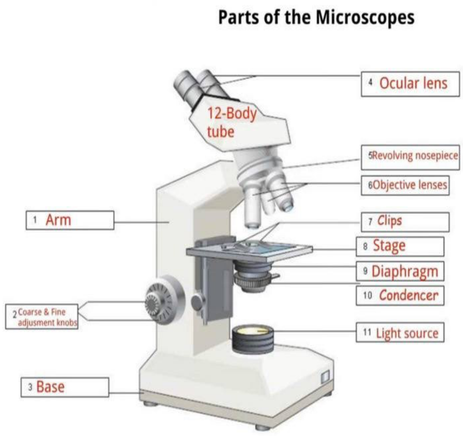
Al-Mustaqbal University
College of Pharmacy

Practical Physiology
2nd class
2023-2024
1st course

EXPERIMENT 1
GENERAL PHYSIOLOGY OF BLOOD



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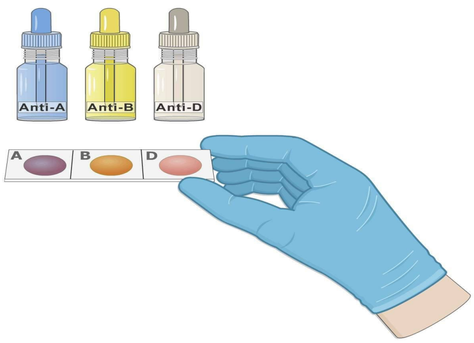

Introduction to Hematology

The branch of science that deals with the study of blood is called hematology. The word hematology is derived from the Greek *haema* = blood; logy = study of.

Blood is a fluid connective tissue that flows through blood vessels of the cardiovascular system. Blood consists of cells and cell fragments pieces, collectively called the **formed elements**, carried in an extracellular fluid called blood **plasma**.

Physical Features and PH

The blood is denser and more viscous than water, slightly alkaline, and sticky to touch. It clots on standing, leaving behind serum. The total volume of blood in an adult of 70 kg is about 5.5 L or 8 per cent of the body weight (5–6 L in an average adult male weighing 70kg, and 4–5 L in a female), Figure 1. The interplay of various hormones that control salt and water excretion in the urine keep the blood volume remarkably constant.

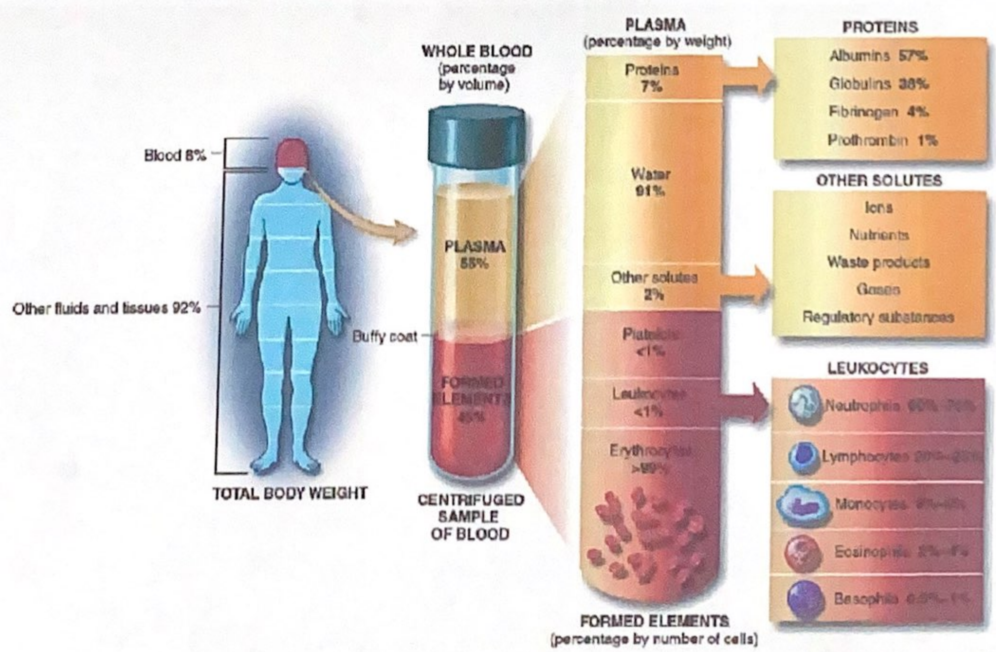

Figure 1: The Composition of Whole Blood.

The normal blood pH is tightly regulated to stay within a narrow range of 7.35 to 7.45, making it **slightly alkaline**. A variety of factors affect blood pH including what is ingested, vomiting, diarrhea, lung function, endocrine function, kidney function, and urinary tract infection. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too alkaline.

Functions of blood

Blood has three general functions:

1. Transportation: Blood transports respiratory gases (O_2 and CO_2) between lungs and body cells, in addition to nutrients, waste products and different regulatory molecules (like hormones) inside the body.

2. Regulation: Circulation of blood helps maintain the homeostasis of the body. It regulates the body temperature by transporting heat from the tissues (mainly liver and muscles) to the skin from where it can be lost. Its buffers regulate pH of the body fluids, while its osmotic pressure regulates water content of cells through the actions of its dissolved proteins and ions.

3. Protection: The blood protects the body either by clotting and thus, preventing excessive bleeding or can fight diseases or pathogenic intruders.

Composition of Whole Blood

A sample of blood is approximately 55 percent plasma and 45 percent formed elements (Figure 1).

Plasma is approx. 91.5% water and 8.5% solutes, most of which are proteins like albumins (regulate the osmotic pressure of blood), globulins, and fibrinogen. Antibodies (or immunoglobulins) belong to the group of globulins, and play important role during certain immune responses. Besides proteins, other solutes like electrolytes, hormones, nutrients, waste products, and gases are also present.

The formed elements are organized into three groups of cells and pieces of cells: red blood cells, white blood cells, and platelets. When stained, each group is easy to identify with a microscope. Development of blood cells is called *hemopoiesis*. In adults, hemopoiesis occurs in the bone marrow.

Red blood cells (RBCs), also called erythrocytes, are the most abundant of all blood cells. RBCs are biconcave discs that are noticeably thin in the center (Figure 2-a). In their mature form, they are red and lack a nucleus. The biconcave shape gives each RBC more surface area than a flat-faced disc would have, an important feature that allows rapid gas exchange between the blood and the tissues of the body. Their shape also allows RBCs to flex and squeeze through narrow capillaries.

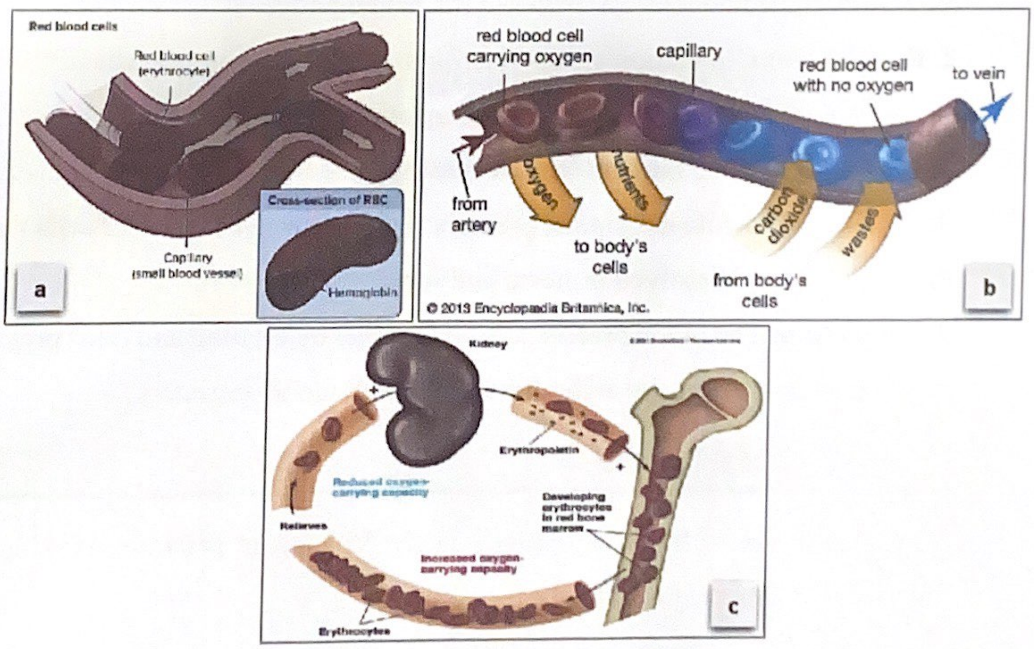

Figure 2: Appearance, movement, production, and function of RBCs.

They contain large amount of hemoglobin (Hb) molecules, which play an important role in the transport of respiratory gases (Figure 2b). RBCs live around 120 days within the circulation. The ruptured cells are removed and destroyed by macrophages in the spleen and liver. Ruptured RBCs must be replaced by new cells by a process called **erythropoiesis** in the bone marrow (Figure 2c).

The major function of RBCs is to transport blood gases (Figure 2-b).

White blood cells (WBCs) have nuclei and all cellular organelles and are classified as either granular or agranular, depending on whether they contain cytoplasmic granules that can be visualized by staining (Figure 3). *Granular leukocytes* include neutrophils (60-70% of WBCs), eosinophils (2-4% of WBCs) and basophils (0.5-1% of WBCs), depending on the type of dyes staining their granules. These granular leukocytes are also called polymorphonuclear leukocytes because the nuclei are complex and branch into two to five lobes. *Agranular leukocytes* include lymphocytes (20-25% of WBCs) and monocytes (3-8% of WBCs).

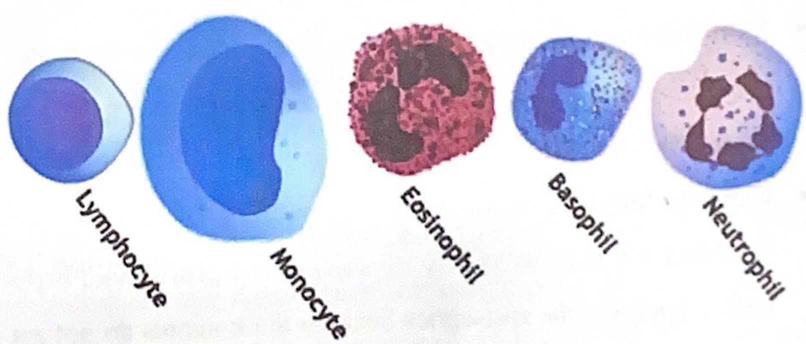

Figure 3: WBCs.

WBCs lack hemoglobin and therefore do not transport blood gases. Most WBCs are phagocytes, scavenger cells that engulf foreign bodies and other unwanted materials circulating in the blood and destroy them, and are therefore part of the immune system.

Platelets are small, irregularly disc-shaped cellular fragments (Figure 4). They have a short life span (4-9 days) in the circulation and then are removed by fixed macrophages in the spleen or liver.

Figure 4: Blood platelets.

Platelets lack a nucleus and other organelles but contain many vesicles which promote blood clotting upon the release of their content. Platelets additionally help stop blood loss by forming a platelet plug in the damaged vessels.

Collection of Blood Samples

Blood samples will be collected for experiments from the subjects themselves. While collecting blood, a number of factors needed to be considered:

1. Asepsis

The term asepsis refers to the condition of being free from septic or infectious material—bacteria, viruses, etc. Puncturing the skin always poses the danger of infection. In order to achieve asepsis, the following aspects should be considered:

- Sterilization of Equipments: All the instruments to be used for collecting blood— syringes, needles, lancets, and cotton and gauze swabs—should preferably be sterile.
- Cleaning/Sterilization of Skin: The selected area must be washed and scrubbed if it is grossly dirty. If washed, the area should be allowed to dry before applying the antiseptics because these agents do not act well on wet skin. At least 2–3 sterile cotton/gauze swabs soaked in 70% alcohol should be used to clean and scrub the area.

2. Sources and Amount of Blood Sample

- ***Capillary blood:*** The skin and other tissues are richly supplied with capillaries, so when a drop or a few drops of blood are required, blood from a skin puncture is adequate. Capillary blood is obtained from the fingertip, ear lobe, heel, or big toe (Figure 5).

  In adults and older children, capillary blood is generally obtained from a skin puncture made on the tip of the middle or ring finger, or on the lobe of the ear. In infants and young children in whom the fingers are too small for a prick, the medial or lateral side of the pad of the big toe or heel is used.

Figure 5: Capillary blood collection sites.

- ***Venous blood:*** When larger amounts (few ml) are needed as for complete hematological and biochemical investigations, venous blood is obtained with a syringe and needle by puncturing a superficial vein (venipuncture).

Notes:

✓ Capillary blood is also called "peripheral blood" as it comes out of the peripheral vessels (capillaries) in contrast to venous blood.
✓ Venous blood is always preferred for clinical tests.

- ***Arterial blood:*** When arterial blood is needed for special tests such as blood pH, an artery is punctured with a syringe and needle. This, however, is not a routine procedure.
- ***Cardiac catheterization:*** Blood from a heart chamber, taken through a cardiac catheter, may be required for special tests.

3. Containers for Blood Sample

A container is a receptacle into which blood is transferred before sending it to the laboratory. Clean and dry glass tubes, blood bags, collection bottles, etc.

are the usual ones in use. A container may or may not contain an anticoagulant depending on whether a sample of blood/plasma, or serum is required (Figure 6).

- For a sample of whole blood or plasma: The blood is transferred to a container containing a suitable anticoagulant. This is to prevent clotting of blood.
- For a sample of serum: No anticoagulant is used. The blood is allowed to clot in the container and serum is collected as described later.

Obviously, capillary blood does not require a container or anticoagulant.

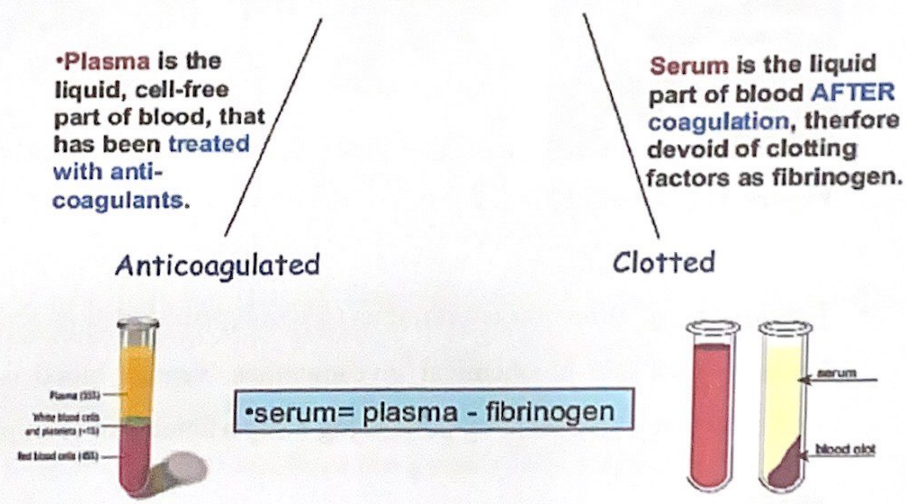

Figure 6: Differences between serum and plasma blood samples.

4. Commonly used anticoagulants

Anticoagulants are substances employed to delay, suppress, or prevent clotting of blood.

The commonly used anticoagulants include: EDTA (Ethylenediamine tetra-acetic acid), trisodium citrate, double oxalate, sodium fluoride, and heparin (Figure 7). The use of fluoride and heparin is limited to pH, blood glucose and gas analysis.

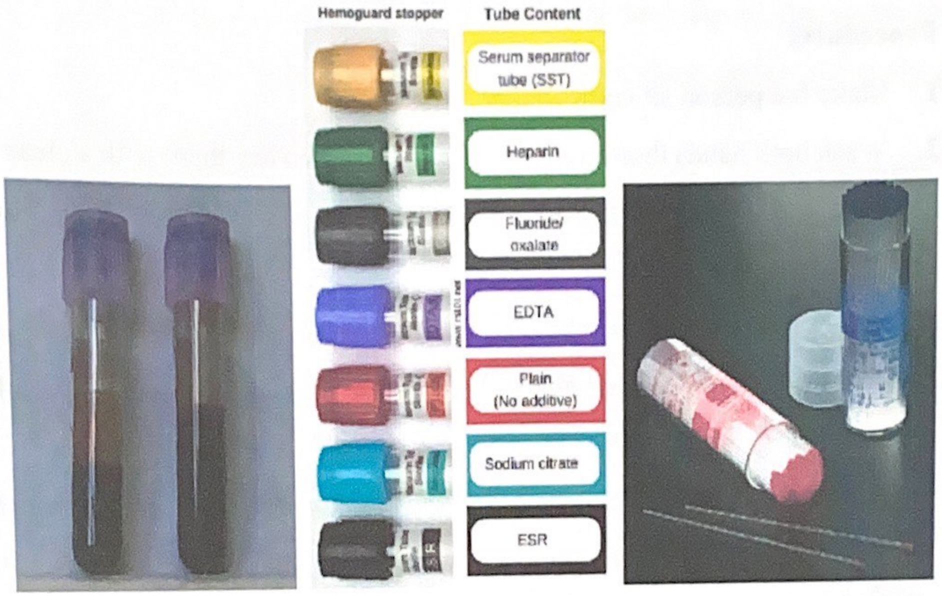

Figure 7: (Left) Two tubes of EDTA-anticoagulated blood. Left tube: After standing, the RBCs have settled at the bottom of the tube. Right tube: contains freshly drawn blood. (Center) blood collection tubes, color coding, and additives. (Right) Capillary tubes.

Collection of capillary blood (skin-prick method)

When a finger is punctured, blood in the capillaries will ooze out. This blood should be collected and utilized quickly before clotting, as there are no anticoagulants added. Sometimes, special heparinized capillary tubes may be used for special investigations (Figure 7). From infants, blood sample may be collected conveniently from the heel or the big toe.

Materials:

- Hand soap
- Paper towels
- Gloves (and other protective wear as necessary)
- Disposable sterile alcohol prep pad
- Disposable sterile blood lancet
- Dry gauze pads
- Appropriate micro-collection tubes, pipette or slide

Procedure:

1. Make the person sit comfortably.
2. Wash both hands thoroughly with soap, and then dry them with a clean paper towel. Wear disposable gloves and safety glasses while collecting and examining blood.
3. Select the fingertip suitable for puncture: The site for skin-prick should be clean and free from edema, infection, skin disease, callus, or circulatory defects.
4. Clean and vigorously rub the ball of the finger with suitable disinfectant (70% alcohol): warming the skin by rubbing before puncture improve circulation and ensure free flow of blood.
5. Wipe the area with dry, sterile gauze pad.

   Note: Allow the alcohol to dry by evaporation for the following reasons:

   a) Sterilization with alcohol/spirit is effective only after it has dried by evaporation.

   b) The thin film of alcohol can cause the blood drop to spread sideways along with alcohol so that it will not form a satisfactory round drop.

   c) The alcohol may cause hemolysis of blood.
6. Hold the finger firmly and puncture the skin with a sterile lancet. Do a quick, single and sufficiently deep (3-5 mm) stroke to puncture to allow free flow of blood. Flow of blood could be enhanced by gentle pressure at the proximal part of the finger to obstruct venous return. Squeezing or milking the finger will dilute the blood with tissue fluid.

   Note: The placement of the lancet should be on the side of the ball of the finger perpendicular to the lines of the fingerprint
7. Allow the blood to flow freely and discard the first drop by wiping it away with a dry gauze pad.
8. Obtain the required amount of blood (Figure 8) directly using appropriate instrument, pipette or slide.

9. Leave the punctured site undisturbed for the bleeding to stop on its own and if it continues to bleed, cover the area with clean gauze and apply gentle pressure until bleeding stops.

Figure 8: Drawing blood into capillary tubes.

Disposal of Materials and Disinfection of Work Space

1. Dispose of all blood-contaminated materials in the appropriate biohazard box. A box for sharp objects may be available to dispose of the lancets, syringe needles, toothpicks, and microscope slides.
2. Your instructor may ask you to disinfect your workstation with a bleach solution. If so, wear gloves and safety glasses while wiping the surfaces clean.
3. Lastly, remove your gloves and dispose of them in the regular trash. Remember to wash your hands after disposing of all materials.