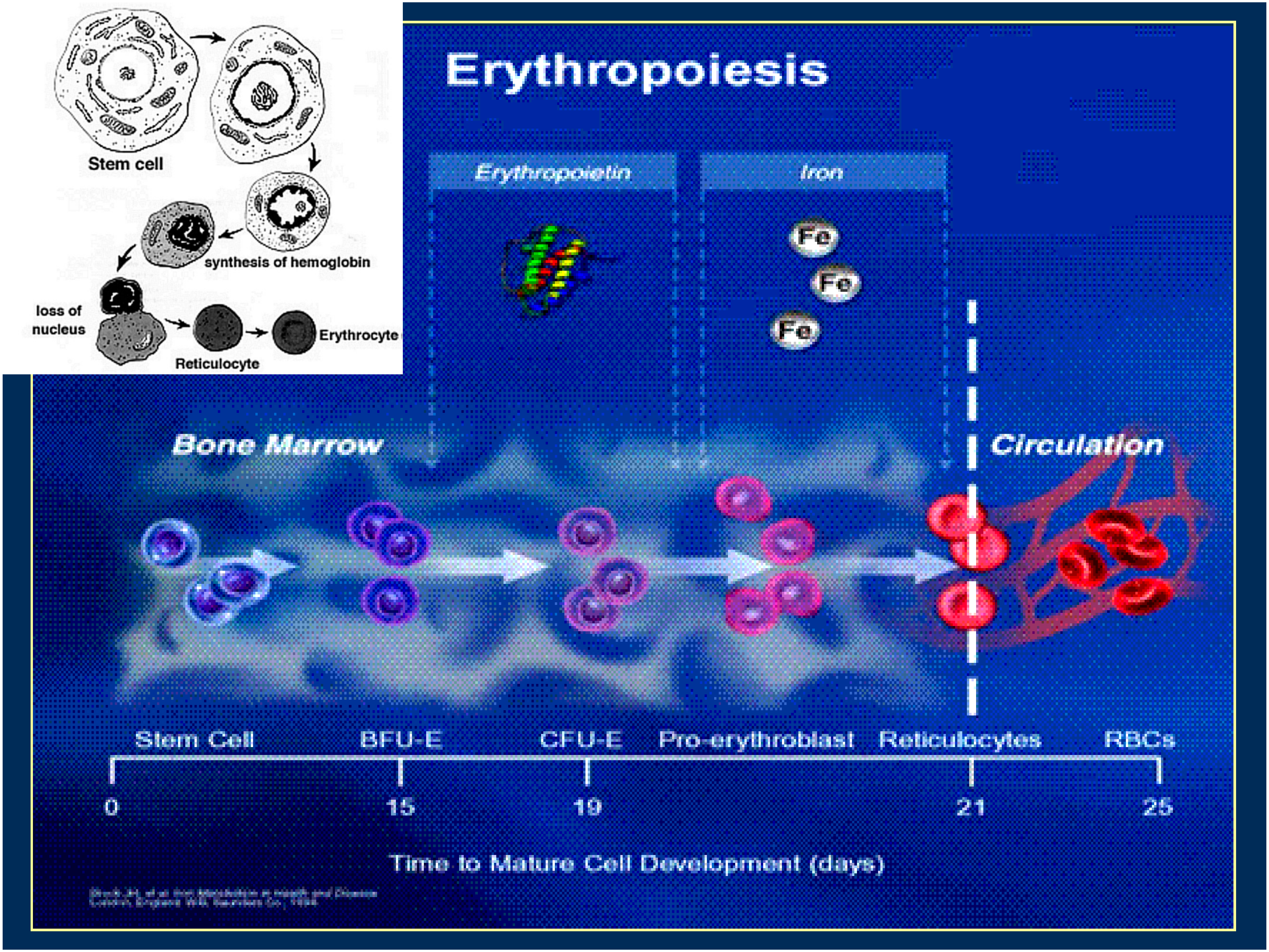
Erythropoiesis

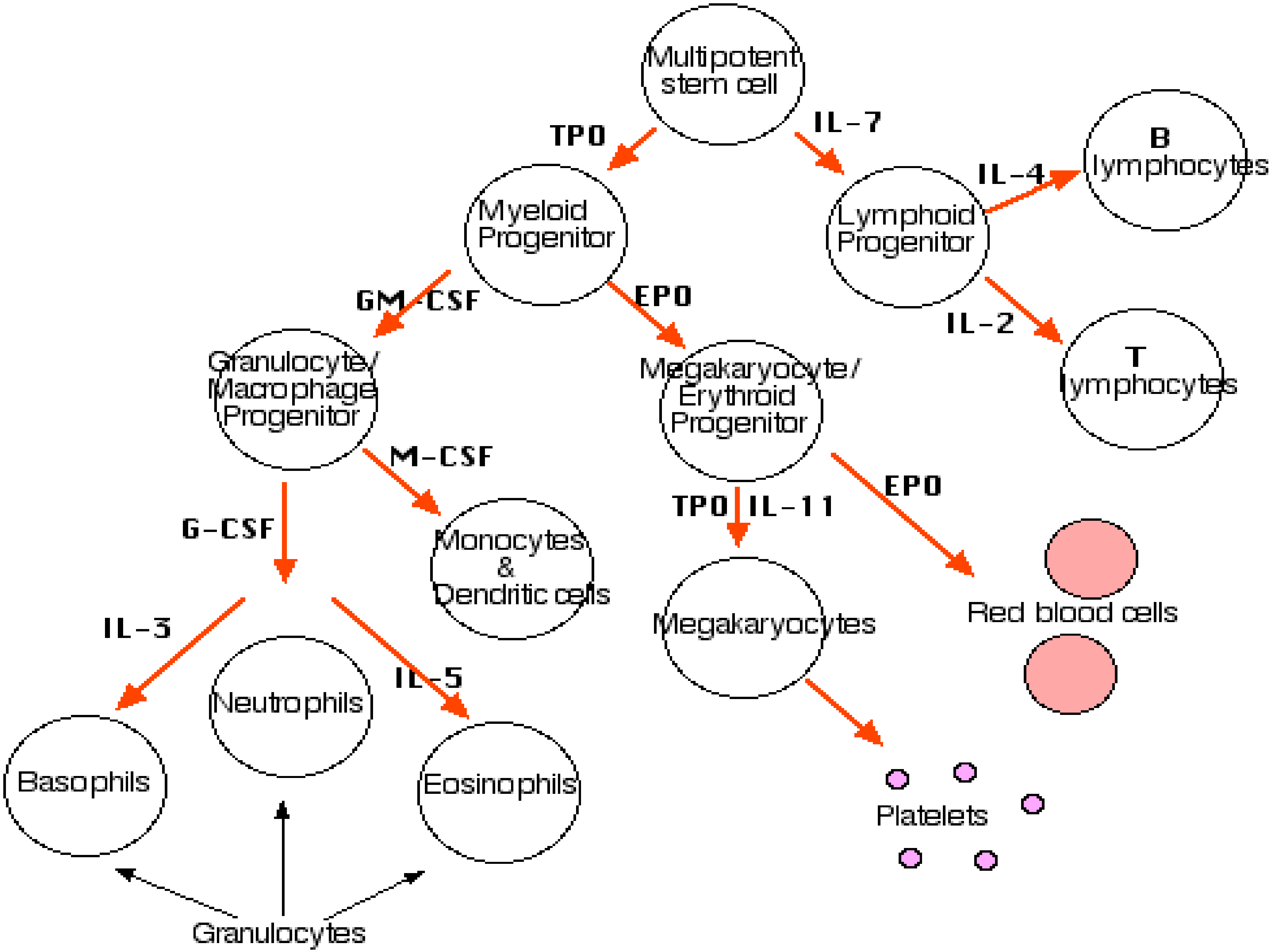





Reticulocytes
Red Blood
Cells


Red Bone Marrow - H&E
normoblasts
erythroblast


Erythropoiesis
Stem cell
synthesis of hemoglobin
loss of nucleus
Reticulocyte
Erythrocyte
Erythropoietin
Iron
Fe
Fe
Fe
Bone Marrow
Circulation
Stem Cell
BFU-E
CFU-E
Pro-erythroblast
Reticulocytes
RBCs
0
15
19
21
25
Time to Mature Cell Development (days)


Multipotent stem cell
TPO
IL-7
Myeloid Progenitor
Lymphoid Progenitor
IL-4
B lymphocytes
GM-CSF
EPO
IL-2
T lymphocytes
Granulocyte/ Macrophage Progenitor
Megakaryocyte/ Erythroid Progenitor
M-CSF
EPO
G-CSF
TPO
IL-11
Monocytes & Dendritic cells
Megakaryocytes
Red blood cells
IL-3
IL-5
Neutrophils
Basophils
Eosinophils
Platelets
Granulocytes

FACTORS REGULATING ERYTHROPOIESIS

- **SINGLE MOST IMPORTANT REGULATOR: "TISSUE OXYGENATION"**
- **BURST PROMOTING ACTIVITY**
- **ERYTHROPOIETIN**
- **IRON**
- **VITAMINS:**
  - **Vitamin B_{12}**
  - **Folic Acid**
- **MISCELLANEOUS**

Regulation and Requirements for Erythropoiesis

- Circulating erythrocytes – the number remains constant and reflects a balance between RBC production and destruction
  - **Too few RBCs leads to tissue hypoxia**
  - **Too many RBCs causes undesirable blood viscosity**

- Erythropoiesis is hormonally controlled and depends on adequate supplies **of iron, amino acids, and B vitamins**

Hormonal Control of Erythropoiesis

- **Erythropoietin (EPO) release by the kidneys is triggered by:**
  - Hypoxia due to decreased RBCs
  - Decreased oxygen availability
  - Increased tissue demand for oxygen

- **Enhanced erythropoiesis increases the**:
  - RBC count in circulating blood
  - Oxygen carrying ability of the blood

Erythropoietin Mechanism

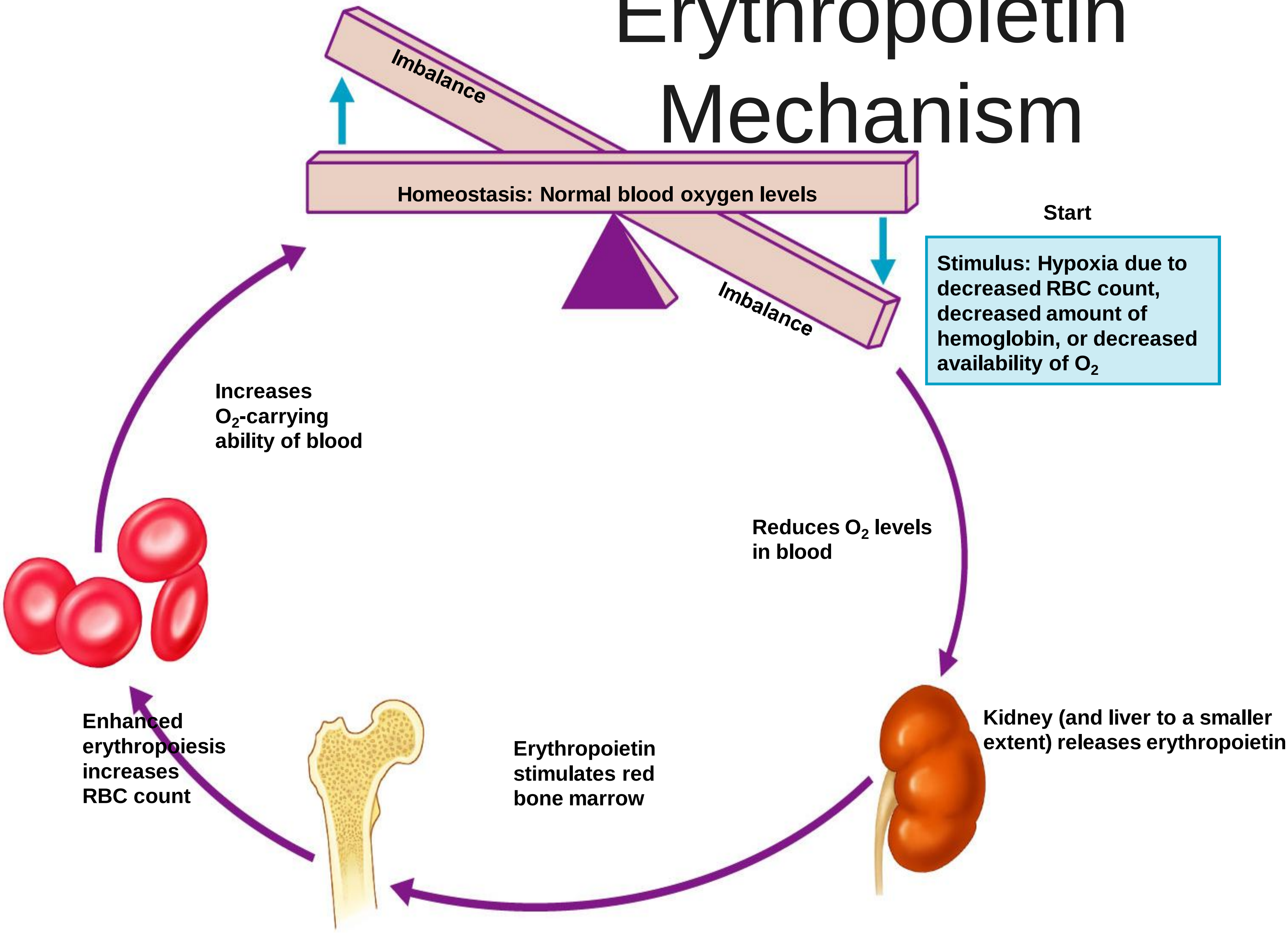


Figure 17.6

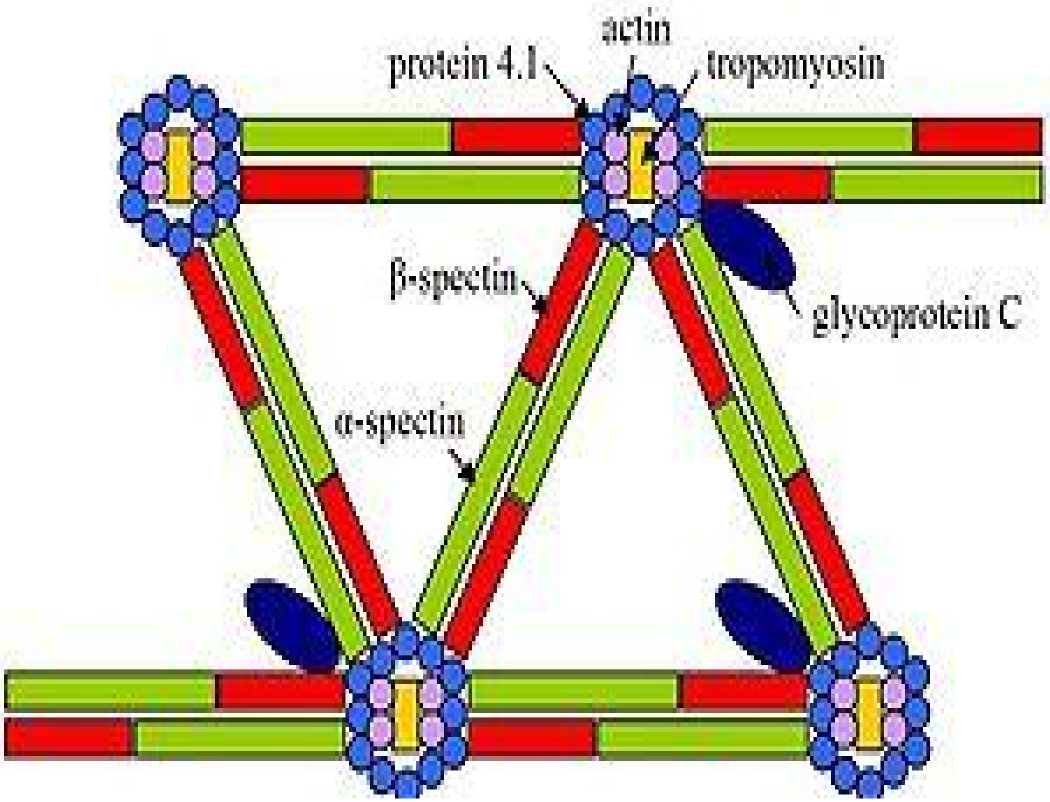
Erythrocytes (RBCs)

- Erythrocytes are an example of the complementarity of structure and function
- **Structural characteristics contribute to its gas transport function**
  - **Biconcave** shape has a huge surface area relative to volume
  - Erythrocytes are more than **97% hemoglobin**
  - **ATP is generated anaerobically**, so the erythrocytes do not consume the oxygen they transport

Erythrocytes (RBCs)

- Biconcave discs, anucleate, essentially no organelles
- Filled with hemoglobin (Hb), a protein that functions in gas transport
- Contain the plasma membrane protein **spectrin** and other proteins that:
  - Give erythrocytes their flexibility
  - Allow them to change shape as necessary

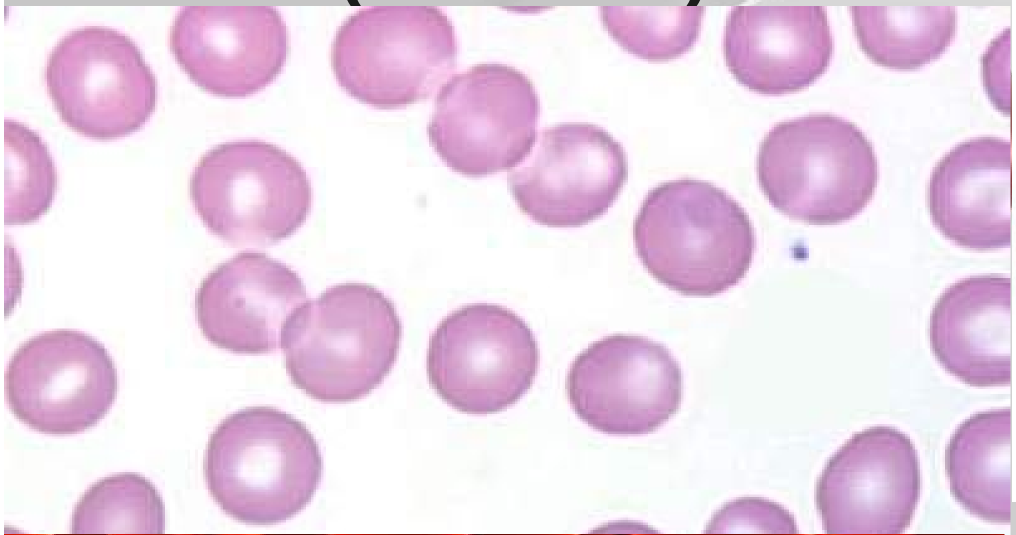
A schematic diagram of **spectrin** and other cytoskeletal molecules

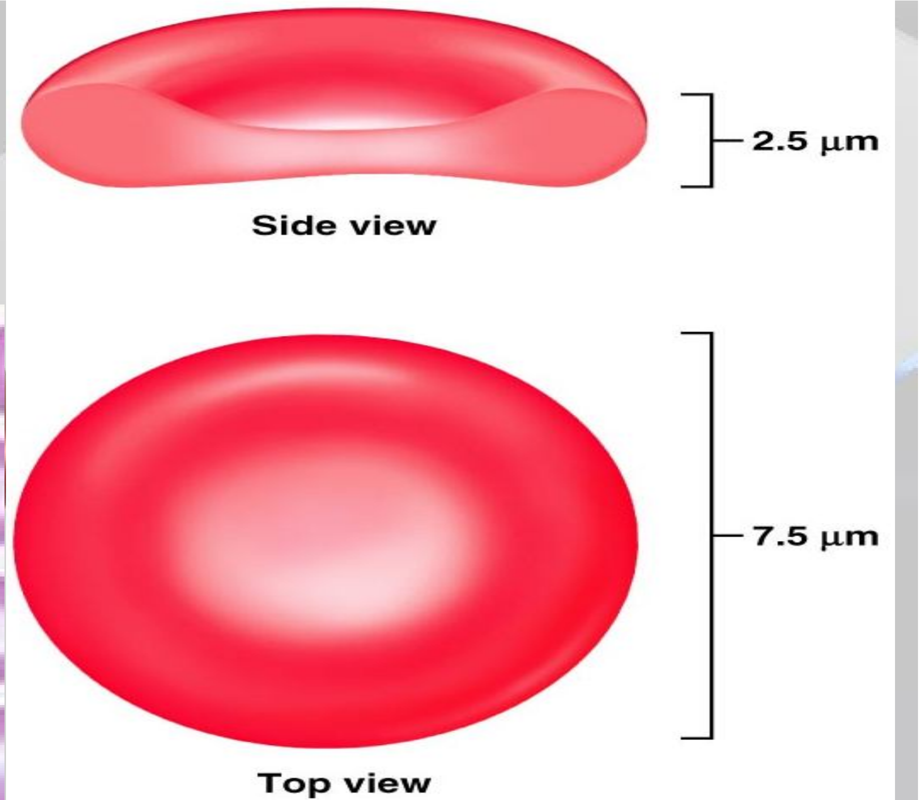


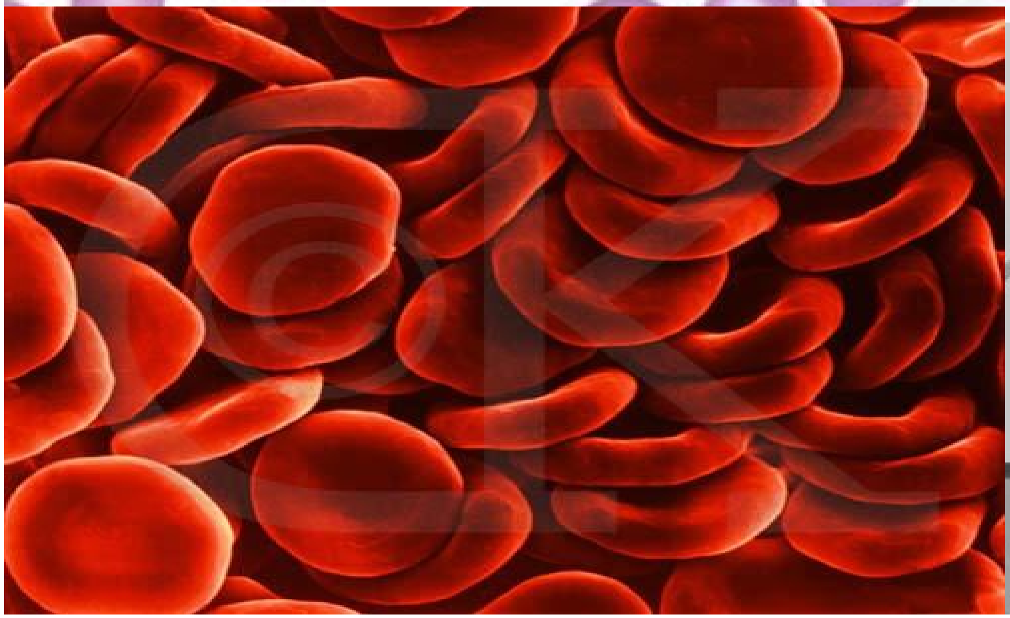
The erythrocyte model demonstrates the importance of the spectrin cytoskeleton in that mutations in spectrin commonly cause hereditary defects of the erythrocyte, including

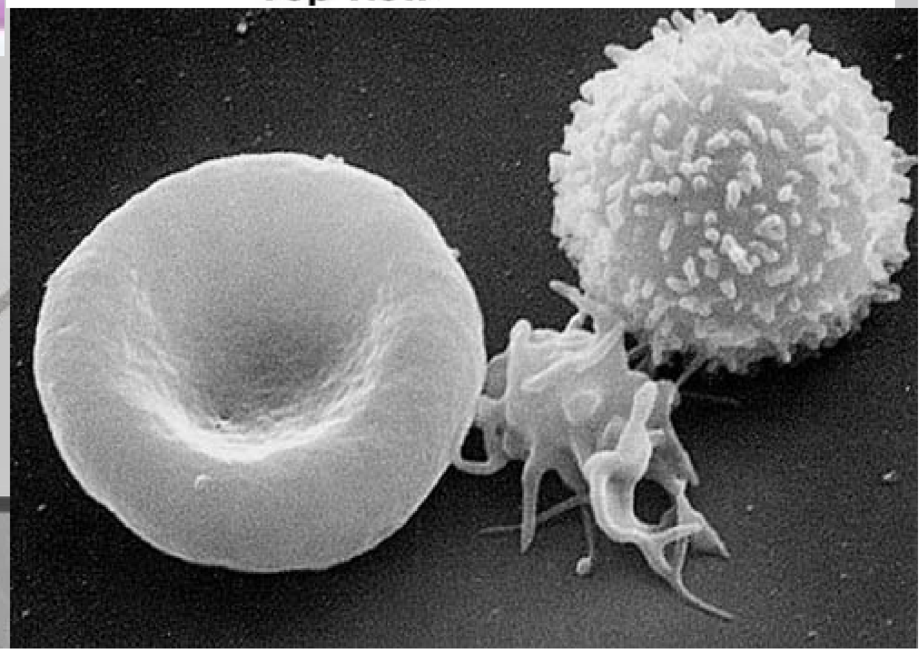
- **hereditary elliptocytosis**
- **Hereditary spherocytosis**

Erythrocytes (RBCs)

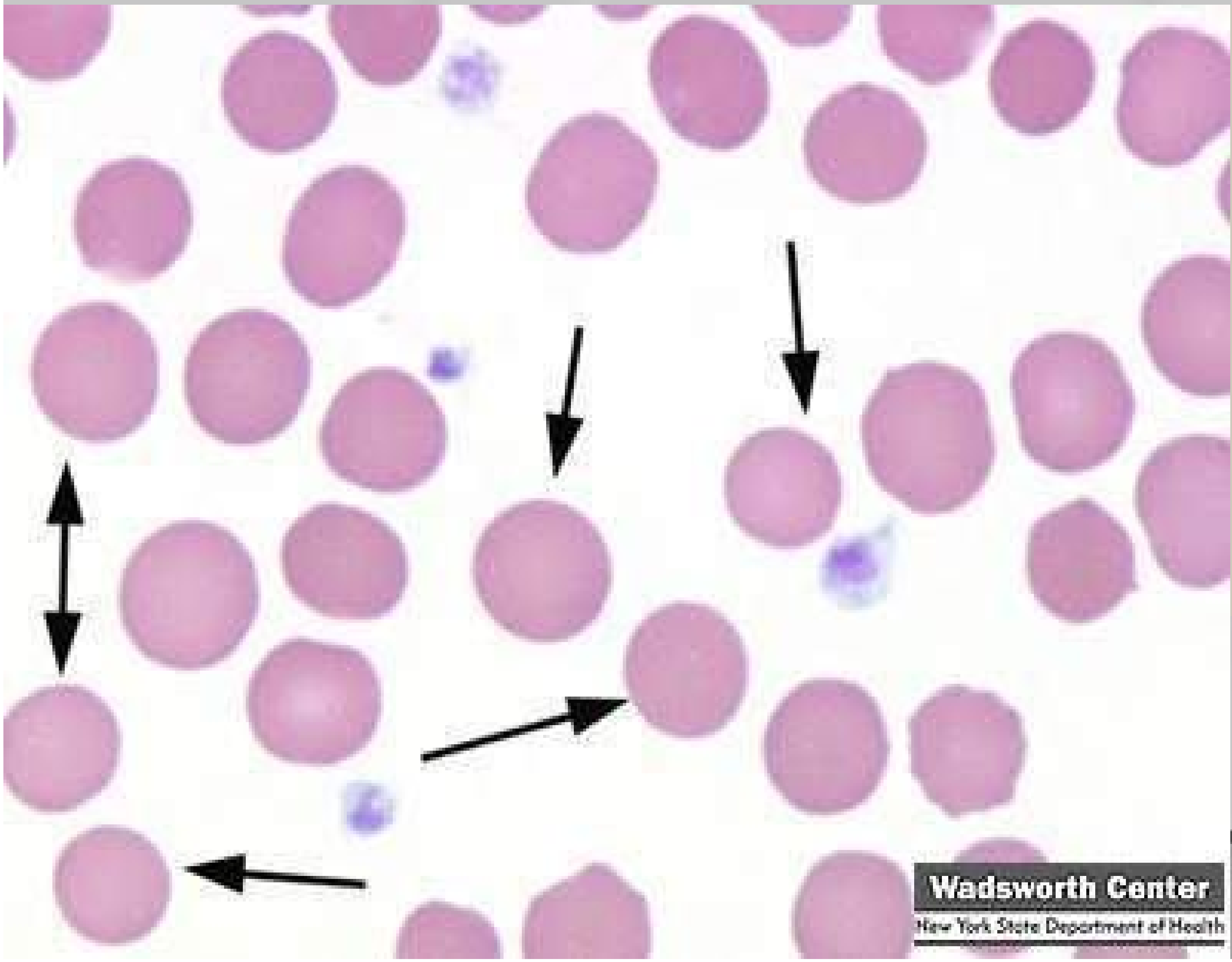






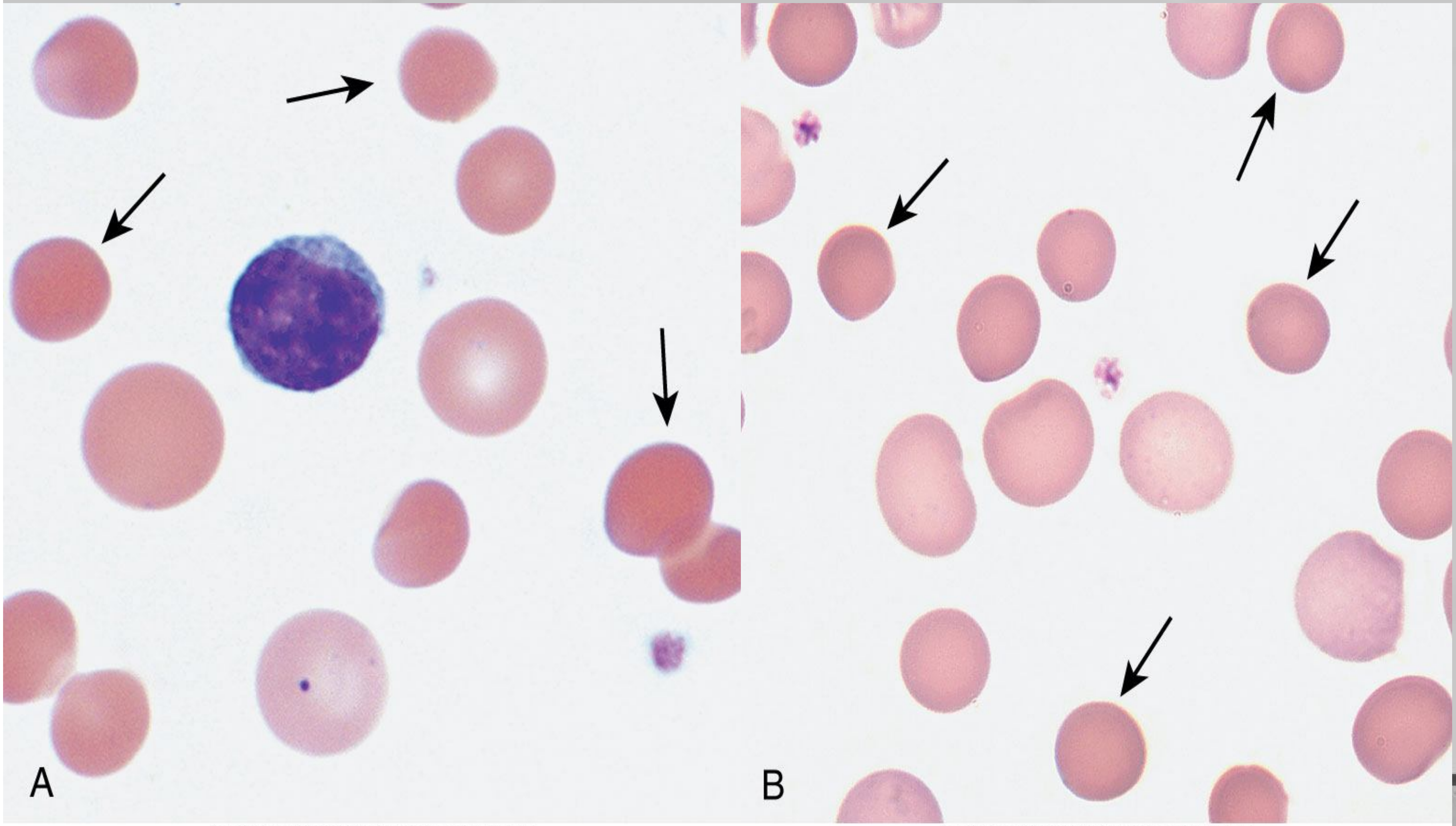


Hereditary Spherocytosis



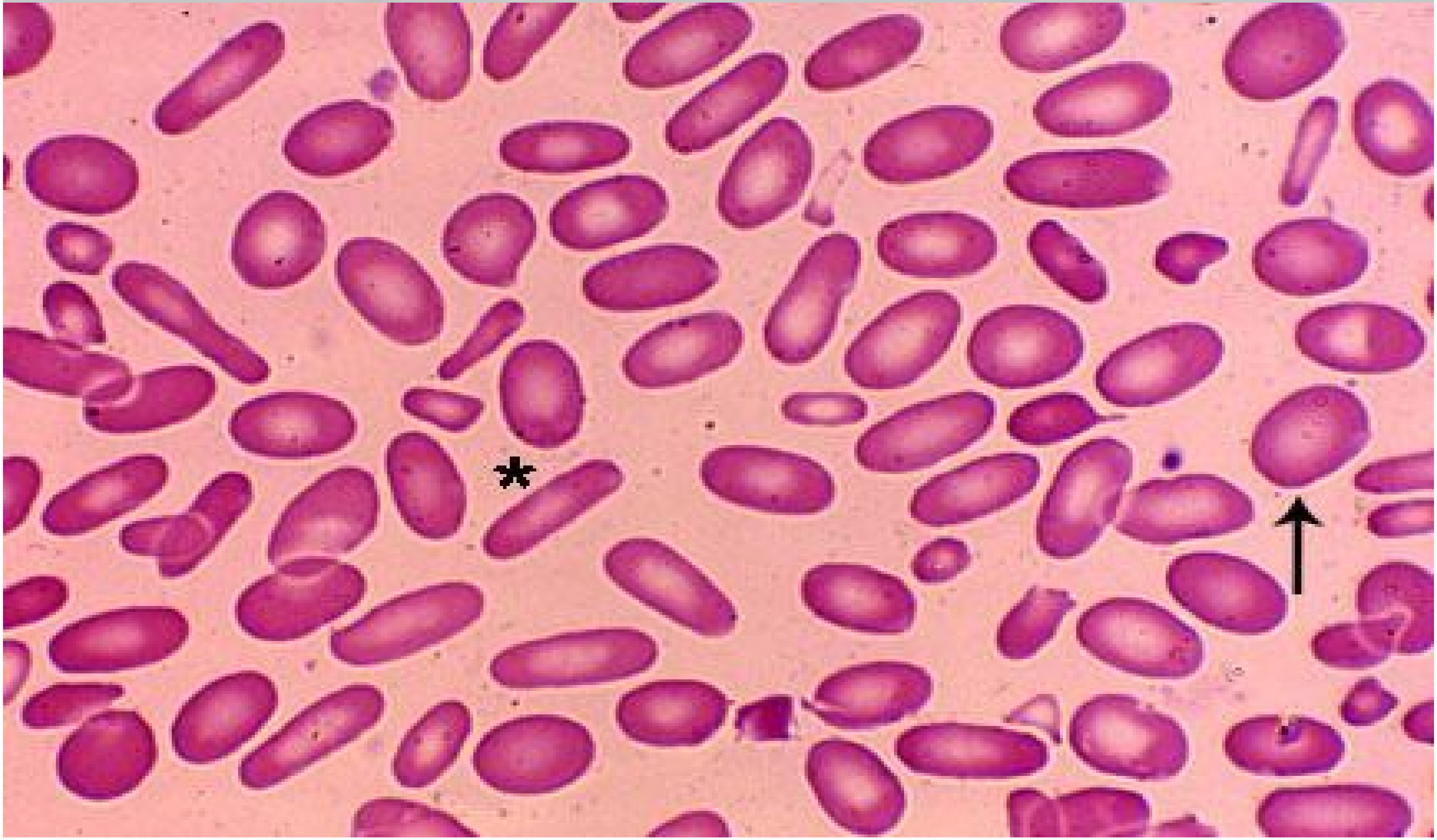
Hereditary spherocytosis (HS) is a familial hemolytic disorder with marked heterogeneity of clinical features, ranging from an asymptomatic condition to fulminant hemolytic anemia.

Spherocytes



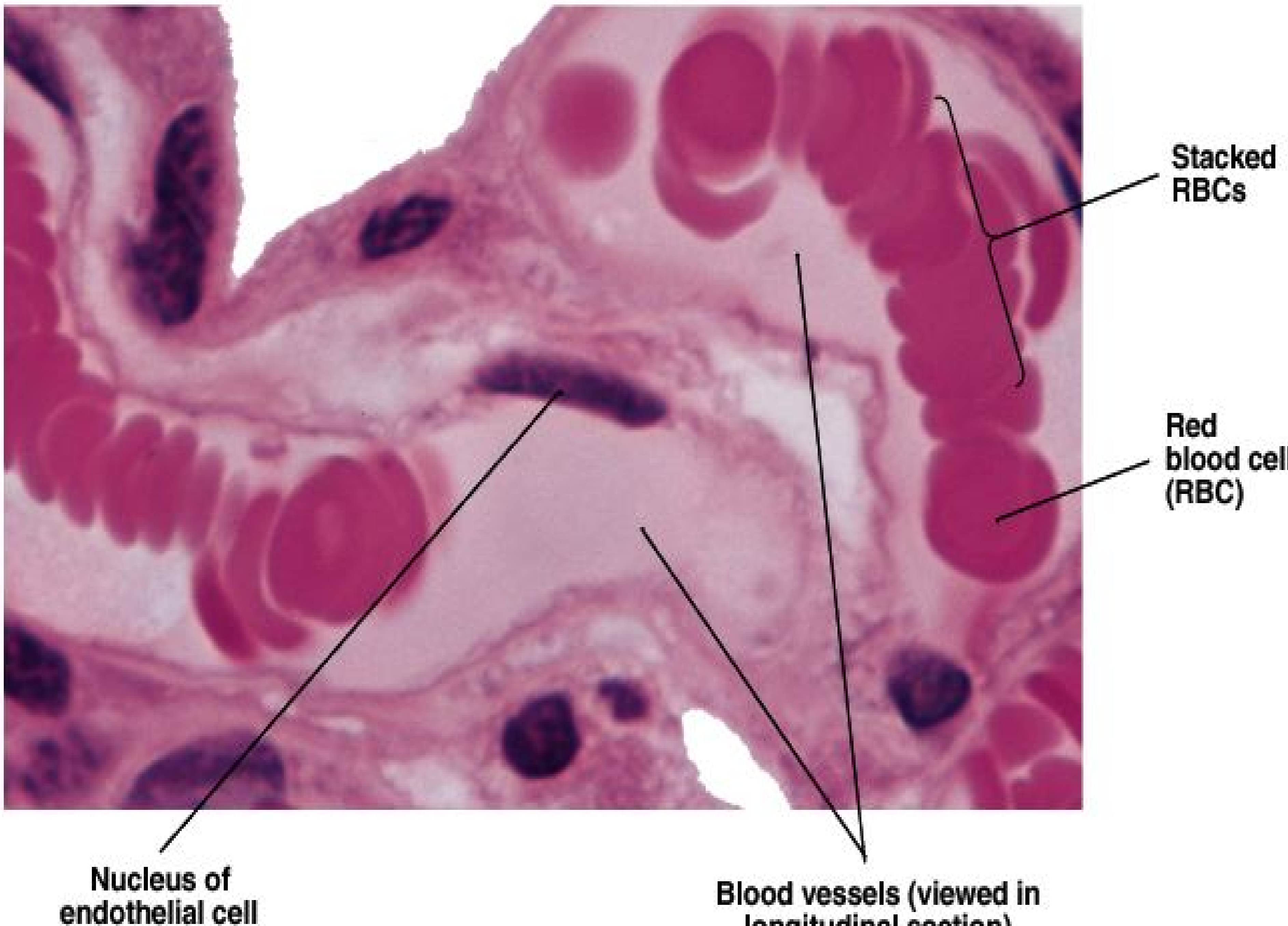
Graphics accessed http://evolvels.elsevier.com/section/default.asp?id=1138_ccalvo7_0001, 2009.

hereditary elliptocytosis

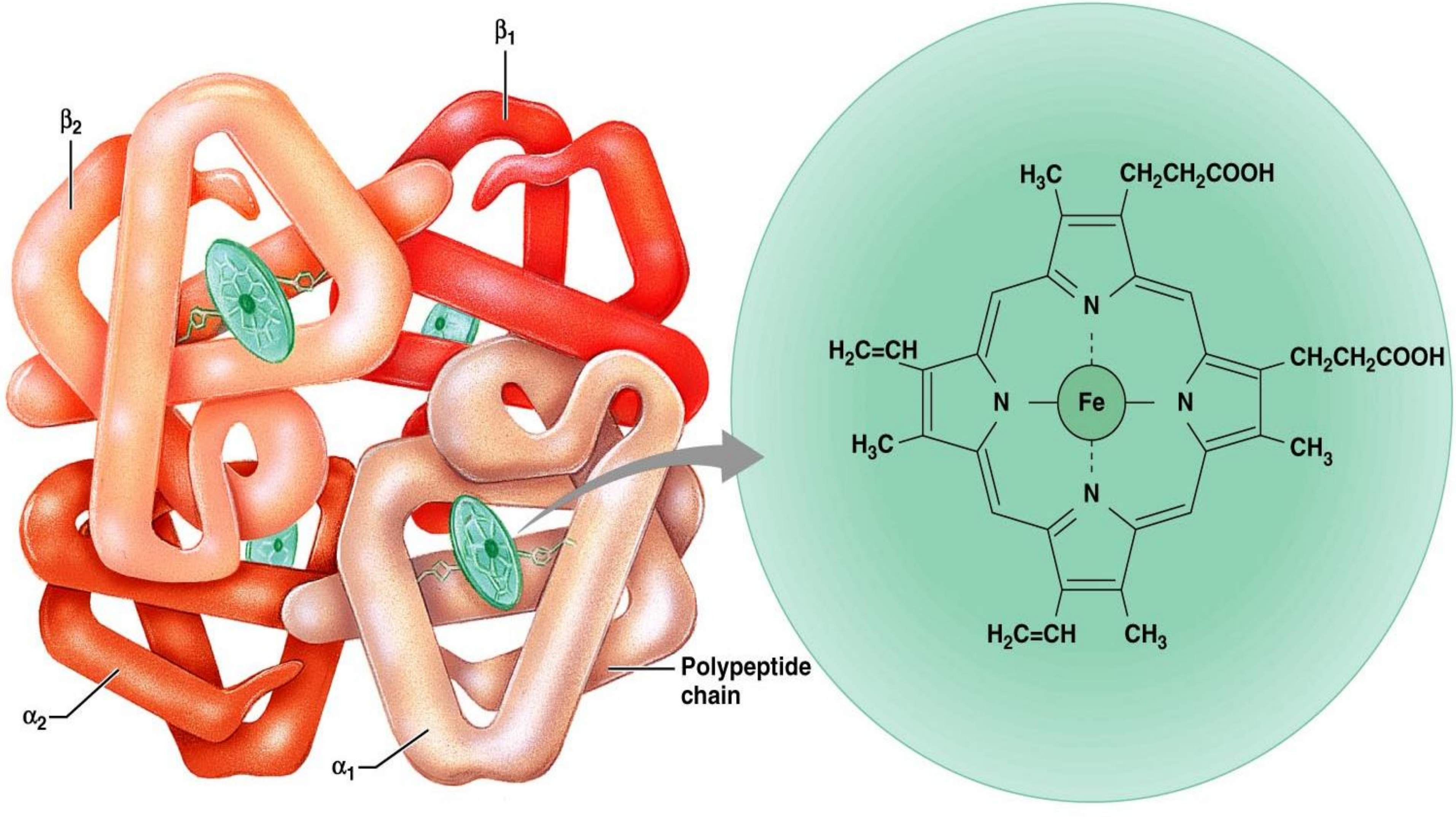


Erythrocyte Function

- RBCs are dedicated to **respiratory gas transport**
- **Hb reversibly binds with oxygen and most oxygen in the blood is bound to Hb**
- **Hb is composed of the protein globin, made up of two alpha and two beta chains, each bound to a heme group**
- **Each heme group bears an atom of iron, which can bind to one oxygen molecule**
- **Each Hb molecule can transport four molecules of oxygen**

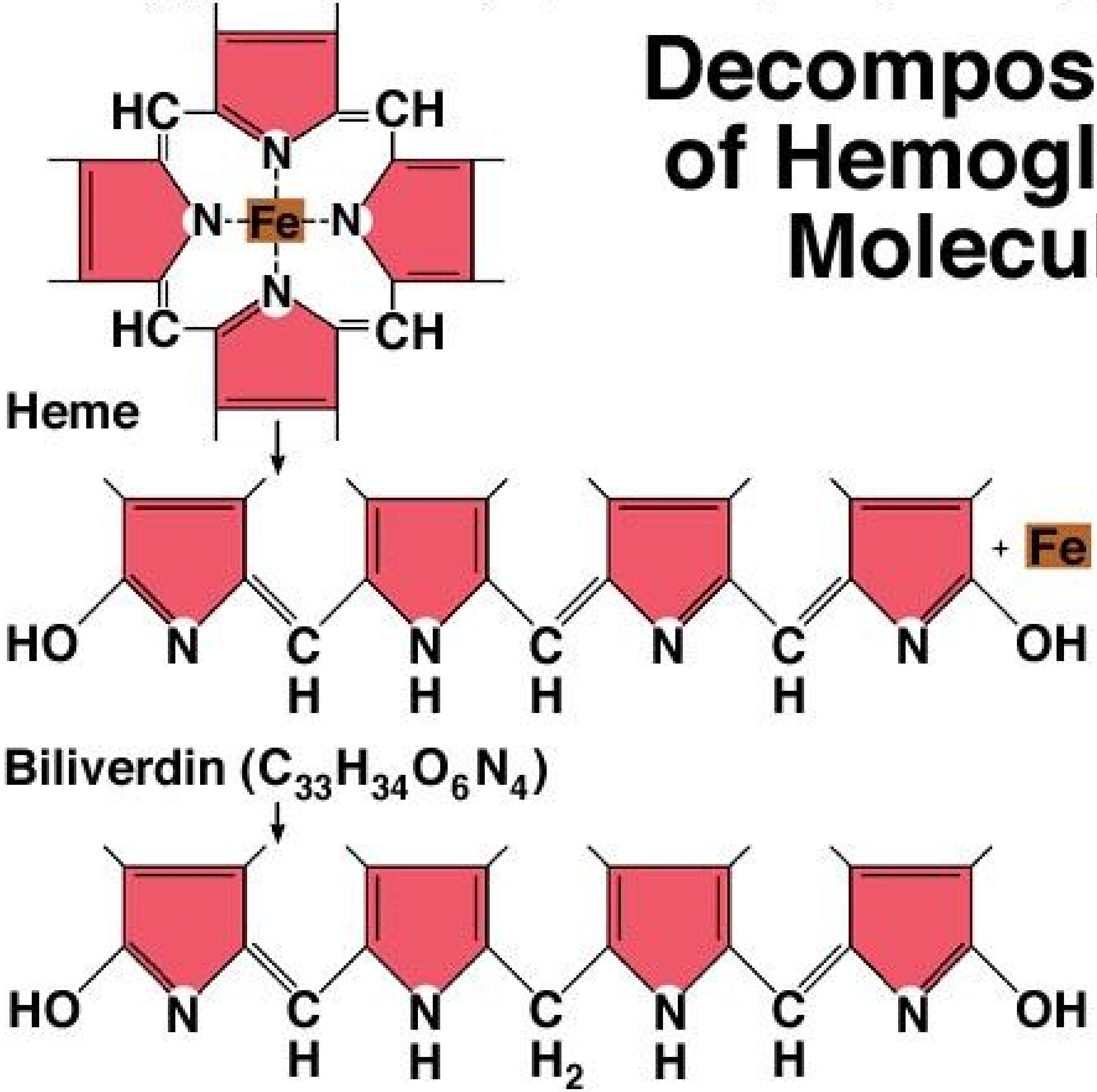

Stacked
RBCs
Red
blood cell
(RBC)
Nucleus of
endothelial cell
Blood vessels (viewed in
longitudinal section)

Structure of Hemoglobin



(a) Hemoglobin **(b) Iron-containing heme group**

Decomposition of Hemoglobin Molecule



Heme

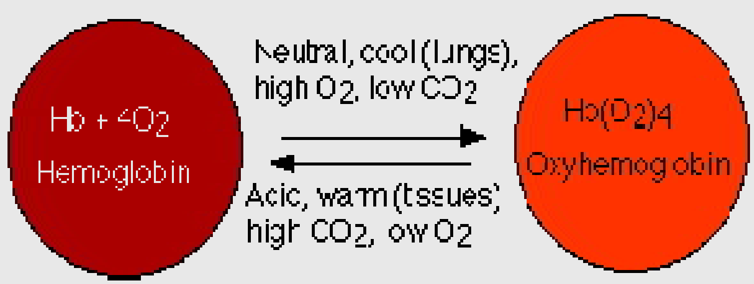
Biliverdin ($C_{33}H_{34}O_6N_4$)

Bilirubin ($C_{33}H_{36}O_6N_4$)

Hemoglobin (Hb)

- **Oxyhemoglobin** – Hb bound to oxygen
  - Oxygen loading takes place in the lungs

- **Deoxyhemoglobin** – Hb after oxygen diffuses into tissues (reduced Hb)
- **Carbaminohemoglobin** – Hb bound to carbon dioxide
  - Carbon dioxide loading takes place in the tissues



- Men: 13.5 to 16.5 g/dl
- Women: 12.1 to 15.1 g/dl
- Children: 11 to 16 g/dl
- Pregnant women: 11 to 12 g/dl

Name of Hemoglobin	Subunit Structure	Time of Expression
Hemoglobin Portland	$\zeta_2\gamma_2$	Embryonic
Hemoglobin Gower I	$\zeta_2\varepsilon_2$	Embryonic
Hemoglobin Gower II	$\alpha_2\varepsilon_2$	Embryonic
Hemoglobin F	$\alpha_2\gamma_2$	Fetal
Hemoglobin Barts	γ_4	Fetal (pathologic Hb secondary to absence of all 4 α globulin genes; fatal in utero)
Hemoglobin A_2	$\alpha_2\delta_2$	Minor adult hemoglobin
Hemoglobin A	$\alpha_2\beta_2$	Major adult hemoglobin

<u>*Other oxygen-binding proteins*</u>

- ***Myoglobin****: Found in the muscle tissue of many vertebrates, including humans, it gives muscle tissue a distinct red or dark gray color. It is very similar to hemoglobin in structure and sequence, but is not a tetramer; instead, it is a monomer that lacks cooperative binding. It is used to store oxygen rather than transport it.*
- ***Hemocyanin****: The second most common oxygen-transporting protein found in nature, it is found in the blood of many arthropods and molluscs. Uses copper prosthetic groups instead of iron heme groups and is blue in color when oxygenated.*
- ***Hemerythrin****: Some marine invertebrates and a few species of annelid use this iron-containing non-heme protein to carry oxygen in their blood. Appears pink/violet when oxygenated, clear when not.*
- ***Chlorocruorin****: Found in many annelids, it is very similar to erythrocruorin, but the heme group is significantly different in structure. Appears green when deoxygenated and red when oxygenated.*
- ***Vanabins****: Also known as* ***vanadium chromagens****, they are found in the blood of sea squirts and are hypothesised to use the rare metal vanadium as its oxygen binding prosthetic group.*
- ***Erythrocruorin****: Found in many annelids, including earthworms, it is a giant free-floating blood protein containing many dozens — possibly hundreds — of iron- and heme-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million daltons.*
- ***Pinnaglobin****: Only seen in the mollusc Pinna squamosa. Brown manganese-based porphyrin protein.*
- ***Leghemoglobin****: In leguminous plants, such as alfalfa or soybeans, the nitrogen fixing bacteria in the roots are protected from oxygen by this iron heme containing oxygen-binding protein. The specific enzyme protected is nitrogenase, which is unable to reduce nitrogen gas in the presence of free oxygen.*
-

ERYTHROPOIETIN

- **A hormone produced by the Kidney.**
- **A circulating Glycoprotein**
- **Nowadays available as Synthetic Epoietin**
- **Acts mainly on CFU – E.**
- **Increases the number of:**
  - **Nucleated precursors in the marrow.**
  - **Reticulocytes & Mature Erythrocytes in the blood.**

VITAMINS

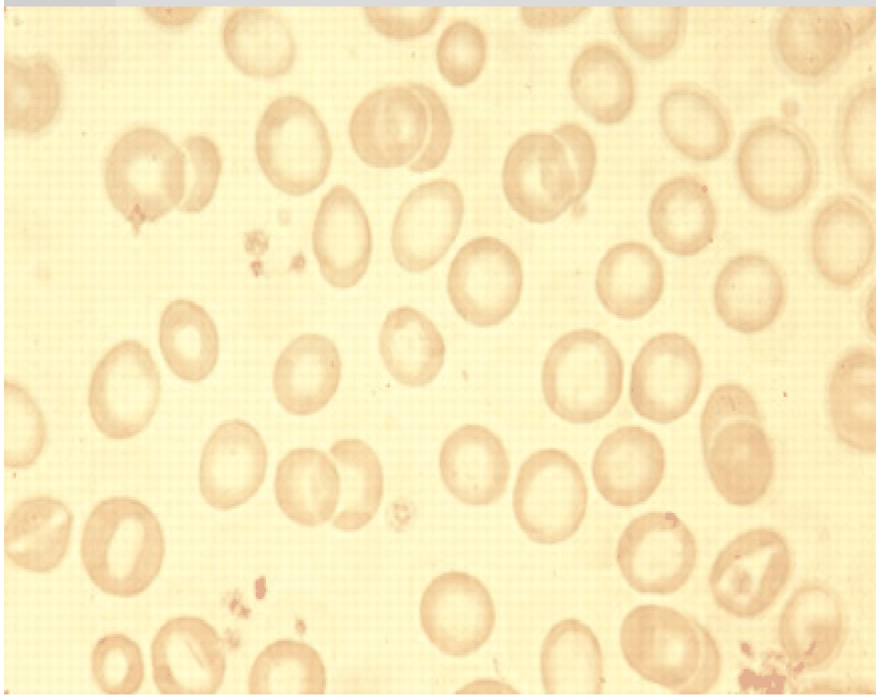
- B_{12}: Cyanocobalamine & Folic Acid:[dna formation]
  - Is also called Extrinsic Factor of Castle.
  - Needs the Intrinsic Factor from the Gastric juice for absorption from Small Intestine.
  - Deficiency causes Pernicious (When IF is missing) or Megaloblastic Anemia.
  - Stimulates Erythropoiesis
  - Is found in meat & diary products.

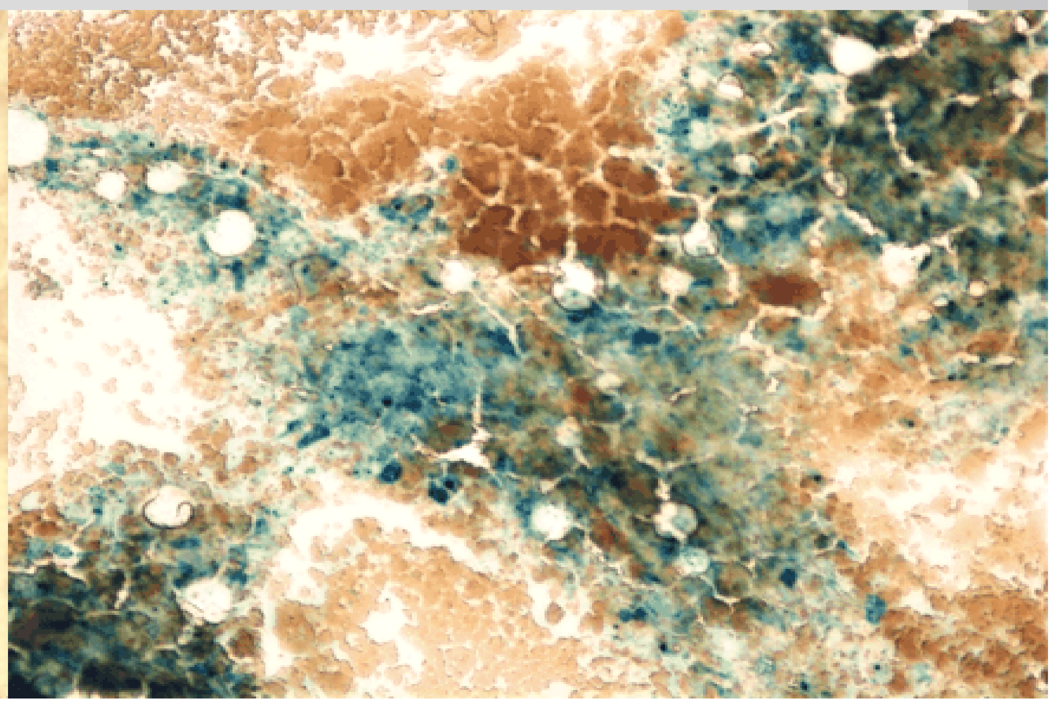
Iron functions

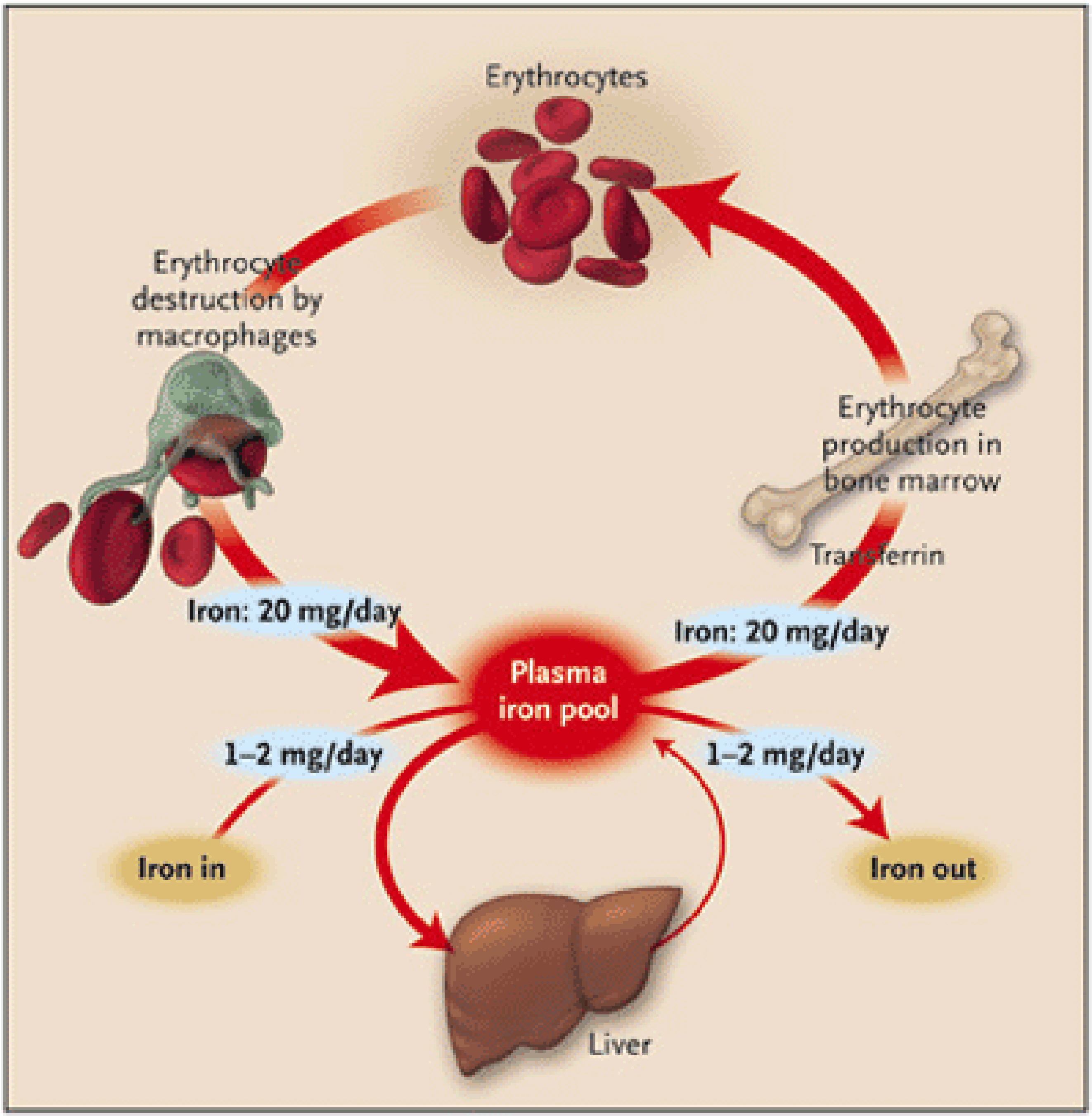
- **Oxygen carriers**
  - hemoglobin
- **Oxygen storage**
  - Myoglobin
- **Energy Production**
  - Cytochromes (oxidative phosphorylation)
  - Krebs cycle enzymes
- **Other**
  - Liver detoxification (cytochrome p450)

IRON

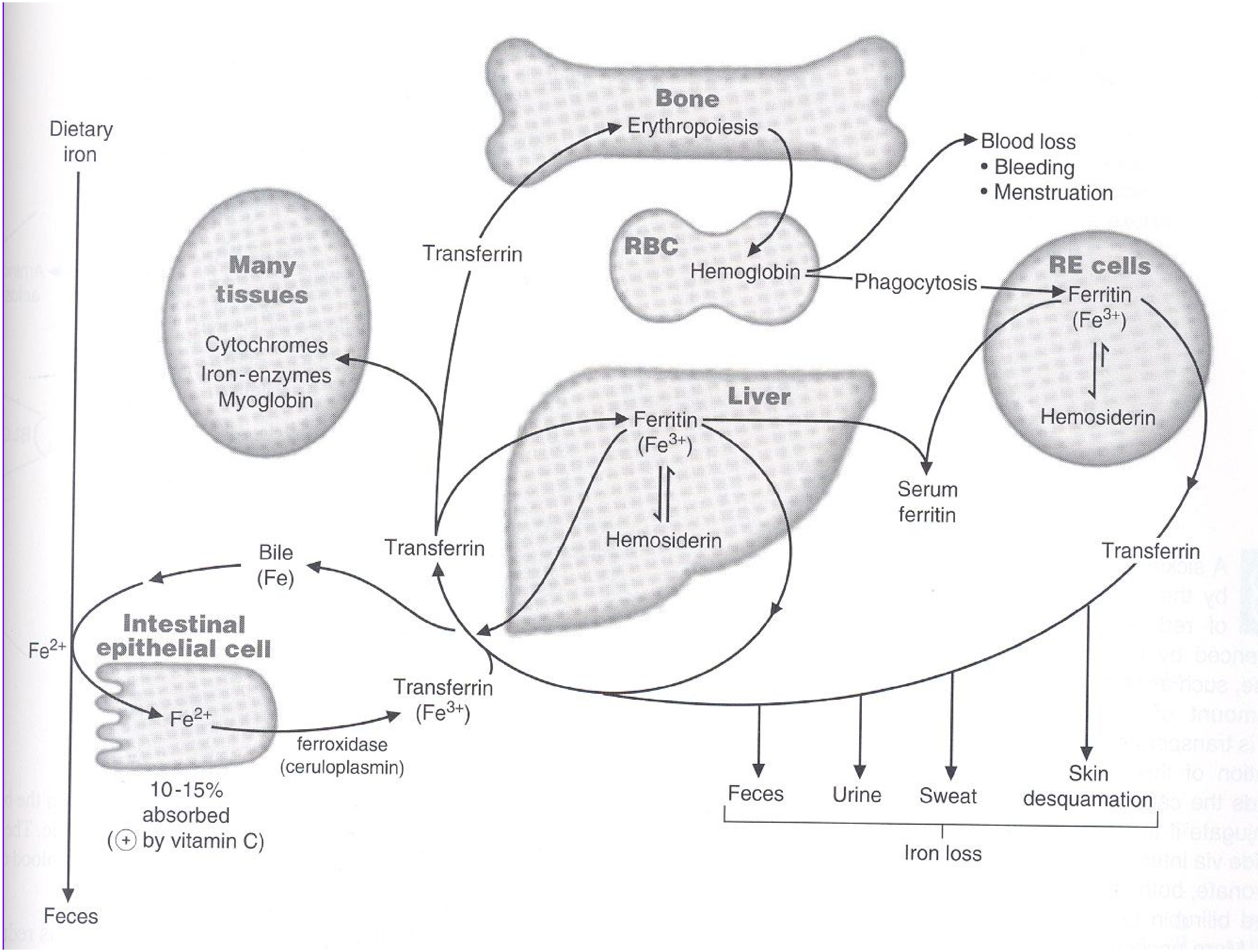
- **Essential for the synthesis of Hemoglobin.**
- **Deficiency causes Microcytic, Hypochromic Anemia.**
- **The MCV, Color Index & MCH are low.**







Erythrocytes
Erythrocyte destruction by macrophages
Erythrocyte production in bone marrow
Transferrin
Iron: 20 mg/day
Iron: 20 mg/day
Plasma iron pool
1–2 mg/day
1–2 mg/day
Iron in
Iron out
Liver


Dietary iron
Bone
Erythropoiesis
Blood loss
• Bleeding
• Menstruation
Transferrin
Many tissues
Cytochromes
Iron-enzymes
Myoglobin
RBC
Hemoglobin
Phagocytosis
RE cells
Ferritin (Fe^{3+})
Hemosiderin
Liver
Ferritin (Fe^{3+})
Hemosiderin
Serum ferritin
Transferrin
Transferrin
Bile (Fe)
Fe^{2+}
Intestinal epithelial cell
Fe^{2+}
Transferrin (Fe^{3+})
ferroxidase (ceruloplasmin)
10-15% absorbed
(+ by vitamin C)
Feces
Urine
Sweat
Skin desquamation
Iron loss
Feces

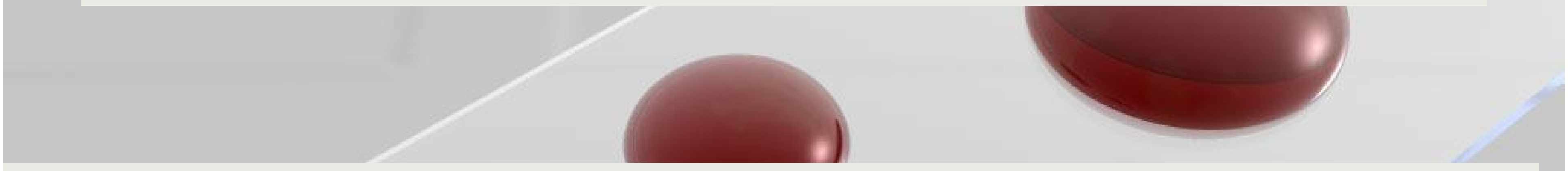
Iron Distribution

- 35 – 45 mg / kg iron in adult male body
- Total approx 4 g
  - Red cell mass as hemoglobin - 50%
  - Muscles as myoglobin – 7%
  - Storage as ferritin - 30%
    - Bone marrow (7%)
    - Reticulo-endothelial cells (7%)
    - Liver (25%)
  - Other Heme proteins - 5%
    - Cytochromes, myoglobin, others
  - In Serum - 0.1%

Iron Transport in Blood

- Red cells
  - As hemoglobin
  - Cannot be exchanged
- Plasma
  - Bound to Transferrin
  - Carries iron between body locations
  - eg between gut, liver, bone marrow, macrophages
  - Iron taken up into cells by transferrin receptors

Transferrin



- Synthesised in the liver.
- Each molecule binds can bind two Fe^{3+} molecules (oxidised)
- Contains 95% of serum Fe.
- Usually about 30% saturated with Fe.
- Production decreased in iron overload.
- Production increased in iron deficiency.
- Measured in blood as a marker of iron status.

Transferrin Testing

- A routine blood test used for iron status
- Also known as TIBC (total iron binding capacity)
- *High levels*:
  - Low body iron stores.
- *Low levels:*
  - High body iron stores.
- *Other conditions*
  - Increase: high oestrogen states (pregnancy, OCP)
  - Decrease: malnutrition, chronic liver disease, chronic disease (eg malignancy), protein-losing states, congenital deficiency, neonates, acute phase (negative reactant).

Iron Toxicity

- Iron can damage tissues
- Catalyzes the conversion of hydrogen peroxide to free-radical ions
- Free-radicals can attack:
  - cellular membranes
  - Proteins
  - DNA
- Iron excess possibly related to cancers, cardiac toxicity and other factors

Dietary Requirements of Erythropoiesis

- **Erythropoiesis requires:**
  - Proteins, lipids, and carbohydrates
  - Iron, vitamin B_{12}, and folic acid
- The body stores iron in Hb (65%), **the liver, spleen, and bone marrow**

- Intracellular iron is stored in protein-iron complexes such as **ferritin and hemosiderin**

- Circulating iron is loosely bound to the transport protein transferrin

Regulation of Erythropoiesis

Stimulates

CFU – E

Proerythroblasts

ERYTHROPOIETIN

Mature Erythrocytes

Decreases

Tissue Oxygenation

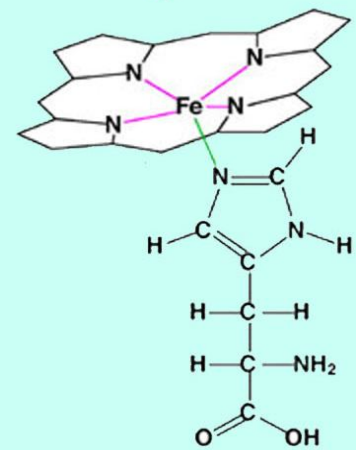
Factors decreasing:

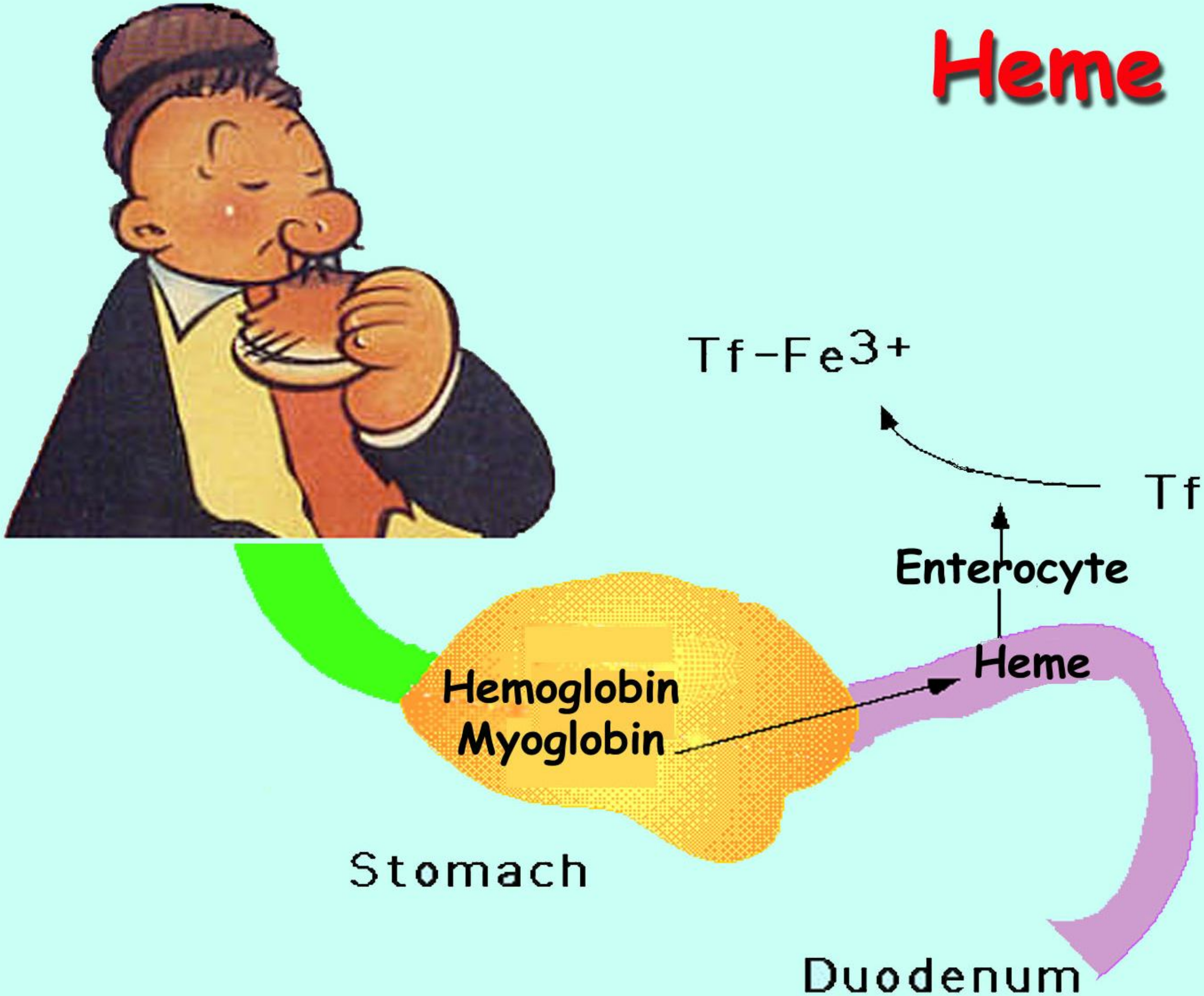
- Hypovolemia
  - » Anemia
- Poor blood flow
- Pulmonary Disease

An example of a Negative feed back mechanism

Iron Absorption

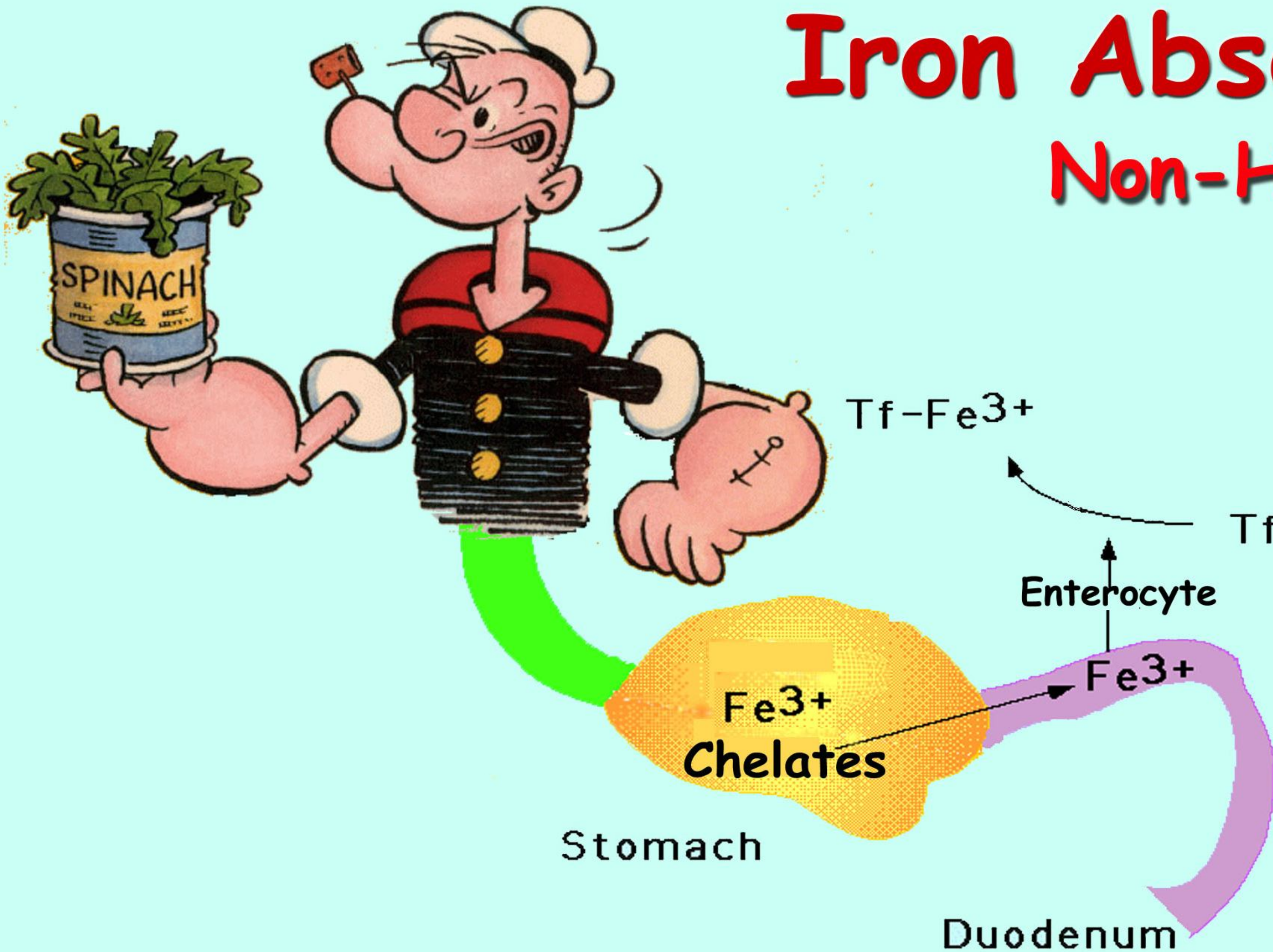
Heme





Iron absorbed as Heme-Iron

Iron Absorption
Non-Heme



Factors That Influence Iron Absorption

Physical State (bioavailability): heme > Fe^{2+} > Fe^{3+}

Inhibitors: phytates, tannins, soil clay, antacids, iron overload

Competitors: cobalt, lead, strontium, manganese, zinc

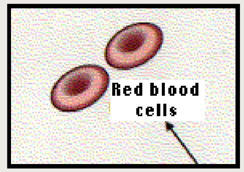
Facilitators: ascorbate, citrate, amino acids, iron deficiency

Fate and Destruction of Erythrocytes

- **The life span** of an erythrocyte is **100–120 days**
- Old RBCs become rigid and fragile, and their Hb begins to degenerate
- Dying RBCs are engulfed by macrophages
- Heme and globin are separated and the iron is salvaged for reuse

Extravascular Pathway for RBC Destruction

(Liver, Bone marrow,
& Spleen)



Phagocytosis & Lysis

Hemoglobin

Globin

Amino acids

Amino acid pool

Heme → → **Bilirubin**

Fe^{2+}

Excreted

Handling of Free (Intravascular) Hemoglobin

Purposes:
1. Scavenge iron
2. Prevent major iron losses
3. Complex free heme (very toxic)

- Haptoglobin: hemoglobin-haptoglobin complex is readily metabolized in the liver and spleen forming an iron-globin complex and bilirubin. Prevents loss of iron in urine.
- Hemopexin: binds free heme. The heme-hemopexin complex is taken up by the liver and the iron is stored bound to ferritin.
- Methemalbumin: complex of oxidized heme and albumin.

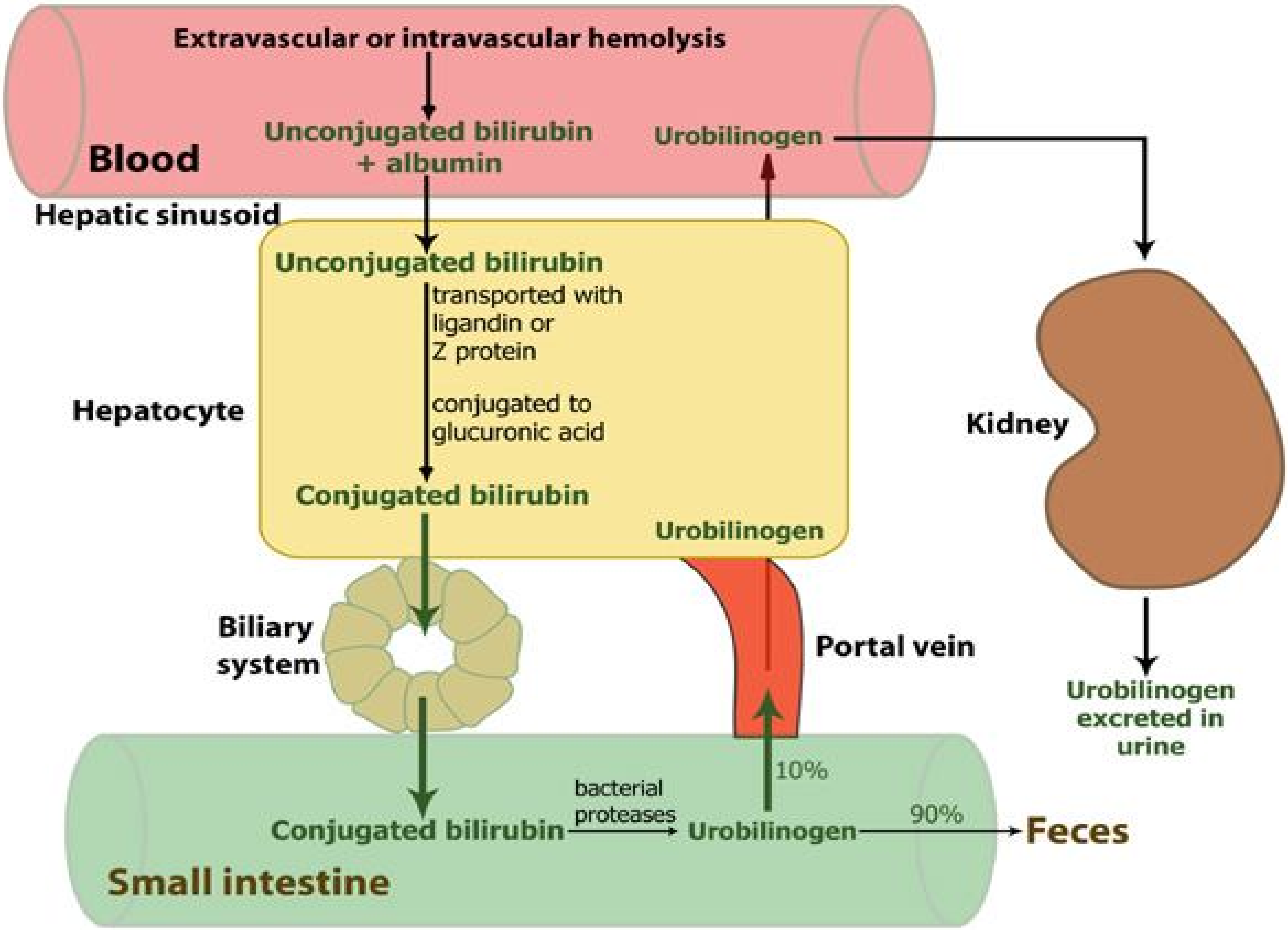
Fate and Destruction of Erythrocytes

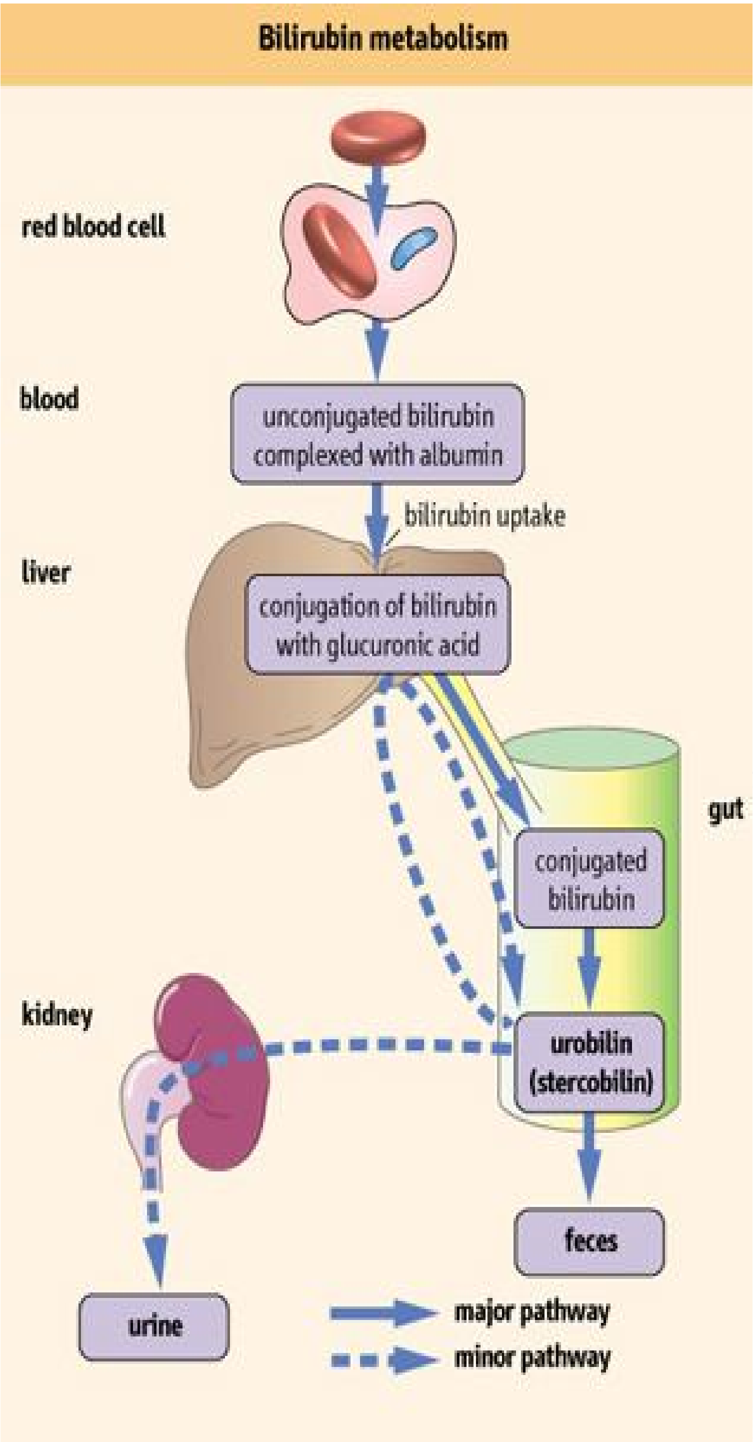
- Heme is degraded to a yellow pigment called ***bilirubin***
- The **liver secretes bilirubin into the intestines as bile**
- The intestines metabolize it into urobilinogen
- This degraded pigment leaves the body in feces, in a pigment called stercobilin

- 75% is derived from RBCs
- In normal adults this results in a daily load of 250-300 mg of bilirubin
- Normal plasma concentrations are less then 1 mg/dL
- Hydrophobic – transported by albumin to the liver for further metabolism prior to its excretion

Fate and Destruction of Erythrocytes

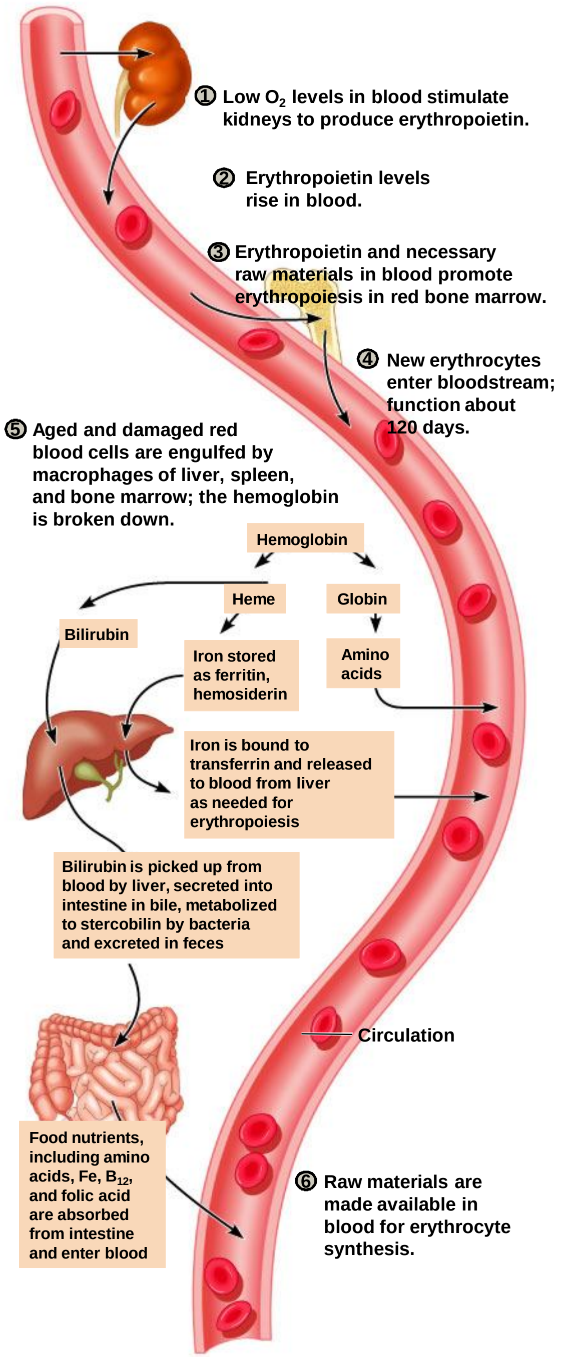
- Globin is metabolized into amino acids and is released into the circulation
- Hb released into the blood is captured by haptoglobin and phagocytized


Extravascular or intravascular hemolysis
Unconjugated bilirubin
+ albumin
Blood
Urobilinogen
Hepatic sinusoid
Unconjugated bilirubin
transported with
ligandin or
Z protein
conjugated to
glucuronic acid
Hepatocyte
Conjugated bilirubin
Urobilinogen
Kidney
Biliary
system
Portal vein
Urobilinogen
excreted in
urine
10%
bacterial
proteases
90%
Conjugated bilirubin
Urobilinogen
Feces
Small intestine



The causes of jaundice

Type	Cause	Clinical example	Frequency
Prehepatic	hemolysis	autoimmune abnormal hemoglobin	uncommon depends on region
intrahepatic	infection	hepatitis A, B, C	common/very common
	chemical/drug	acetaminophen alcohol	common common
	genetic errors: bilirubin metabolism	Gilbert's syndrome Crigler–Najjar syndrome Dubin–Johnson syndrome Rotor's syndrome	1 in 20 very rare very rare very rare
	genetic errors: specific proteins	Wilson's disease α_1 antitrypsin	1 in 200 000 1 in 1000 with genotype
	autoimmune	chronic active hepatitis	uncommon/ rare
	neonatal	physiologic	very common
Posthepatic	intrahepatic bile ducts	drugs primary bilary cirrhosis cholangitis	common uncommon common
	extrahepatic bile ducts	gall stones pancreatic tumor cholangiocarcinoma	very common uncommon rare



① Low O_2 levels in blood stimulate kidneys to produce erythropoietin.
② Erythropoietin levels rise in blood.
③ Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow.
④ New erythrocytes enter bloodstream; function about 120 days.
⑤ Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down.
Hemoglobin
Heme
Globin
Bilirubin
Iron stored as ferritin, hemosiderin
Amino acids
Iron is bound to transferrin and released to blood from liver as needed for erythropoiesis
Bilirubin is picked up from blood by liver, secreted into intestine in bile, metabolized to stercobilin by bacteria and excreted in feces
Circulation
Food nutrients, including amino acids, Fe, B_{12}, and folic acid are absorbed from intestine and enter blood
⑥ Raw materials are made available in blood for erythrocyte synthesis.

Figure 17.7

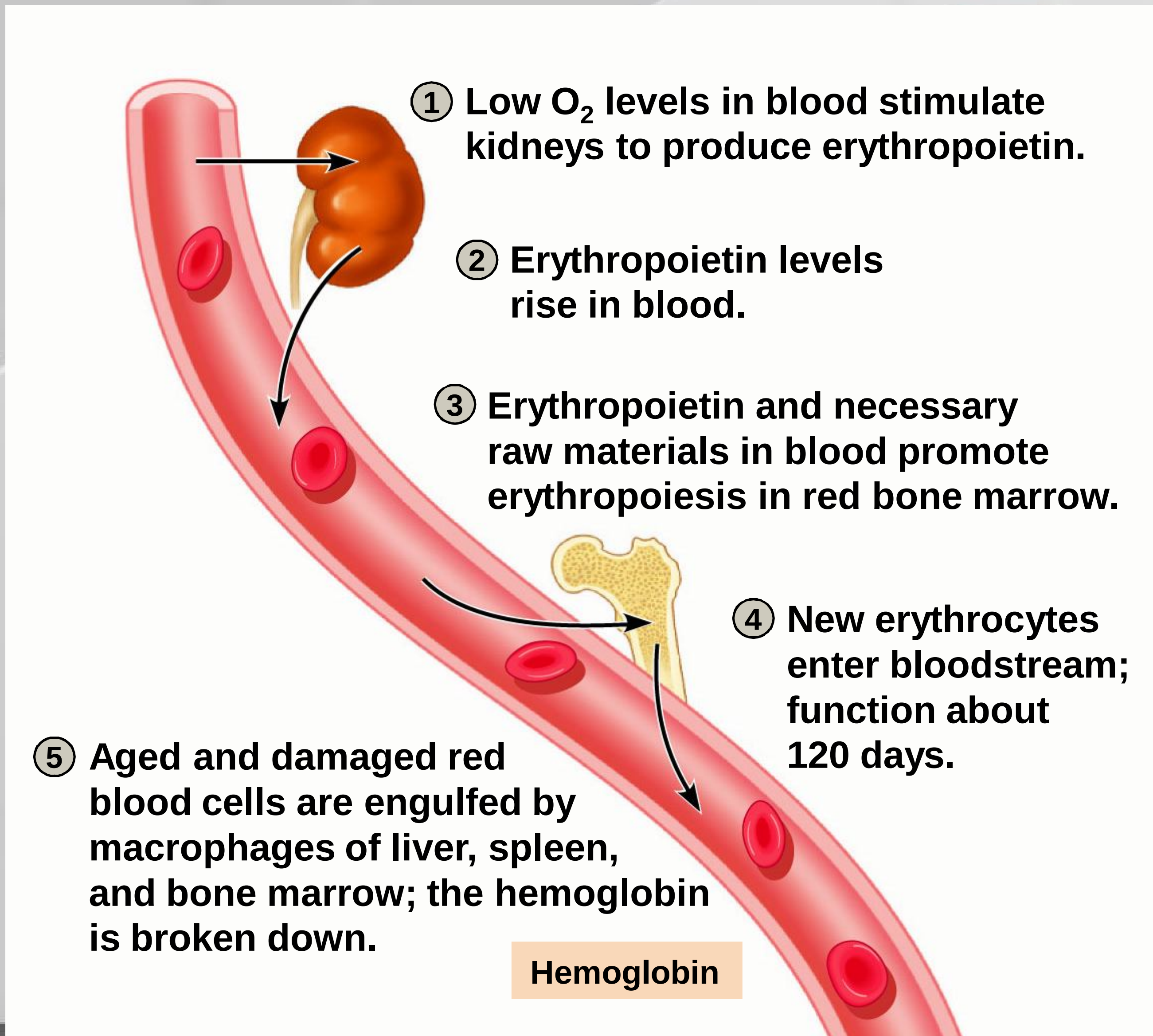

1 Low O_2 levels in blood stimulate kidneys to produce erythropoietin.
2 Erythropoietin levels rise in blood.
3 Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow.
4 New erythrocytes enter bloodstream; function about 120 days.
5 Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down.
Hemoglobin

Figure 17.7

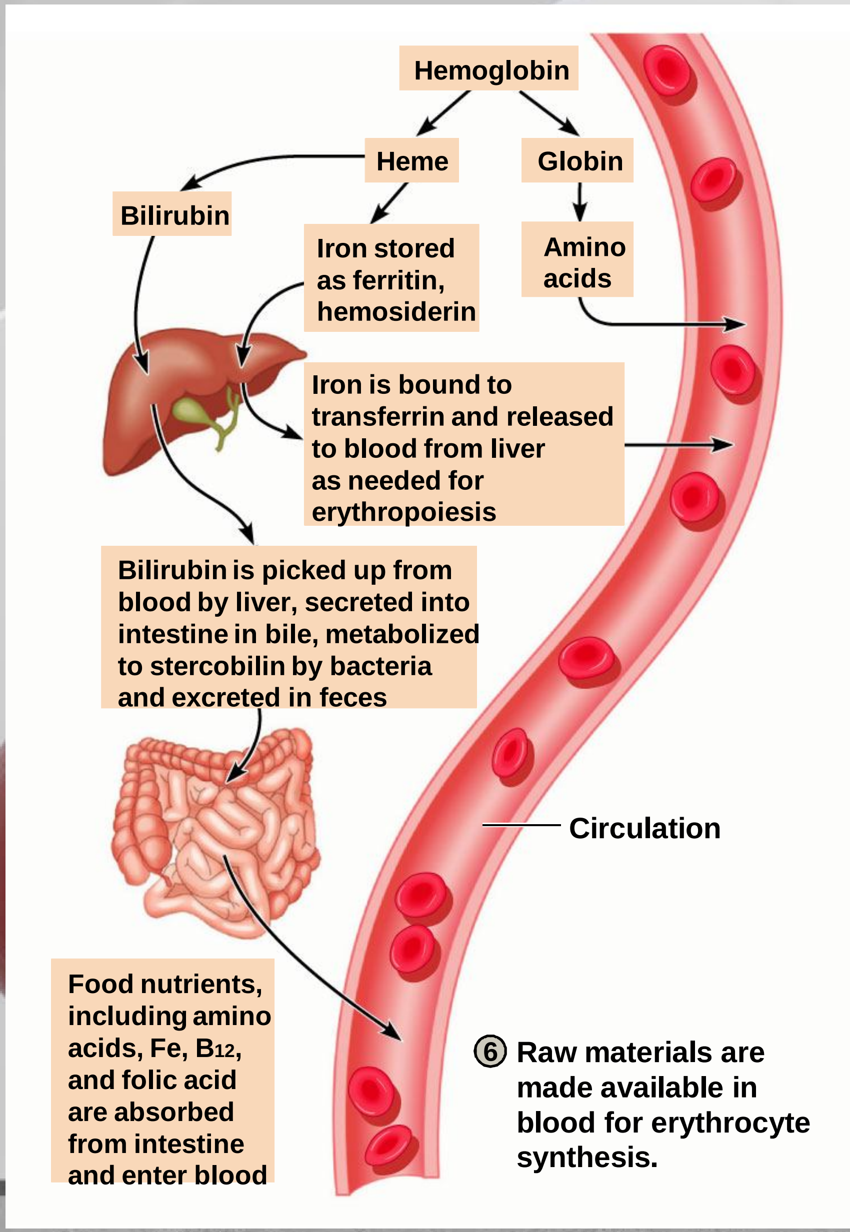
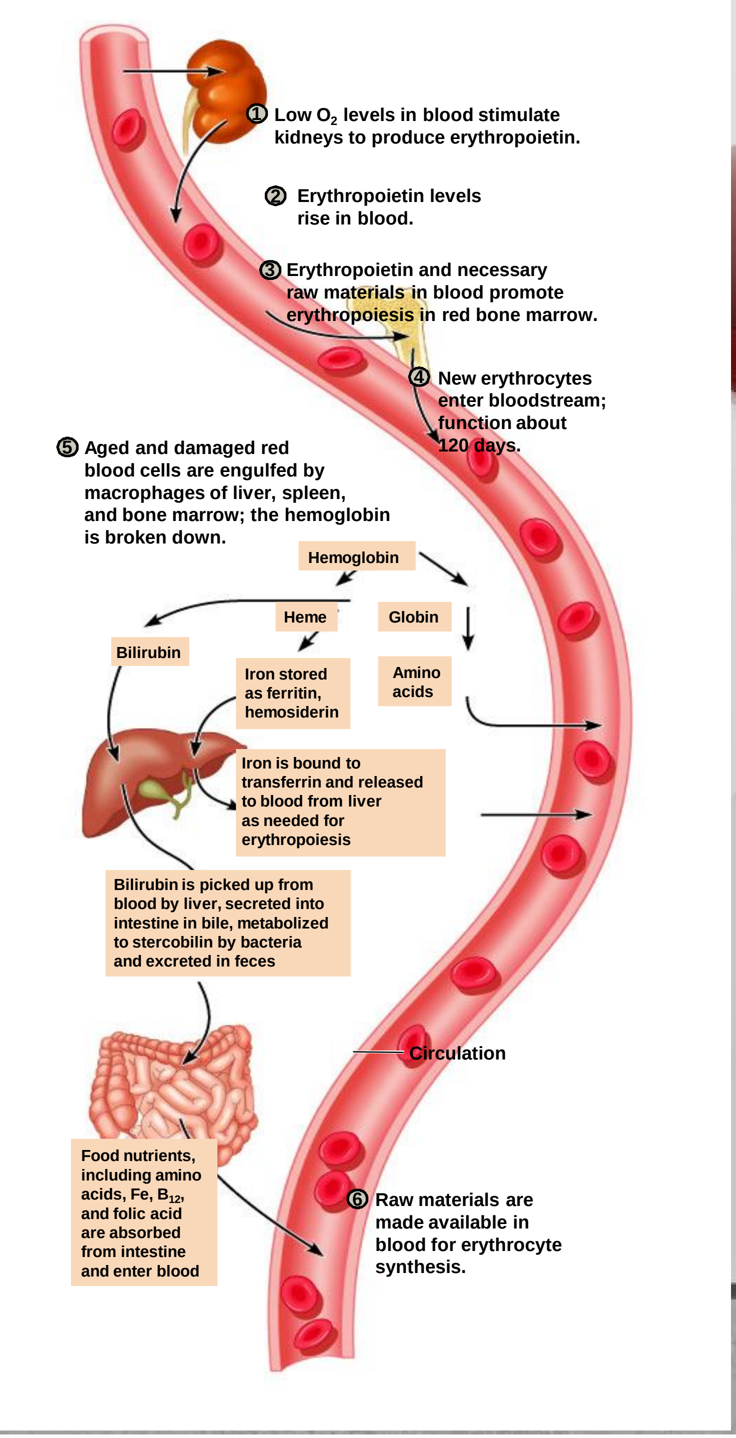

Hemoglobin
Heme
Globin
Bilirubin
Iron stored as ferritin, hemosiderin
Amino acids
Iron is bound to transferrin and released to blood from liver as needed for erythropoiesis
Bilirubin is picked up from blood by liver, secreted into intestine in bile, metabolized to stercobilin by bacteria and excreted in feces
Circulation
Food nutrients, including amino acids, Fe, B12, and folic acid are absorbed from intestine and enter blood
6 Raw materials are made available in blood for erythrocyte synthesis.

Figure 17.7



1 Low O_2 levels in blood stimulate kidneys to produce erythropoietin.
2 Erythropoietin levels rise in blood.
3 Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow.
4 New erythrocytes enter bloodstream; function about 120 days.
5 Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down.
Hemoglobin
Heme
Globin
Bilirubin
Iron stored as ferritin, hemosiderin
Amino acids
Iron is bound to transferrin and released to blood from liver as needed for erythropoiesis
Bilirubin is picked up from blood by liver, secreted into intestine in bile, metabolized to stercobilin by bacteria and excreted in feces
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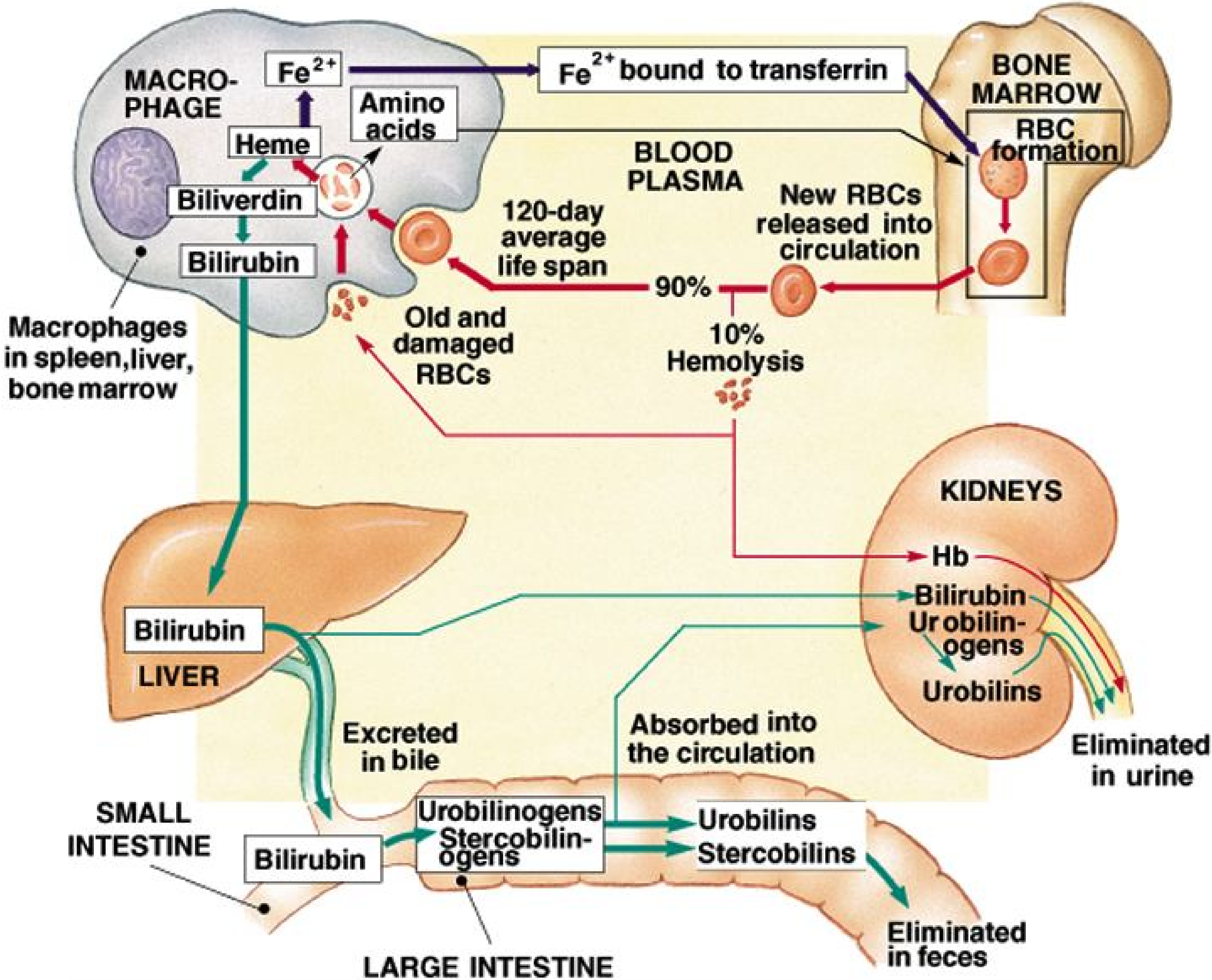
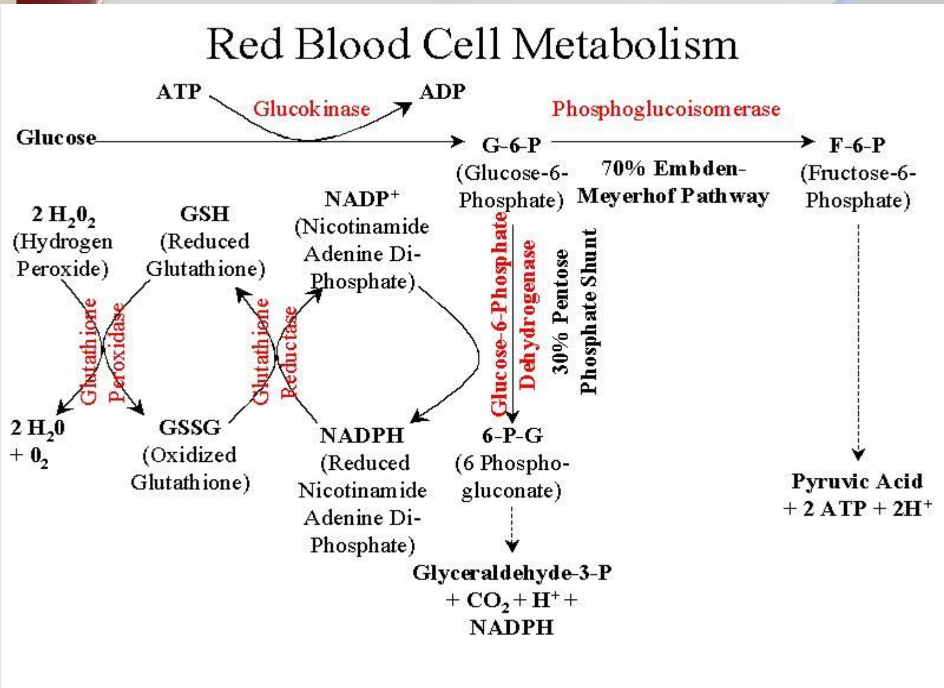


MACRO-PHAGE
Fe^{2+}
Fe^{2+} bound to transferrin
BONE MARROW
RBC formation
Amino acids
Heme
Biliverdin
Bilirubin
BLOOD PLASMA
120-day average life span
New RBCs released into circulation
90%
10% Hemolysis
Old and damaged RBCs
Macrophages in spleen, liver, bone marrow
KIDNEYS
Hb
Bilirubin
Urobilin-ogens
Urobilins
Eliminated in urine
Bilirubin
LIVER
Excreted in bile
Absorbed into the circulation
SMALL INTESTINE
Bilirubin
Urobilinogens
Stercobilin-ogens
Urobilins
Stercobilins
Eliminated in feces
LARGE INTESTINE

RBC Metabolic Needs

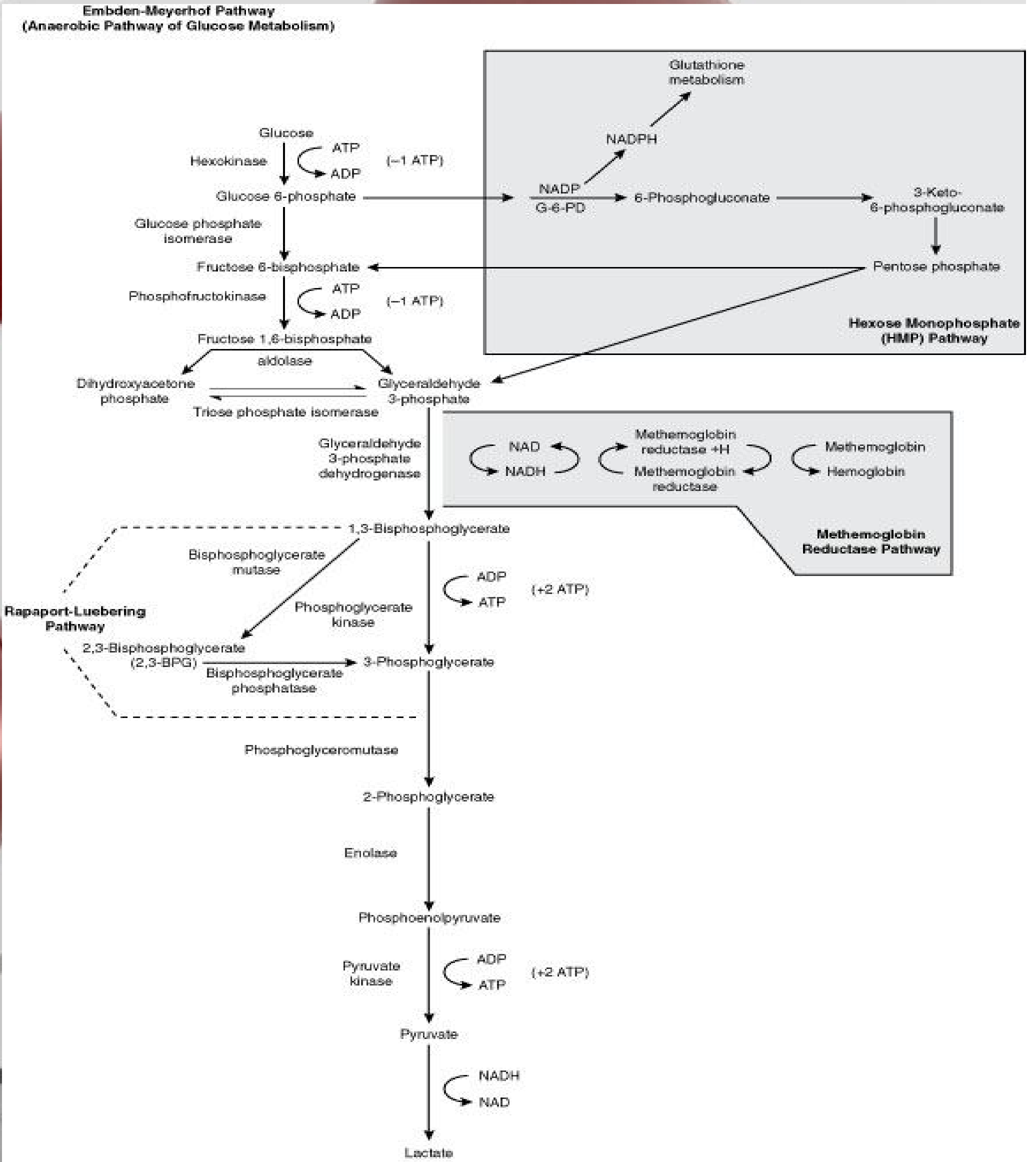
- Intracellular cationic electrochemical gradients
- Maintain membrane
- Maintain HGB (ferrous oxidation state)
- Protect cell proteins from oxidative denaturation
- Glycolysis
- Glutathione synthesis
- Nucleotide salvage



Graphic accessed http://www.as.ua.edu/ant/bindon/ant475/g6pd/img001.jpg, 2001.

RBC Sources of Energy

- **EMP (anaerobic)**
  - glucose to lactate
    - ATP
- **Hexose-monophosphate Shunt (aerobic)**
  - G-6-P to 6-PG
    - NADPH
- **Rapoport-Luebering Shunt**
  - 1,3-diphosphoglycerate to 2,3- DPG
- **Methemoglobin Reductase**
  - G-3-P to 1,3-Bisphosphoglycerate
    - NADH



Graphic accessed http://evolvels.elsevier.com/section/default.asp?id=1138_ccalvo7_0001, 2009.

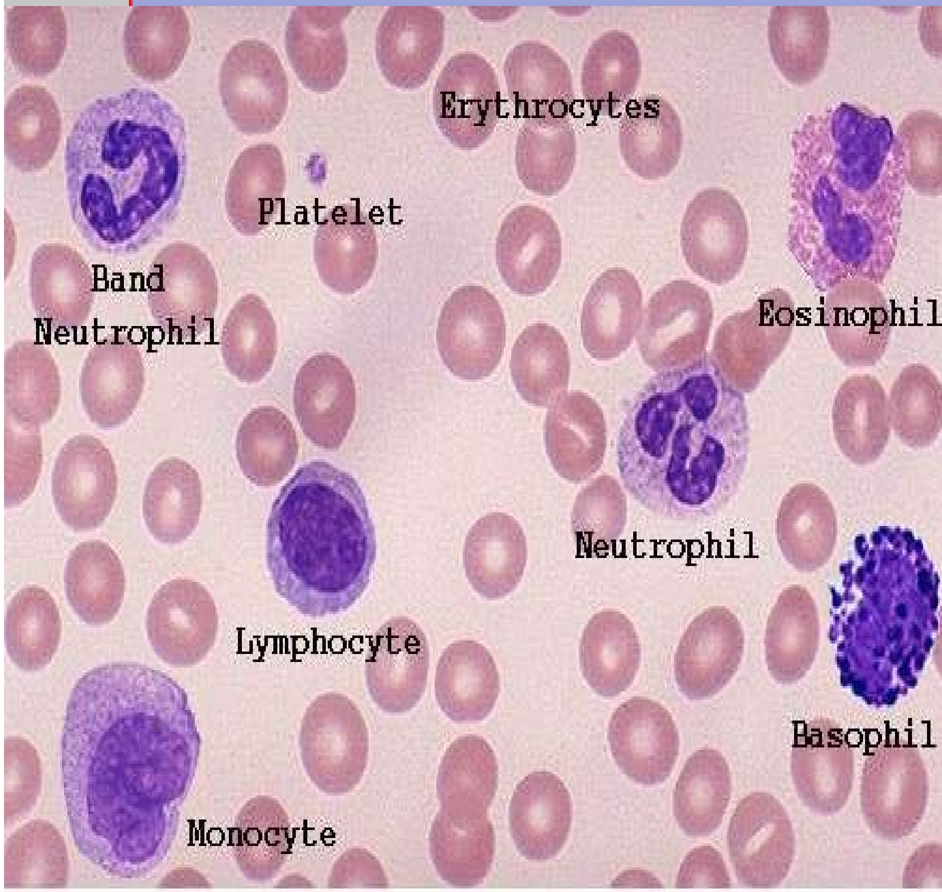
Role of Metabolic Pathways

Pathway	Enzymes	Role	Problems
EMP	Phosphofructokinase Pyruvate kinase (PK)	Produce ATP RBC Shape (ion pumps)	Hemolytic Anemia PK Deficiency
HMS	Glutathione Reductase G6PD	NADPH Production OXY-METH HB Balance	Hemolytic Anemias
RLB	DPG Synthetase	2,3-DPG Production HB Oxygen Affinity	Hypoxia
MHBR	Methemoglobin reductase	Protects HB from Oxidation via NADH	Hemolytic Anemia Hypoxia

Classification of Leucocytes

- LEUCOCYTES 100
  - GRANULOCYTES
    - EOSINOPHILS 1 - 8
    - BASOPHILS 0 - 1
    - NEUTROPHILS 50 -70
  - AGRANULOCYTES
    - MONOCYTES 3 - 8
    - LYMPHOCYTES 30 - 50

White cells, leukocytes



Wright-stained smear of normal blood (x1000)
The RBC's are biconcave discs stained buff-pink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.

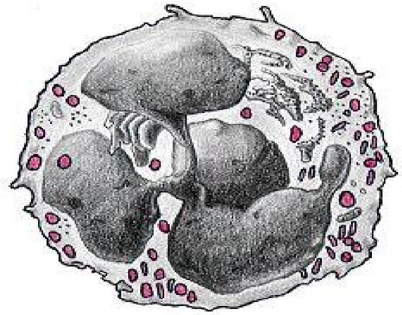


Fig. 8 - Neutrophil

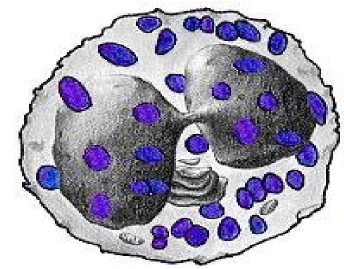
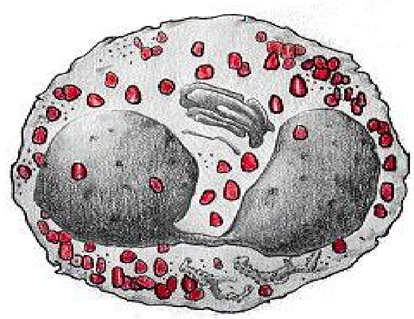


fig. 10 - Basophil



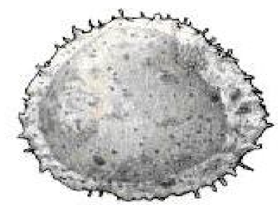


Fig. 11 - Lymphocyte

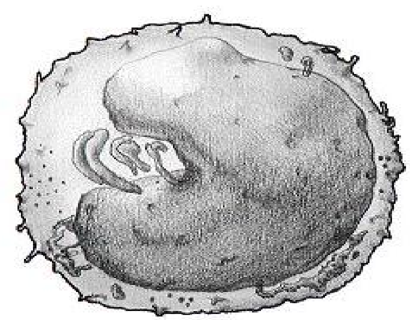
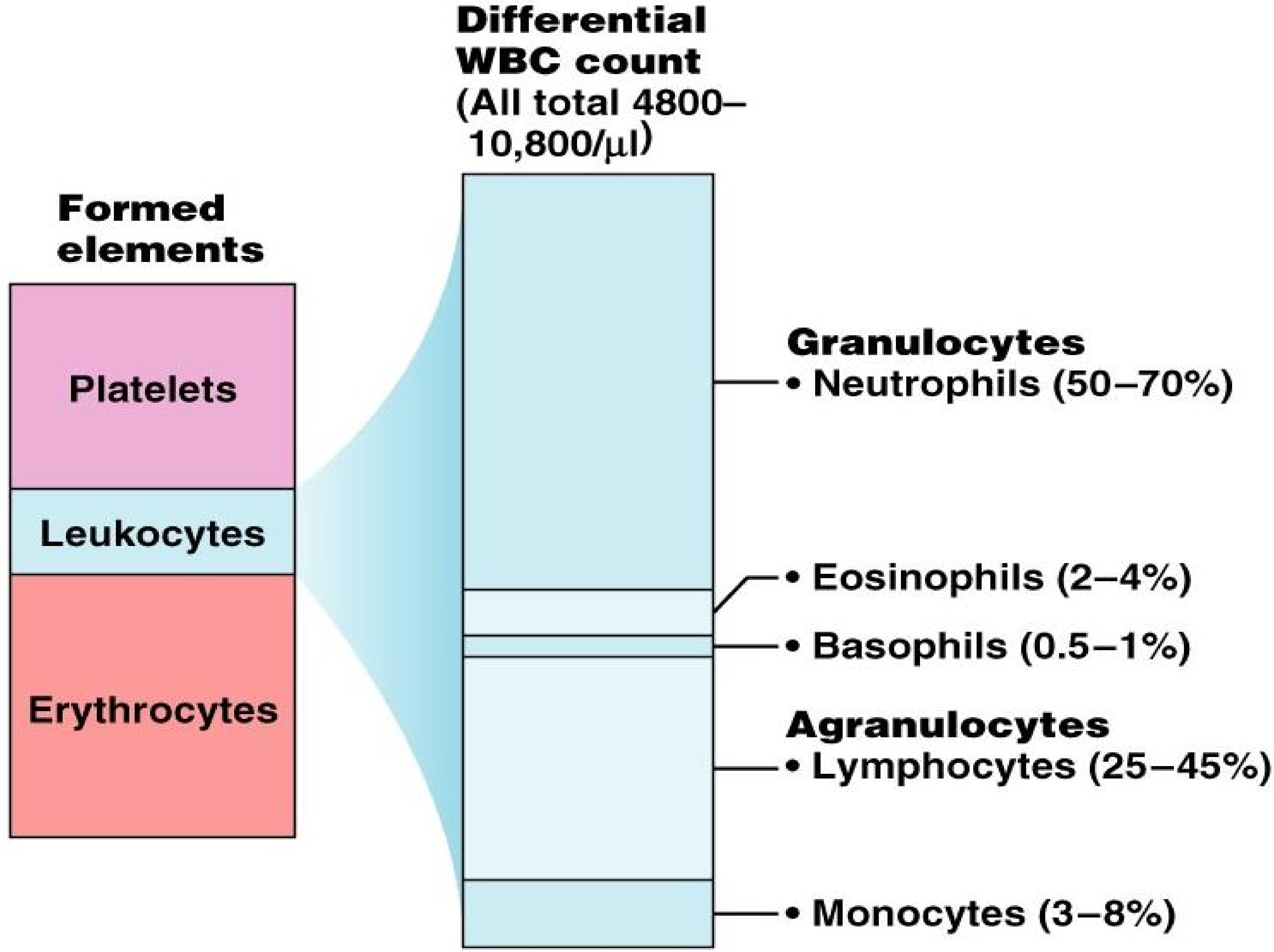


Fig. 12 - Monocyte

Leukocytes (WBCs)

- Leukocytes, the only blood components that are complete cells:
  - Are less numerous than RBCs
  - Make up 1% of the total blood volume
  - Can leave capillaries via diapedesis
  - Move through tissue spaces
- Leukocytosis – WBC count over 11,000 / mm^3
  - Normal response to bacterial or viral invasion


Differential WBC count (All total 4800–10,800/μl)
Formed elements
Platelets
Leukocytes
Erythrocytes
Granulocytes
• Neutrophils (50–70%)
• Eosinophils (2–4%)
• Basophils (0.5–1%)
Agranulocytes
• Lymphocytes (25–45%)
• Monocytes (3–8%)

Granulocytes

- Granulocytes –
  - **neutrophils,**
  - **eosinophils,**
  - **and basophils**

» Contain cytoplasmic granules that stain specifically (acidic, basic, or both) with Wright's stain

» Are larger and usually shorter-lived than RBCs

» Have lobed nuclei

» Are all phagocytic cells

LEUCOCYTES: REVIEW

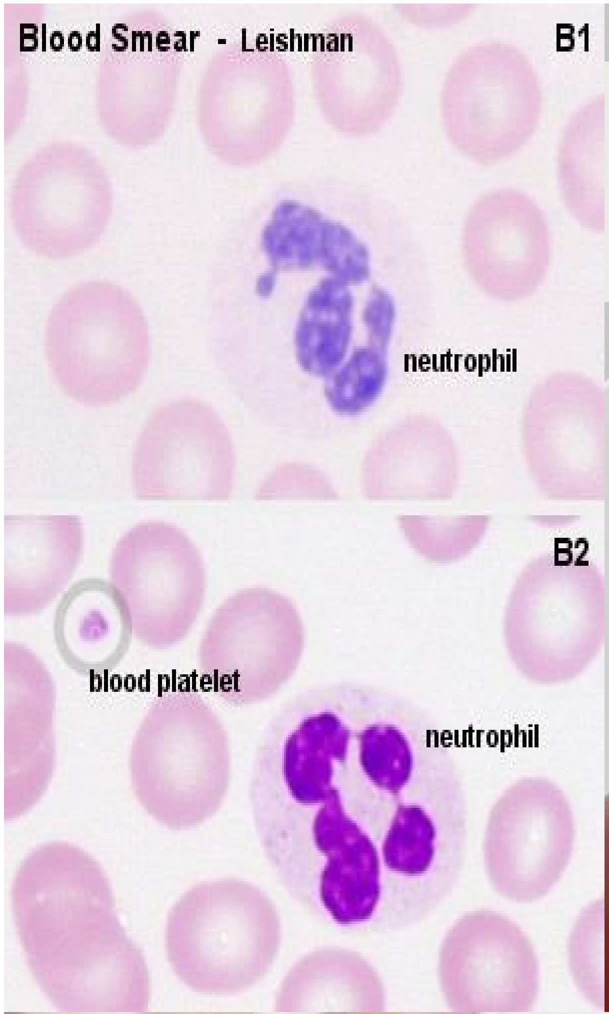
Transient life spans in the blood.

Neutrophils:

- Phagocytic cells
- Act as frontline cells for defence along with Macrophages.
- Show:
    - Diapedesis
    - Amoeboid movement
    - Chemotaxis
    - Phagocytosis.

LEUCOCYTES: REVIEW

- **Chemotaxis: Directed movement**
- **Chemotaxins: Cytotaxins & Cytotaxigens**



Neutrophils

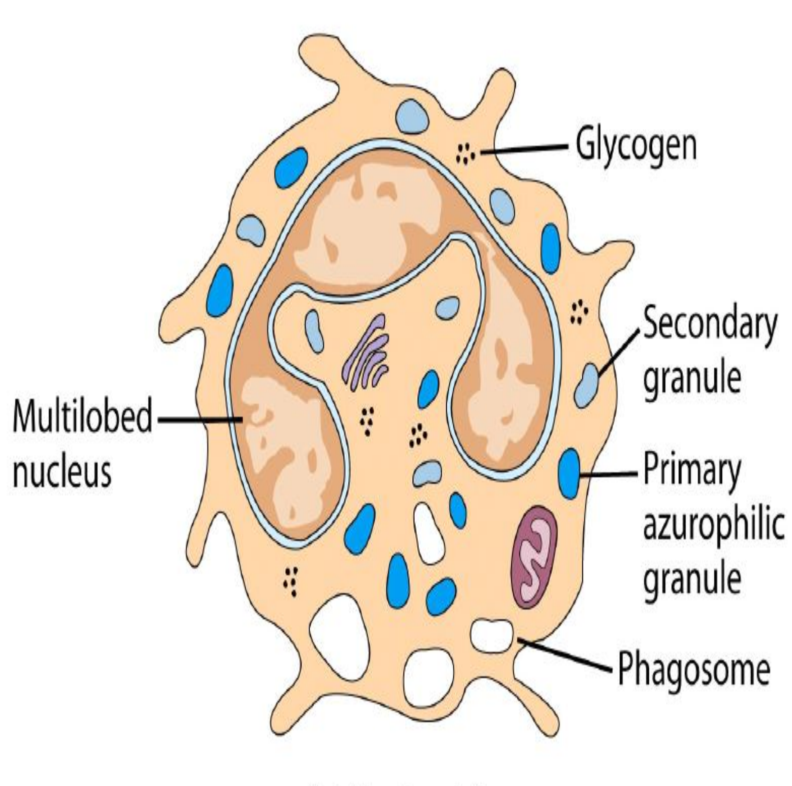
- Neutrophils have two types of granules that:
  - Take up both acidic and basic dyes
  - Give the cytoplasm a lilac color
  - Contain
    - **<u>peroxidases,</u>**
    - **<u>hydrolytic enzymes,</u>**
    - **<u>defensins (antibiotic-like proteins)</u>**
- Neutrophils are our body's bacteria slayers

- Neutrophils also release an assortment of proteins in three types of granules by a process called degranulation:[DON’T HAVE TO KNOW FOR NOW GENERAL INFO]

Granule type Protein

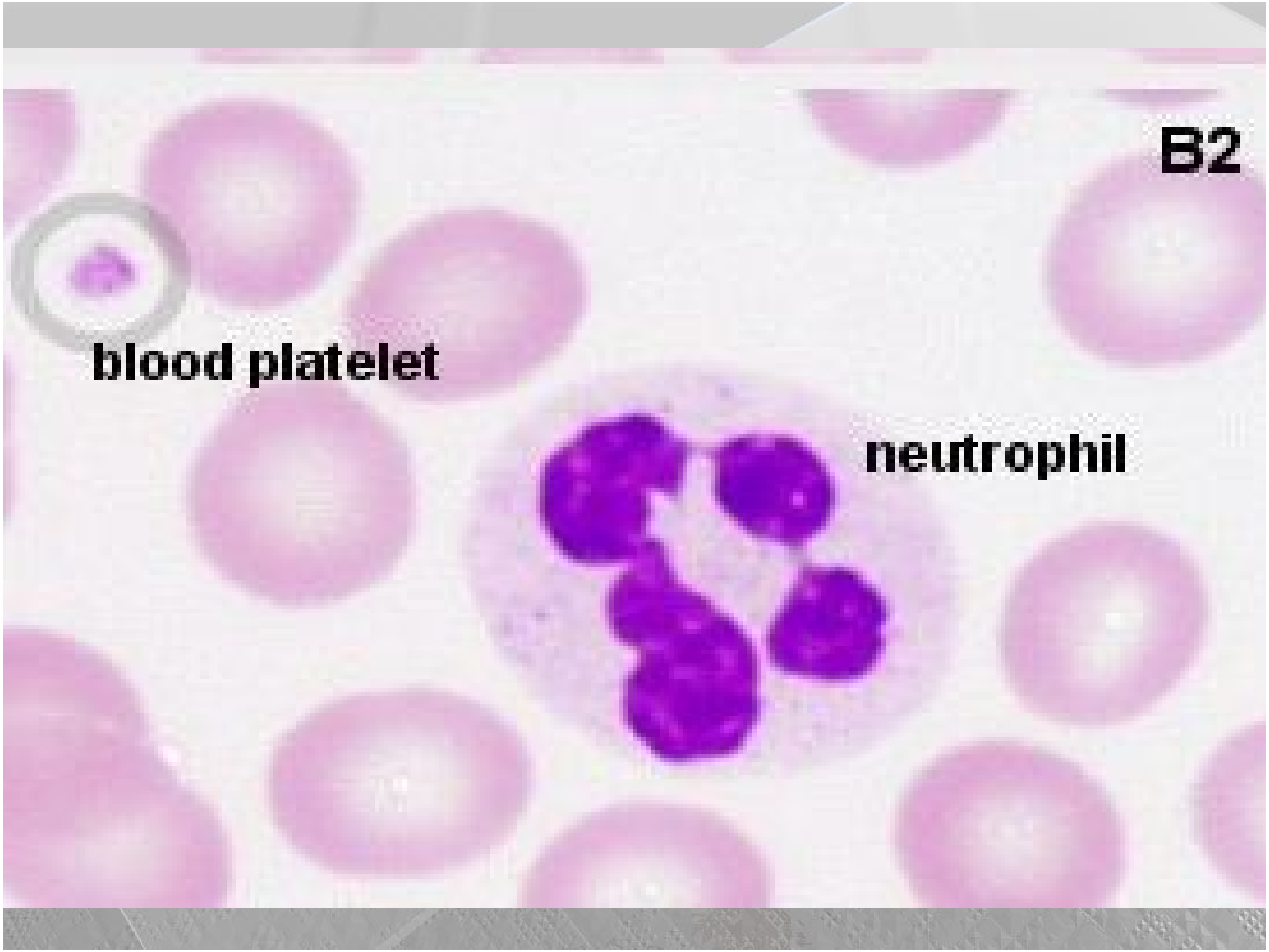
- -**<u>specific granules</u>** (or "secondary granules") Lactoferrin and Cathelicidin
- **<u>azurophilic granules</u>** (or "primary granules") myeloperoxidase, bactericidal/permeability increasing protein (BPI), Defensins and the serine proteases neutrophil elastase and cathepsin G
- **<u>tertiary granules</u>** cathepsin and gelatinase

Neutrophils

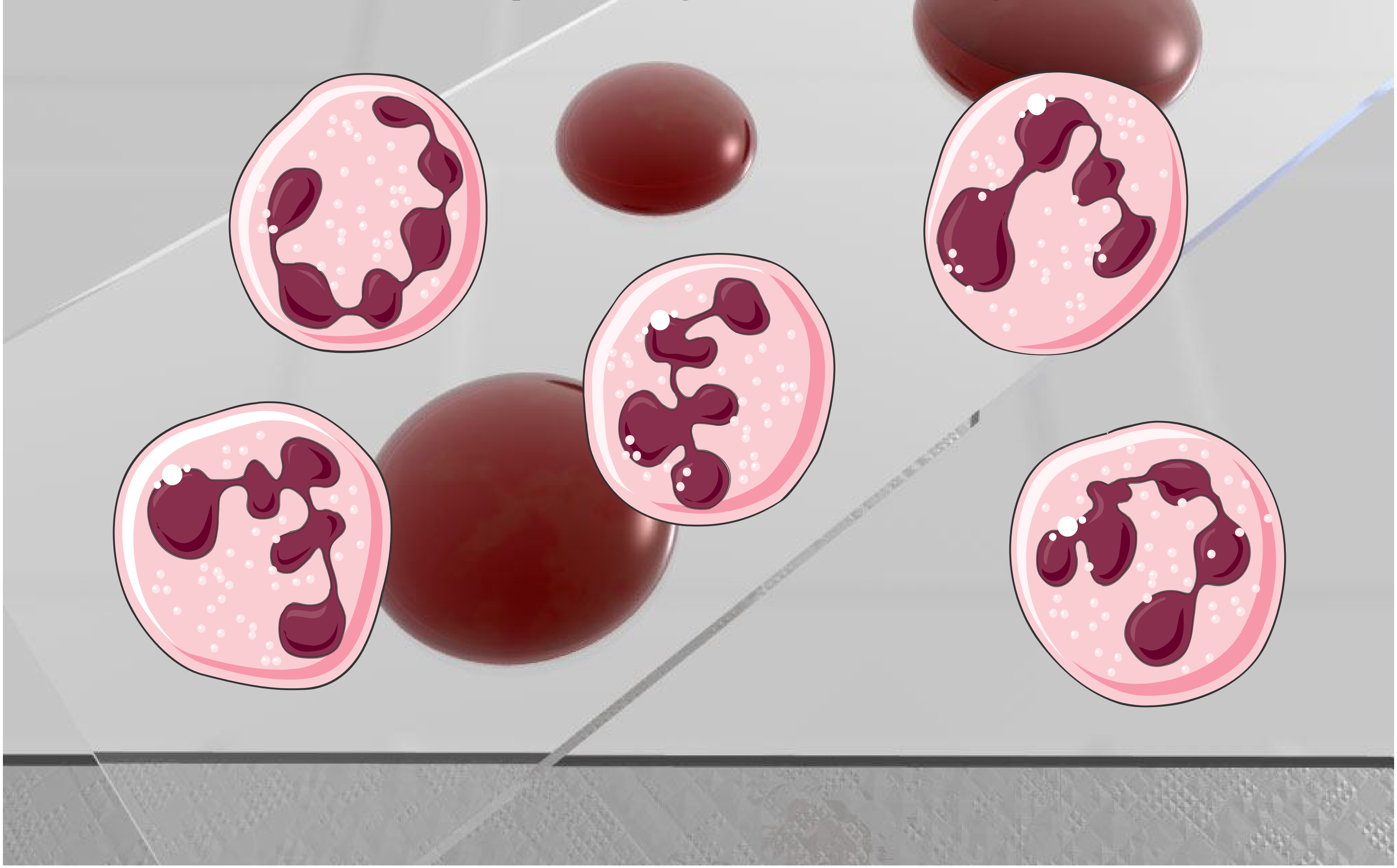


(a) Neutrophil

- Circulate in peripheral blood 7-10 hr before migrating into tissue; live only a few days
- "front line of innate defense"
- increased # (leukocytosis) used as an indicator of infection
- extravasate in inflam rxn
- **attracted by chemotactic factors They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as**
- **-Interleukin-8 (IL-8) and C5a**
- **-interferon gamma (IFN-gamma),**
- **-C5a**.
- active phagocytes; digestive enzyme held in 1° and 2° granules
- Use both O2-dep and O2-indep digestive mech's


B2
blood platelet
neutrophil

Neutrophil granulocytes

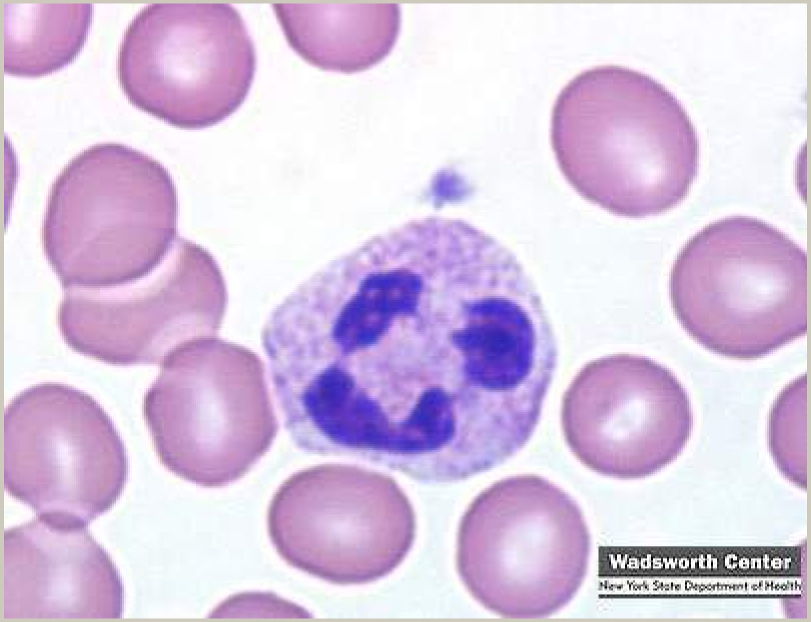


NEUTROPHILIA

- **NEUTROPHILIA: Increased neutrophil count, can be due to:**
  - **Release of stored cells from the bone marrow reserves.**
  - **Bacterial Infections causing increased Neutropoiesis.**
  - **Exercise can cause release of stored neutrophils.**

Segmented Neutrophil

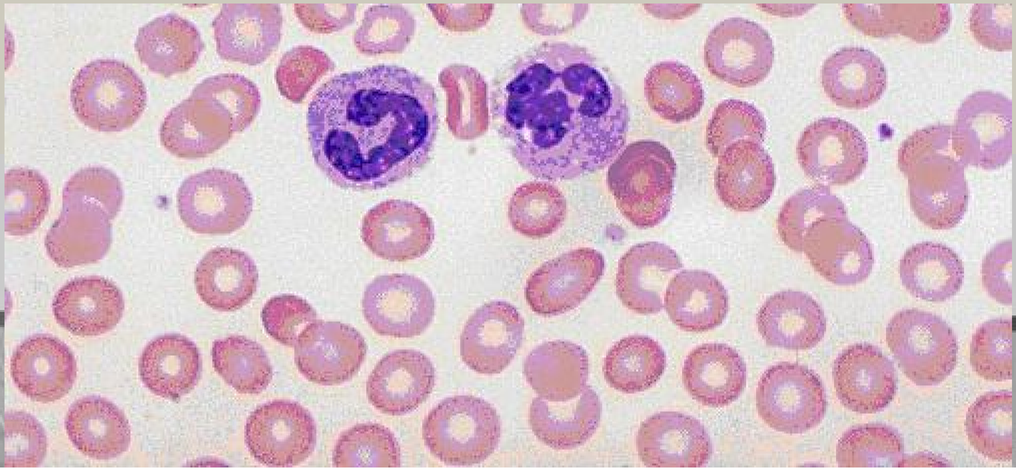
- Neutrophils are produced in bone marrow,
- Released into blood after completing their maturation in marrow, circulate for less than a day, and migrate out of the vessels into tissues or into alveoli and gut lumen.



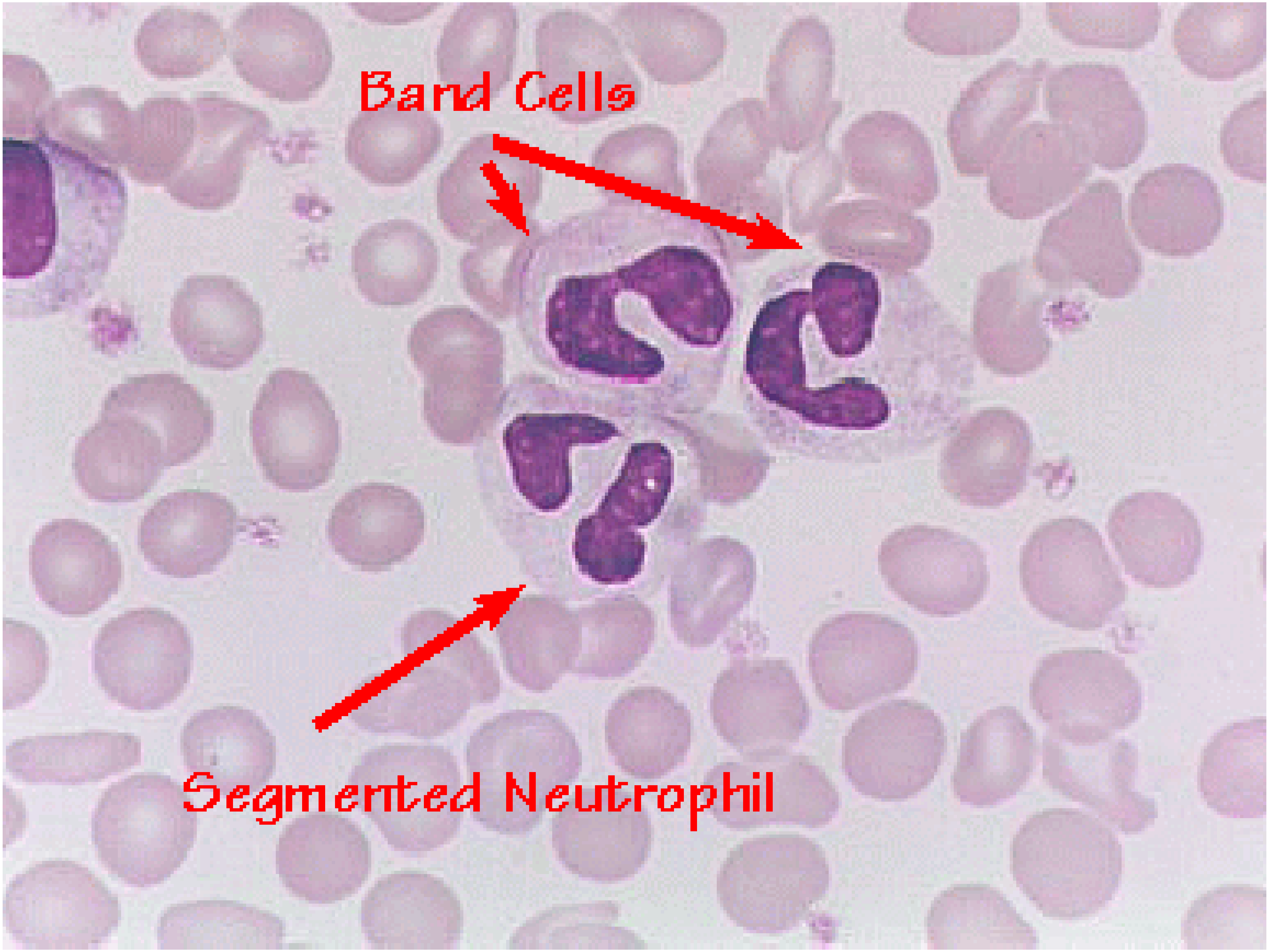
NOTICE THE FILAMENTS
BETWEEN THE LOBES

Band neutrophil

- A **band cell** (**stab cell**) is a cell undergoing granulopoiesis, derived from a metamyelocyte, and leading to a mature granulocyte.
- It is characterized by having a nucleus which is curved, but not lobar.
- Often the term "band cell" implies a neutrophilic lineage. However, the term is not used only with neutrophils.
- **A count of band neutrophils is used to measure inflammation.**



NOTICE NO FILAMENT BETEEN THE LOBES , ONE CONNECTION


Band Cells
Segmented Neutrophil

Cytoplasmic Granules

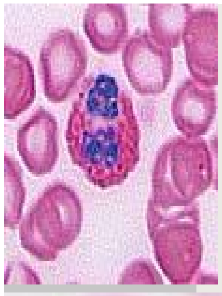
- **Fine, azurophilic (Stain with both Eosin & Methylene blue) in nature.**
- **Contain enzymes such as:**
  - **Cathepsins.**
  - **Phosphatases.**
  - **Nucleases.**
- **Granules serve as lysosomes.**

NEUTROPHILS & MONOCYTES: Functions

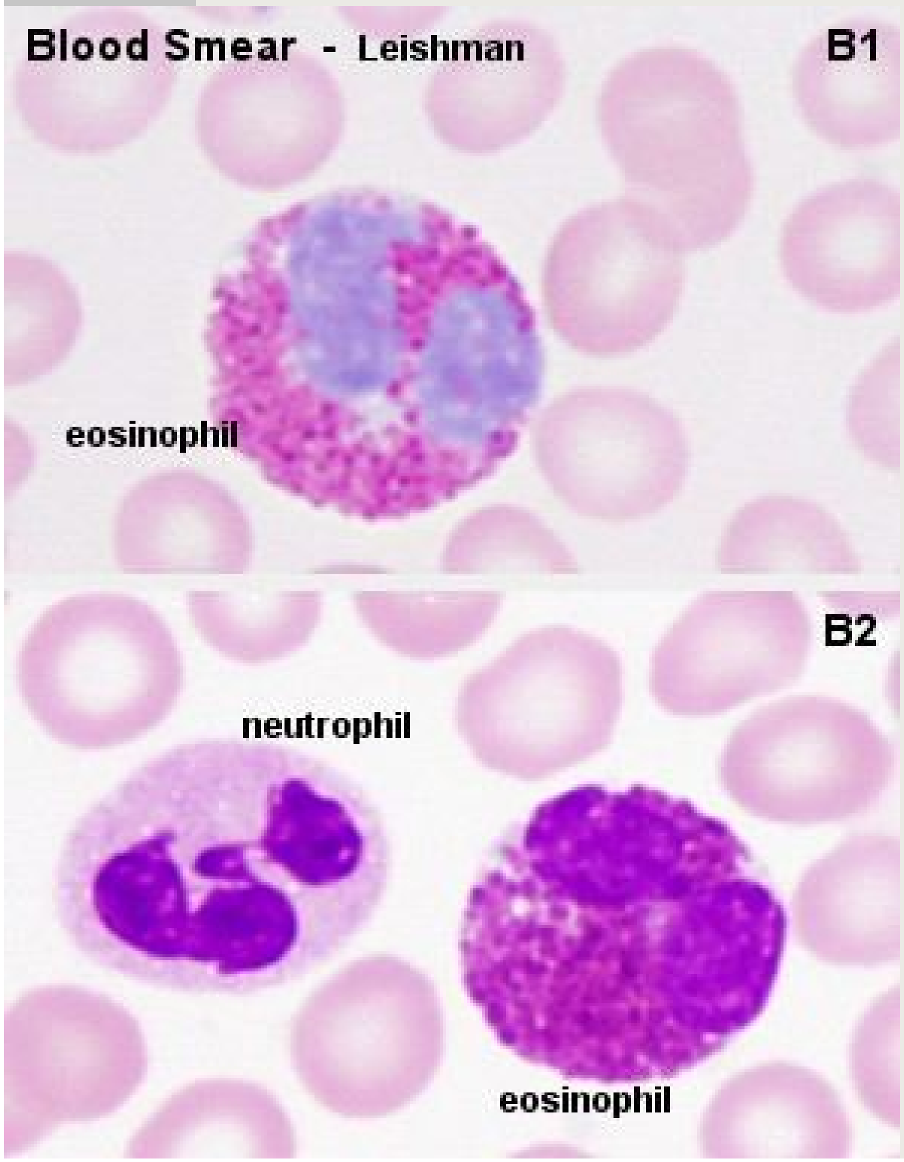
- They seek, attack and destroy invading bacteria, viruses and other injurious agents
- Neutropils attack and destroy bacteria and viruses, even in the blood.
- Monocytes are immature until they enter the tissues. There, they swell up to 80 Microns, develop lysosomes, and become Macrophages, capable of defence.

Neutrophils & Macrophages

- **Diapedesis: They squeeze through the pores of the blood vessels.**
- Amoeboid movement**: They move at rates several times their own length!**
- Chemotaxis**: Directed movement – cells move to wards infected areas.**



Eosinophils



- Eosinophils account for 1–4% of WBCs
  - Have red-staining, bilobed nuclei connected via a broad band of nuclear material
  - Have red to crimson (acidophilic) large, coarse, lysosome-like granules
  - Lead the body's counterattack against parasitic worms
  - Lessen the severity of allergies by phagocytizing immune complexes

EOSINOPHILS

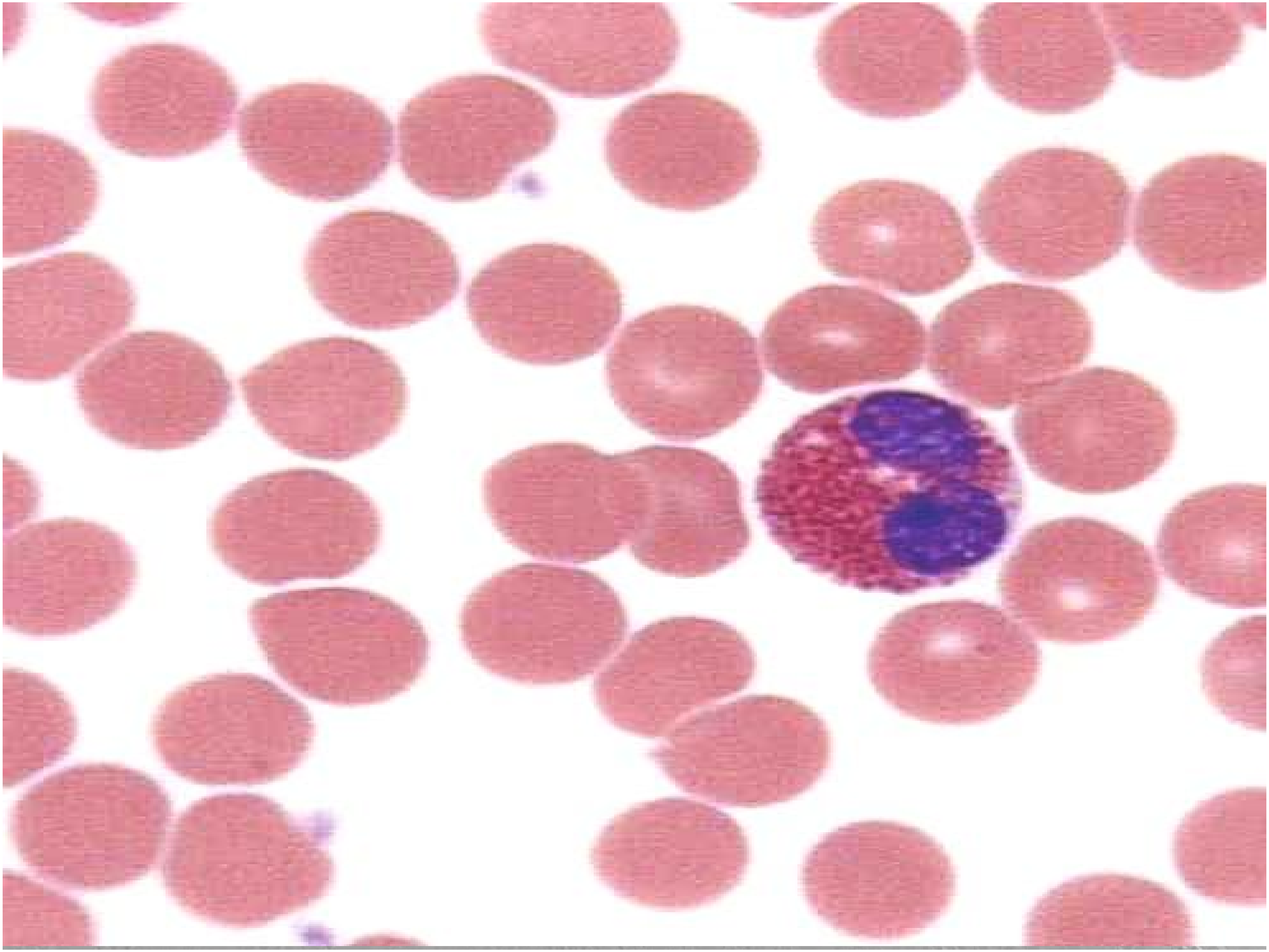
- Small granules contain many mediators:
  - histaminase and
  - eosinophil peroxidase,
  - ribonuclease (RNase),
  - deoxyribonucleases,
  - lipase, plasminogen,.

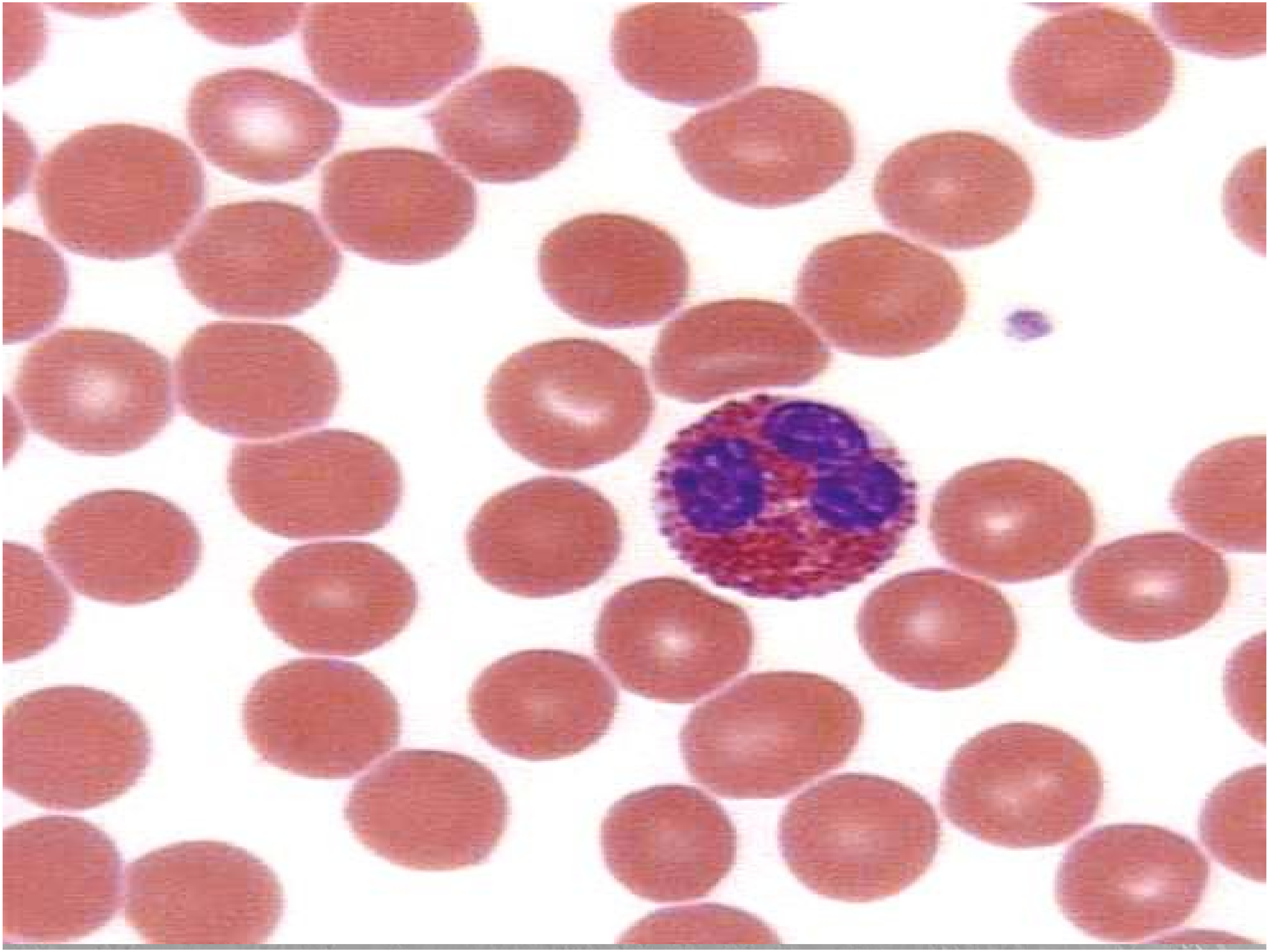
  - These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues

EOSINOPHILS

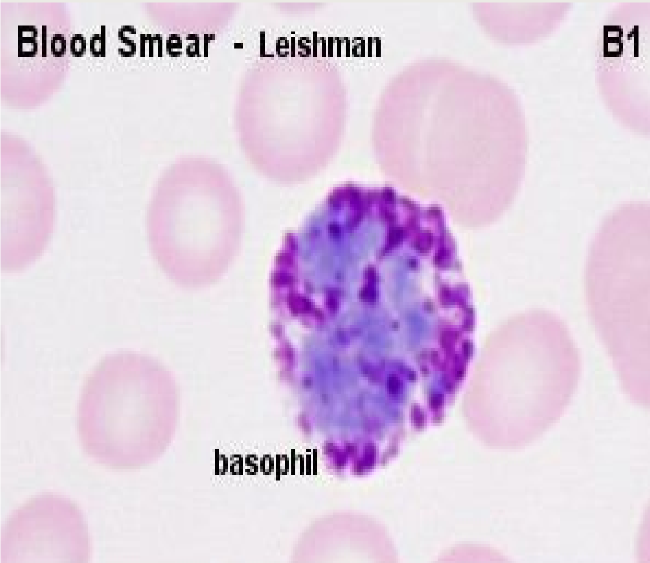
- They are found in the
  - medulla and the junction between the cortex and medulla of the thymus,
  - in the lower gastrointestinal tract,
  - ovary,
  - uterus,
  - spleen,
  - lymph nodes,
  - **but not in the lung, skin, esophagus, or some other internal organs[*vague*] under normal conditions.**

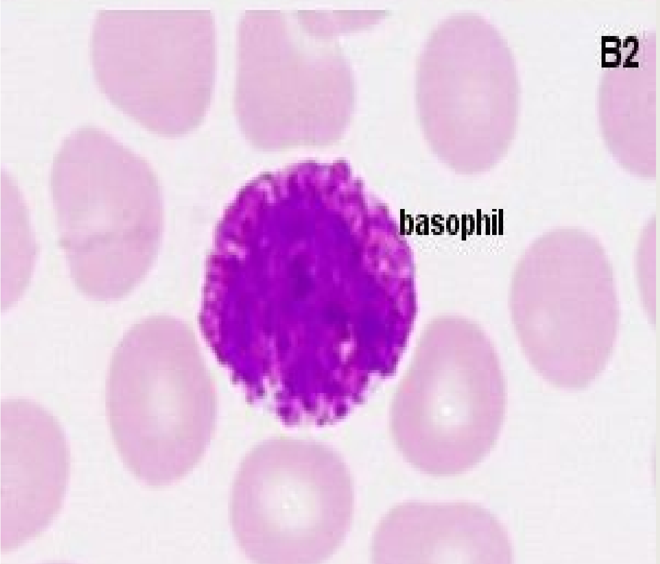
The presence of eosinophils in these latter organs is associated with disease. Eosinophils persist in the circulation for 8–12 hours, and can survive in tissue for an additional 8–12 days in the absence of stimulation



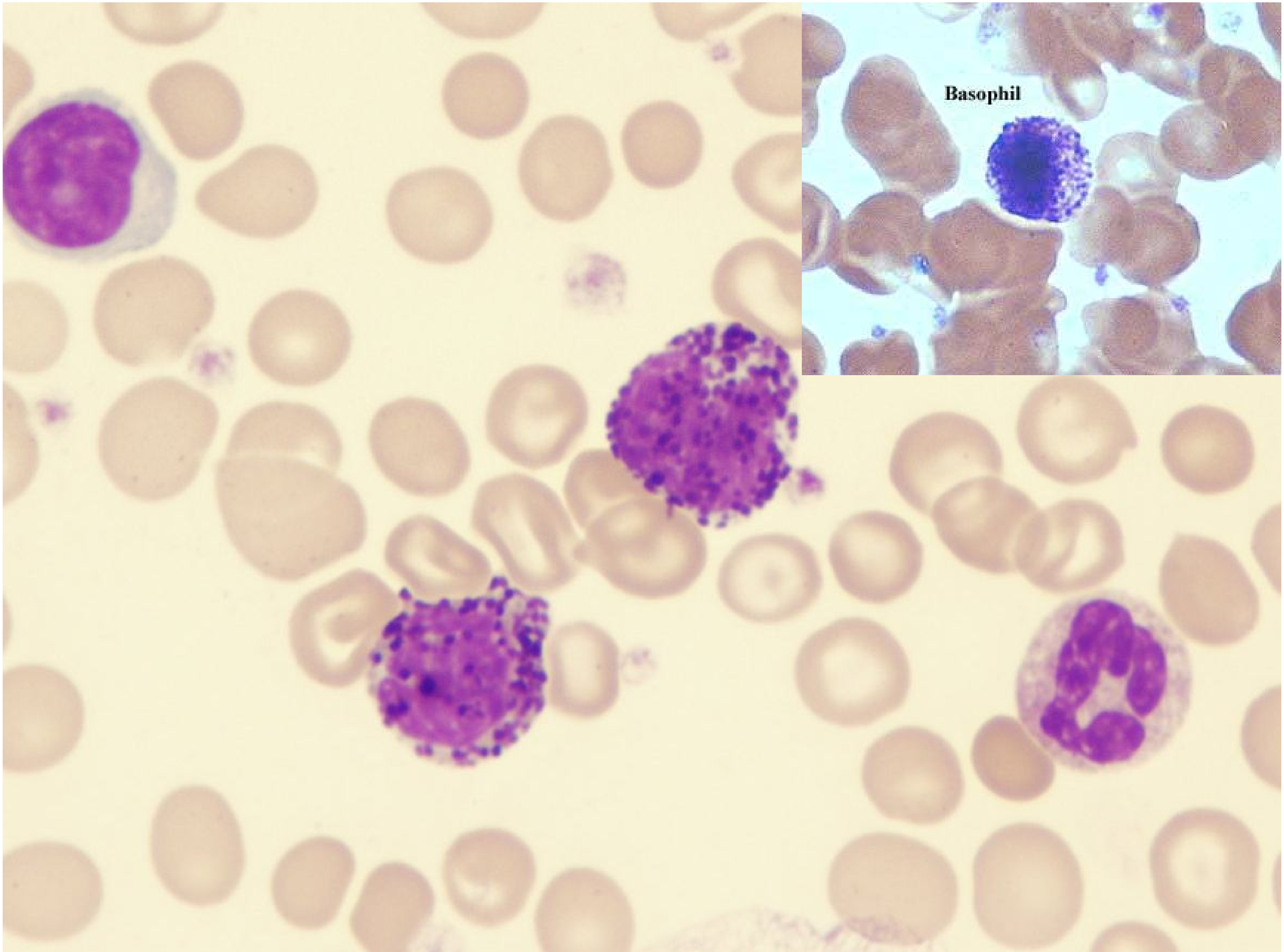


Basophils





- Account for 0.5% of WBCs and:
  - Have U- or S-shaped nuclei with two or three conspicuous constrictions
  - Are functionally similar to mast cells
  - Have large, purplish-black (basophilic) granules that contain histamine
    - **Histamine** – inflammatory chemical that acts as a vasodilator and attracts other WBCs (antihist amines counter this effect)
    - **Heparine**


Basophil

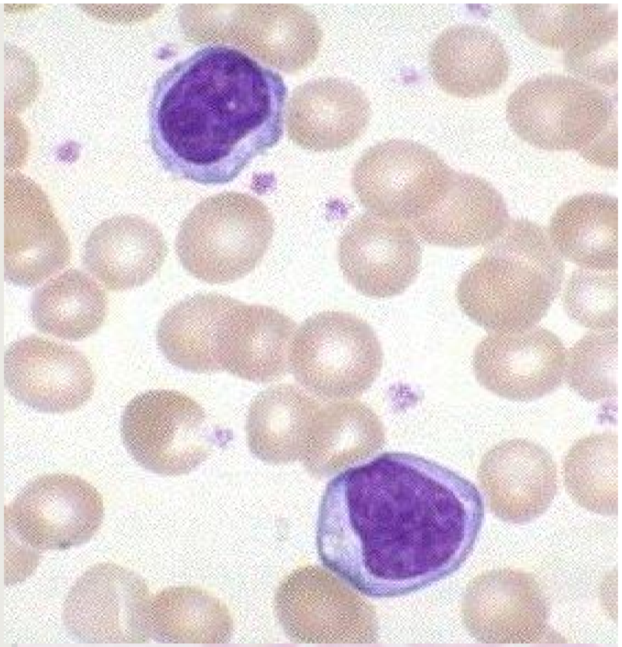
Agranulocytes

- Agranulocytes :
- **lymphocytes and monocytes**:
  - Lack visible cytoplasmic granules
  - Are similar structurally, but are functionally distinct and unrelated cell types
  - Have spherical (lymphocytes) or kidney-shaped (monocytes) nuclei

Lymphocytes

- Account for 25% or more of WBCs and:
  - Have large, dark-purple, circular nuclei with a thin rim of blue cytoplasm
  - Are found mostly enmeshed in lymphoid tissue (some circulate in the blood)

- There are two types of lymphocytes: T cells and B cells
  - *T cells function in the immune response*
  - *B cells give rise to plasma cells, which produce antibodies*



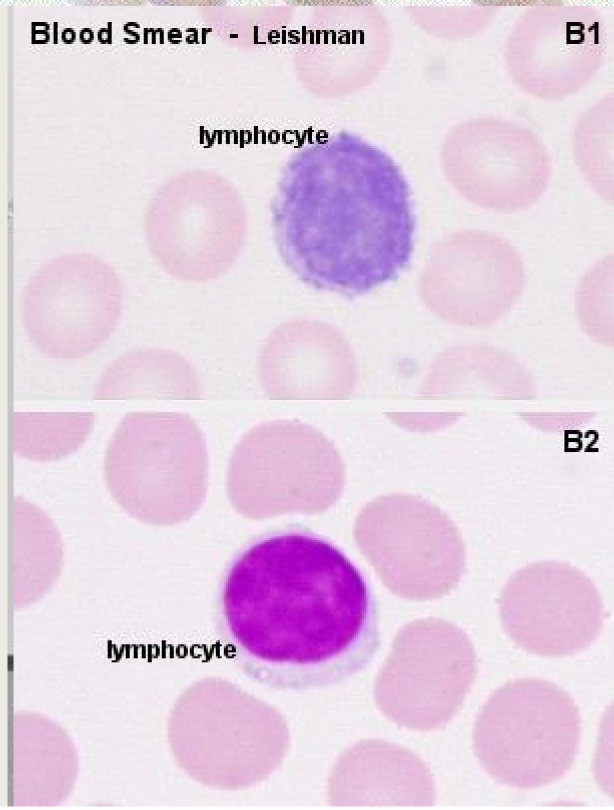
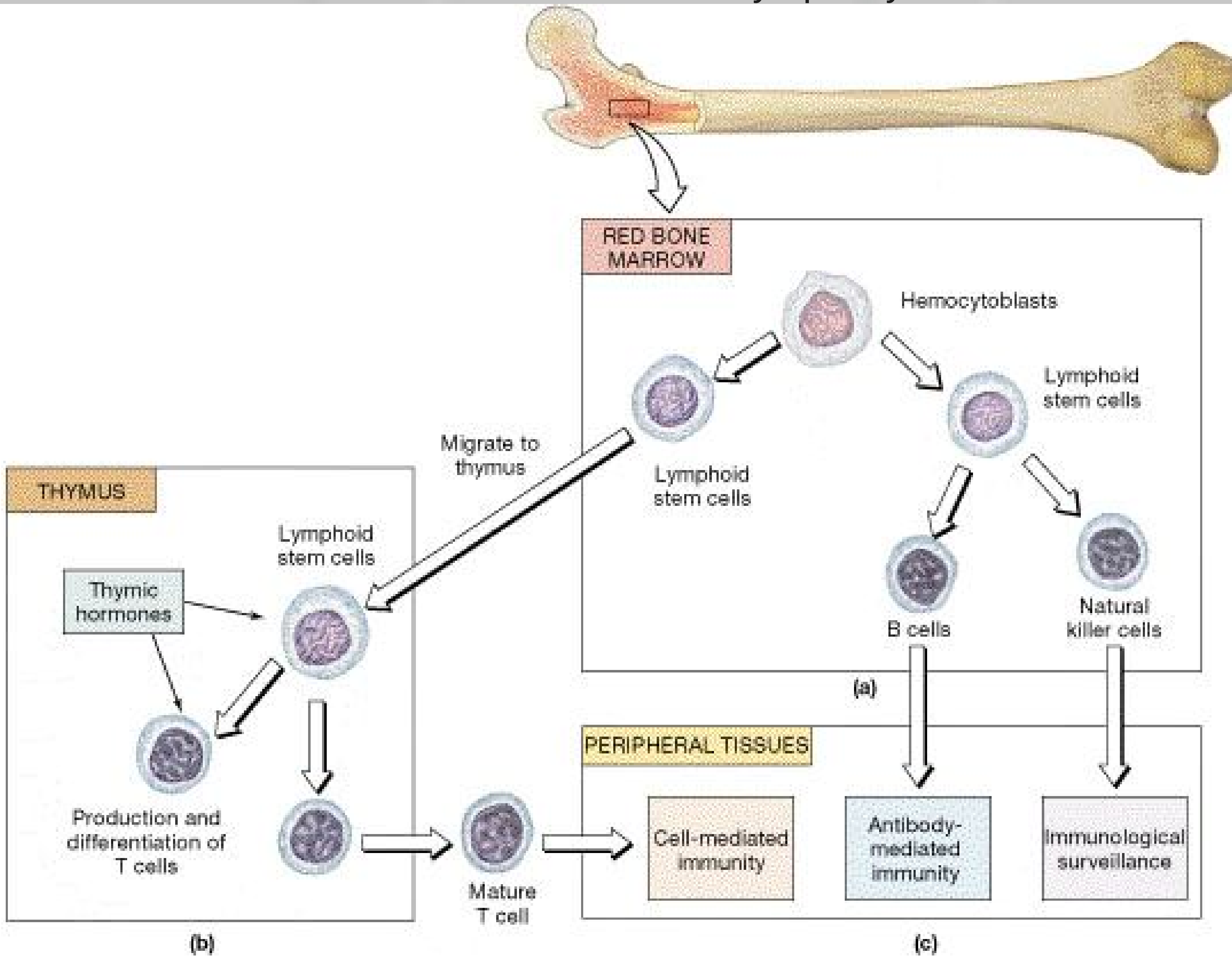


Table 24.1	Types of Lymphocytes	
Cell Type	**Function**	**Type of Antigen Response**
T-LYMPHOCYTE		
Helper T-lymphocyte	Initiates and oversees the immune response	Responds to a single antigen
Cytotoxic T-lymphocyte	Directly kills foreign cells; must be activated by a helper T-lymphocyte first	Responds to a single antigen
Memory T-lymphocyte	A type of cytotoxic T-lymphocyte that has already killed; patrols the body looking for the same antigen again	Responds to a single antigen
Suppressor T-lymphocyte	Helps "turn off" the immune response once it has been activated	Responds to a single antigen
B-LYMPHOCYTE		
Plasma cell	Produces and secretes antibodies	Responds to a single antigen
Memory B-lymphocyte	Remembers an initial antigen attack and mounts a faster, more efficient response should the same antigen type attack again	Responds to a single antigen
NK (NATURAL KILLER) CELL		
NK (natural killer) cell	Kills a wide variety of infected and cancerous cells	Responds to multiple antigens

Derivation and Distribution of Lymphocytes



Cells of Immune System

Stem cells of bon marrow

differentiate into

cytokines (IL-&, IL-3)

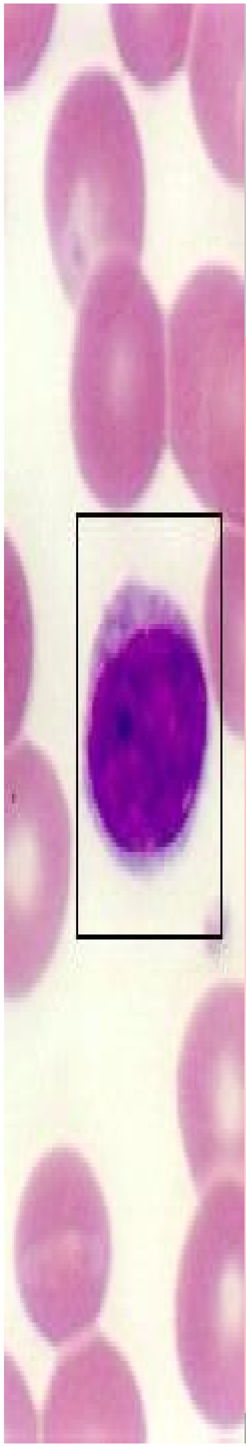
colony stimulating factor

Lymphoid series

Myeloid series

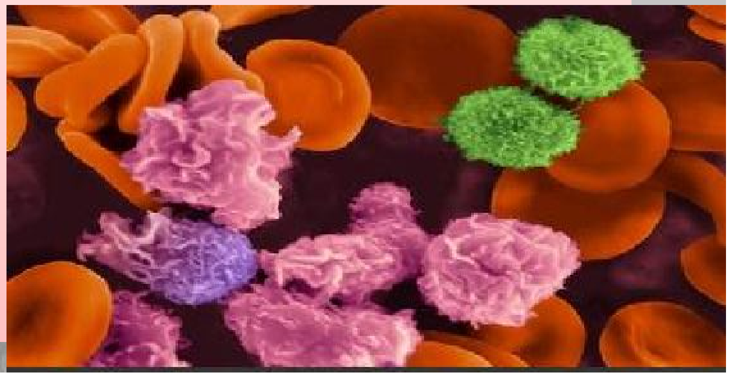
B-lymphocytes **T-lymphocytes** NK

monocytee-macrophages dendritic cells eosinophils mast cells

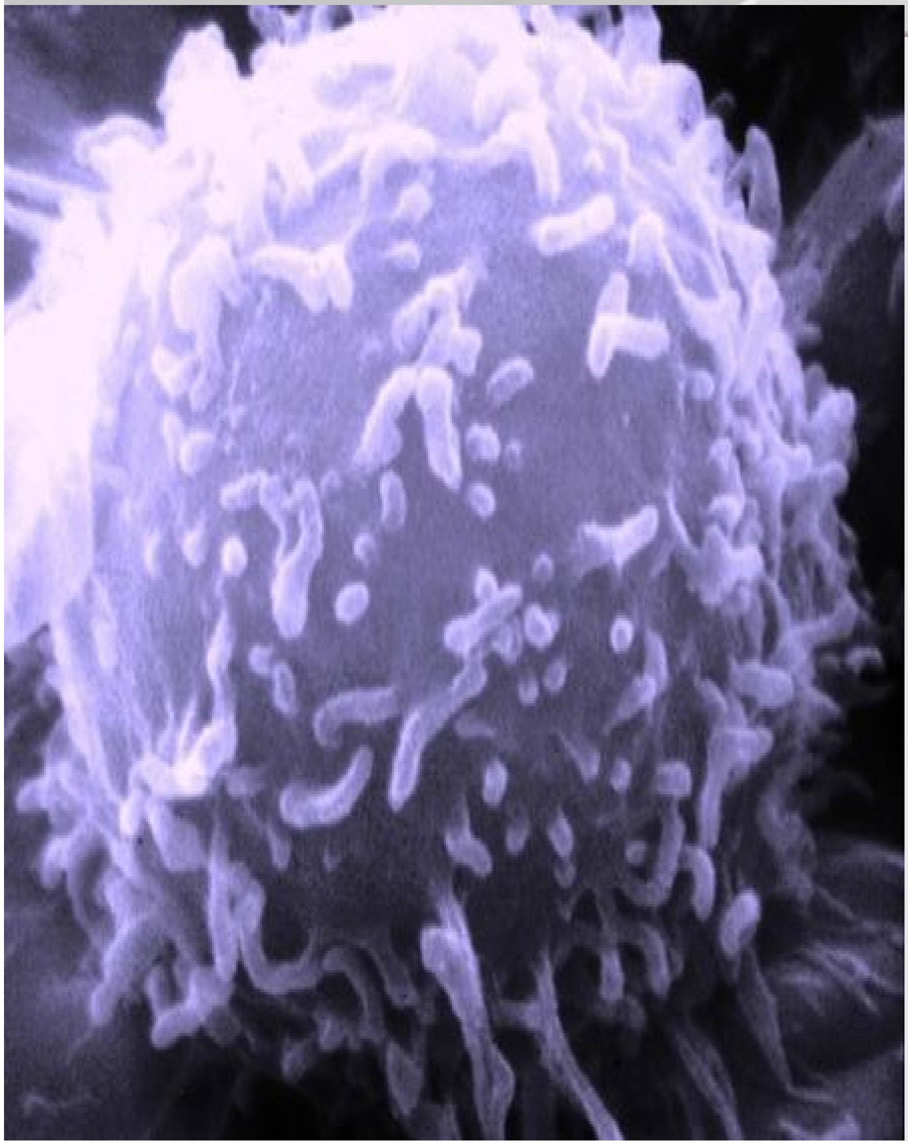


LYMPHOCYTES: IMMUNOCYTES

- Physiological Classification:T and B
  - 'T' LYMPHOCYTES :
    - Thymus trained or schooled cells
    - Responsible for Cell mediated immunity.
    - Provide protection against intracellular pathogens



Lymphocytes



- T cells and B cells protect the body against antigens
- Antigen – anything the body perceives as foreign
  - Bacteria and their toxins; viruses
  - Mismatched RBCs or cancer cells

Lymphocytes

- T cells
  - Manage the immune response
  - Attack and destroy foreign cells
- B cells
  - Produce plasma cells, which secrete antibodies
  - Antibodies immobilize antigens

T-Lmphocytes

Immature T-cells express both CD4 and CD8 (DP)

As they mature

* T-cell with TCRs that have affinity to bind to MHC class II will become helper T-cells with CD4 molecule only

* T-cell with TCRs that have affinity to bind with MHC class I will become cytotoxic T-cells with CD8 molecule only

The Life Of The B Cell

- B lymphocytes are formed within the bone marrow and undergo their development there

They have the following functions:

- To interact with antigenic epitopes, using their immunoglobulin receptors
- To subsequently **develop into plasma cells, secreting large amounts of specific antibody**, or
- To **circulate as memory cells**
- To present antigenic peptides to T cells

B-lympocytes

* Immature B cells express IgM receptors on the surface

* Mature B cells express IgM, IgD molecules on surfaces

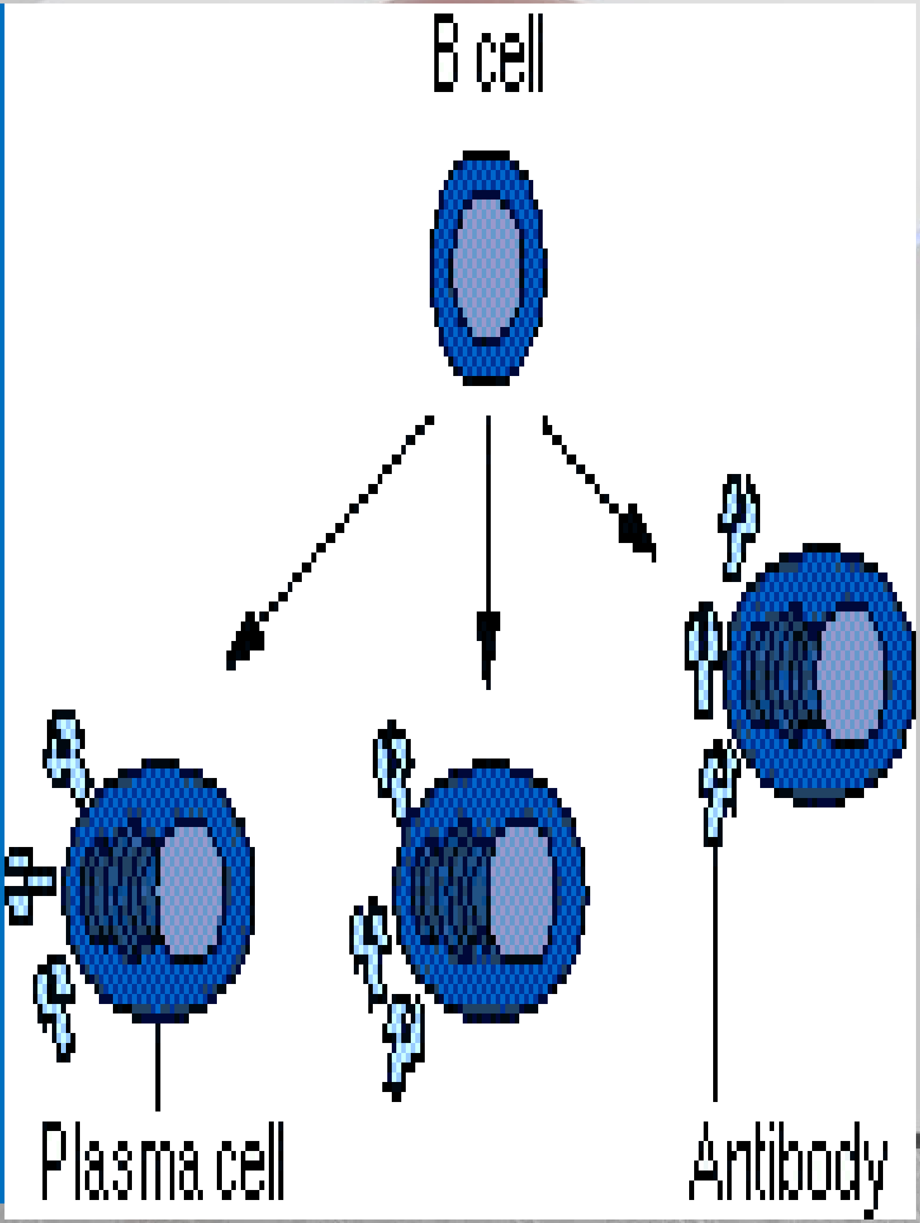
* IgM and IgD molecules serve as receptors for antigens

* Memory B-cells express IgG or IgA or IgE on the surface

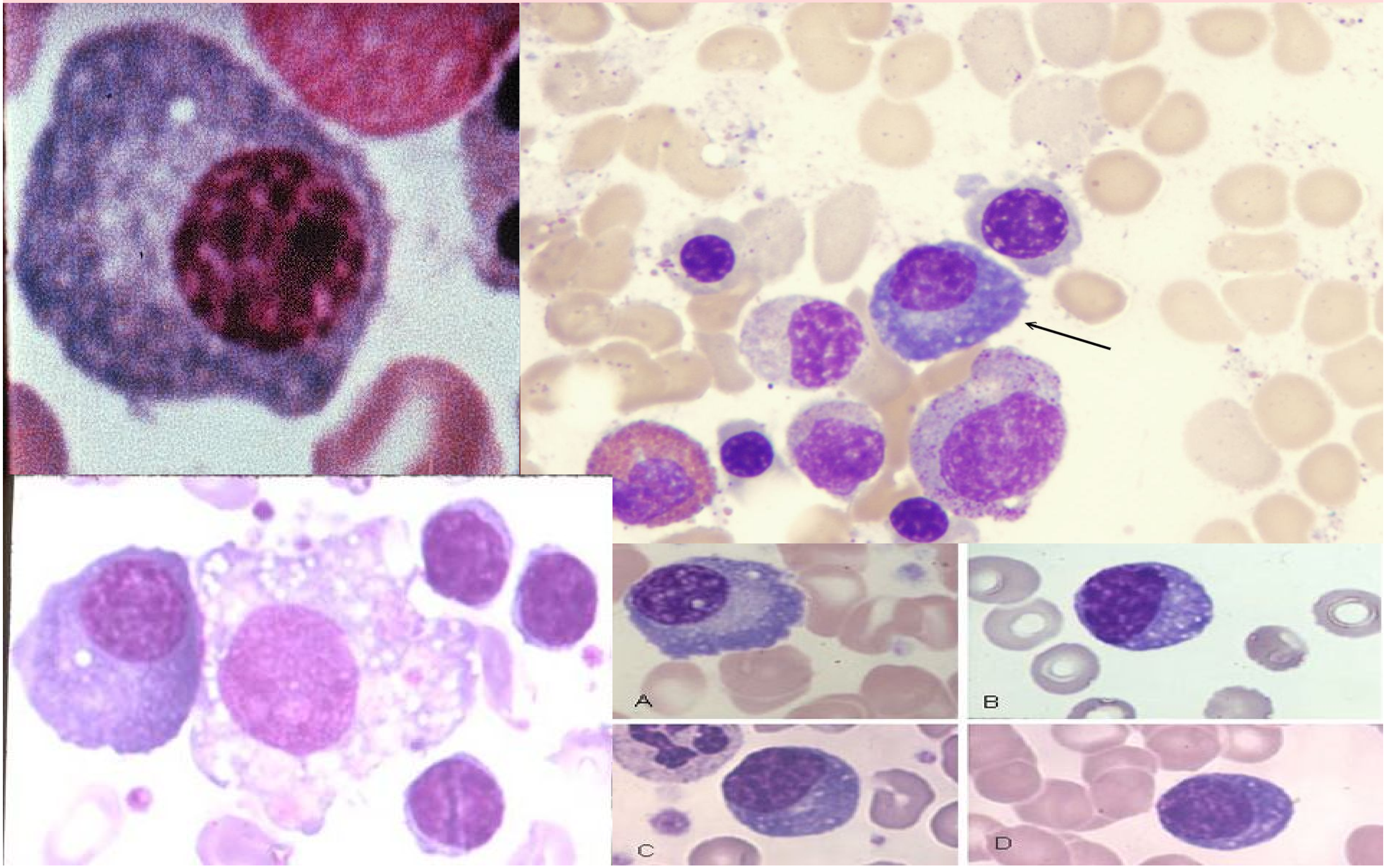
* B-cells bear receptors for Fc portion of IgG and a receptor for C3 component of the complement

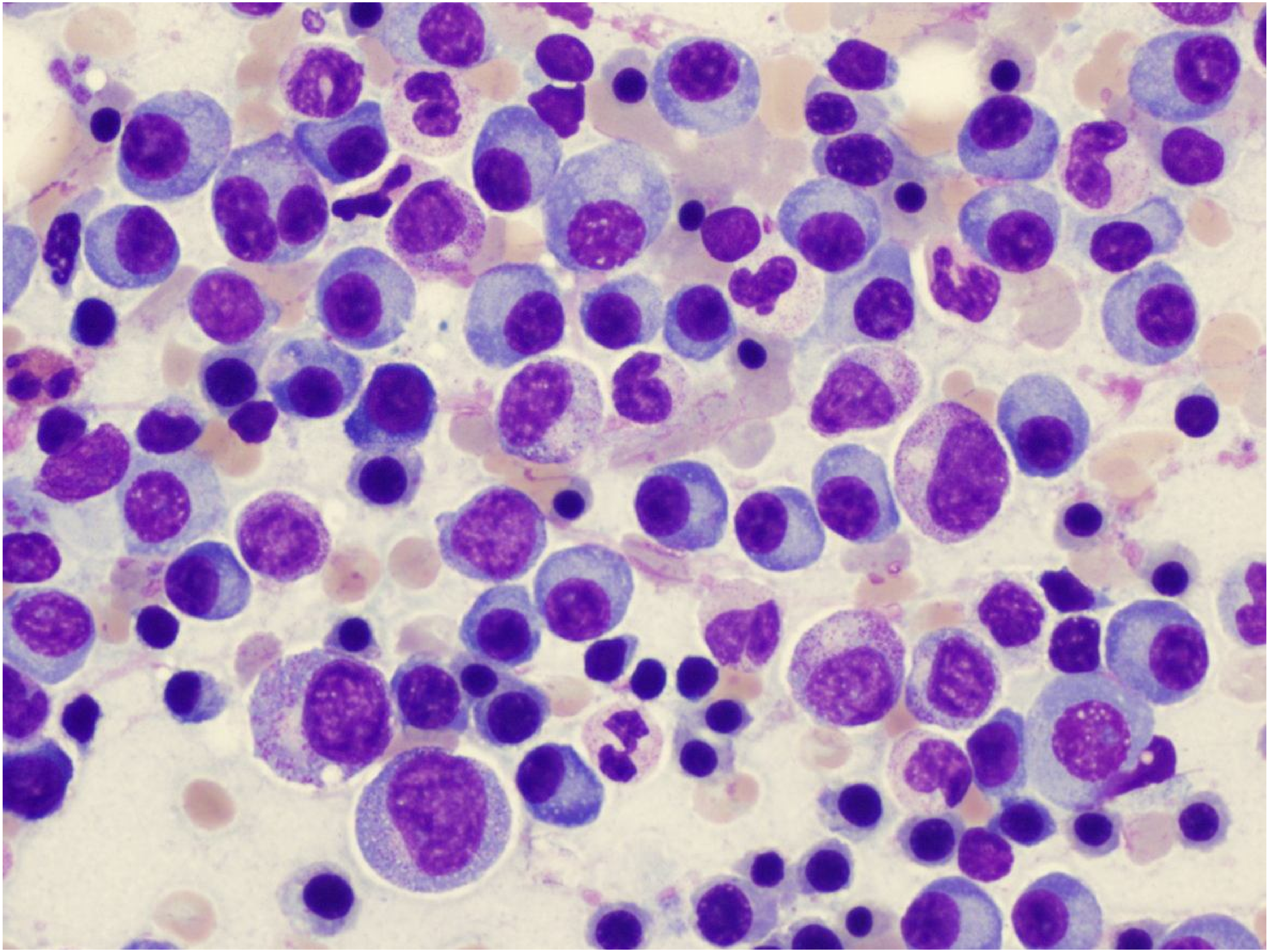
* They express an array of molecules on their surfaces that are important in B-cells interactions with other cells such as MHC II, B7 and CD40

* B cells become plasma cells, which produce antibodies when a foreign antigen triggers the immune response

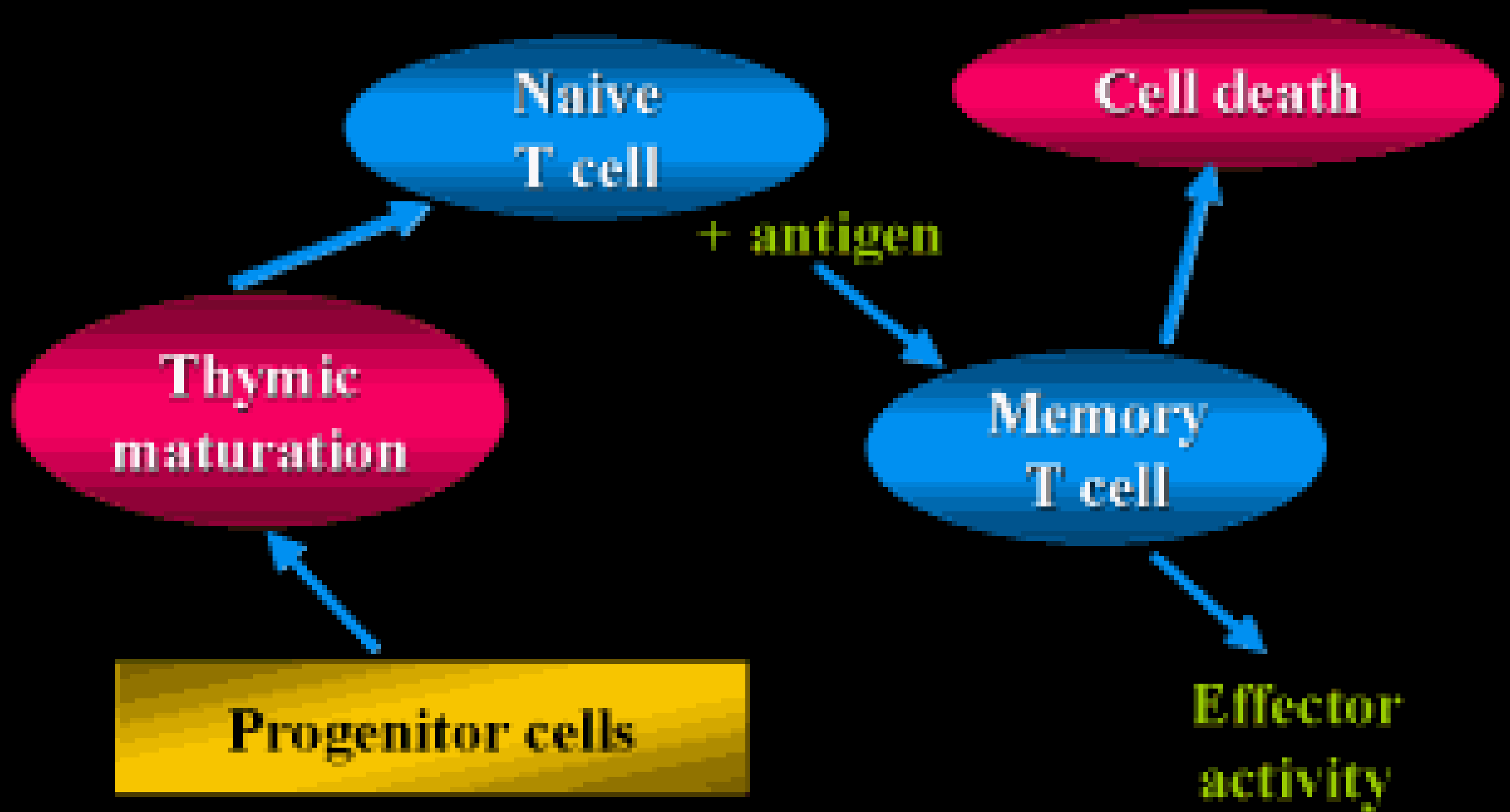


B lymphocytes , plasma cells
plasma B cells, plasmocytes, effector B cells

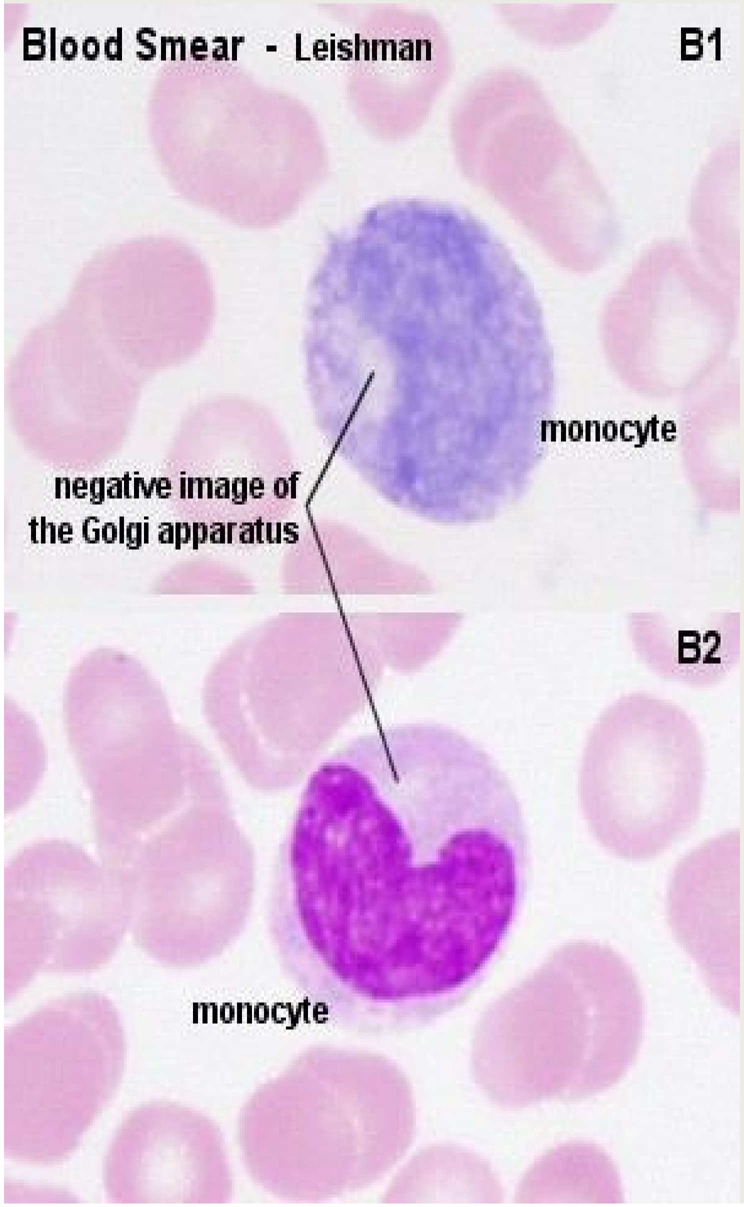




T-Lymphocyte Development and Function



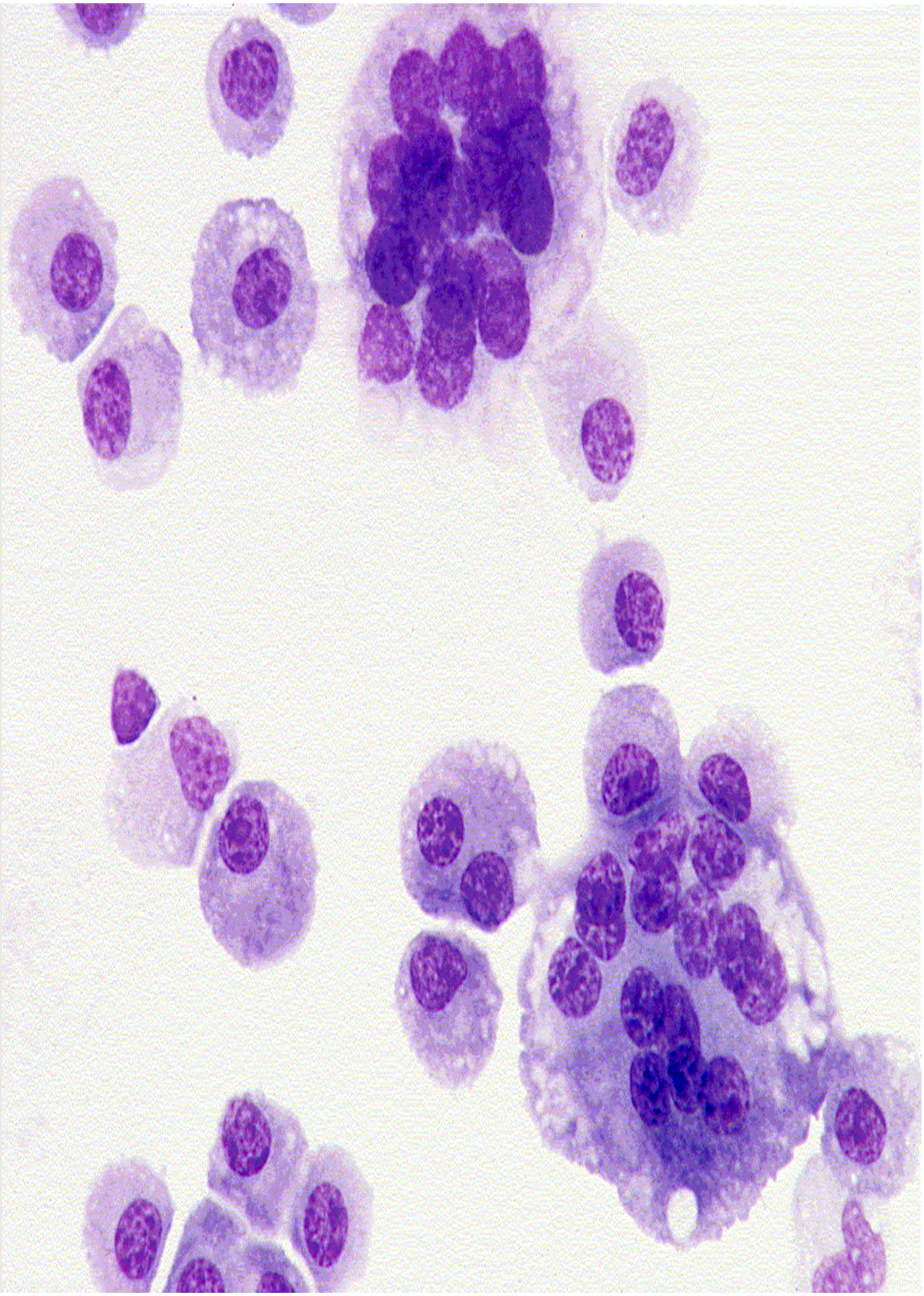
Monocytes



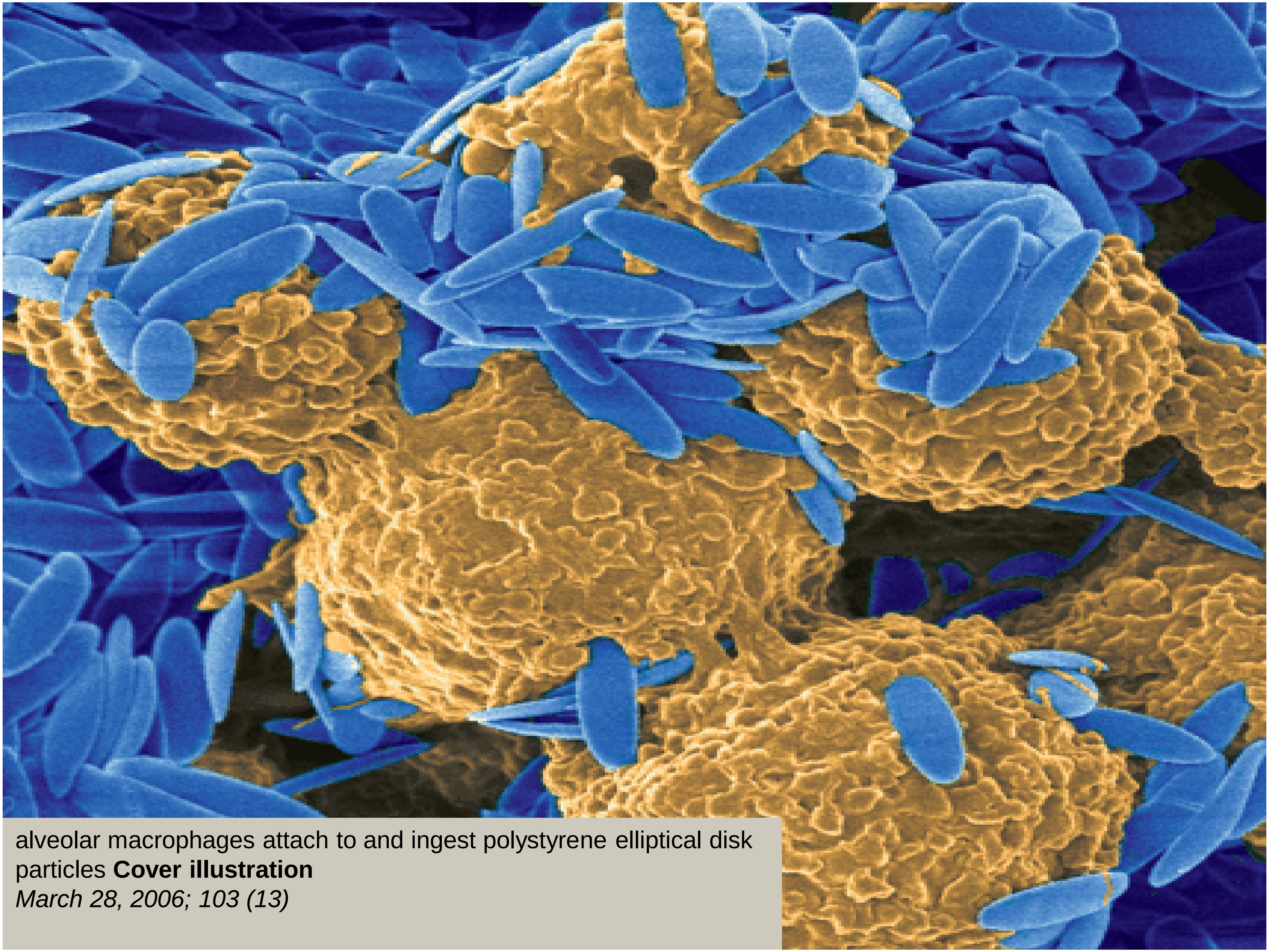
- Monocytes account for 4–8% of leukocytes
  - They are the largest leukocytes
  - They have abundant pale-blue cytoplasms
  - They have purple-staining, **U- or kidney-shaped nuclei**
  - **They leave the circulation, enter tissue, and differentiate into macrophages and dendritic cells**

Macrophages

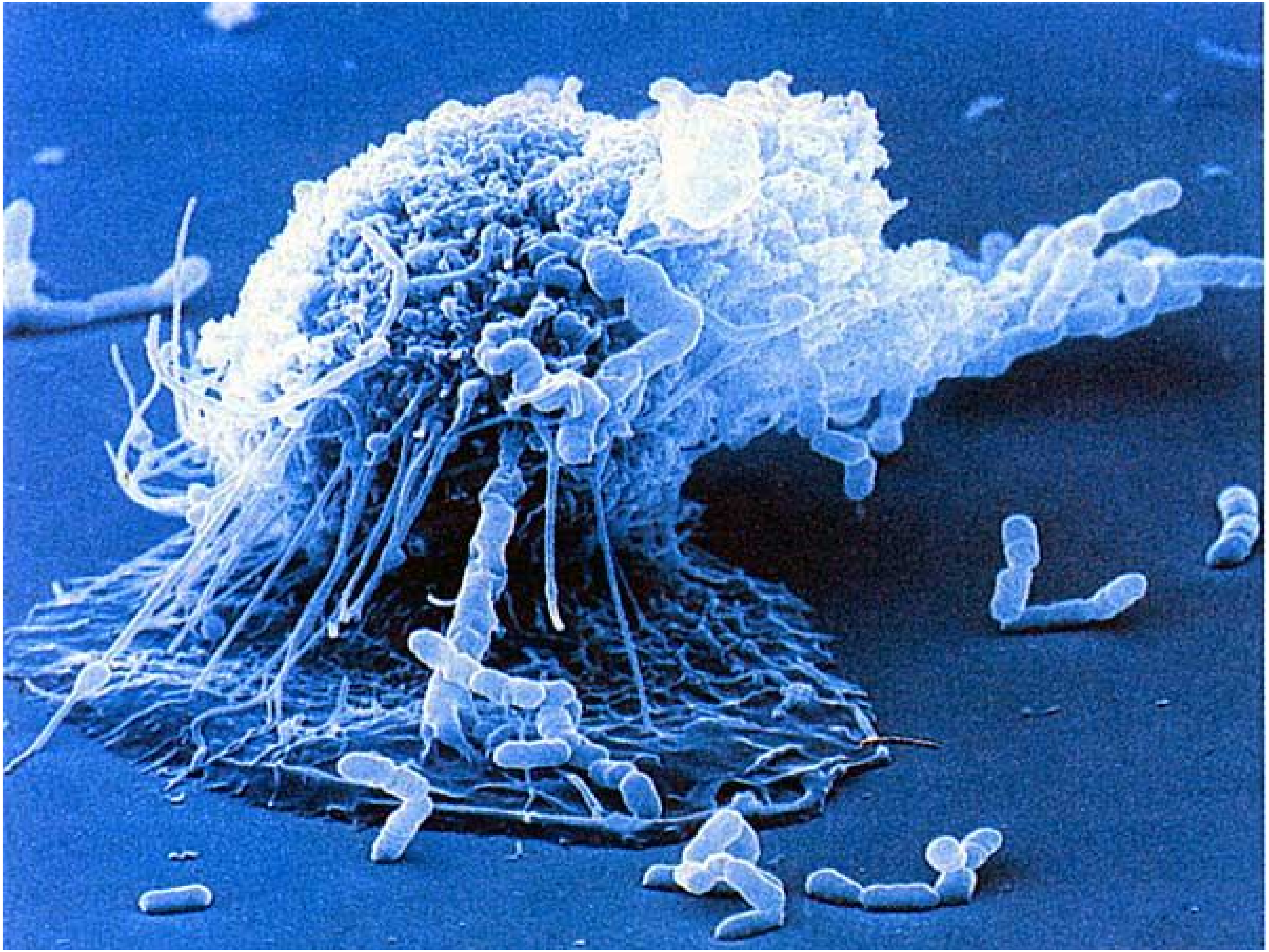
- Macrophages:
  - Are highly mobile and actively phagocytic
  - Activate lymphocytes to mount an immune response
  - Will have different names depending on the location



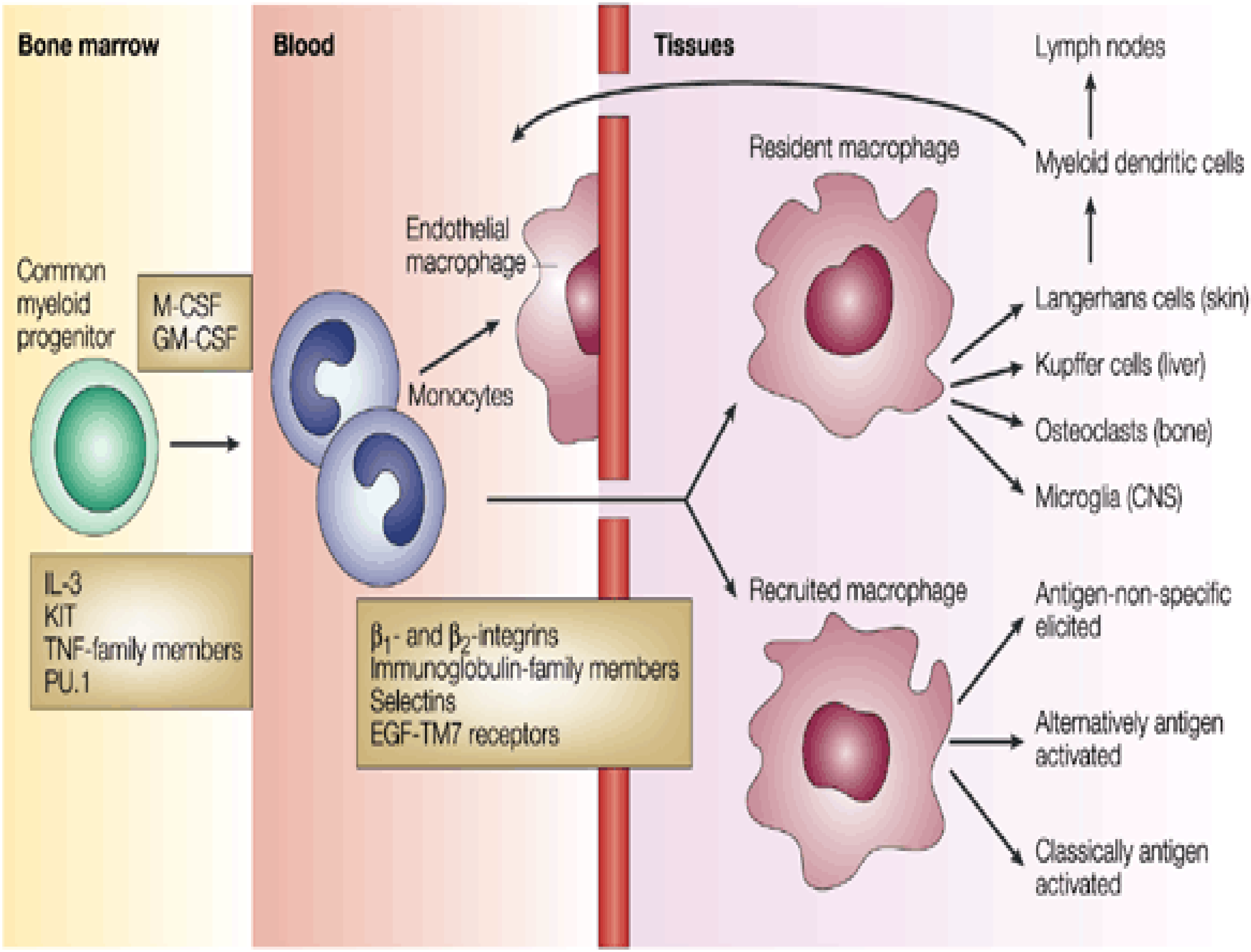
An activated macrophage phagocytosing bacteria upon contact
Photo: courtesy of Dennis Kunkel



alveolar macrophages attach to and ingest polystyrene elliptical disk particles **Cover illustration**
March 28, 2006; 103 (13)

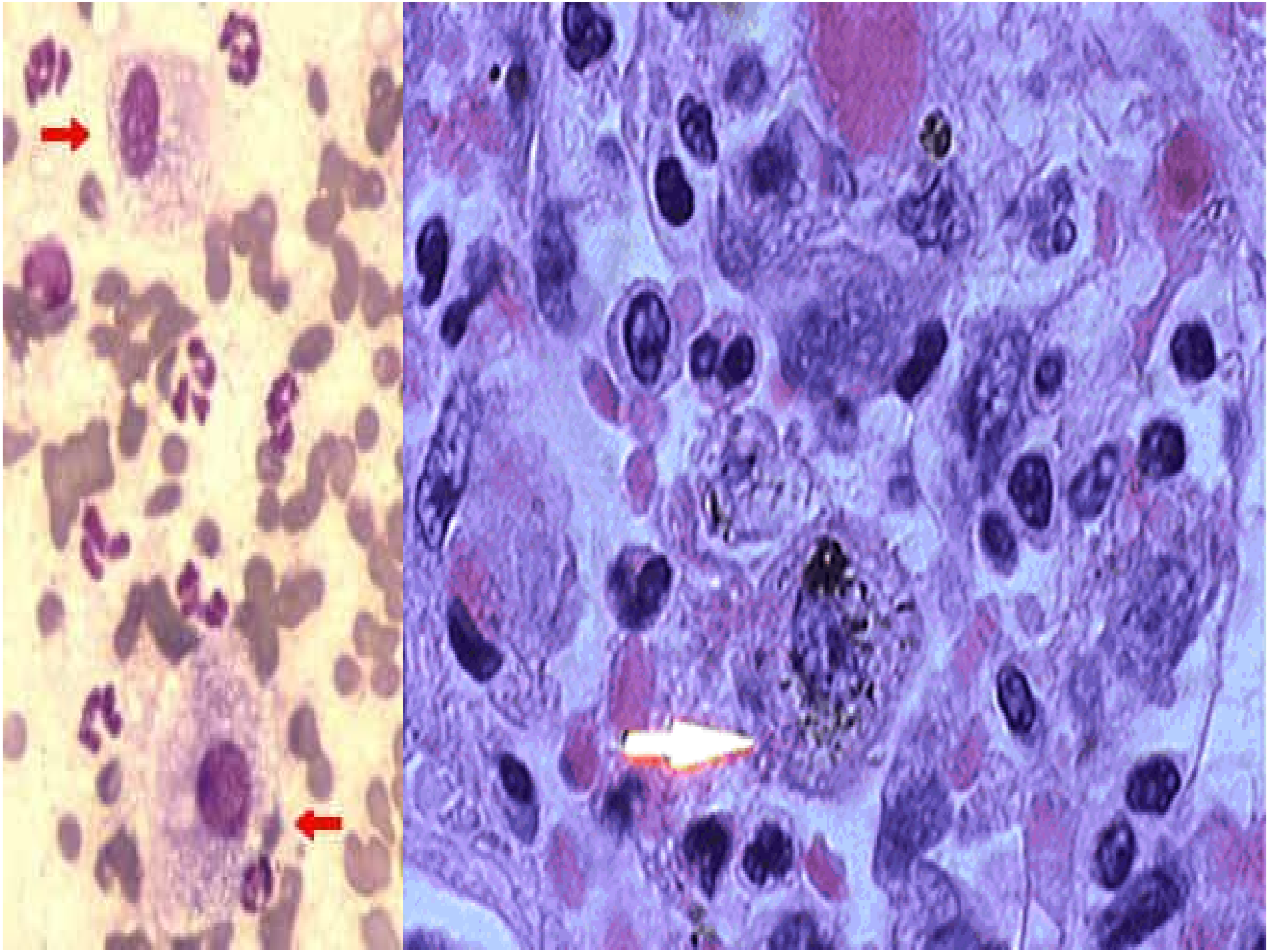


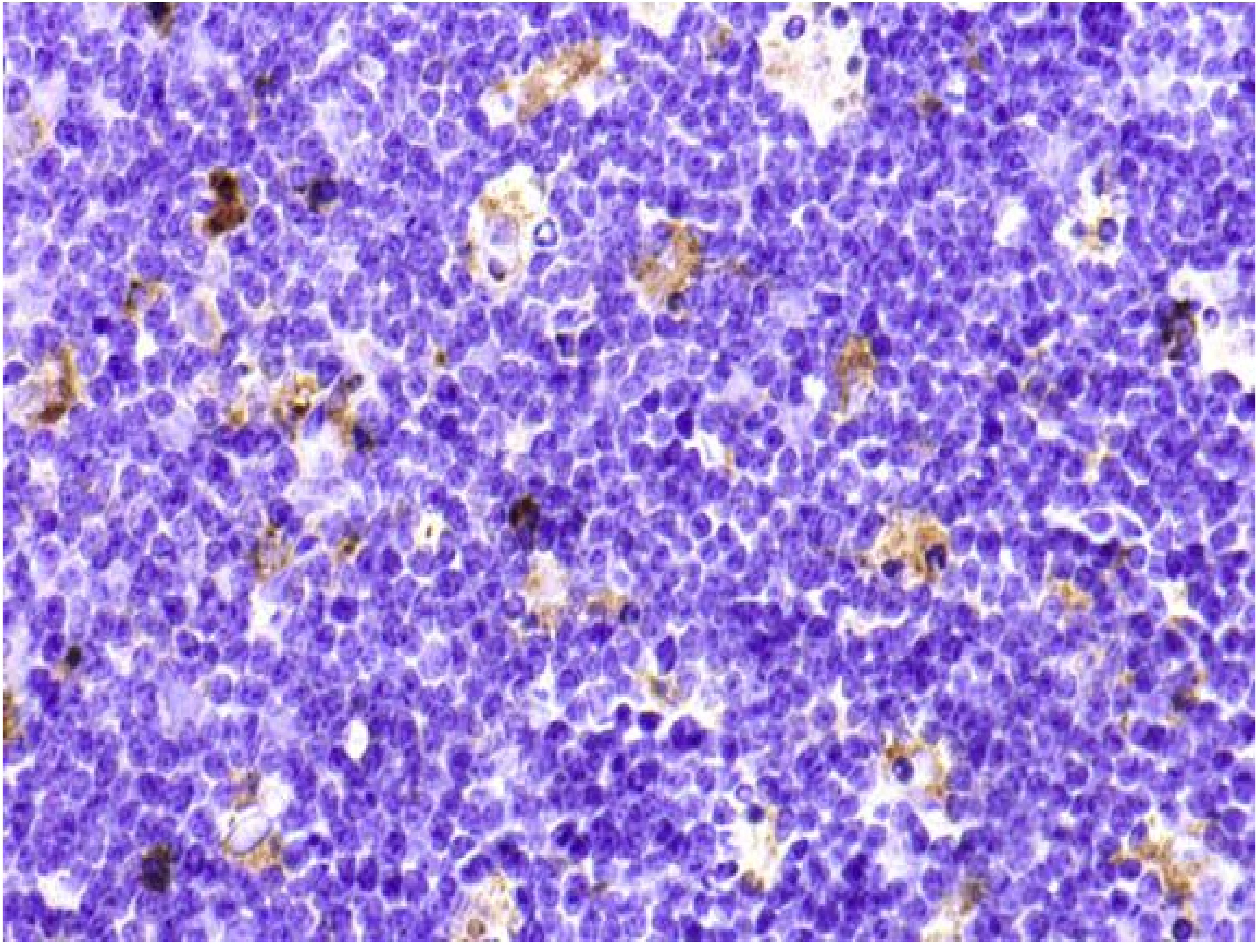
- Normal macrophages **include macrophages located in tissues that include:**
● connective tissue –histiocytes
● liver sinusoids – Kupffer's cells
● lung – alveolar macrophages
● lymph nodes – free and fixed macrophages
● spleen – free and fixed macrophages
● bone marrow – fixed macrophages
● serous fluids –pleural and peritoneal macrophages
● skin – histiocytes, Langerhans's cell

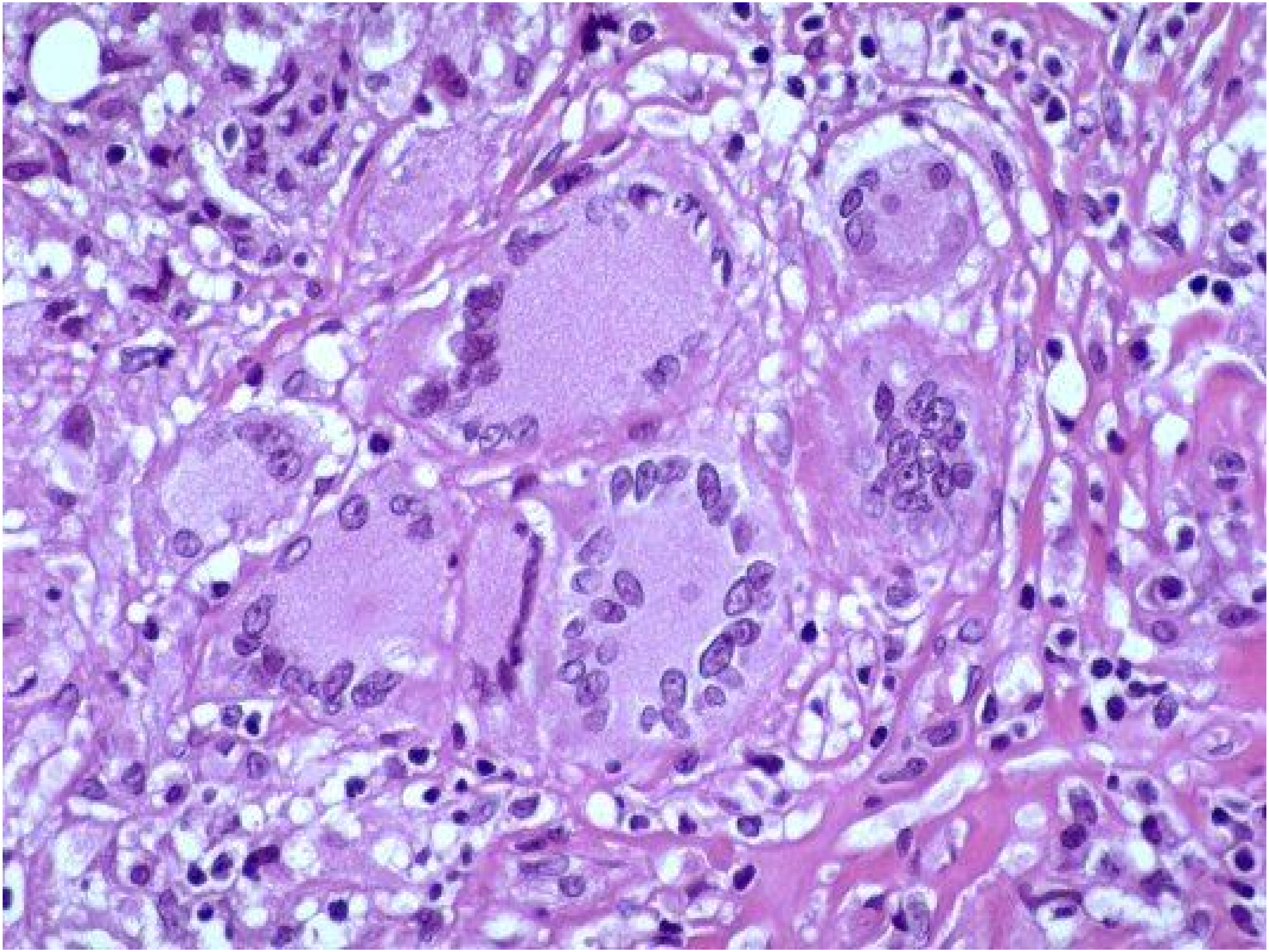


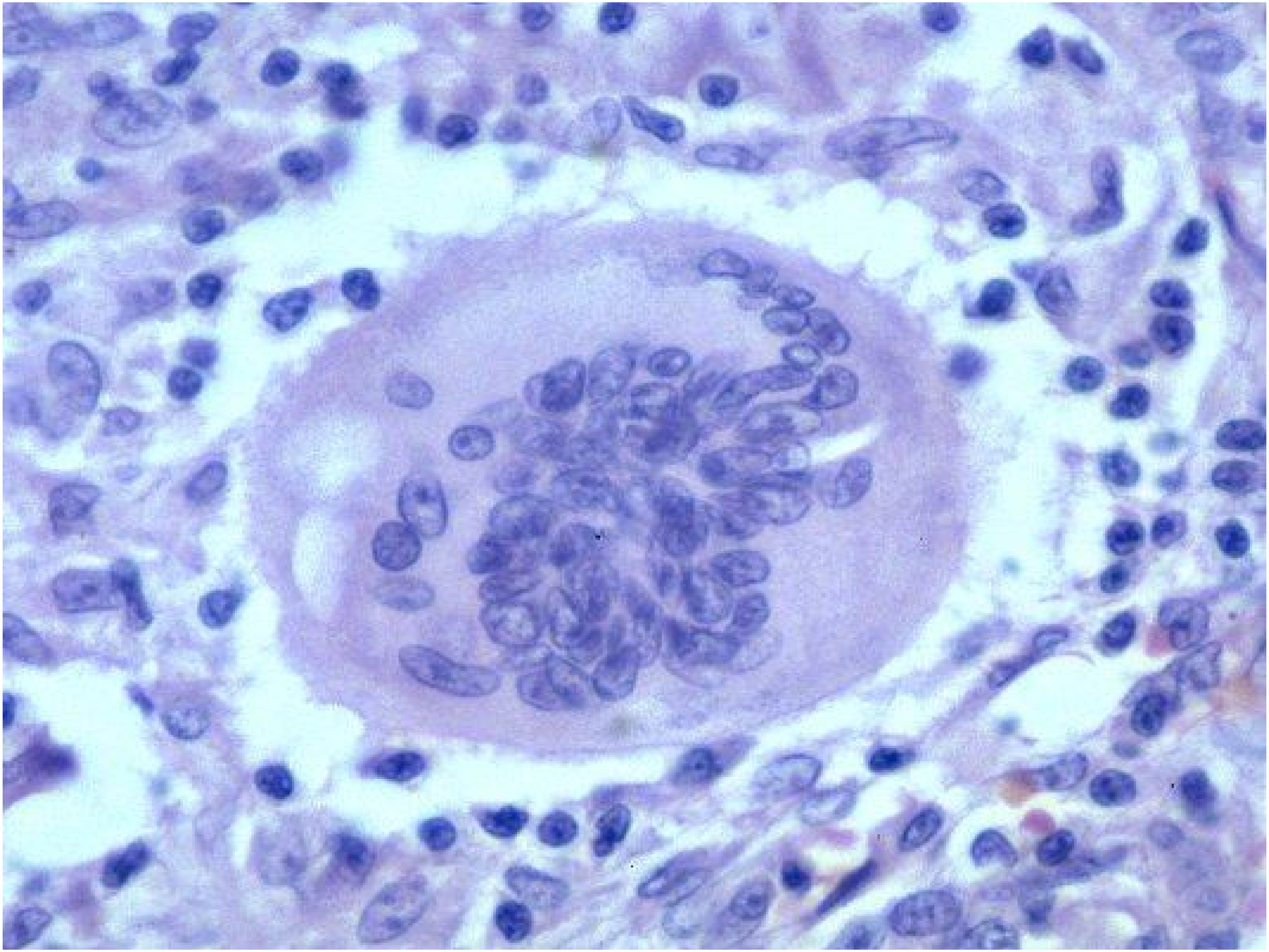
Bone marrow
Blood
Tissues
Lymph nodes
Resident macrophage
Myeloid dendritic cells
Endothelial macrophage
Common myeloid progenitor
M-CSF
GM-CSF
Monocytes
Langerhans cells (skin)
Kupffer cells (liver)
Osteoclasts (bone)
Microglia (CNS)
IL-3
KIT
TNF-family members
PU.1
Recruited macrophage
β_1- and β_2-integrins
Immunoglobulin-family members
Selectins
EGF-TM7 receptors
Antigen-non-specific elicited
Alternatively antigen activated
Classically antigen activated


Alveolar macrophages
("dust cells")
20 um
20 um

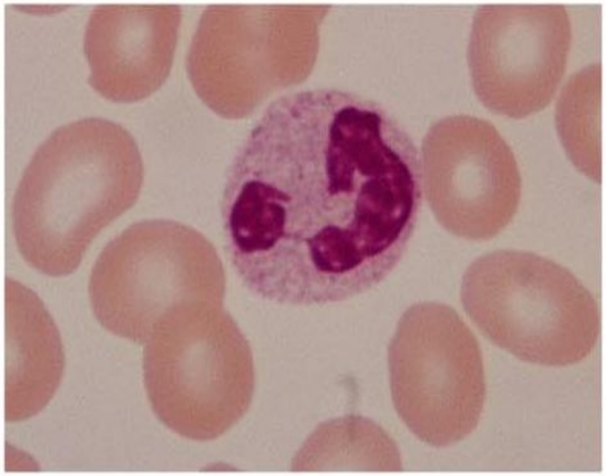




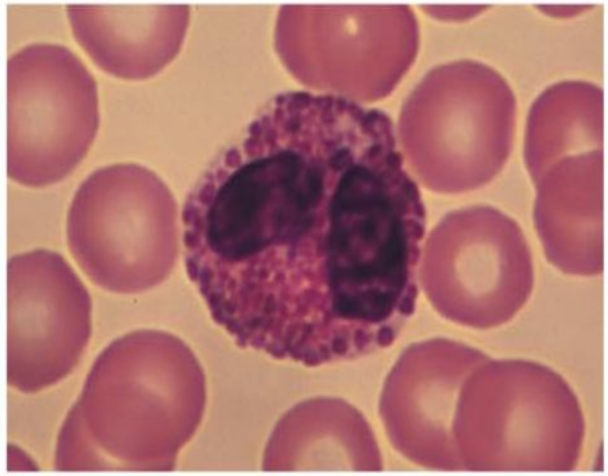




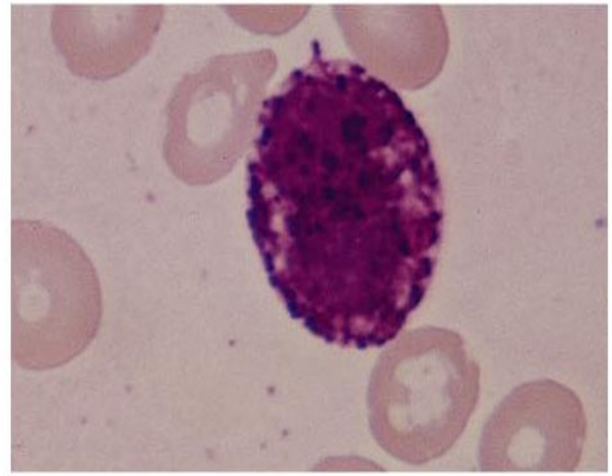
Leukocytes



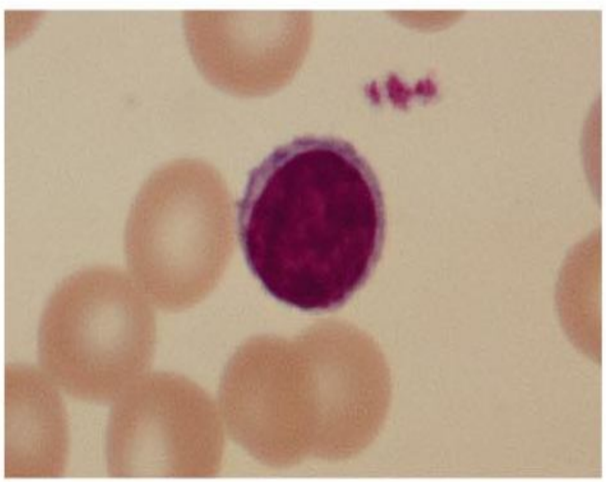
(a)



(b)



(c)



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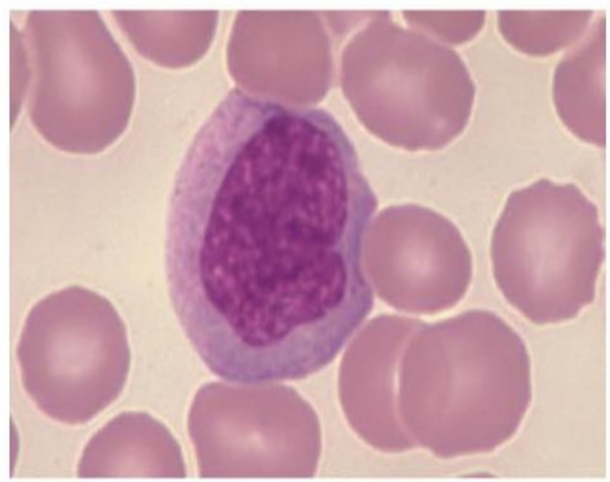


(e)

Summary of Formed Elements

TABLE 17.2 Summary of Formed Elements of the Blood

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 μm	4–6 million	D: about 15 days LS: 100–120 days	Transport oxygen and carbon dioxide
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Granulocytes					
▪ Neutrophil		Nucleus multilobed; inconspicuous cyto-plasmic granules; diameter 10–12 μm	3000–7000	D: about 14 days LS: 6 hours to a few days	Phagocytize bacteria
▪ Eosinophil		Nucleus bilobed; red cytoplasmic granules; diameter 10–14 μm	100–400	D: about 14 days LS: about 5 days	Kill parasitic worms; destroy antigen-antibody complexes; inactivate some inflammatory chemicals of allergy
▪ Basophil		Nucleus lobed; large purplish-black cyto-plasmic granules; diameter 10–14 μm	20–50	D: 1–7 days LS: a few hours to a few days	Release histamine and other mediators of inflammation; contain heparin, an anticoagulant

*Appearance when stained with Wright's stain.

Summary of Formed Elements

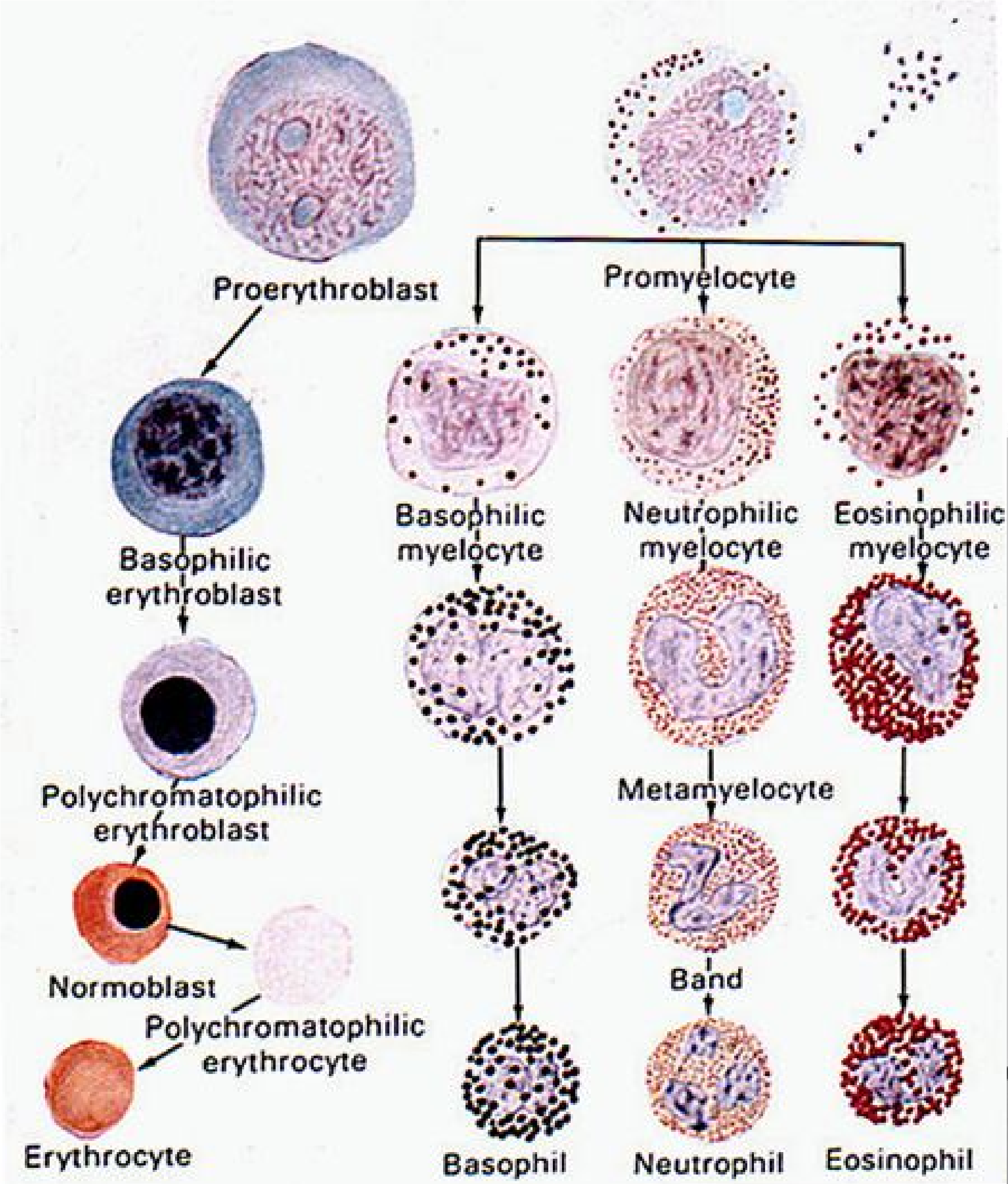
TABLE 17.2 Summary of Formed Elements of the Blood *(continued)*

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Agranulocytes					
▪ Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 μm	1500–3000	D: days to weeks LS: hours to years	Mount immune response by direct cell attack or via antibodies
▪ Monocyte		Nucleus U or kidney shaped; gray-blue cytoplasm; diameter 14–24 μm	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 μm	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

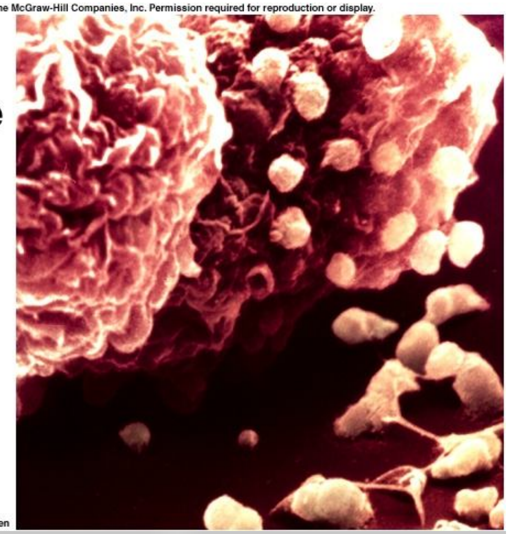
*Appearance when stained with Wright's stain.

Production of Leukocytes

- **Leukopoiesis is stimulated by interleukins and colony-stimulating factors (CSFs)**
  - Interleukins are numbered (e.g., IL-1, IL-2), whereas CSFs are named for the WBCs they stimulate (e.g., granulocyte-CSF stimulates granulocytes)
- **<u>Macrophages and T cells are the most important sources of cytokines</u>**
- Many hematopoietic hormones are used clinically to stimulate bone marrow



Mast cell Releases Histamine Granules



Formation of Leukocytes

- All leukocytes originate from **hemocytoblasts**
- Hemocytoblasts differentiate into myeloid stem cells and lymphoid stem cells
- Myeloid stem cells become myeloblasts or monoblasts
- Lymphoid stem cells become lymphoblasts
- Myeloblasts develop into eosinophils, neutrophils, and basophils
- Monoblasts develop into monocytes
- Lymphoblasts develop into lymphocytes

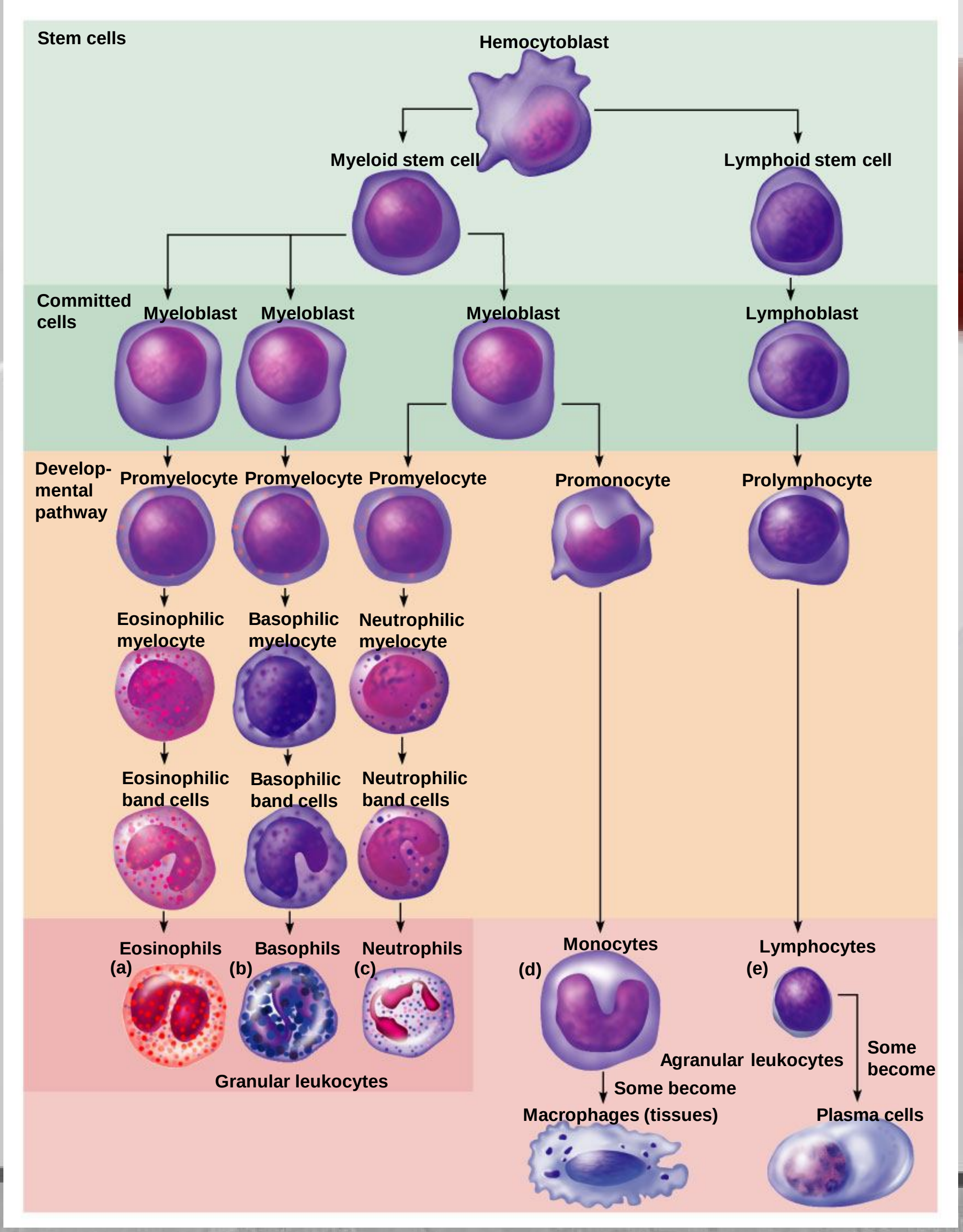
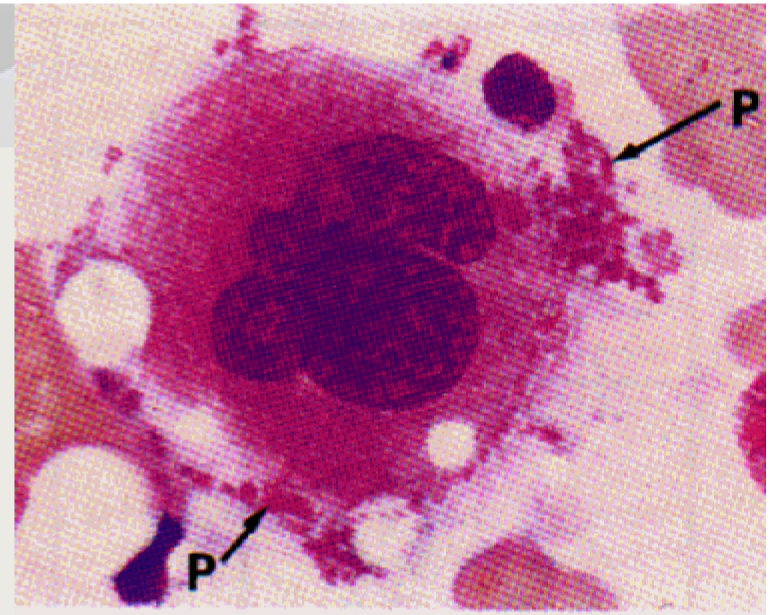

Stem cells
Hemocytoblast
Myeloid stem cell
Lymphoid stem cell
Committed cells
Myeloblast
Myeloblast
Myeloblast
Lymphoblast
Develop-mental pathway
Promyelocyte
Promyelocyte
Promyelocyte
Promonocyte
Prolymphocyte
Eosinophilic myelocyte
Basophilic myelocyte
Neutrophilic myelocyte
Eosinophilic band cells
Basophilic band cells
Neutrophilic band cells
Eosinophils
(a)
Basophils
(b)
Neutrophils
(c)
Granular leukocytes
Monocytes
(d)
Lymphocytes
(e)
Agranular leukocytes
Some become
Some become
Macrophages (tissues)
Plasma cells

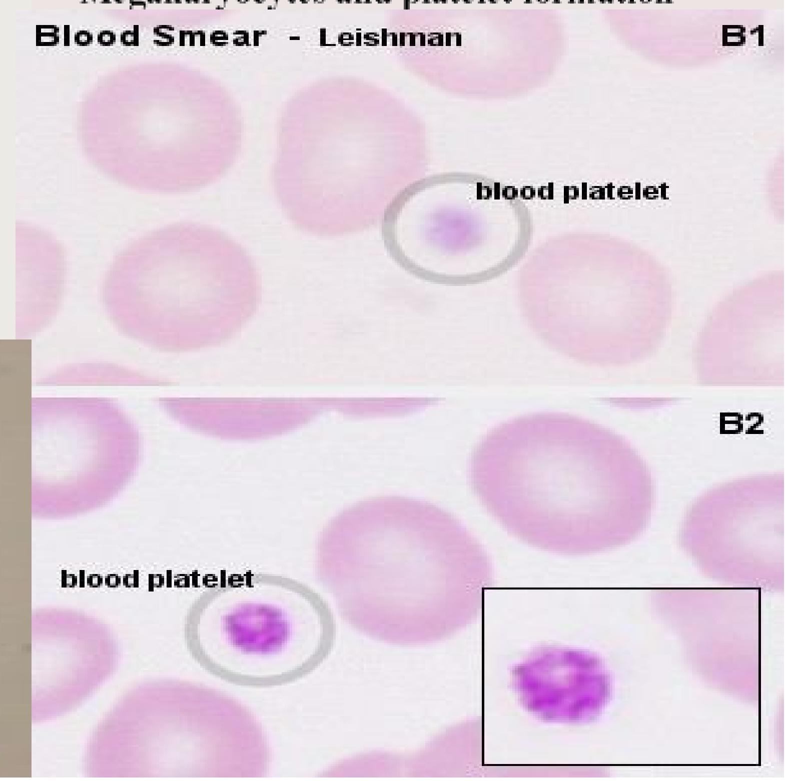
Figure 17.11

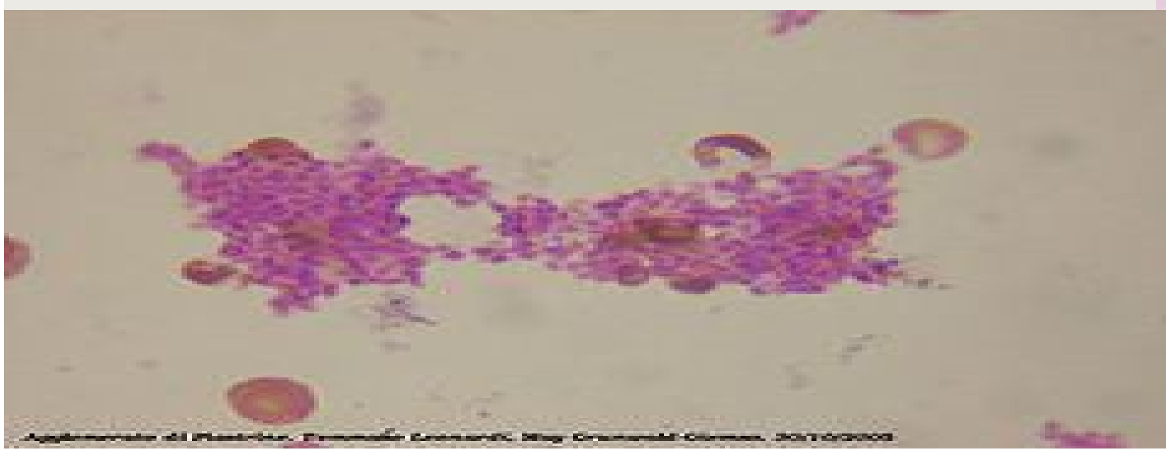
Platelets

- Platelets are fragments of **megakaryocytes** with a blue-staining outer region and a purple granular center
- Their granules contain
  - **serotonin, Ca^{2+},**
  - **enzymes, ADP,**
  - **and platelet-derived growth factor (PDGF)**
- Platelets function in the clotting mechanism by forming a temporary plug that helps seal breaks in blood vessels
- **Platelets not involved in clotting are kept inactive by NO and prostacyclin**



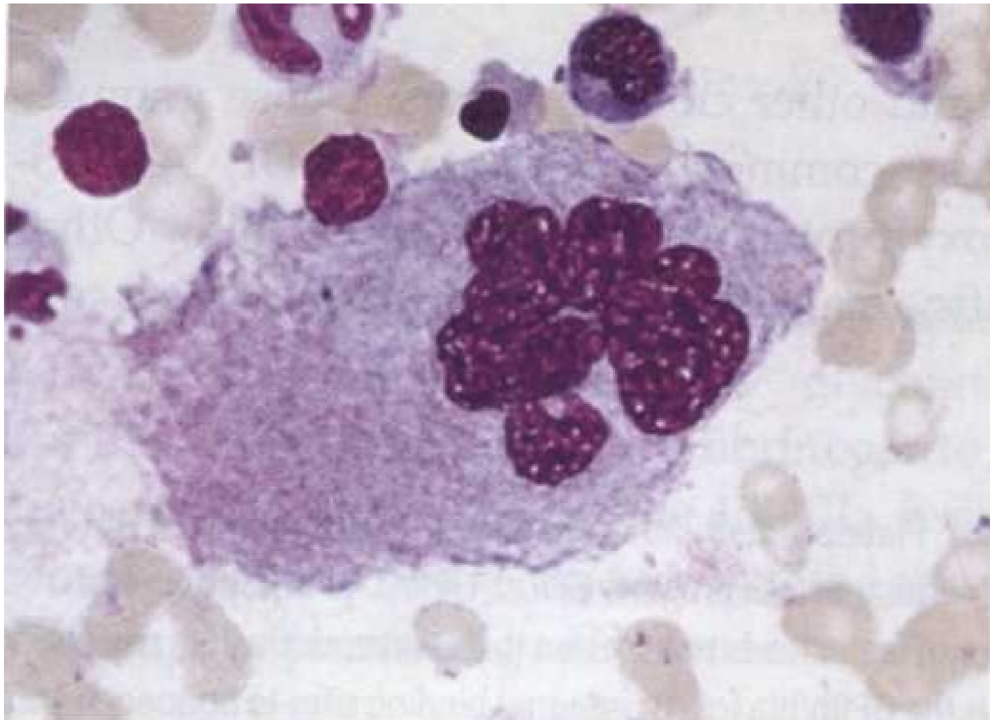
Megakaryocytes and platelet formation

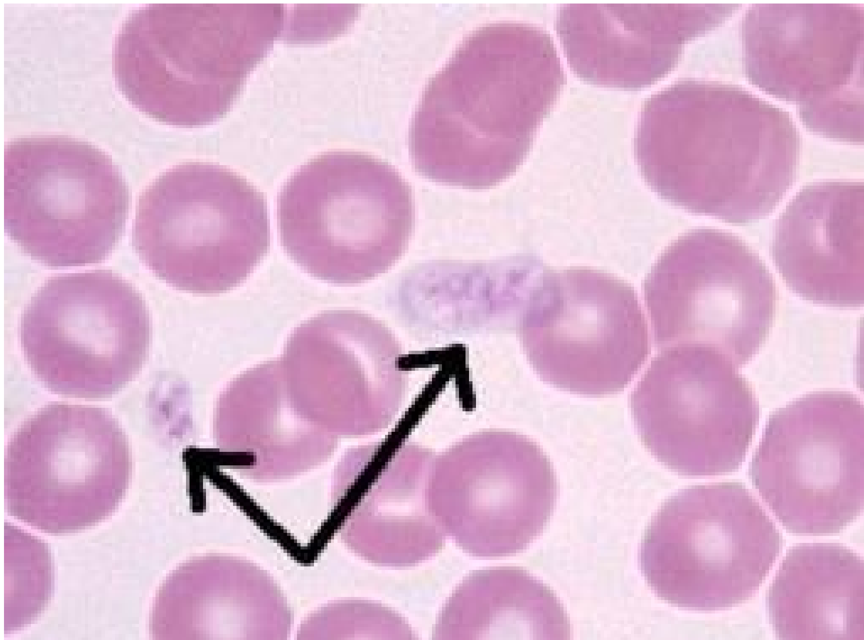
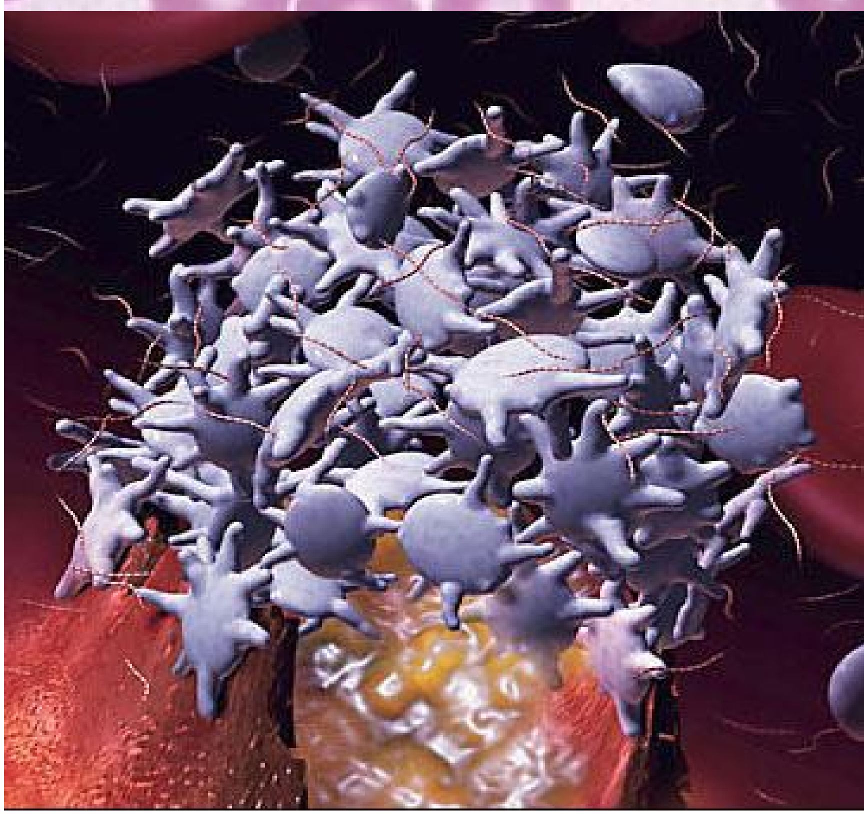
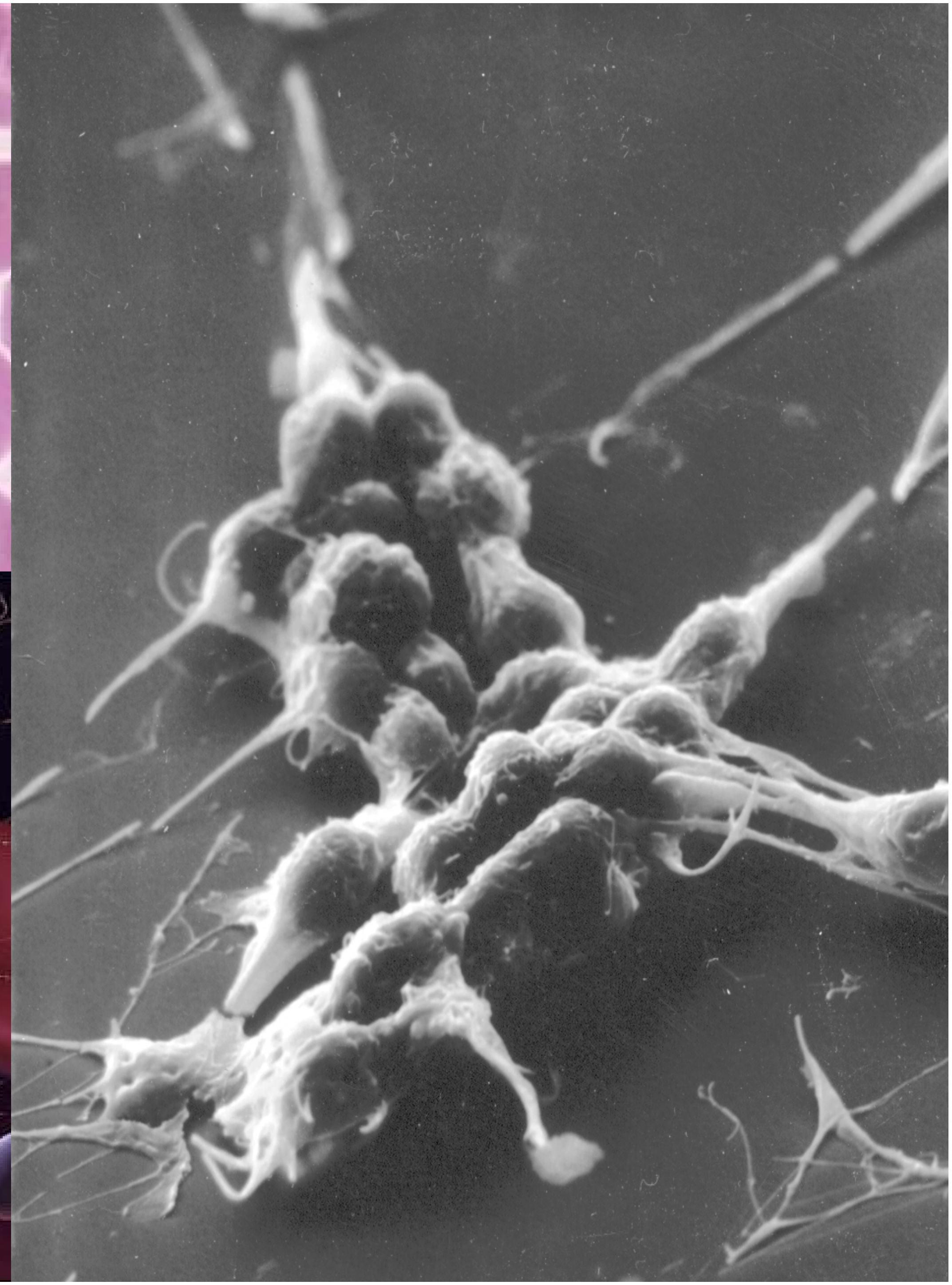




PLATELET FUNCTION STUDIES

- Bleeding Time
- Platelet Count
- Platelet Aggregation Studies



Cytoplasm includes active proteins such as:

- Actin.
- Myosin.
- Thrombesthenin.

- The normal concentration of platelets in the blood is between 150,000 and 300,000 per microliter.
- do not have nuclei and cannot reproduce .

In their cytoplasm are such active factors as

- (1) actin and myosin molecules,
  - as well as still another contractile protein, thrombosthenin, that can cause the platelets to contract;
- (2) residuals of both the endoplasmic reticulum and the Golgi apparatus that synthesize various enzymes and store large quantities of calcium ions;
- (3) mitochondria and enzyme systems that are capable of forming ATP and ADP;
- (4) enzyme systems that synthesize prostaglandins, which are local hormones that cause many types of vascular and other local tissue reactions;
- (5) fibrin-stabilizing factor;
- (6) a growth factor that can cause vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus causing cellular growth that helps repair damaged vascular walls.

- The cell membrane of the platelets is also important.

- On its surface is a coat of glycoproteins that causes it to avoid adherence to normal endothelium and yet to adhere to injured areas of the vessel wall, especially to injured endothelial cells and even more so to any exposed collagen from deeper in the vessel wall.

- In addition, the membrane contains large amounts of phospholipids that play several activating roles at multiple points in the blood-clotting process.

Thrombopoietin:

- Regulator of platelet production.
- Produced by the liver and kidneys.
- Levels are increased in thrombocytopenia,and reduced in thrombocytosis.
- It increases the no. & rate of maturation of the megakaryocytes.

Functions of platelets

- Platelets assist in **hemostasis, the arrest of bleeding**.
- Serotonin is a potent vasoconstrictor.
- The release of serotonin from thrombocytes, which adhere to the walls of a damaged vessels, is sufficient to close even small arteries.
- Platelets, which come into contact with collagenous fibers in the walls of the vessel (which are not usually exposed to the blood stream), swell, become "sticky" and activate other platelets to undergo the same transformation.
- This cascade of events results in the formation of a platelet plug (or platelet thrombus). Finally, activating substances are released from the damaged vessel walls and from the platelets.
- These substances mediate the conversion of the plasma protein prothrombin into thrombin.
- Thrombin catalyzes the conversion of fibrinogen into fibrin, which polymerizes into fibrils and forms a fibrous net in the arising blood clot.
- Platelets captured in the fibrin net contract leading to clot retraction, which further assists in hemostasis.

Genesis of Platelets

- The stem cell for platelets is the **hemocytoblast**
- The sequential developmental pathway is as shown.

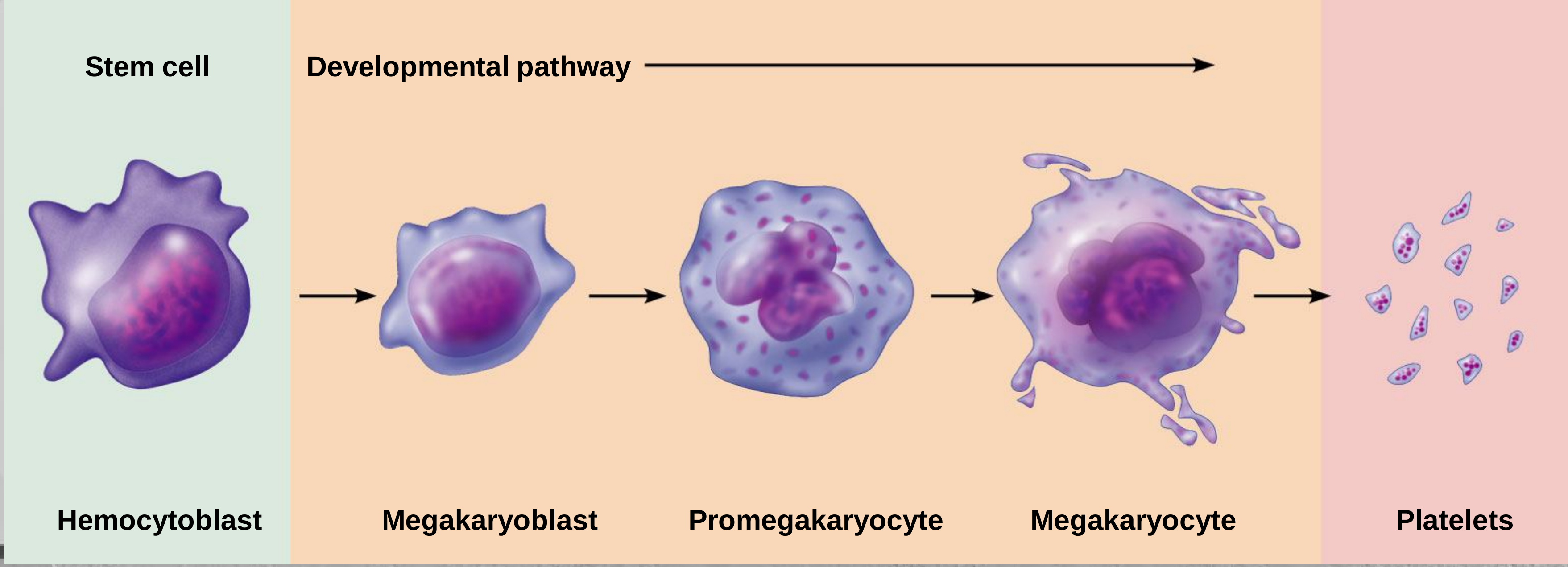
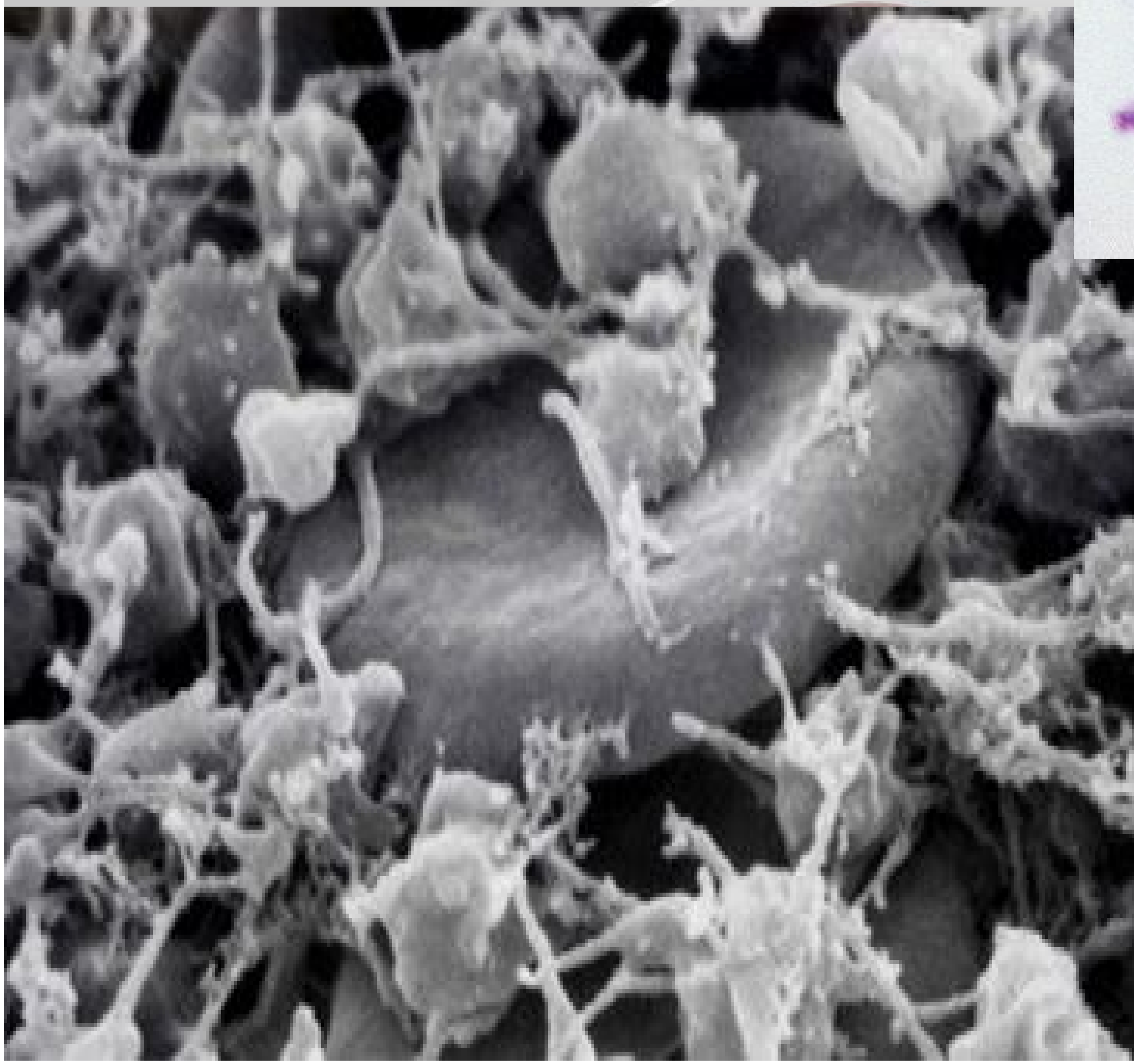


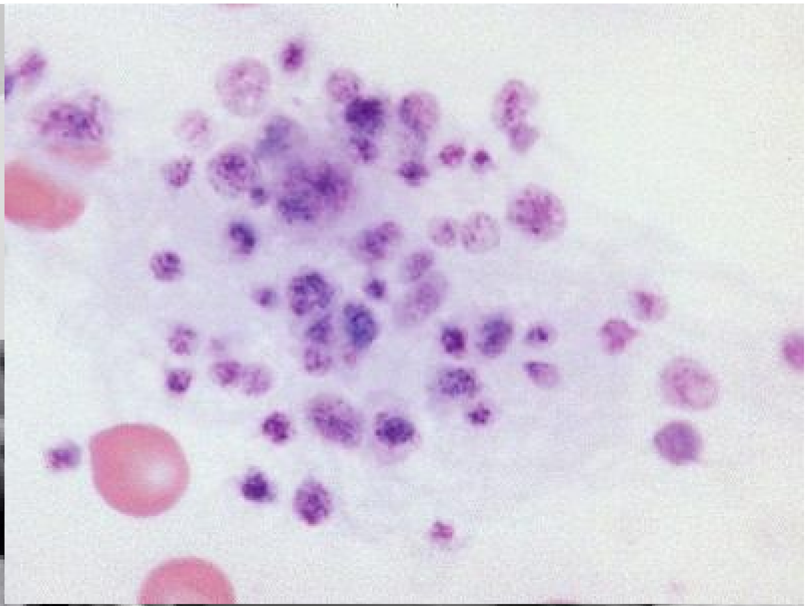
Figure 17.12

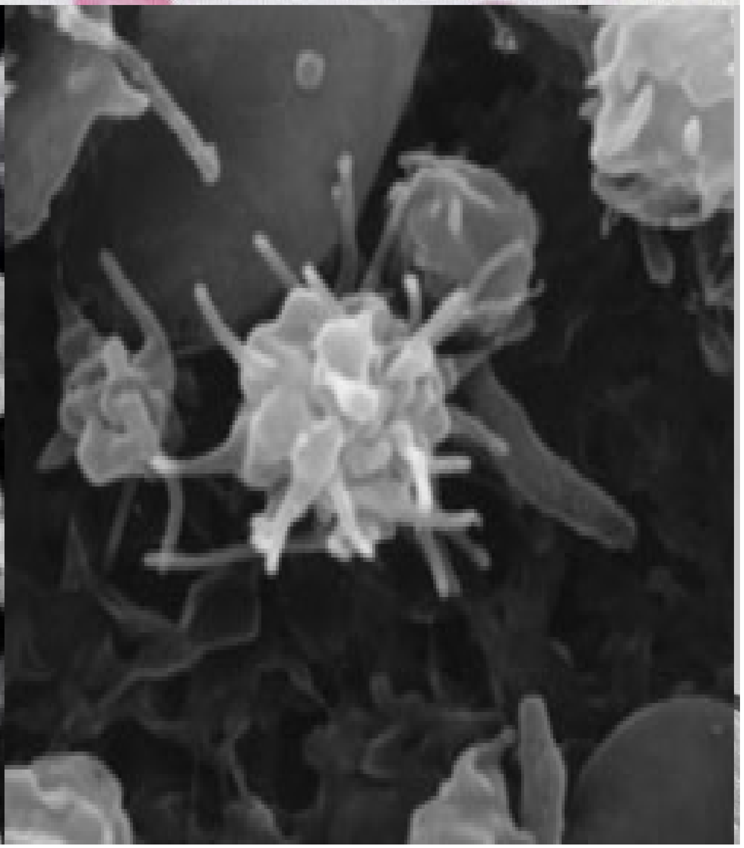
PLATELET CIRCULATION

- ❖ Normal count is 250,000.
- ❖ Normal life span 7-10 days.
- ❖ About 1/3 are trapped in the spleen.

Activated platelets







Platelet adhesion:
Binding to collagen

Platelet activation:
Release of contents, surface receptor expression