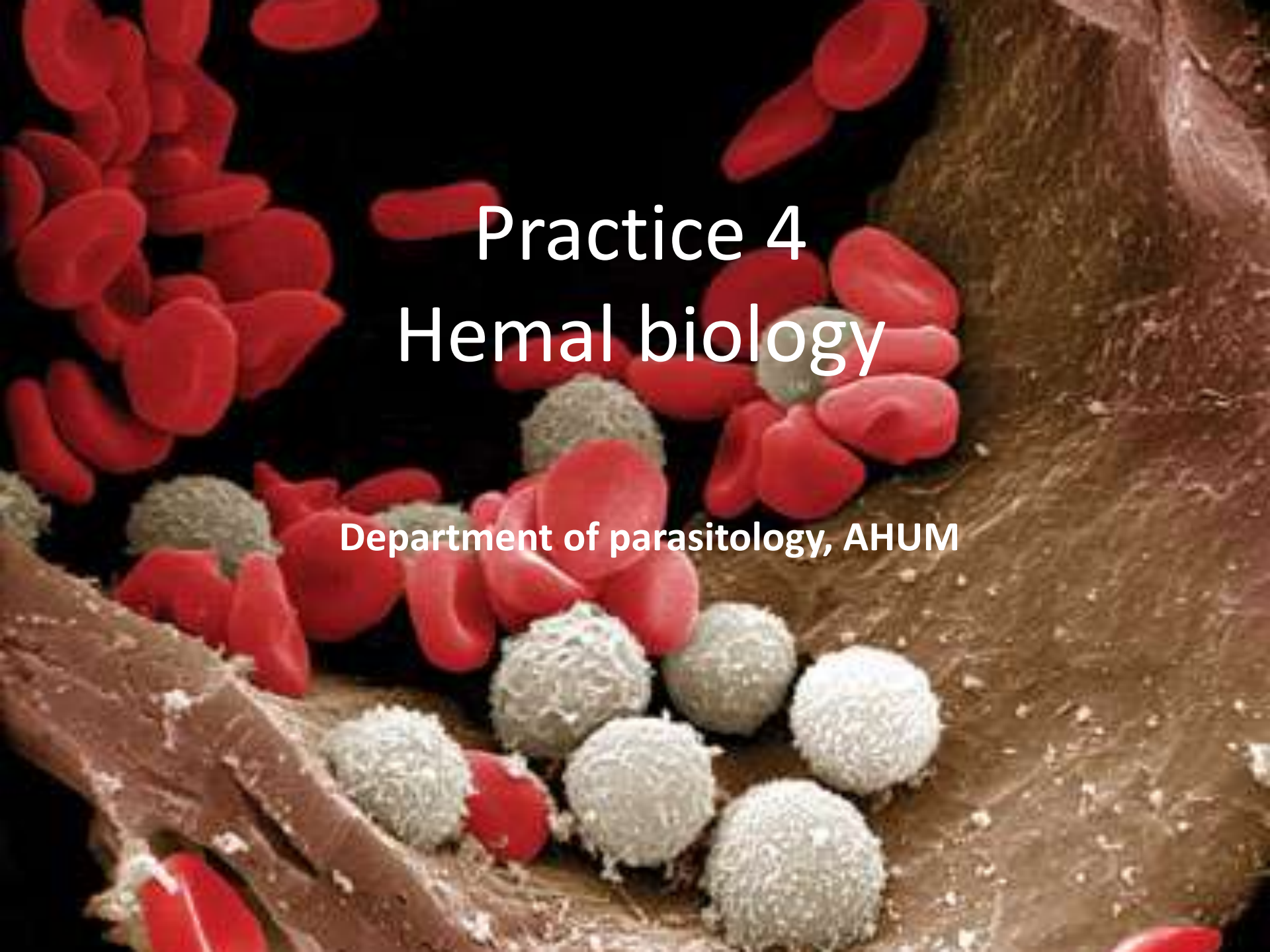


Practice 4
Hemal biology

Department of parasitology, AHUM

Definition

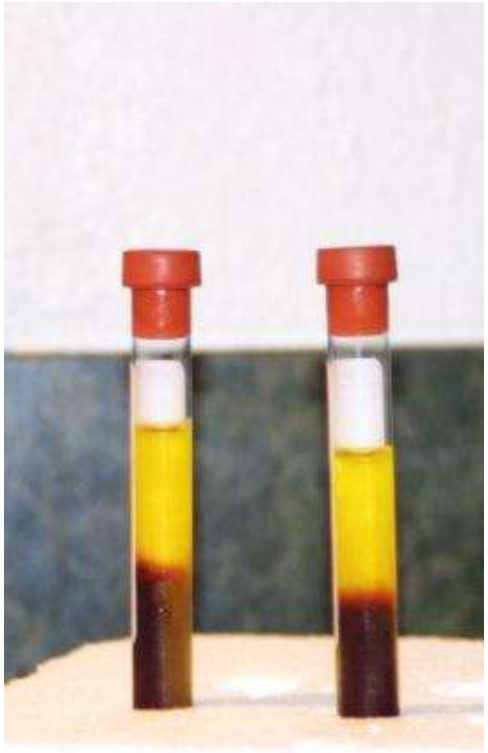
- Hemal biology：Study of normal and pathologic aspects of blood

Contents

1. blood components
2. hematopoiesis
3. blood function
4. white blood cell count
5. White blood cell differential
6. homework
7. case study

1. Blood components

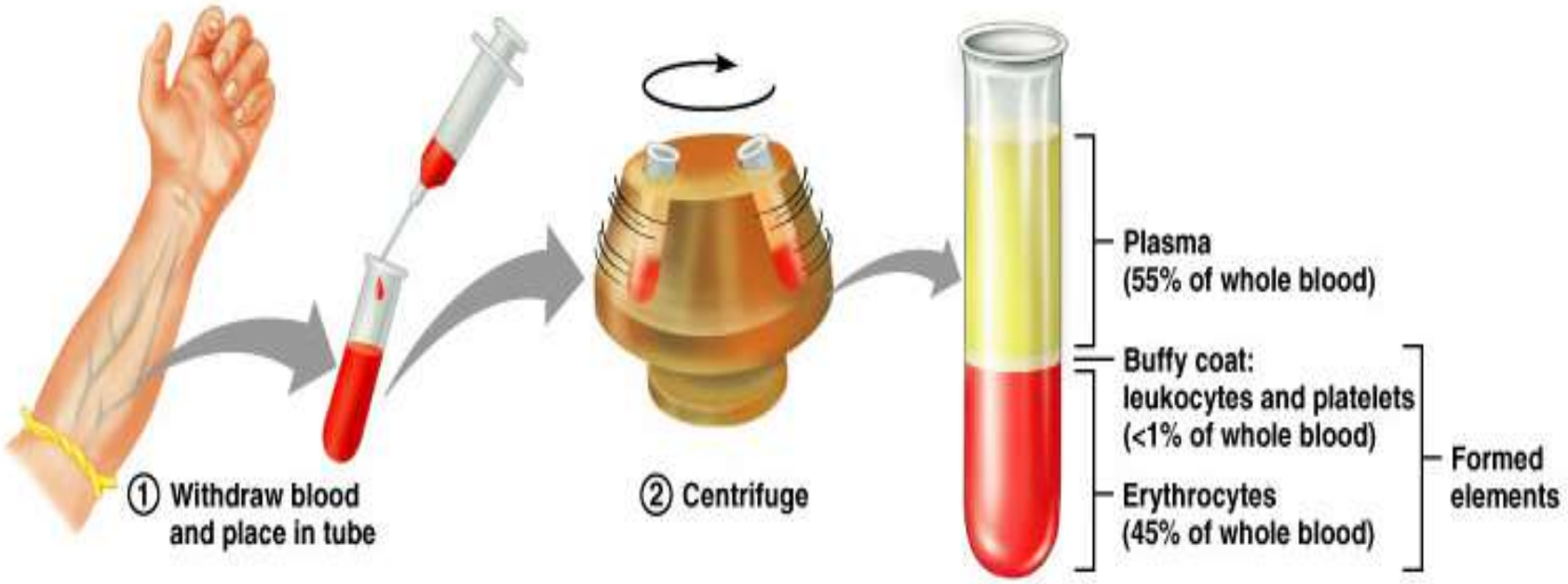
- Adults have 4-6 L of blood

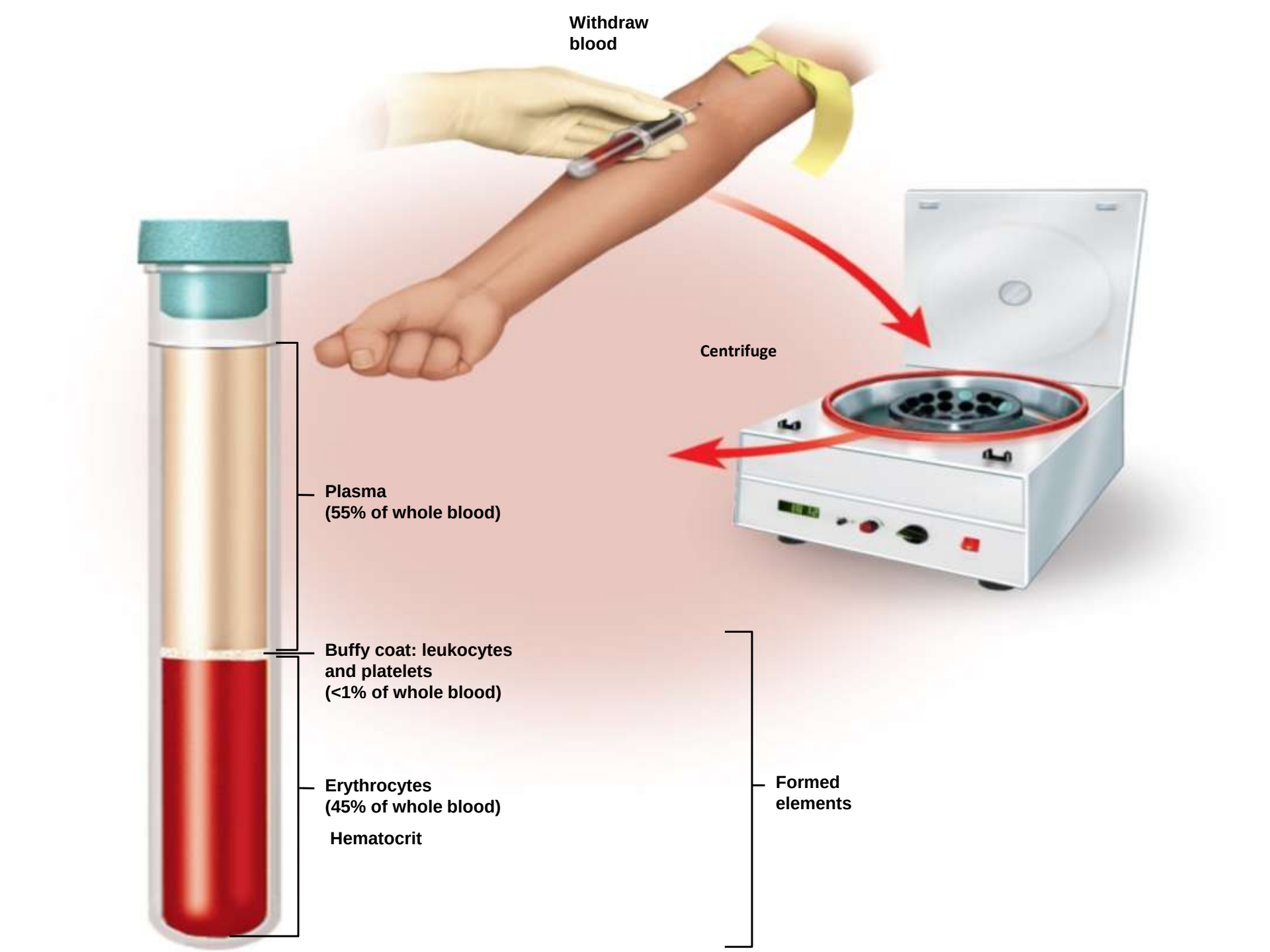


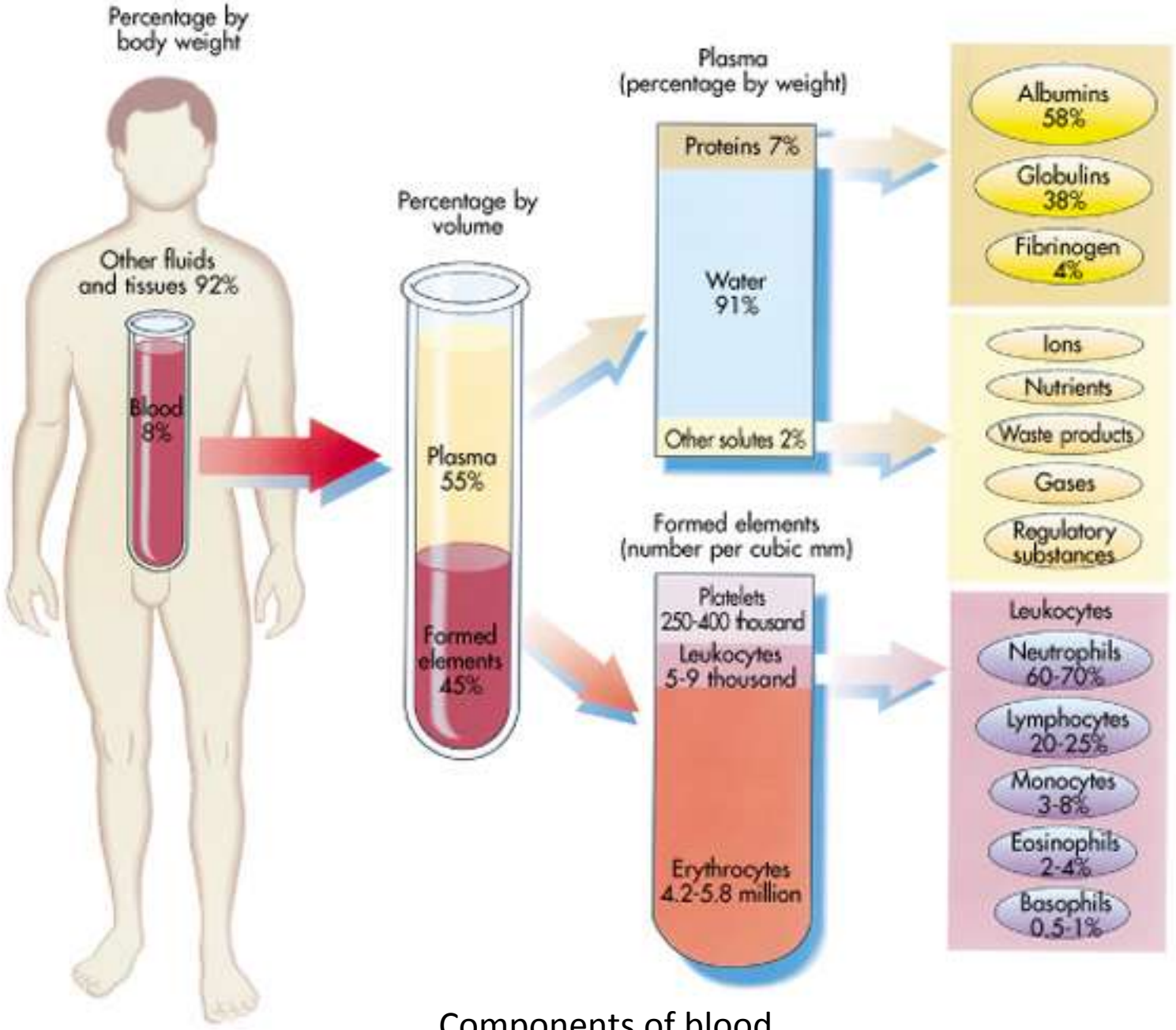
- A liquid connective tissue
  - plasma – matrix of blood
    - a clear, light yellow fluid
  - formed elements - blood cells and cell fragments
    - red blood cells, white blood cells, and platelets

1. Blood components

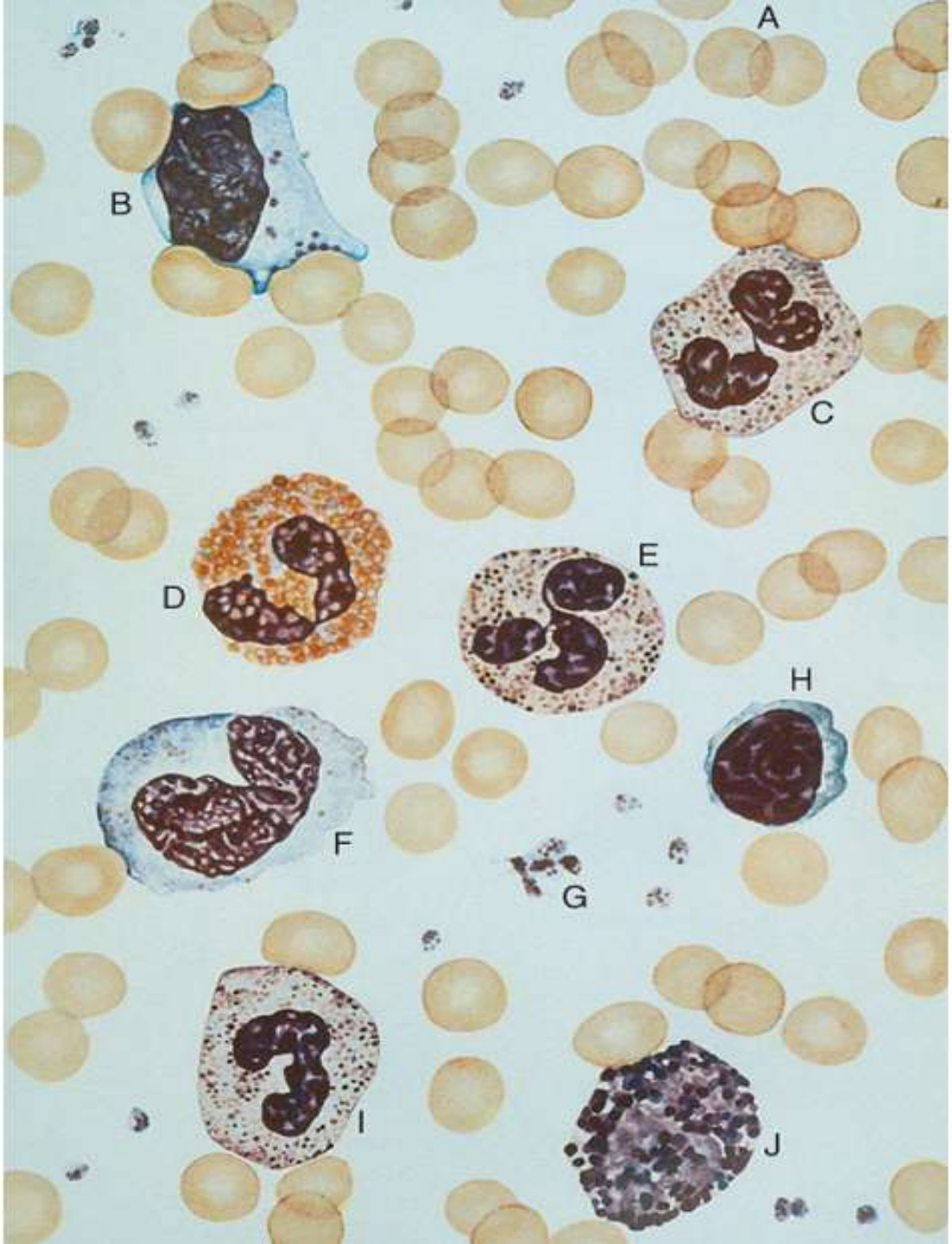
- Blood cells (formed elements) suspended in plasma




Withdraw blood
Centrifuge
Plasma (55% of whole blood)
Buffy coat: leukocytes and platelets (<1% of whole blood)
Erythrocytes (45% of whole blood)
Hematocrit
Formed elements



Components of blood


A
B
C
D
E
F
G
H
I
J

1. Blood components

- plasma (55%):
  - water (90% of total plasma)
  - protein (7%)
    - albumen, globulin (antibodies), hormones, enzymes, cytokines
    - blood coagulation factor: prothrombin, thrombin, fibrinogen
  - other (3%)
    - nutrients: fats, vitamins, carbohydrates (glucose)
    - electrolytes
    - gases: oxygen, carbon dioxide
    - metabolic waste products: creatinine, urea nitrogen, uric acid, etc.

1. Blood components

- formed elements (45%)
  - red blood cell (RBC, erythrocyte): male: 4-5$\times 10^6$/μl, female: 3.5-4.5$\times 10^6$/μl
    - hemoglobin (Hb, male: 12-16g/dl, female:11-15g/dl, newborn:17-20g/dl)
    - hematocrit (Hct, male: 42-52%, female: 37-47%):
    - reticulocyte (Ret, immature erythrocyte): adult: 0.5-1.5%, newborn: 2.0-6.0%
    - Mean Corpuscular Volume: MCV, 80-100 fl
    - Mean Cellular Haemoglobin Concentration: MCHC, 32-36 g/dl

1. Blood components

Mean Corpuscular Volume (MCV):

$$\text{MCV (fl)} = \frac{\text{Hct (\%)}}{\text{RBC (x } 10^6/\mu\text{l)}} \times 10$$

Mean Cellular Haemoglobin Concentration (MCHC):

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{Hct (\%)}} \times 100$$

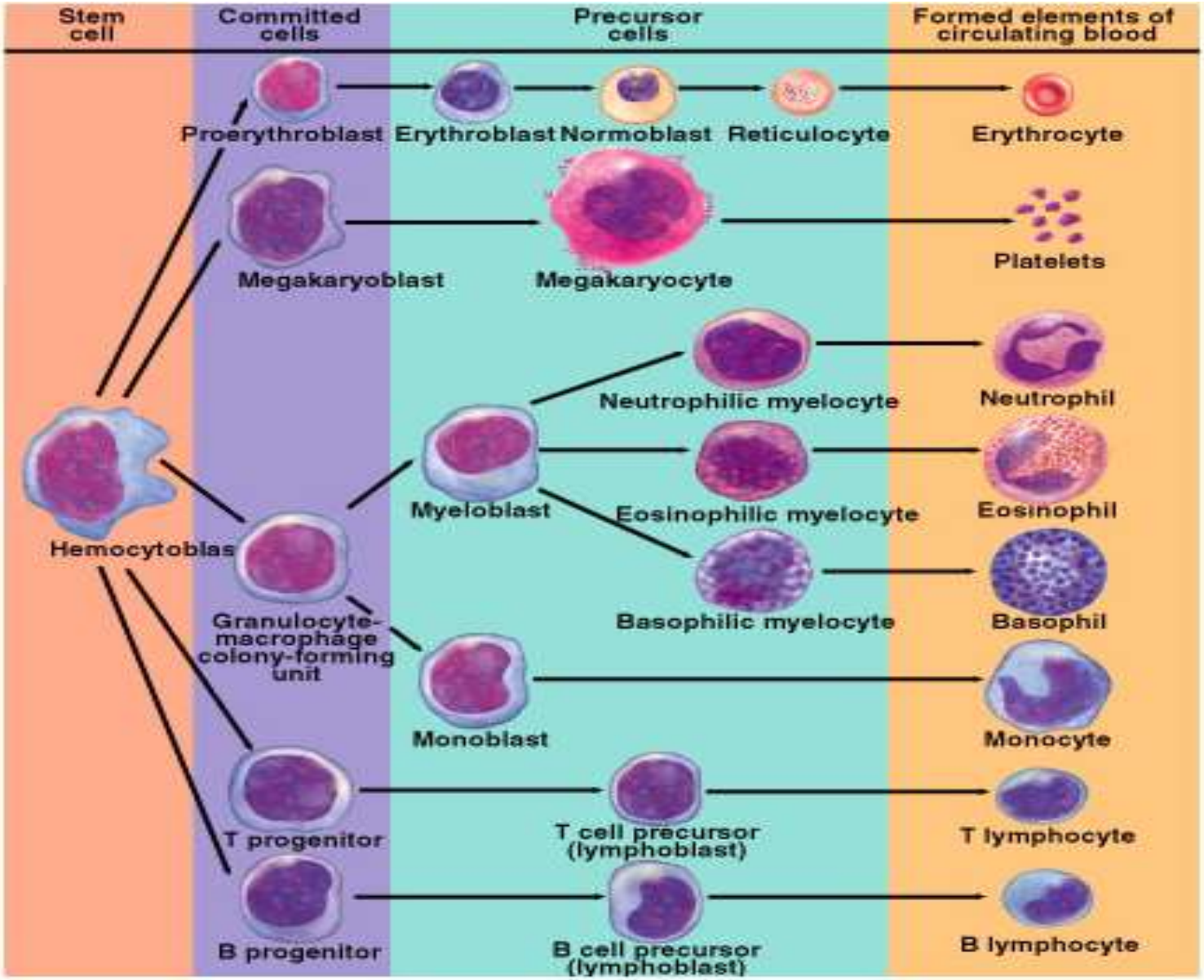
1. Blood components

- white blood cell (WBC, leukocyte): 4×10^3-1×10^4/μl
  - granulocyte: granules, lobed nuclei, phagocytic
    - neutrophil: 50-70%
    - eosinophil: 0.5-3%
    - basophile: 0-1%
  - agranulocyte
    - lymphocyte: 20-30%
    - monocyte: 3-8%
- Platelet (thrombocytes): $100\text{-}280 \times 10^3$/μl

2. Hematopoiesis

- bone marrow: formation of blood cells occurs mostly in red bone marrow in adult. All cells arise from same blood stem cell (pluripotent hematopoietic stem cell), then become colony-forming units (CFUs), precursor cells, and mature cell
- In certain cases, immature and/or abnormal forms of the cells may be present in the blood and may be detected with a blood differential, so further bone marrow aspiration may be necessary to give an accurate diagnosis (metastatic or hematological malignancies)

2. Hematopoiesis



3. Blood function

- protection
  - inflammation
  - limit spread of infection
  - destroy microorganisms and cancer cells
  - neutralize toxins
  - initiate clotting
- transport: nutrients, oxygen, carbon dioxide, hormones, enzymes, etc
- regulation: regulates fluid balance, acid-base balance, the temperature of the body

3. Blood function

- RBC
  - carry oxygen via hemoglobin from lungs to peripheral tissues and carbon dioxide away from peripheral tissues to lungs
  - reticulocyte: number increases in hemolytic anemia, decreases in aplastic anemia, radiation and chemotherapy

3. Blood function

- WBC
  - leucocytes are an important part of the body's immune system and also have a role in inflammation, allergic responses, and protect against pathogens, abnormal cells (cancer cells), remove toxins and wastes

3. Blood function

- Neutrophils
  - phagocytize and provide a first line of defense in acute infections
  - increase in bacterial infection
  - phagocytize bacteria and fungi
  - release antimicrobial chemicals

3. Blood function

- lymphocytes
  - lymphocytosis: viral infections, antigen stimulation
  - lymphocytopenia: early stages of bacteria infection

3. Blood function

- Monocytes
  - increase in viral infections and inflammation
  - differentiate into macrophages and dendritic cells in the tissue
  - phagocytize pathogens and debris
  - “present” antigens to activate other immune cells
  - take part in tissue repair

3. Blood function

- Eosinophils
  - increase in parasitic infection (release enzymes destroy parasites) or allergic reaction
  - phagocytize antigen-antibody complexes, allergens

3. Blood function

- Basophils
  - increase in chicken pox, sinusitis, diabetes and immunolgically mediated hypersensitivity reactions
  - secrete histamine, cytokines, heparin, inflammatory chemicals

3. Blood function

- Platelets
  - hemostasis (vascular spasm, platelet plug, coagulation): blood clotting and blood vessel repair
  - phagocytize bacteria
  - attract WBCs to sites of inflammation

4. WBC count

- significance of WBC count
  - diagnose an infection, immune disorders (autoimmune disorder, immune deficiency, and inflammation), blood disorders (anemia, leukemia), etc
  - monitor the progression of conditions such as those named above
  - monitor the body's response to various treatments

4. WBC count

- objective of the experiment
  - learn to use hemocytometer and master the method of cell counting
- requirements for the experiment
  - 0.02M acetic acid, alcohol cotton ball
  - rubber leash, puncture needle, vacuum anticoagulant tube, cuvette
  - a clean hemocytometer, cover slip, pipetting tube, microscope

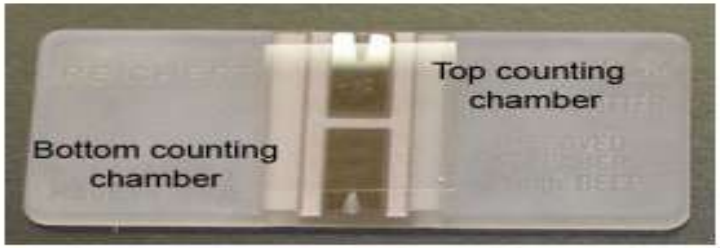
4. WBC count

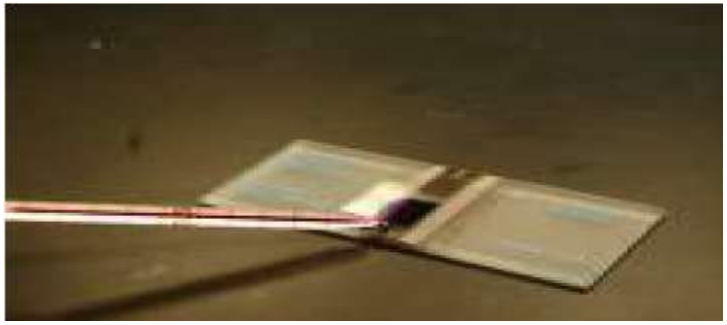
- protocol of the experiment
  - draw blood from ulnar vein with a vacuum anticoagulant tube, mix sample well by inversion
  - take 0.02M acetic acid 380 µl into a cuvette
  - take blood 20 µl into the cuvette
  - shake the cuvette to mix the blood and acetic acid
  - get a clean hemocytometer, place the cover slip onto the counting gab, put the tip of the pipetting tube close to the edge of the cover slip, put a drop of solution into the crevice between the clivue and cover slip, to make it infiltrate to the counting gab
  - put the microscope on low power objective, count the number of the cells in the 4 big squares of each corner of the counting gab
  - count one by one, if the cells are on the lines “count the upper but not the lower, the left but not the right”

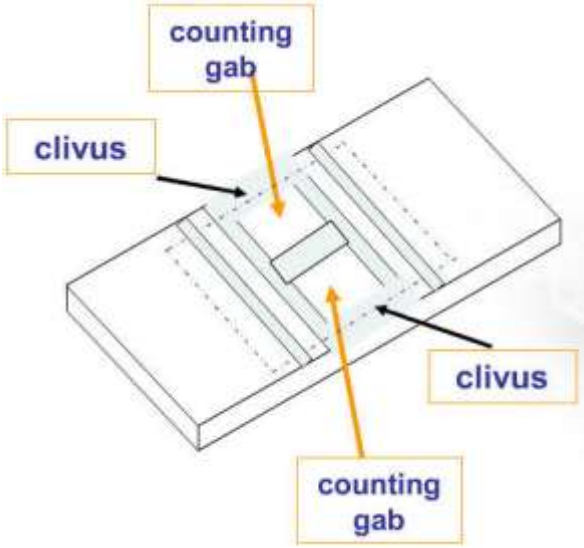
4. WBC count

- calculate the number of white blood cells per milliliter using the following equation:
  - WBCs（number/ml）= (total number of cells in the four big squares/four)×20×10×1, 000
- normal value
  - adult: 4×10^3-1×10^4/μl
  - child: 5×10^3-1.2×10^4/μl
  - newborn: 1.5×10^4-2.0×10^4/μl

4. WBC count


Top counting chamber
Bottom counting chamber

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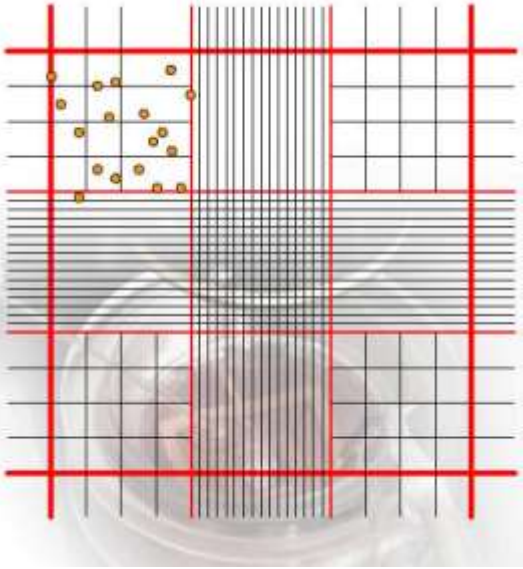
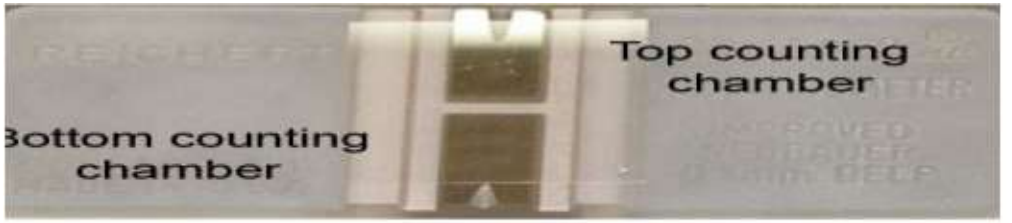
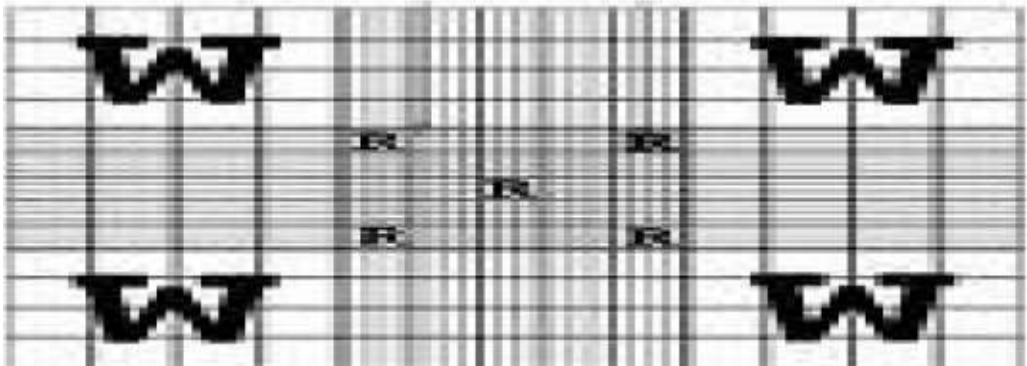
clivus
clivus
counting gab




Top counting chamber
Bottom counting chamber



4. WBC count

- High white blood cell count
  - Definition: a high white blood cell count (leukocytosis): the count more than 10,000 white blood cells per microliter of blood
  - Causes
    - Infections: bacteria, viruses, fungi and parasites
    - Inflammation or inflammatory conditions: rheumatoid arthritis, vasculitis or inflammatory bowel disease
    - Allergic responses (e.g., allergies, asthma)
    - Leukemia, myeloproliferative neoplasms: causing abnormally high production of WBCs
    - Tissue necrosis (e.g., such as trauma, burns, surgery or heart attack)
    - Pregnancy in the final month and labor
    - Severe emotional or physical stress
    - Intense exercise
    - Drug (e.g., corticosteroids, epinephrine)

4. WBC count

- Low white blood cell count
  - Definition: a low white blood cell count (leucopenia): the count lower than 4,000 white blood cells per microliter of blood.
  - Causes
    - Bone marrow damage and disorders
    - Autoimmune disorders—the body attacks and destroys its own WBCs (e.g., Systemic lupus erythematosus)
    - Overwhelming infections that use up white blood cells faster than they can be produced (e.g., Sepsis)
    - Diseases of the immune system, such as HIV, which destroy T lymphocytes, immune deficiency
    - Malnutrition (Vitamin B12 or folate deficiency)
    - Hypersplenism
    - Parasitic diseases (leishmaniasis)

5. White blood cell differential

- significance of the experiment
  - WBC differential: estimates percentages of each type of WBCs
  - a WBC differential determines the specific types of WBCs that are affected, and give further clues as to the cause of some diseases
  - identify the presence of abnormal formed elements to help make a diagnosis

5. White blood cell differential

- objectives of the experiment
  - realize the principle of slide staining with Wight's stain
  - master the appropriate technique for staining a blood smear with Wight's stain
  - learn how to observe the formed elements present in a slide, identify the presence of abnormal populations of blood cells and conduct a WBC differential count

5. White blood cell differential

- principle of the experiment
  - Wright's stain is made by mixing methylene blue dye with eosin in a methanol diluent
  - basic components of the cell, such as hemoglobin or certain inclusions or granules, will unite with the acidic portion of the stain, eosin, and are said to be eosinophilic. These components are stained varying shades of pink or red
  - acidic cell components, such as nucleic acids, reactive cytoplasm, etc. take up the basic dye components, methylene blue, and stain blue or purple
  - buffer pH is 6.4-6.7

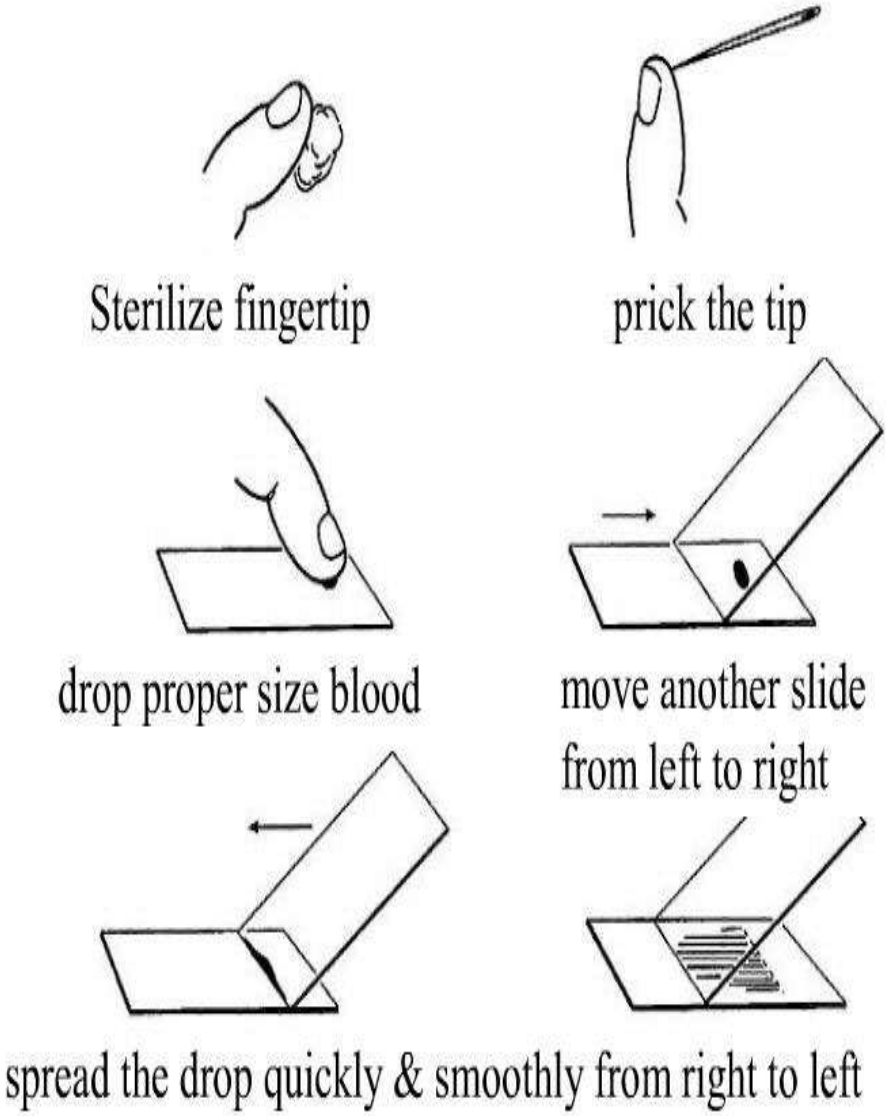
5. White blood cell differential

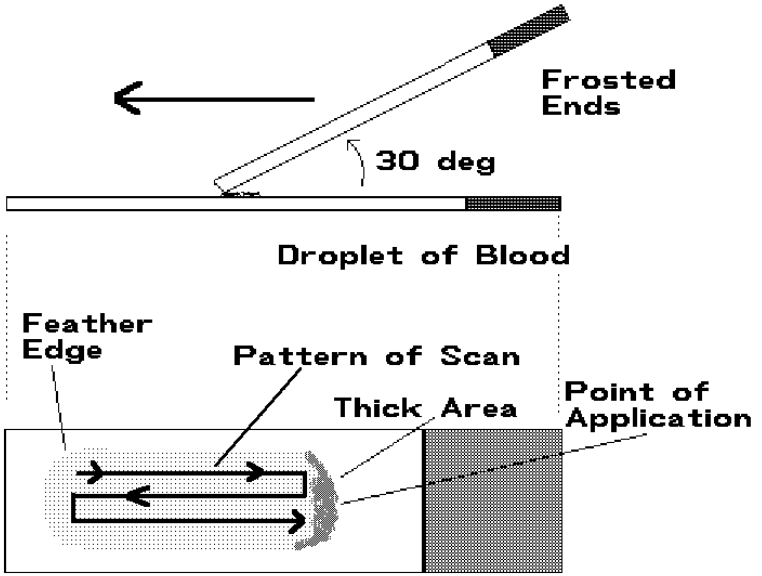
- requirements for proper smear preparation
  - Wright's stain, PBS buffer, rosin oil, zylene, alcohol cotton ball
  - rubber leash, puncture needle, vacuum anticoagulant tube
  - clean glass slide, spreader slide and coverslip, red pencil, microscope

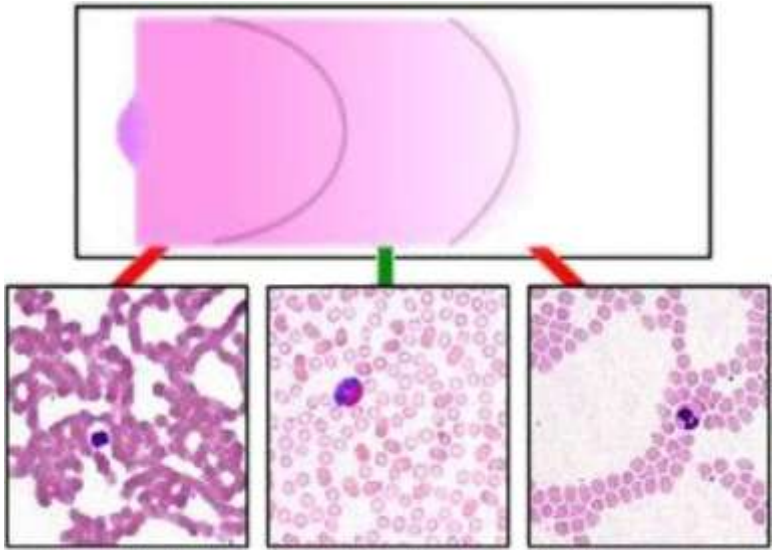
5. White blood cell differential

- protocol of the experiment
  - place a drop of blood at the right side of the slide
  - place the spreader slide onto the lower slide in front of the blood drop, and pull the slide back until it touches the drop
  - push the spreader slide forward at approximately a 30 degree angle, using a rapid, even motion
  - when the smear is completely dried, mark the stained area using a red pencil
  - completely cover the marked area with enough Wright's stain and keep it for 1-2 minutes
  - add PBS buffer onto the blood smear slide, after 5 minutes, wash the slide for 30 seconds with tap water
  - allow slide to dry at room temperature before examination
  - observe under a microscope with 100X objective
  - observe the formed elements, and calculate the percentage of each type of leukocytes found in the slide

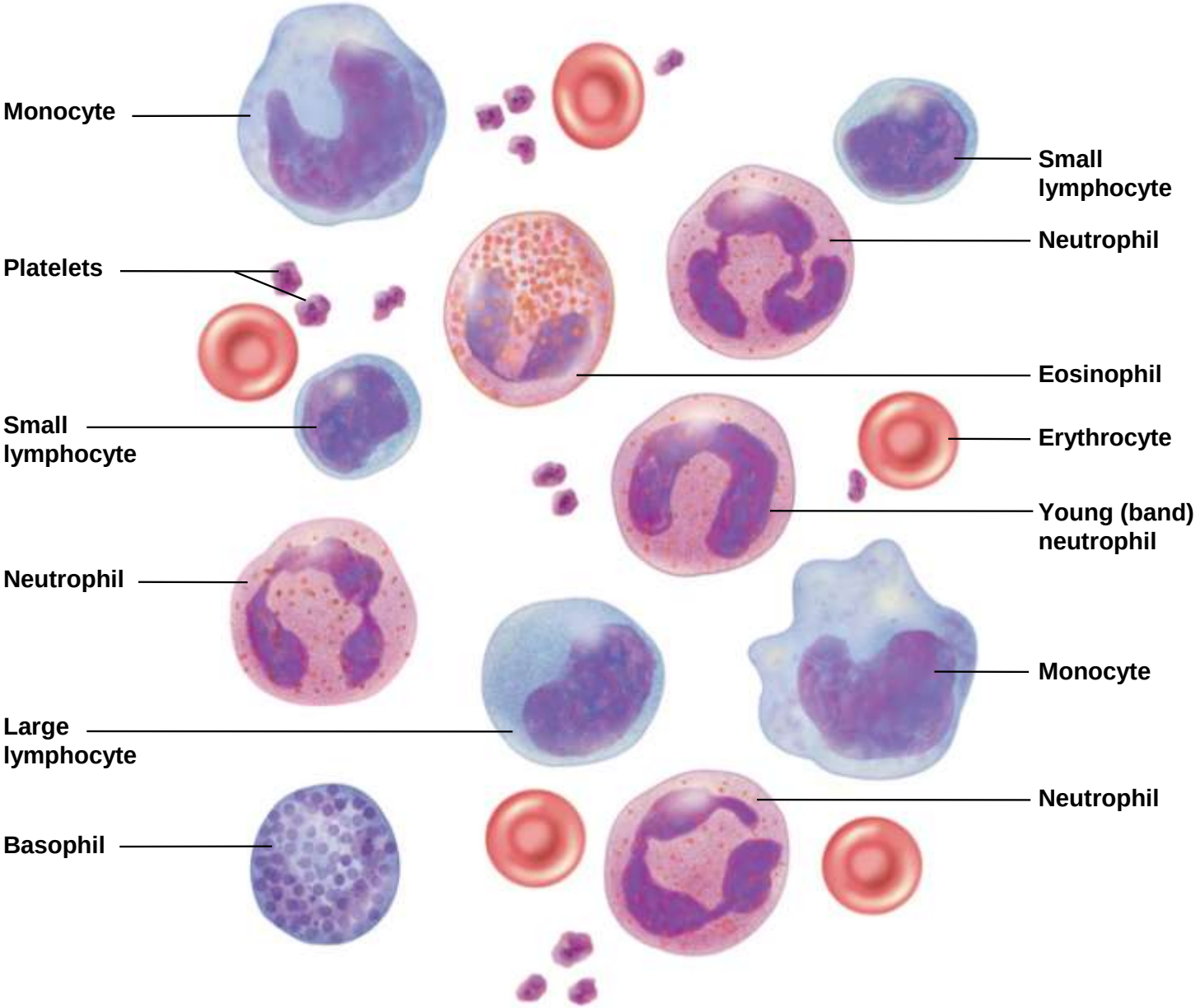
5. White blood cell differential

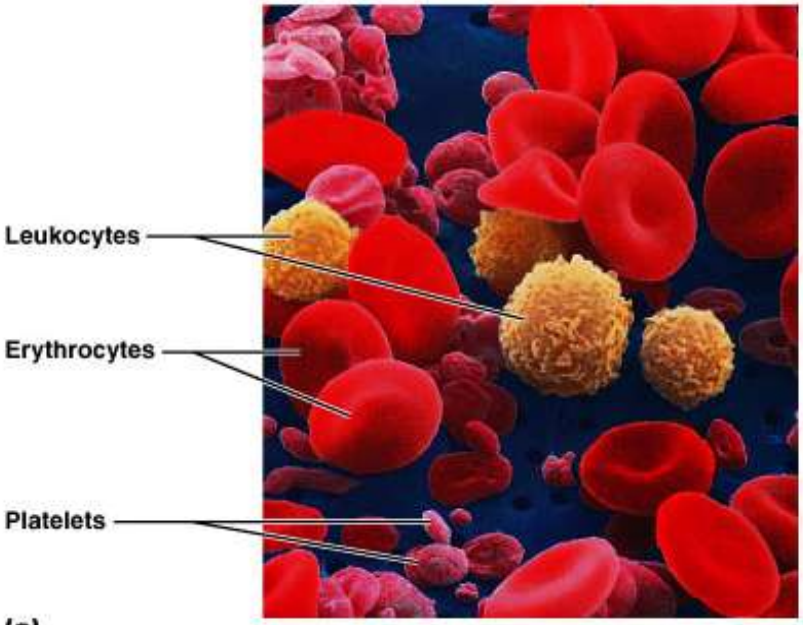




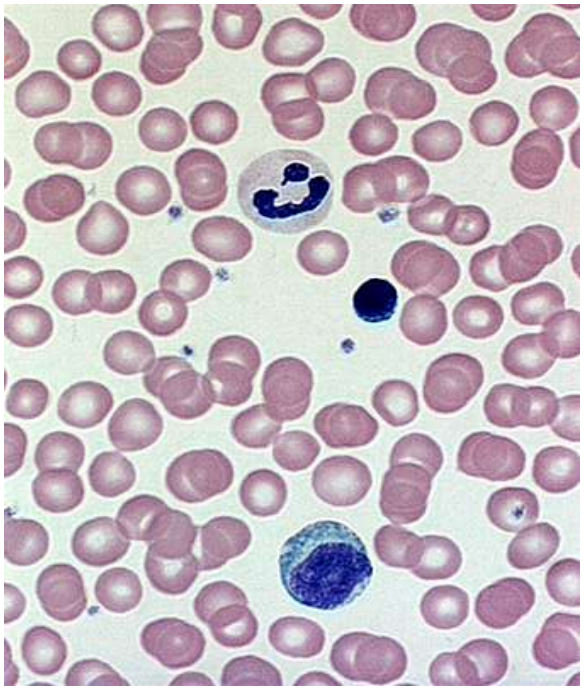


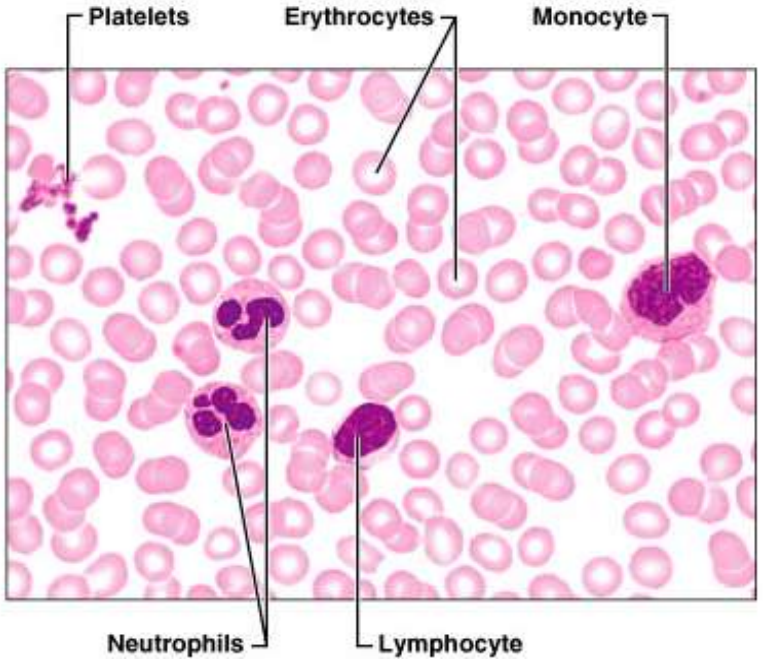
5. White blood cell differential

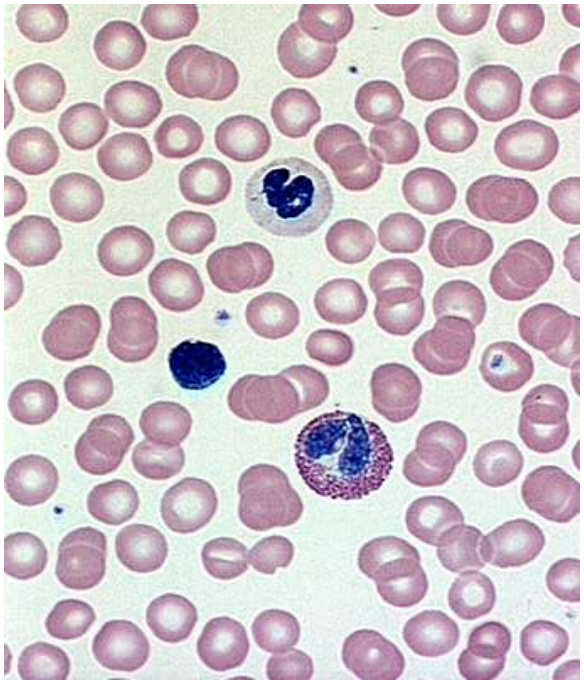



Leukocytes
Erythrocytes
Platelets

(a)

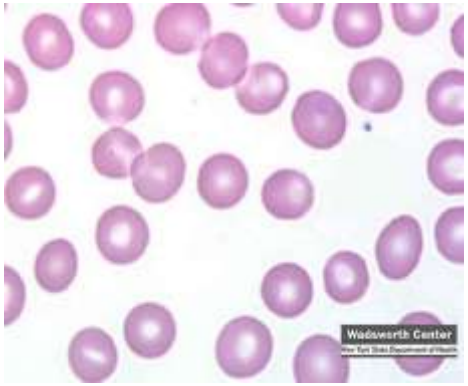


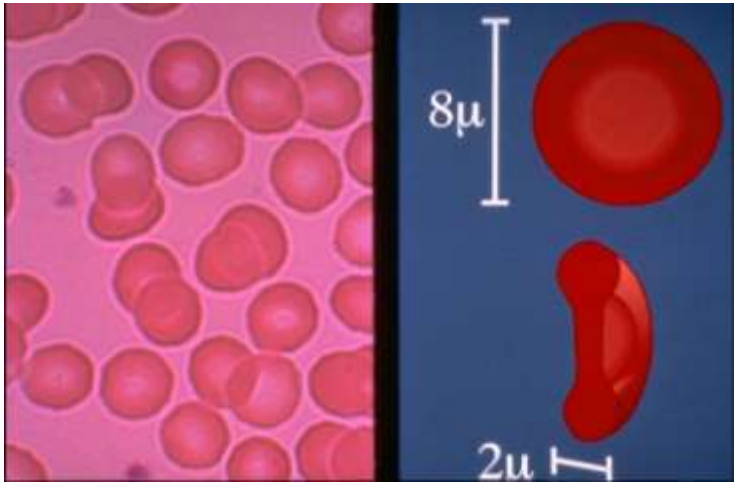

Platelets
Erythrocytes
Monocyte
Neutrophils
Lymphocyte

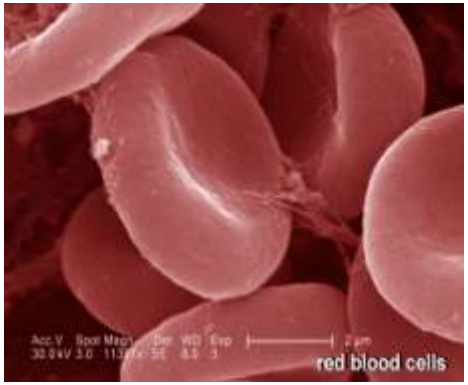


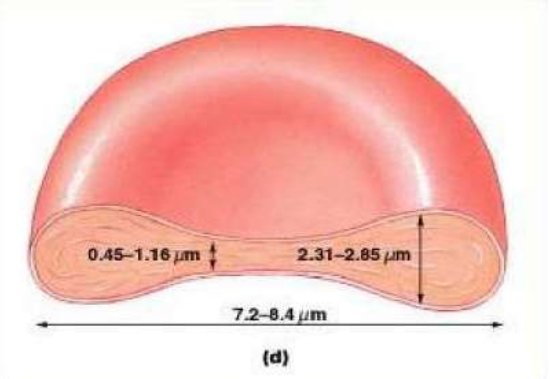
5.1. Red blood cells

- biconcave discs and flexible
- plasma membrane but no nuclei or organelles



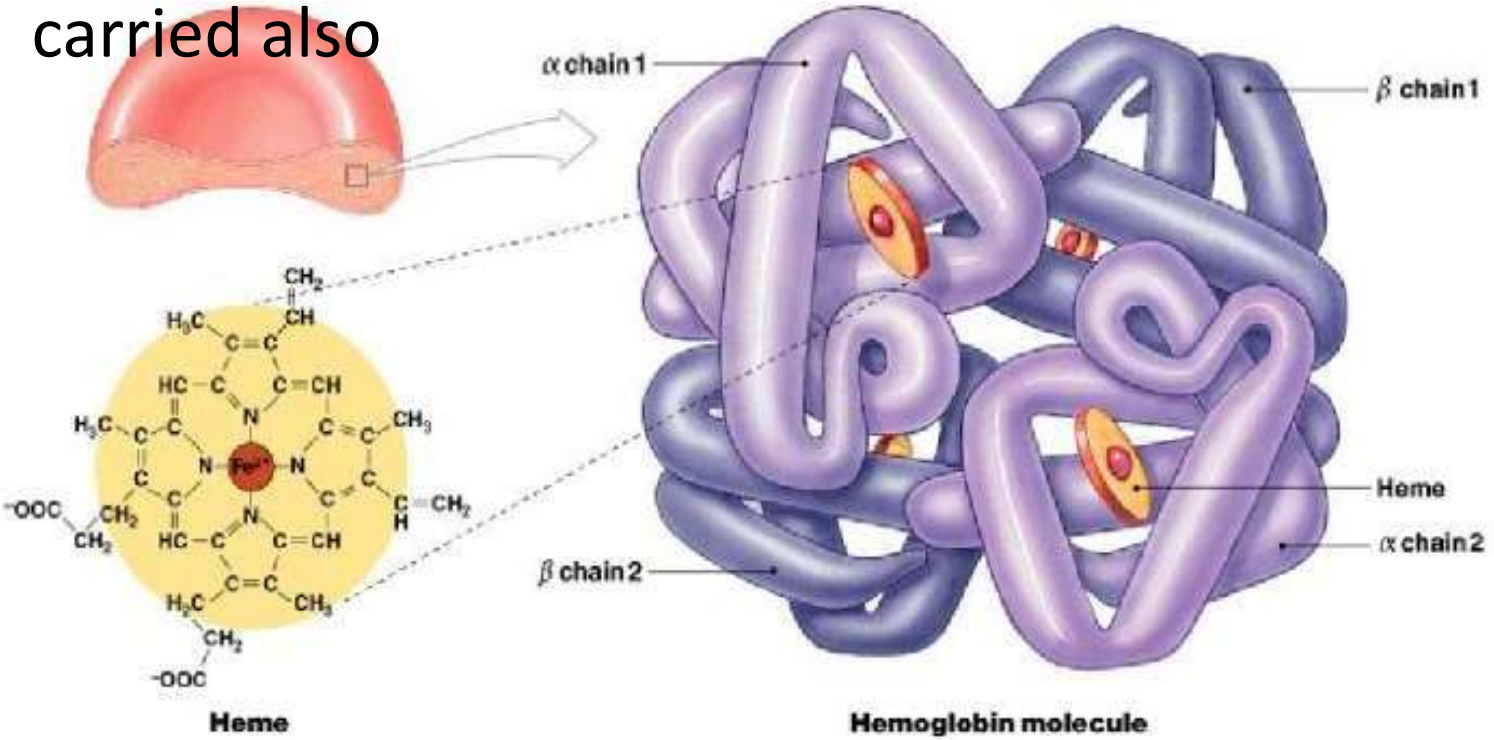






5.1. Red blood cells

- hemoglobin molecules
  - oxygen carrying protein
  - 4 chains of amino acids, each with a heme and a iron atom which is binding site for oxygen; CO2 carried also



Anemia

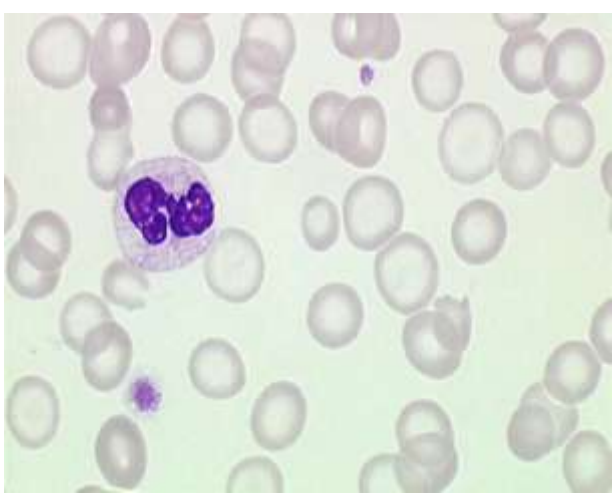
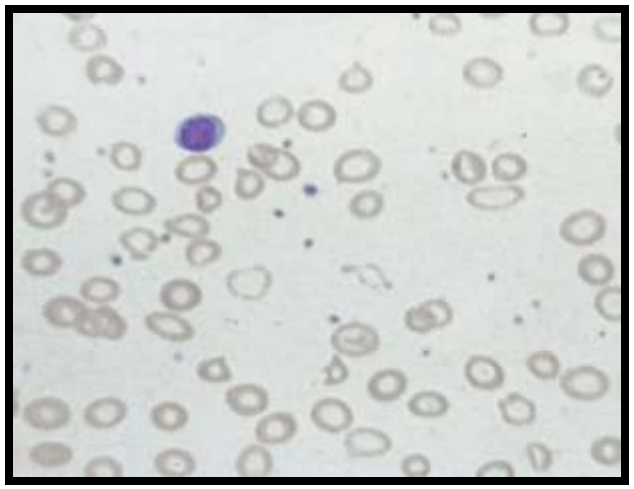
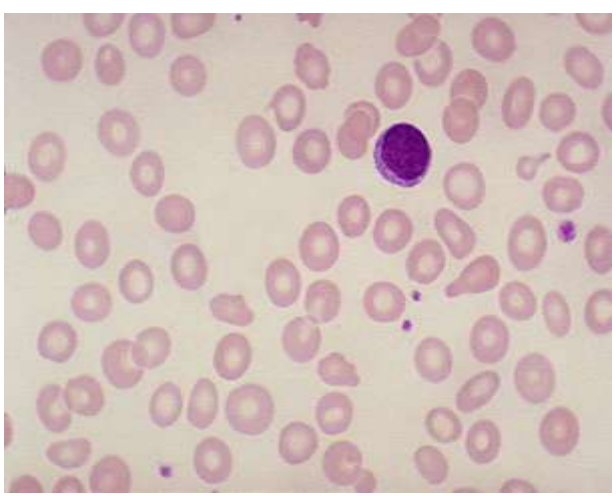
- definition: a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin or a decrease in percentage of hematocrit in the blood
- symptoms: weakness, or fatigue, general malaise, pica, and sometimes poor concentration
- signs: pallor, jaundice
- consequence: hypoxia (lack of oxygen) in organs

Anemia

- anemia classification
  - MCV: microcytic (iron deficiency), normocytic (acute blood loss, hemolysis), marcocytic anemia (VitB12 or folate deficiency)
  - MCHC: hypochromic, normochromic, hyperchromic amemia (hereditary spherocytosis, sickle cell disease)
  - causes:
    - inadequate synthesis, increased destruction, loss

Anemia

- peripheral blood smear
  - microcytic, hypochromic anemia (MCV< 80 fl, MCHC< 32 g/dl, Hb< 11 g/dl)

Iron deficiency anemia

Anemia

- Anemia causes:
  - inadequate erythropoiesis or hemoglobin synthesis
    - Nutritional deficiency
      - Iron-deficiency anemia
      - VitB12- or folate-deficiency anaemia
    - Bone marrow disorders
      - Aplastic anemia
      - Pure red cell aplasia

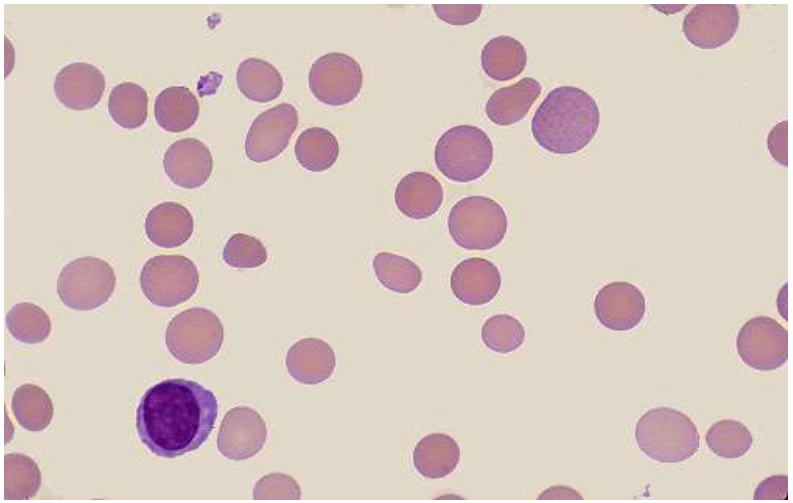
Anemia

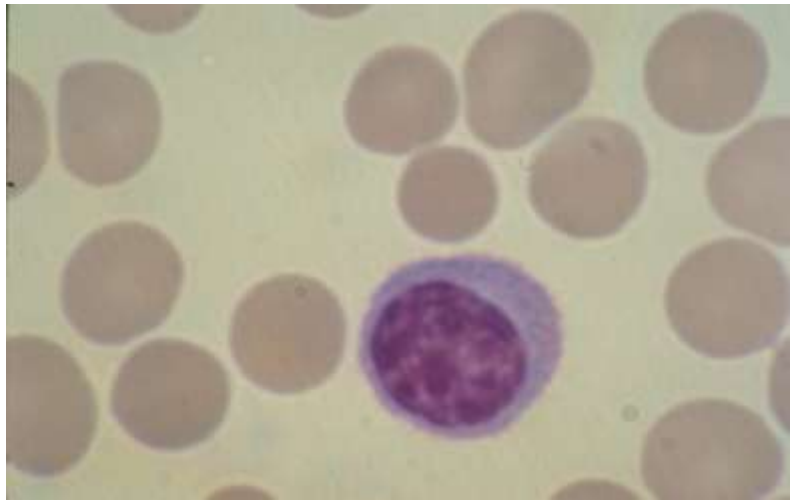
- increased destruction of RBC (hemolytic anemia)
  - membrane defects
    - hereditary spherocytosis
    - hereditary stomatocytosis
    - paroxysmal nocturnal hemoglobinuria
  - hemoglobin defects
    - thalassemia
    - sickle-cell anemia
  - enzyme defect
    - Glucose-6-phosphate dehydrogenase (G6PD) deficiency
  - immune
    - transfusion reaction
    - hemolytic disease of the newborn
  - nonimmune
    - burn, sulfanilamide, lead poisoning, malaria infection

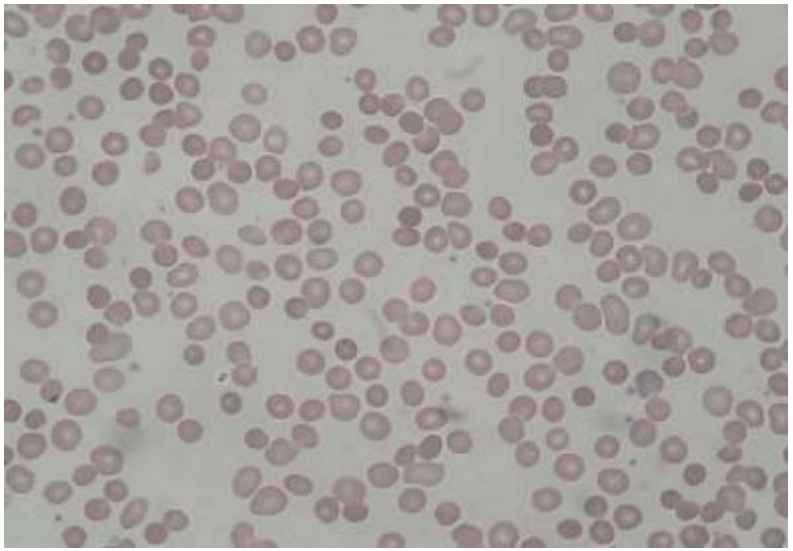
Anemia

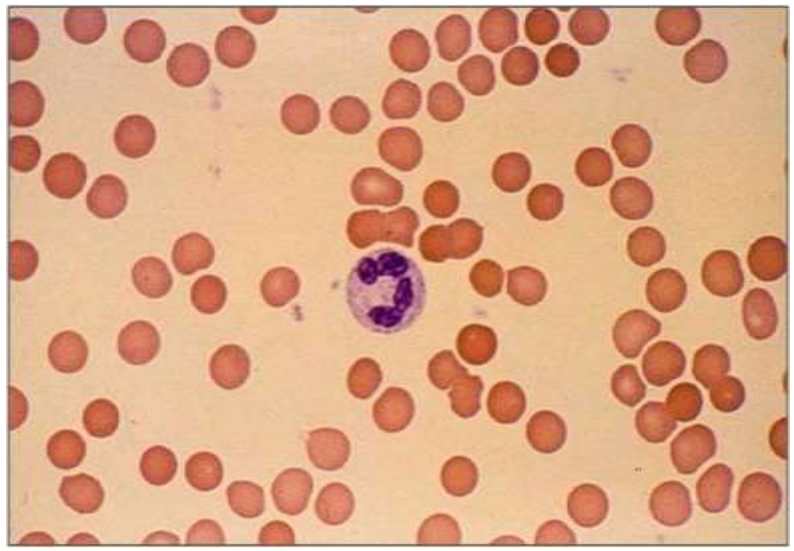
- RBCs loss or dilution
  - trauma
  - chronic disease
    - hookworm disease
    - gastrointestinal ulcer
    - haemorrhoids

Anemia



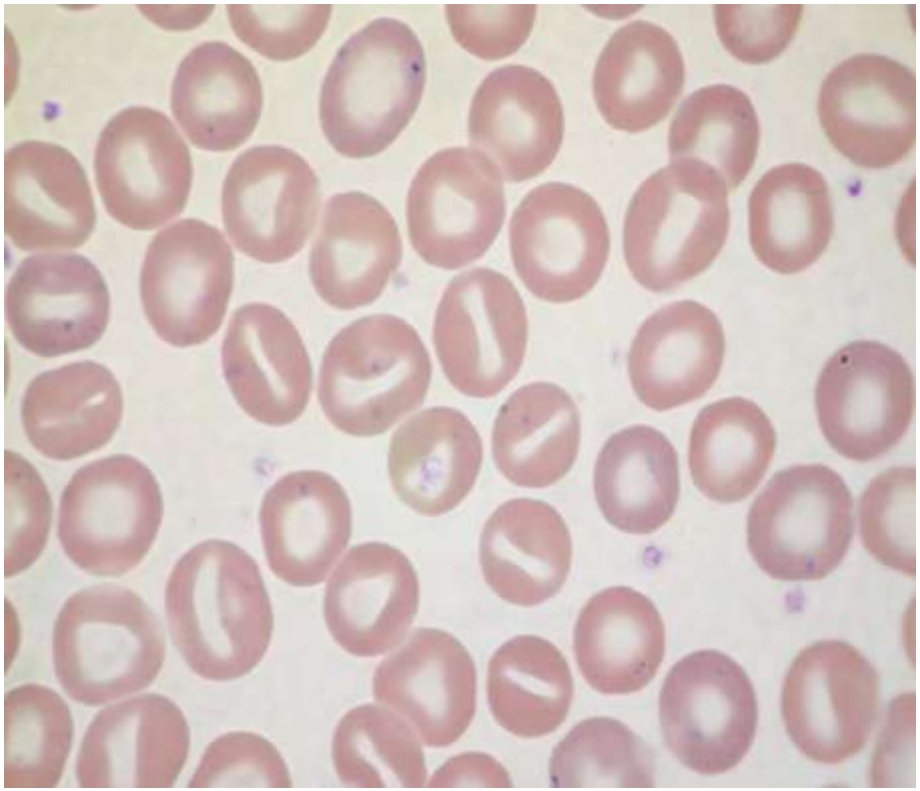


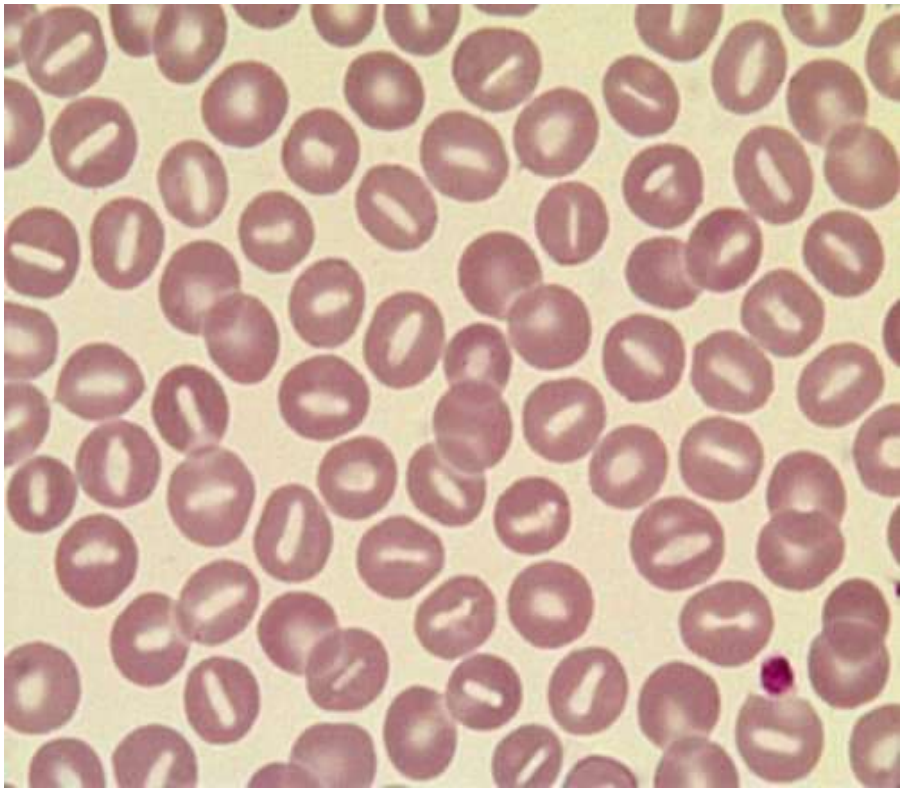




hereditary spherocytosis (spherocytes)

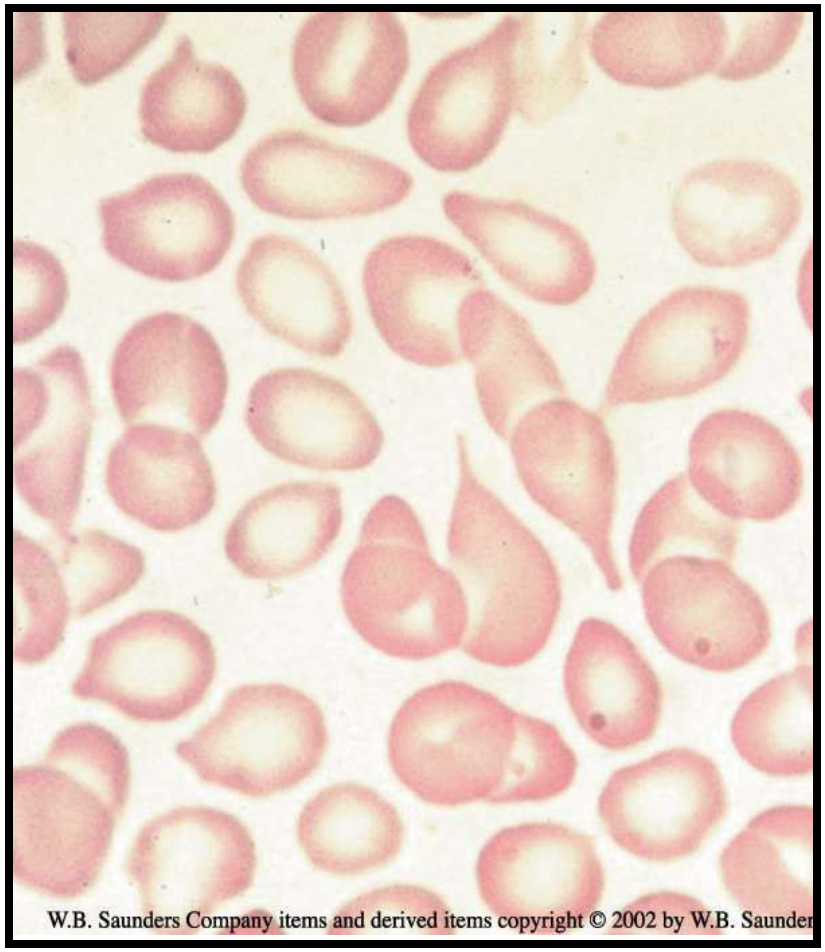
Anemia



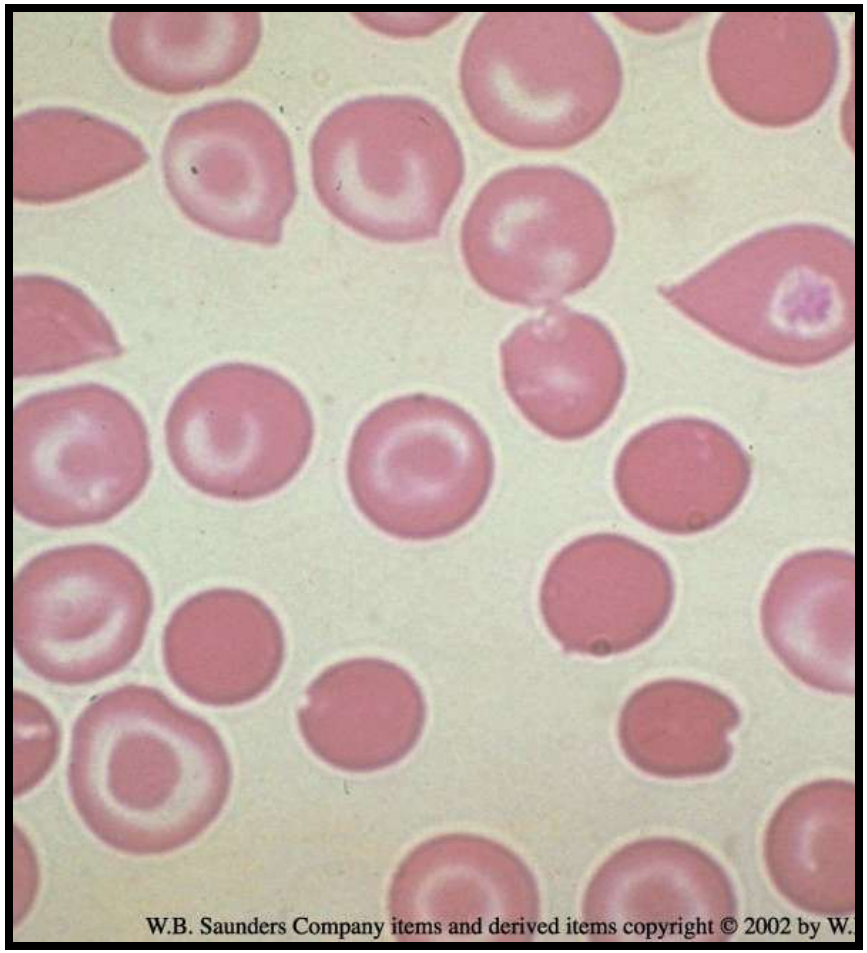


hereditary stomatocytosis (stomatocytes)

Anemia



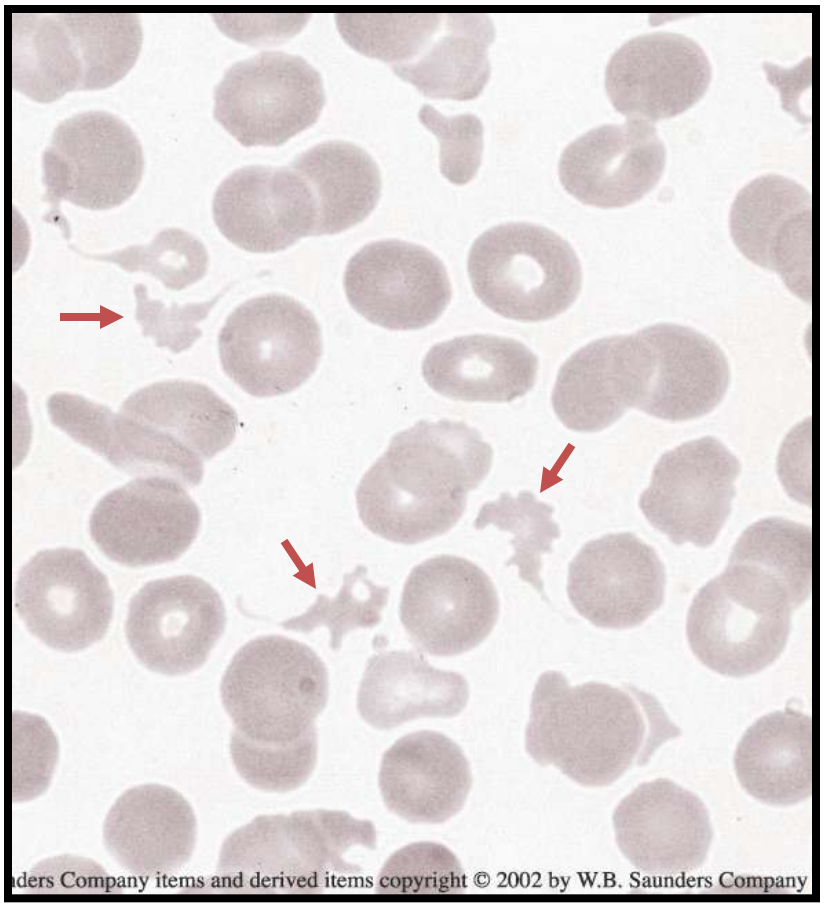
thalassemia (tear drop cells)



thalassemia (target cells)

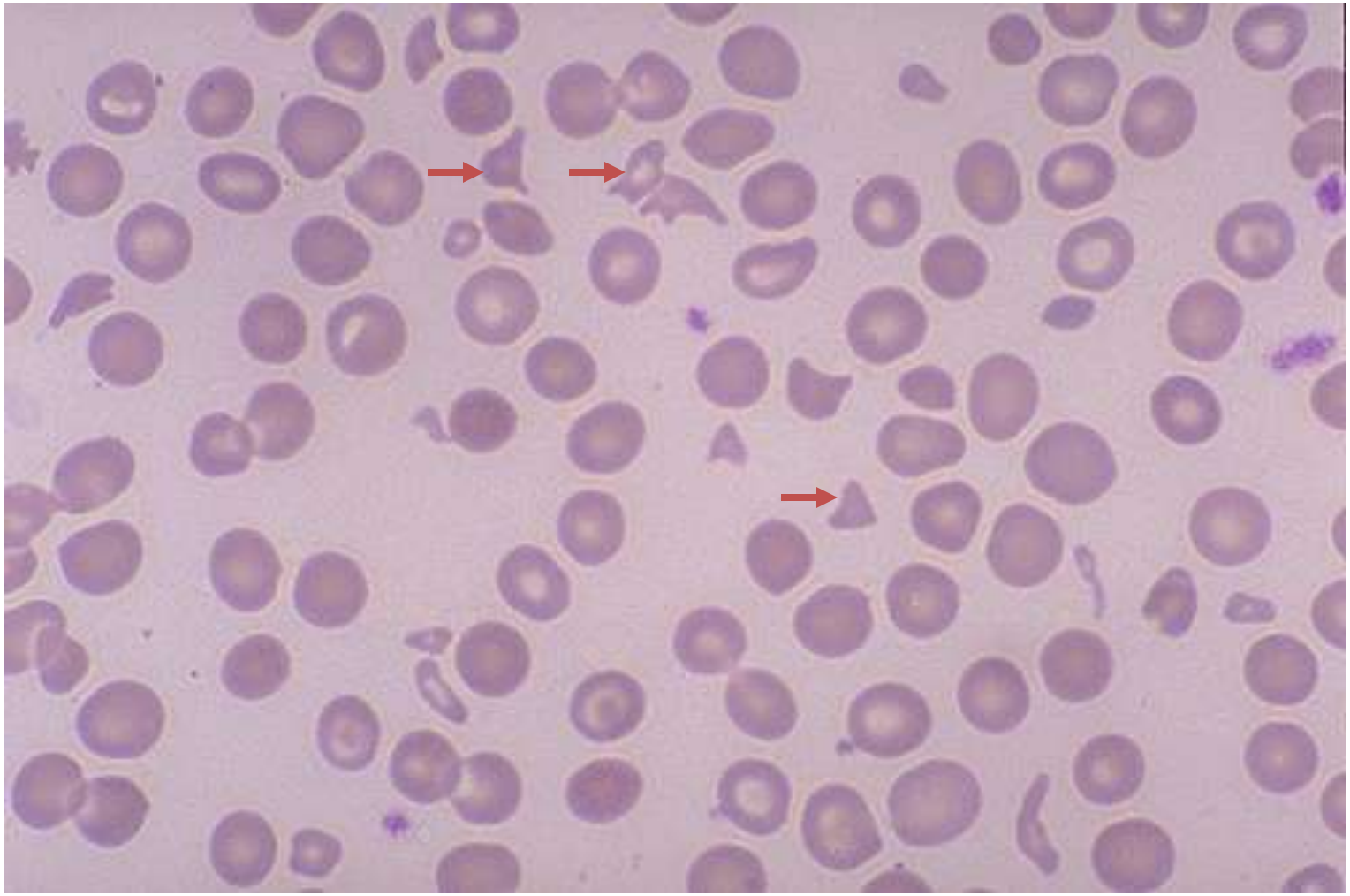
Anemia

sickle cell anemia (sickle cells)



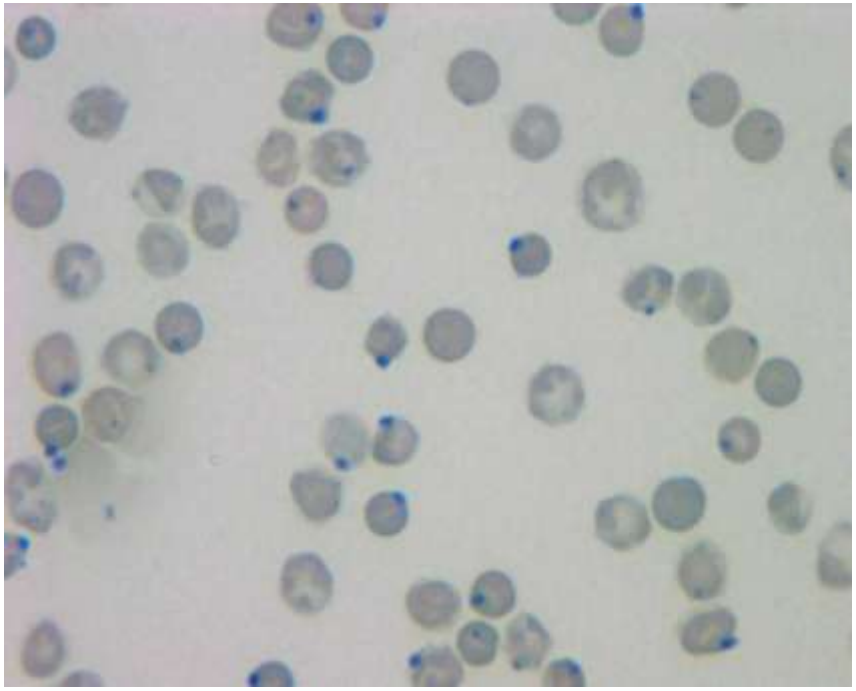
hemolytic anemia (schistocytes)

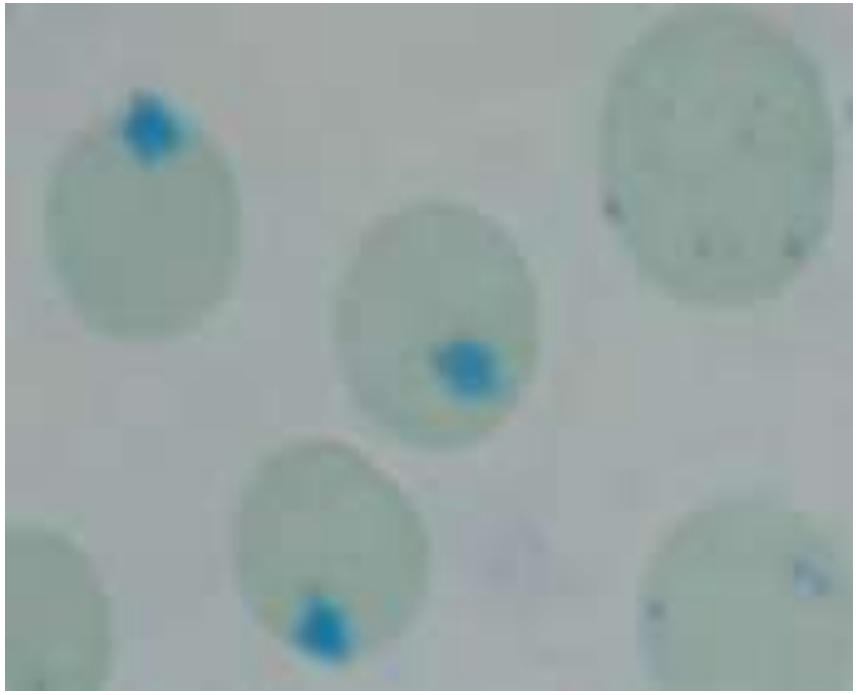
Anemia



hemolytic anemia (schistocytes)

Anemia

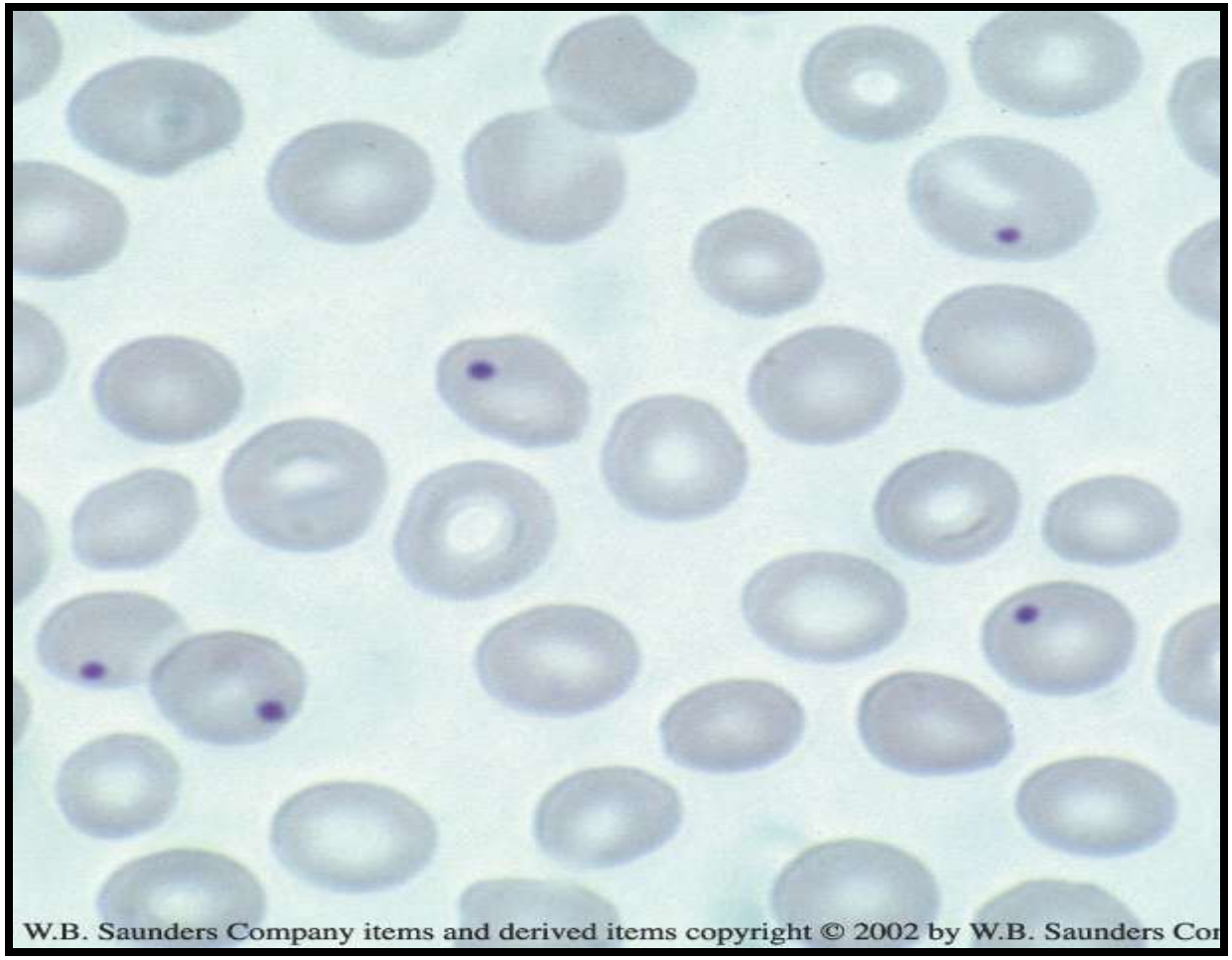




heinz bodies

Heinz bodies appear as small blue round inclusions when stained with new methylene blue. They are composed of denatured hemoglobin, found in G6PD deficiency and thalassemia anemia.

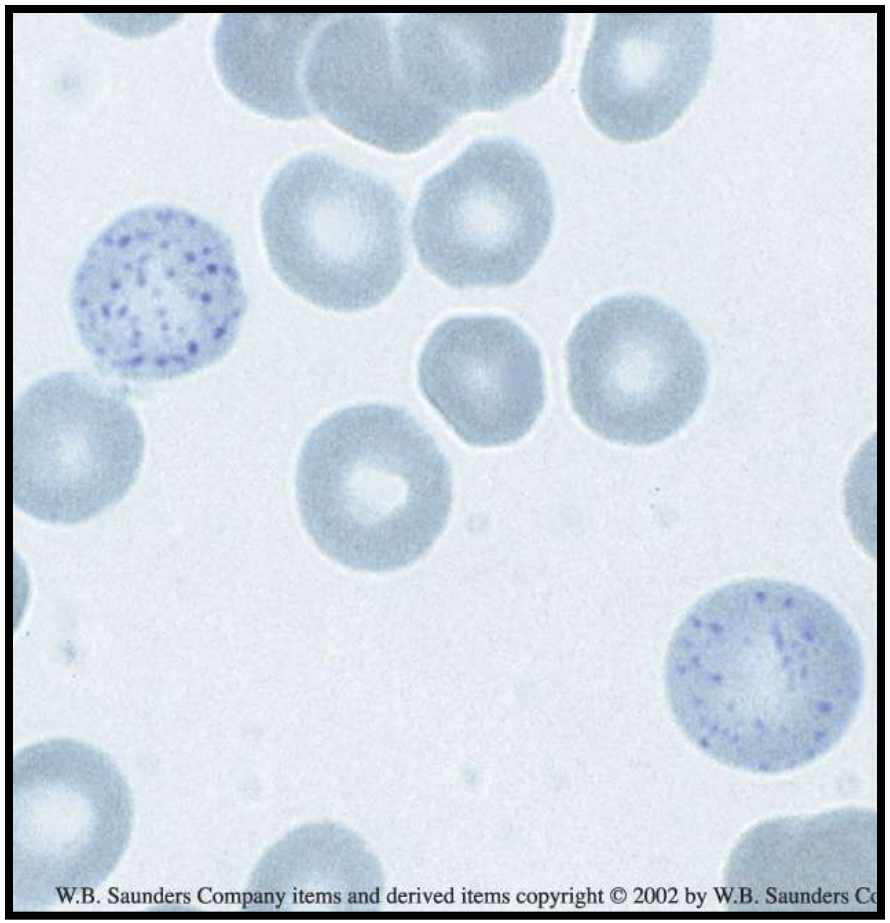
Splenectomy



howell-jolly bodies

Howell-jolly bodies appear as round, purple inclusions in RBCs, and are composed of DNA, commonly seen in in patients with splenectomy.

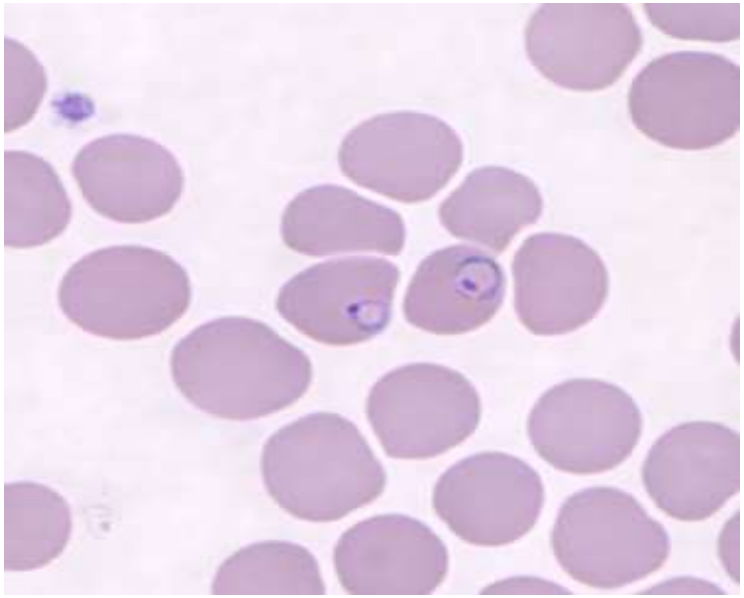
Lead poisoning



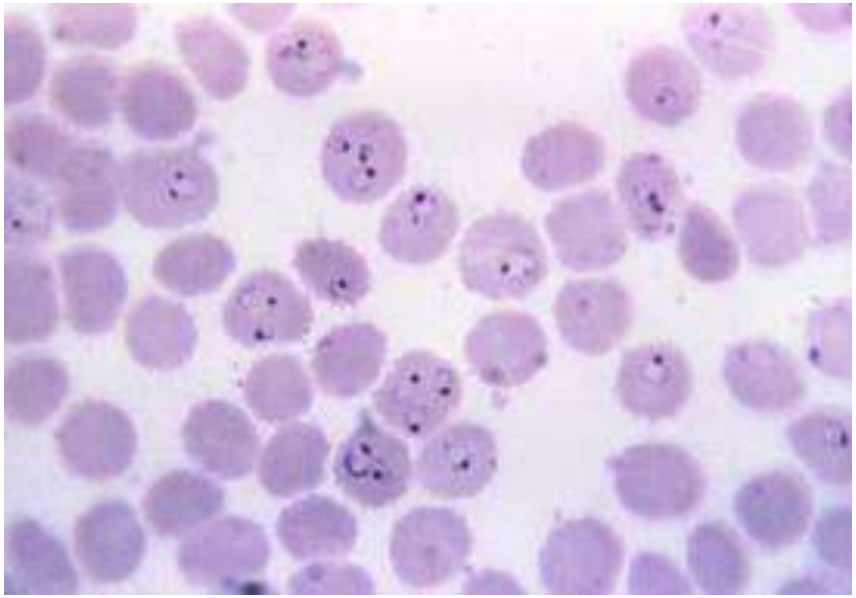
basophilic stipplings

Basophilic stipplings appear as numerous, small purple inclusions in RBCs, and are aggregates of ribosomal RNA, most commonly seen in lead poisoning.

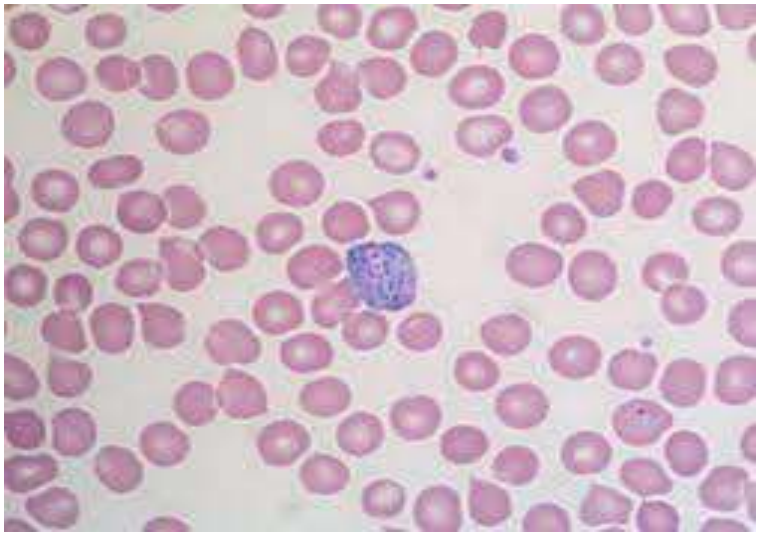
Anemia



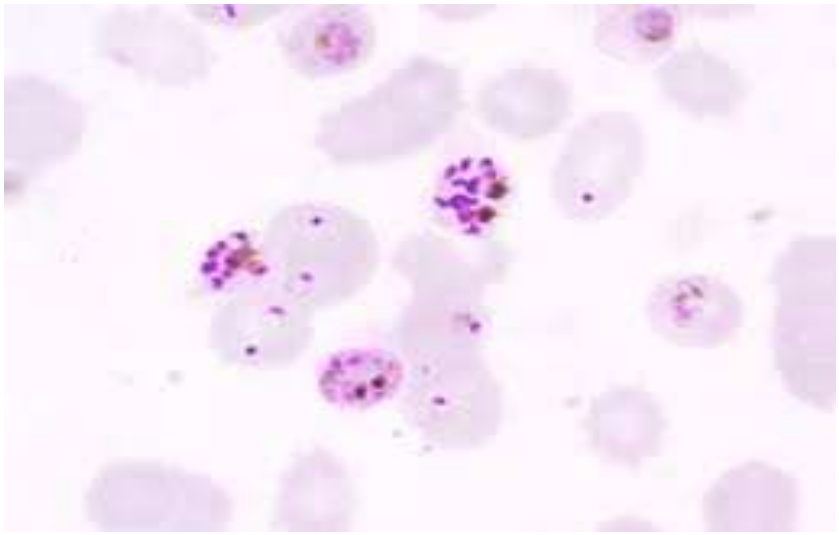
Ring form trophozoite of plasmodium vivax



Ring form trophozoite of plasmodium falciparum



Immature schizont of plasmodium vivax

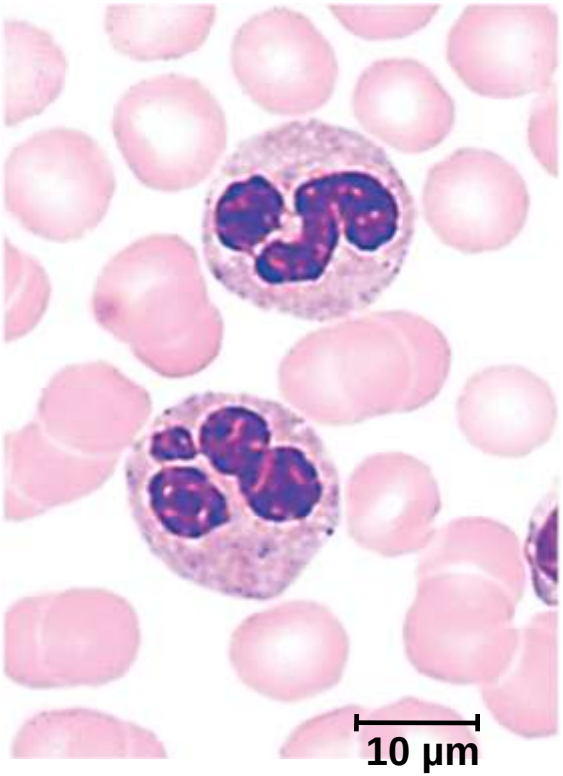


mature schizont of plasmodium vivax

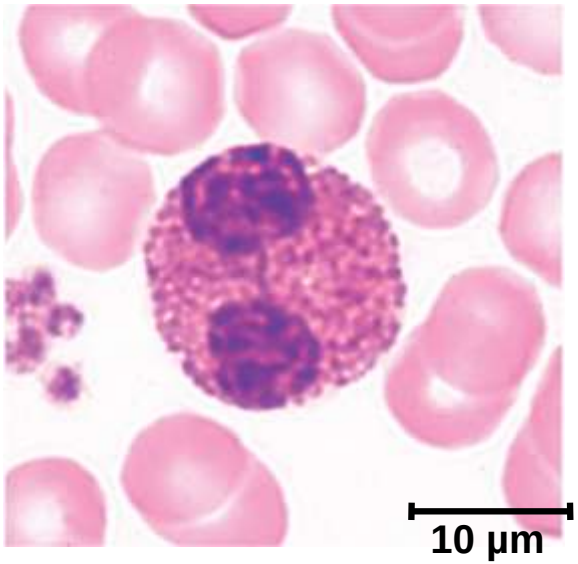
5.2. Leukocytes (WBCs)

- granulocytes
  - neutrophils
    - with a multi-lobed (2-6) nucleus and very fine, neutral-stained cytoplasmic granules (enzymes)
  - eosinophils
    - large rosy-orange granules; bilobed nucleus
  - basophils
    - large, abundant, violet granules (obscure a large S-shaped nucleus)
- agranulocytes
  - lymphocytes
    - variable amounts of bluish cytoplasm (scanty to abundant); ovoid/round, uniform dark violet nucleus
  - monocytes
    - largest WBC; ovoid, kidney-, or horseshoe- shaped nucleus

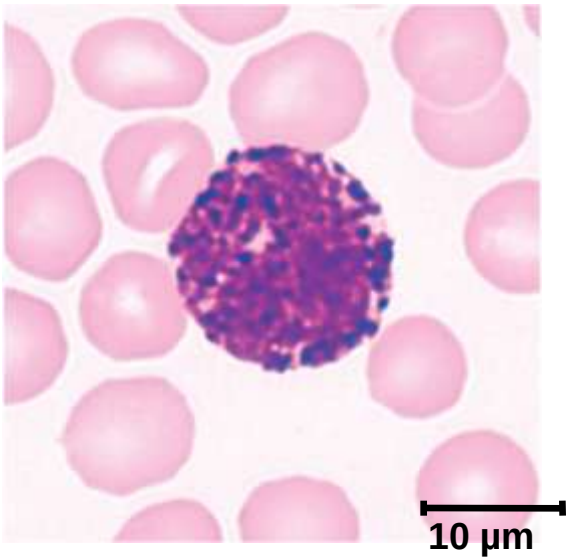
Granulocytes



neutrophils

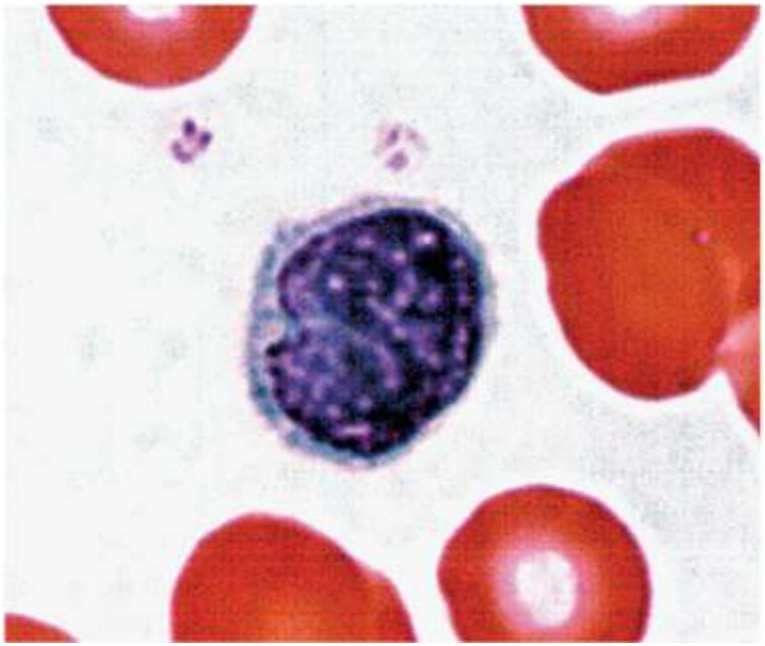


eosinophil



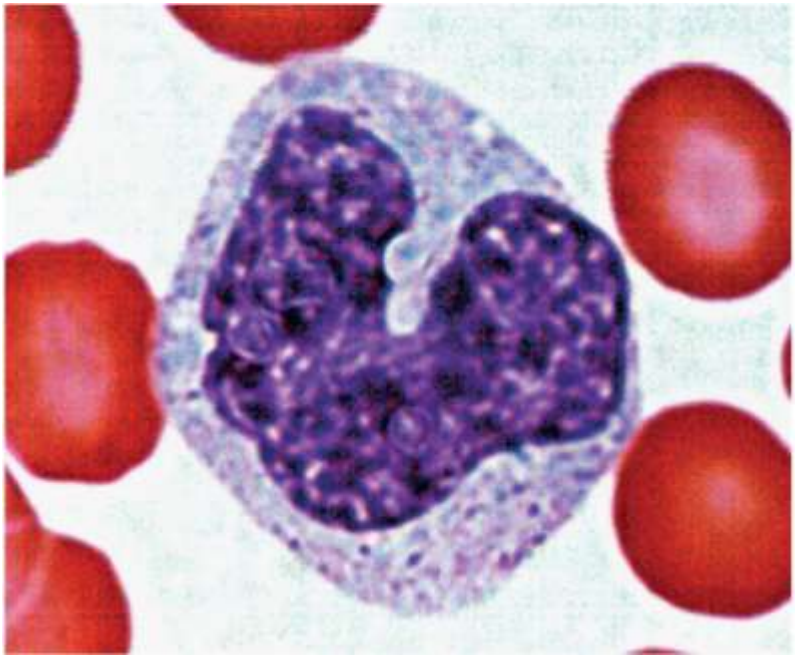
basophil

Agranulocytes



lymphocyte

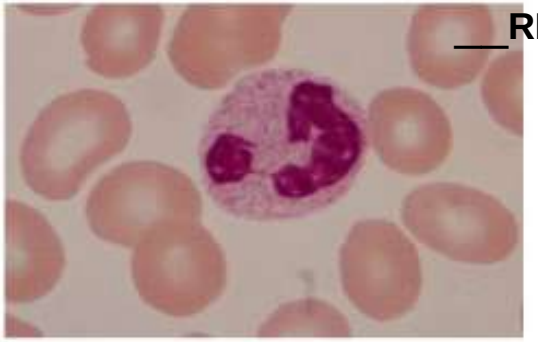
10 µm



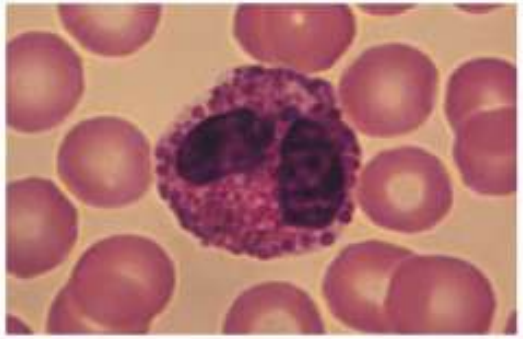
monocyte

10 µm

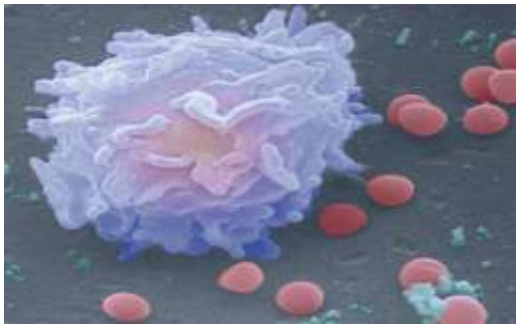
Leukocytes

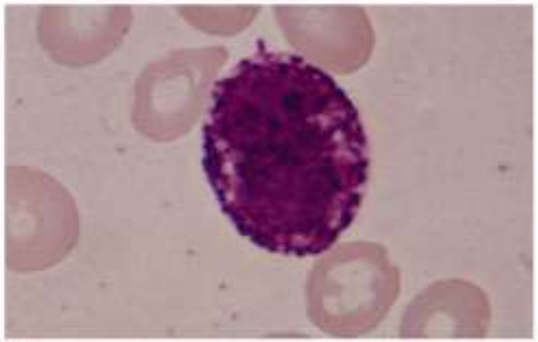


(a) neutrophil

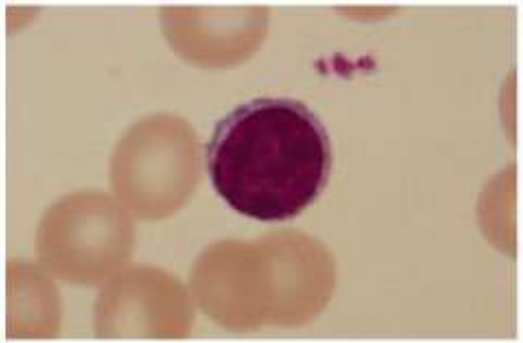


(b) eosinophil

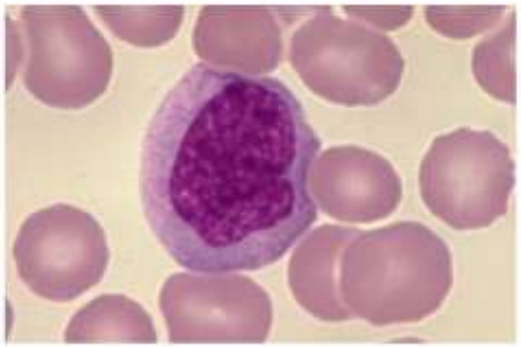




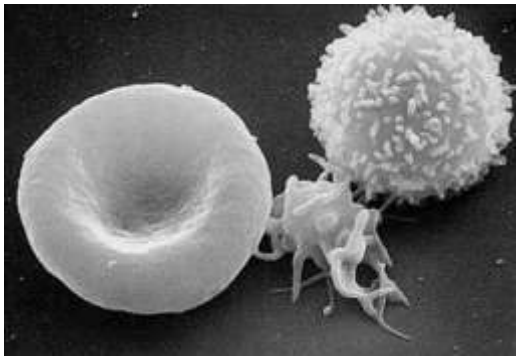
(c) basophil

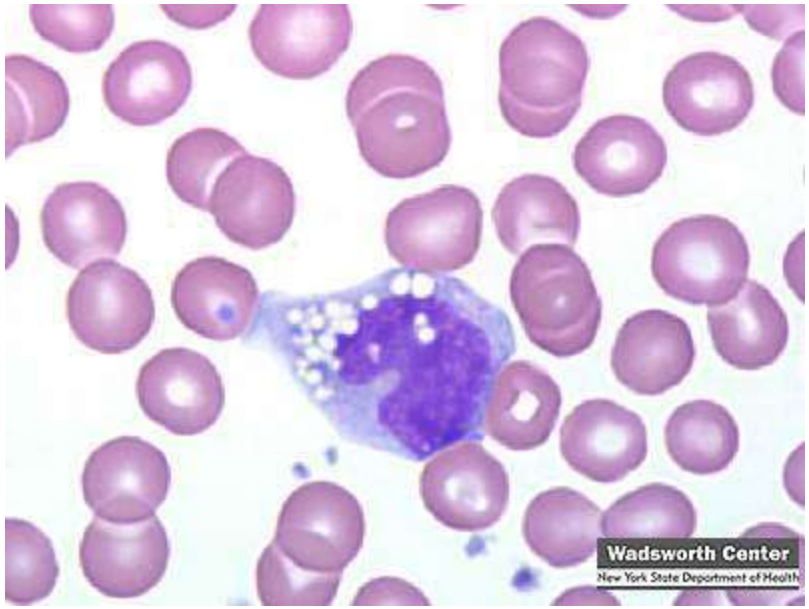


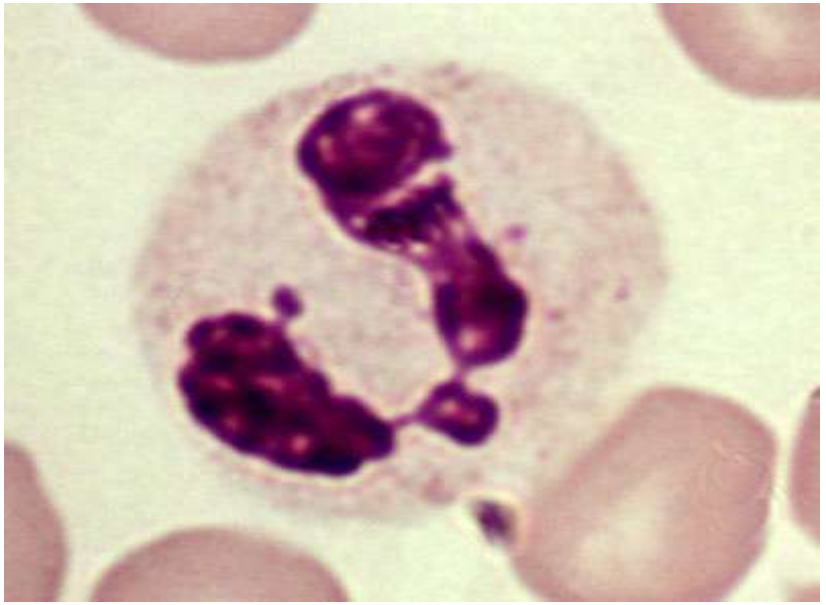
(d) small lymphocyte

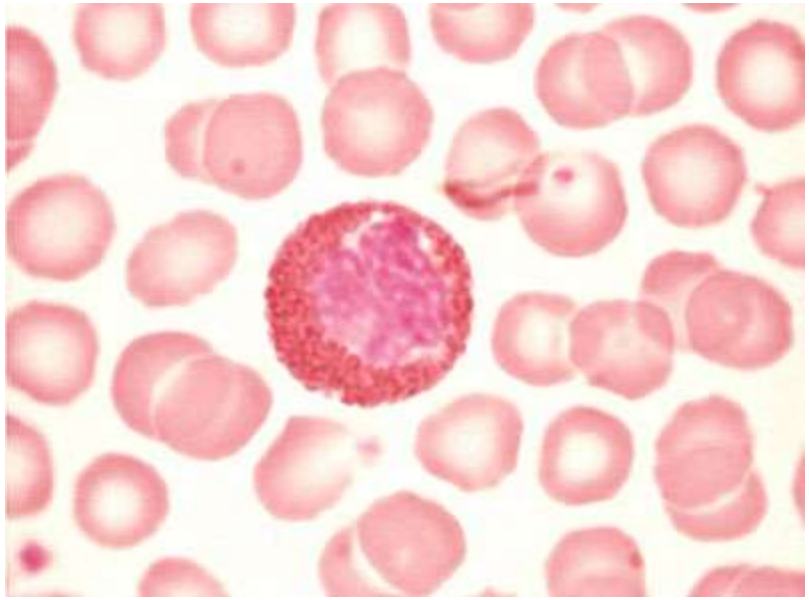


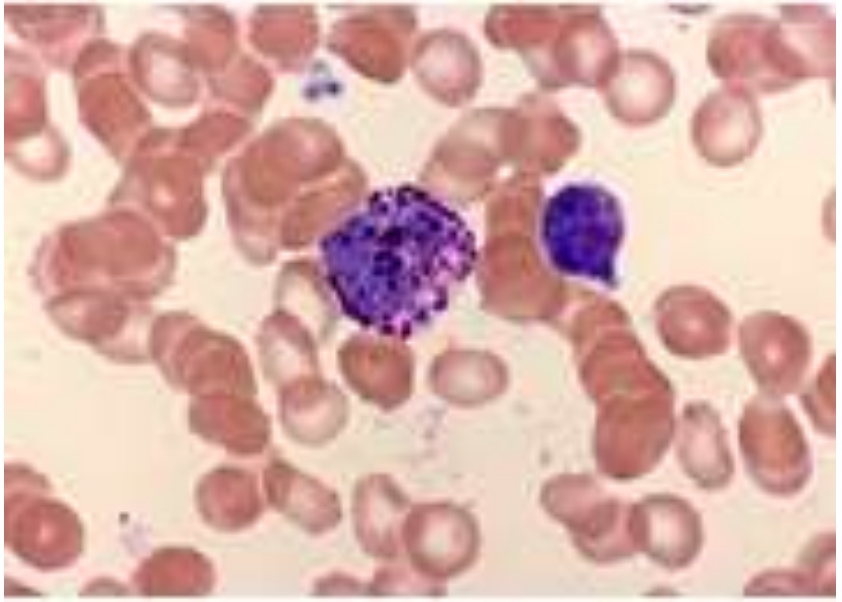
(e) monocyte

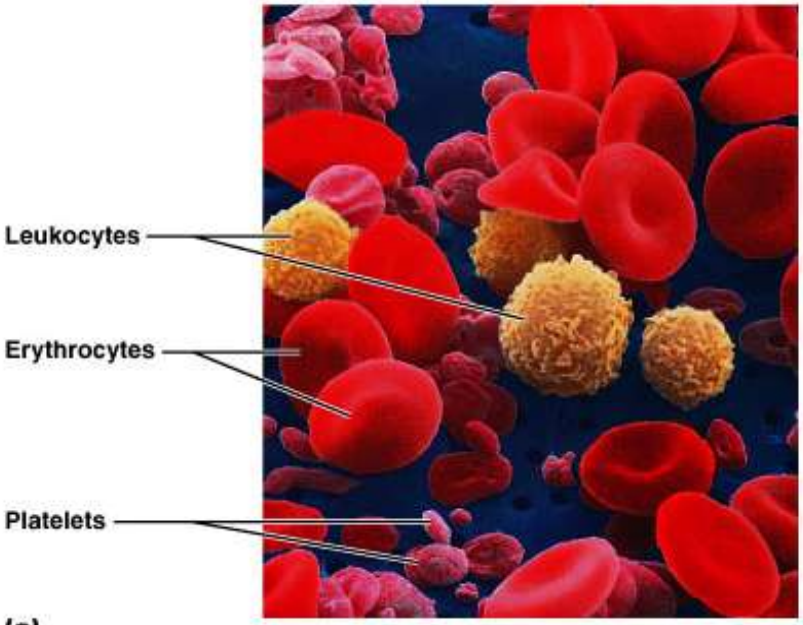



Wadsworth Center
New York State Department of Health

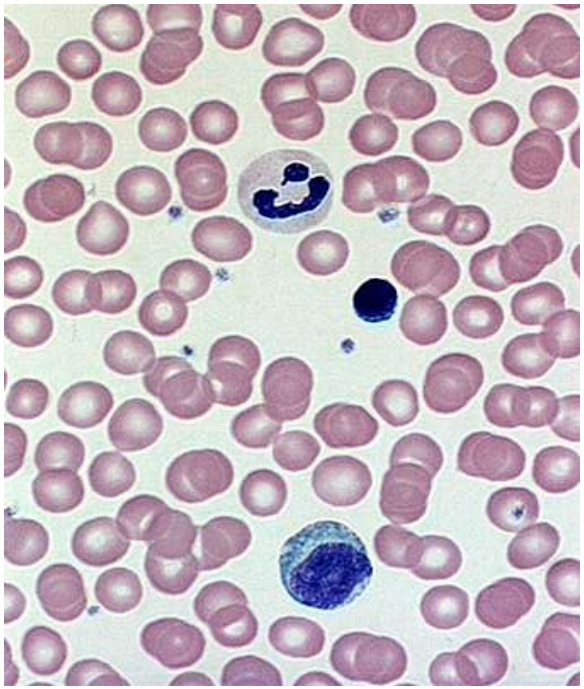


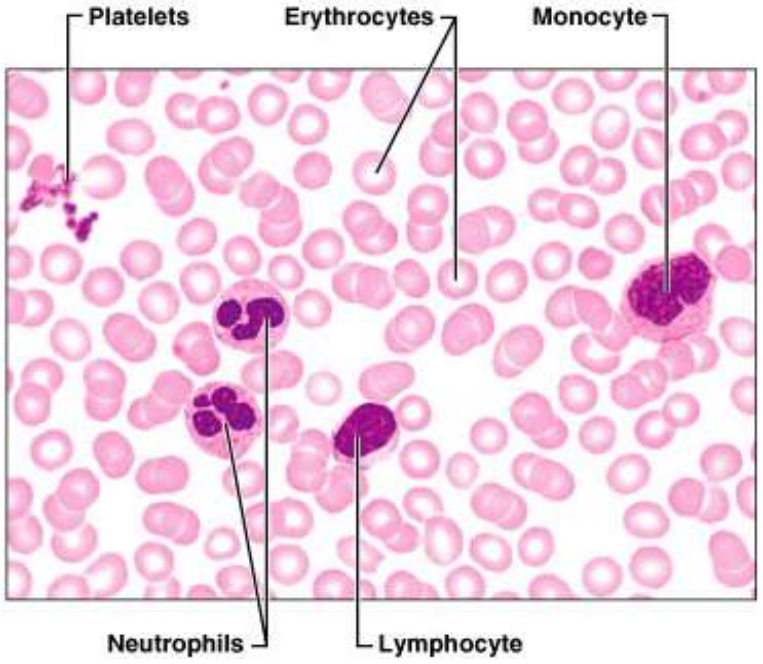


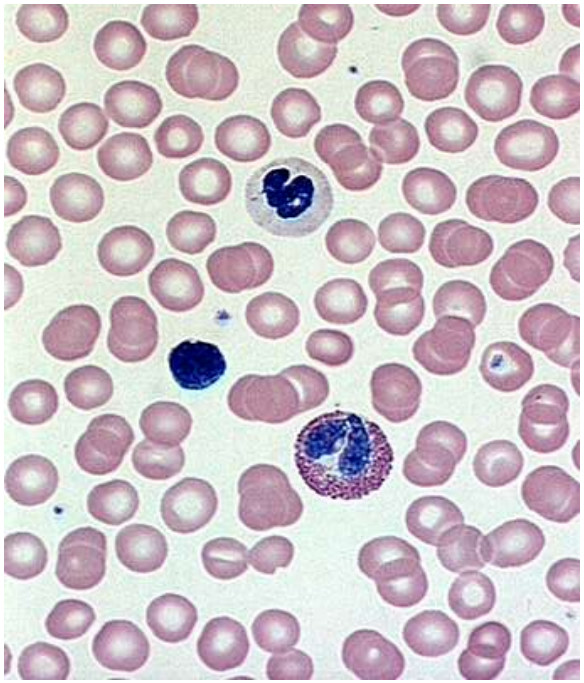


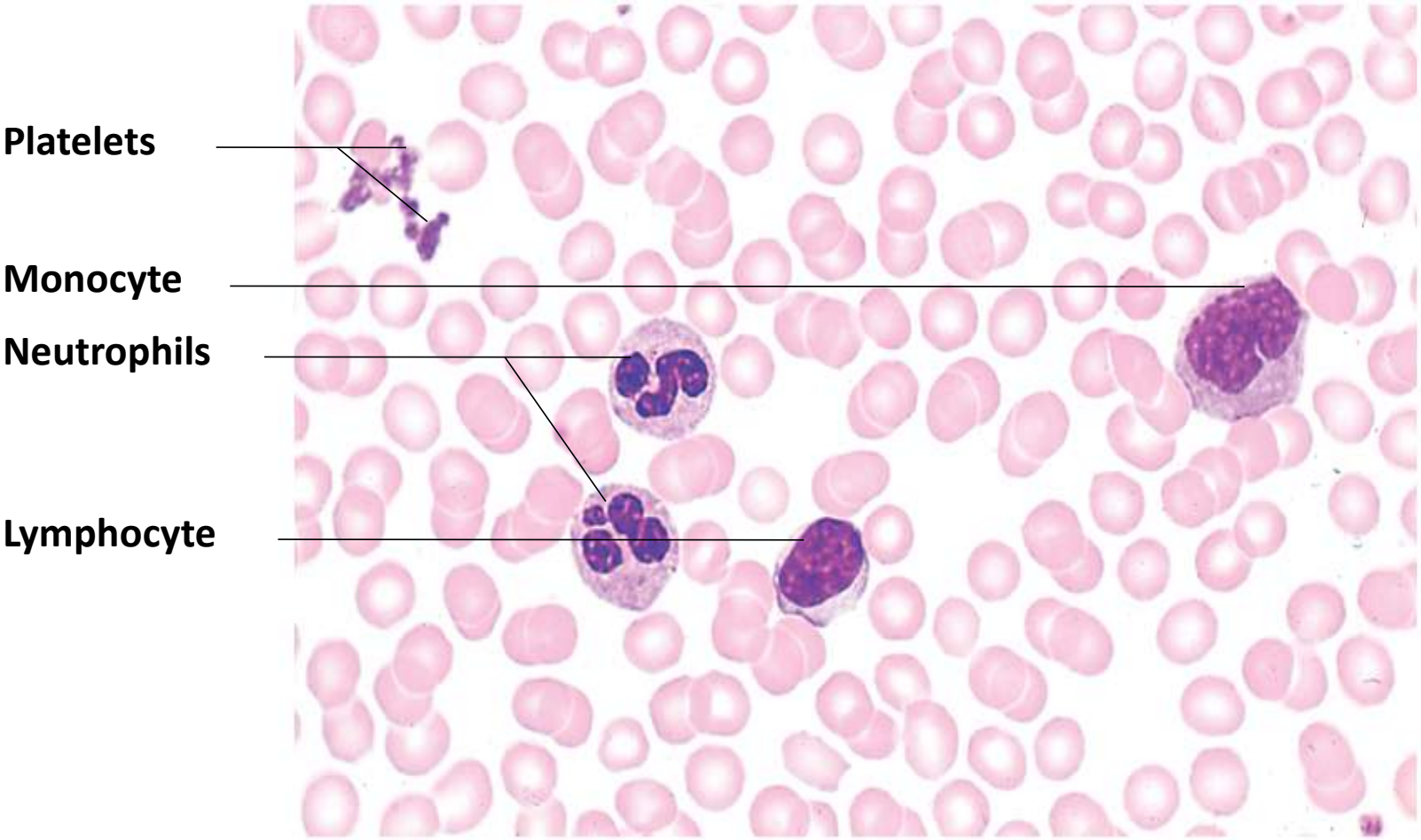

Leukocytes
Erythrocytes
Platelets

(a)

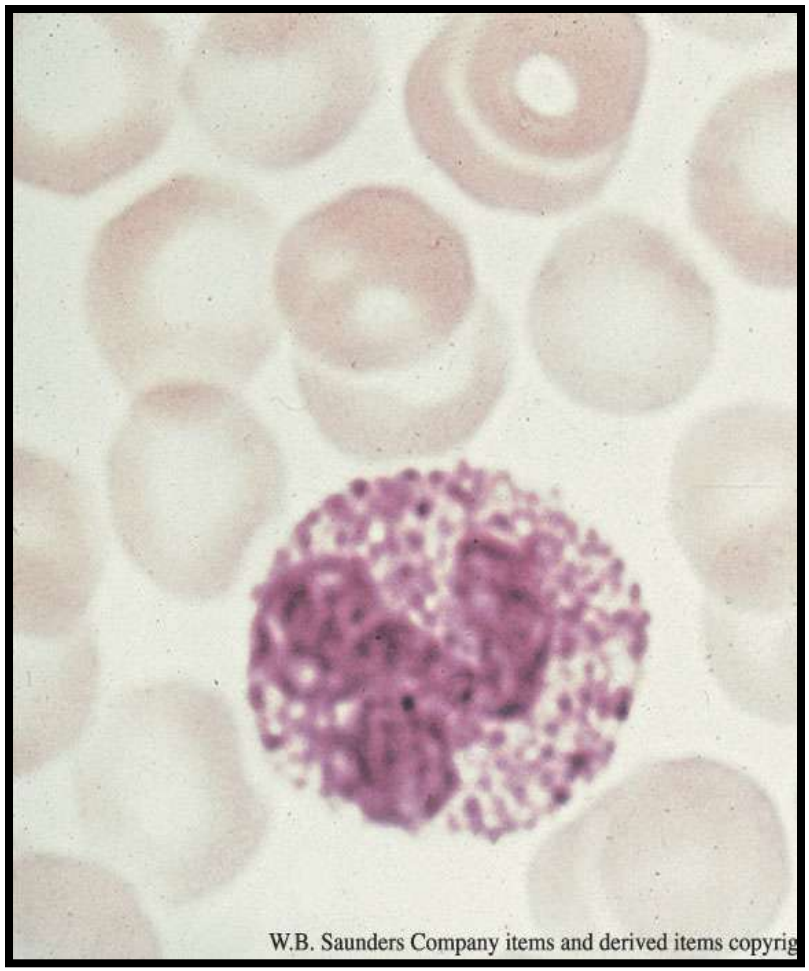



Platelets
Erythrocytes
Monocyte
Neutrophils
Lymphocyte




Platelets
Monocyte
Neutrophils
Lymphocyte

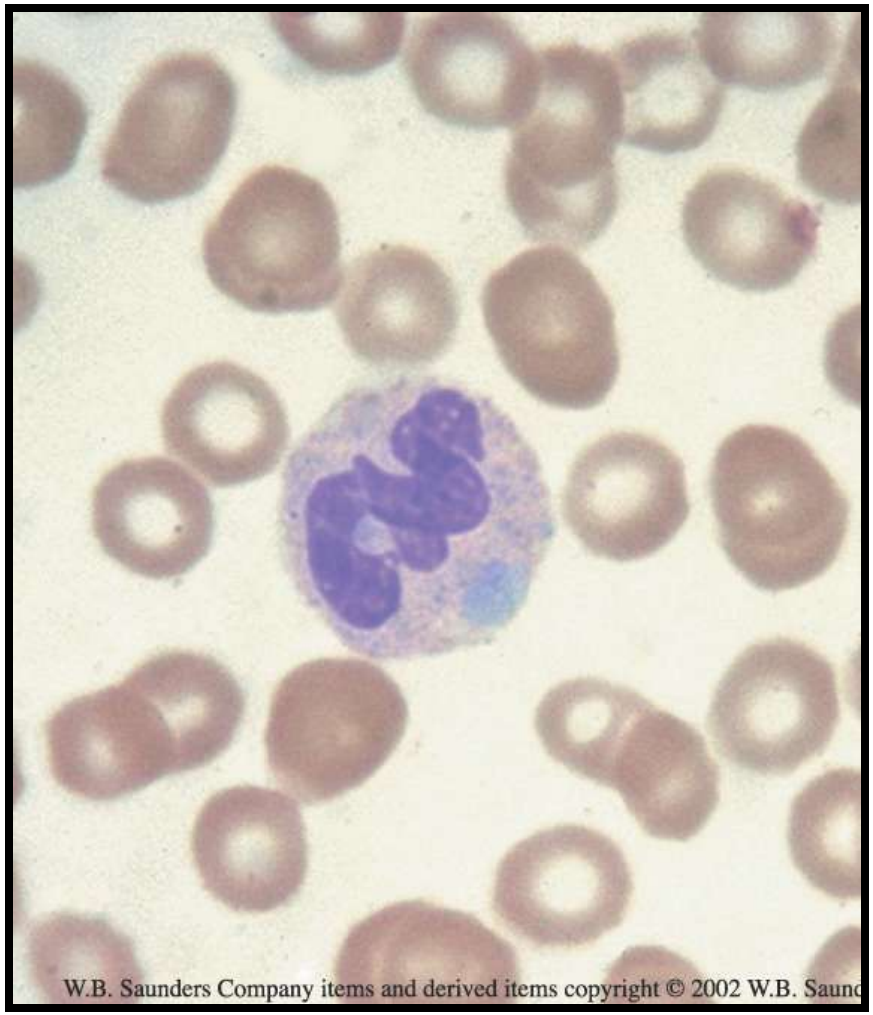
Toxic granulation



neutrophil

Increased basophilic granules in neutrophils, seen in severe infections, burns, malignancies, and pregnancy. Distinguish from basophils.

Dohle bodies

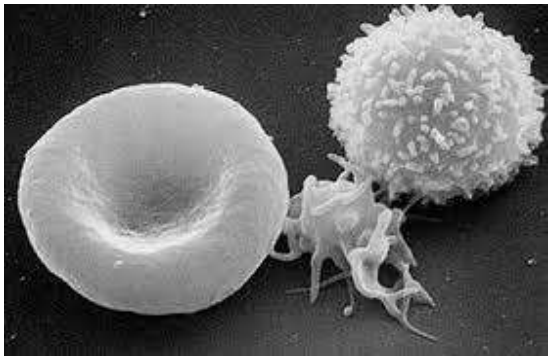


neutrophil

Sky blue inclusions in cytoplasm of neutrophils, seen in infections, burns, myeloproliferative disorders, and pregnancy. Composed of RER and glycogen granules.

5.3. Platelets

- platelets - small fragments of megakaryocyte cells
  - 2-4 μm diameter
  - pseudopods
  - contain “granules”
  - thrombocytosis: thrombosis
  - thrombocytopenia: idiopathic thrombocytopenic purpura



5.3. Platelets

- Petechiae in patient with acute idiopathic thrombocytopenic purpura (AITP)

6. work

- calculate the number of white blood cells per milliliter in blood
- calculate the percent of 5 types of white blood cells in blood

Cell Type	Cell Number	%
Neutrophils		
Lymphocytes		
Monocytes		
Eosinophils		
Basophils		
Total		**100**

- Draw a picture of formed elements you observed in blood smear slide

7. Case study

- A 56-year-old male farmer was evaluated for 2 months of black stools. He had fatigue and dizziness for 3 weeks with a weight loss. He denied abdominal pain, or diarrhea. No abnormal past history. He was afebrile. Physical examination is unremarkable except pallor.
- Laboratory studies show:
  - RBC count: 3.2×10^6/μl (4-5×10^6/μl)
  - Hemoglobin value: 7.0 g/dl (12-16 g/dl)
  - Hematocrit: 24.8% (42-52%)
  - MCV: 77.5 fl (80-100 fl)
  - MCHC: 28.2 g/dl (32-36 g/dl)
  - Peripheral blood smear: many microcytic hypochromic RBCs
  - Reticulocyte: 1.2%（0.5-1.5%）
  - WBC count: 5.6×103 (4×10^3-1×10^4/μl)
  - WBC differential: eosinophil was 6% (0.5-3%), other types of WBC were normal
  - Platelet count: 120×10^3/μl (100-280×10^3/μl)
  - Fecal occult blood (OB) test: strong positive
  - Stool microscopy: numerous hookworm eggs
  - Liver and renal function and tumor markers were normal
- What is the most likely diagnosis? Why?