COLLEGE NAME & CODE : Periyar Arts College, Cuddalore-01 & 105
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UNIT-I

Blood: Definition, Characters, Composition.

Collection of Blood –

Capillary Blood: from Adults / Infants, Examinations employed. Advantages/Disadvantages

Venous blood: from Adults / Infants, Examinations employed, Advantages/Disadvantage.

Anticoagulants: Definition - Type: Wintrobes /EDTA /Heparin /Citrate, Concentration, Examinations, Advantages /Disadvantage.

BLOOD

DEFINITION

- Blood is a connective tissue consisting of **plasma** (fluid matrix) and **formed elements**.
- Blood is the most common body fluid that transports substances from one part of the body to the other.
    - i.e., *Blood, fluid that transports oxygen and nutrients to the cells and carries away carbon dioxide and other waste products.*

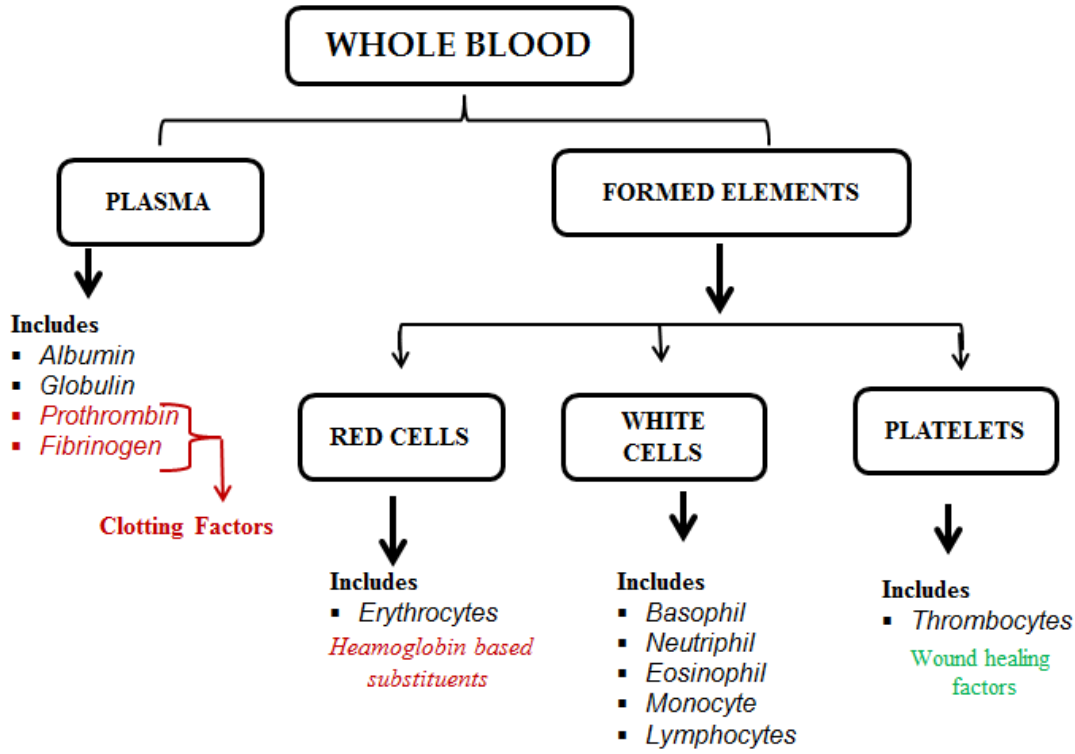
- The plasma constitutes 55% of the total blood volume.
- The remaining 45% is the formed elements that consist of blood cells.
- The average blood volume is about 5000ml (5L) in an adult weighing 70 Kg.

CHARACTERS

- It is a tissue because it is a collection of similar specialized cells that serve particular functions.
- These cells are suspended in a liquid matrix (plasma), which makes the blood a fluid.
- In the lungs, blood acquires oxygen and releases carbon dioxide transported from the tissues.
- The kidneys remove excess water and dissolved waste products.
- Nutrient substances derived from food reach the bloodstream after absorption by the gastrointestinal tract.
- Glands of the endocrine system release their secretions into the blood, which transports these hormones to the tissues in which they exert their effects.
- Many substances are recycled through the blood;

    for e.g., old red cells - iron released - plasma - new red cell production -reused.
- Hemocyanin, a copper-containing protein chemically unlike hemoglobin, is found in some crustaceans. Hemocyanin is blue in colour when oxygenated and colourless when oxygen is removed.
- Some annelids have the iron-containing green pigment chlorocruorin, others the iron-containing red pigment hemerythrin.
- In many invertebrates the respiratory pigments are carried in solution in the plasma, but in higher animals, including all vertebrates, the pigments are enclosed in cells; if the pigments were freely in solution, the pigment concentrations required would cause the blood to be so viscous as to impede circulation.

COMPOSITION



A. PLASMA

- Plasma mainly consists of water (80- 92%) in which the plasma proteins, inorganic constituents (0.9%), organic constituents (0.1%) and respiratory gases are dissolved.
- The four main types of plasma proteins synthesized in the liver are
    i. albumin,
    ii. globulin,
    iii. prothrombin and
    iv. fibrinogen

B. FORMED ELEMENTS

- Red blood cells/corpuscles (erythrocytes) **(RBC)**, white blood cells/corpuscles (Leucocytes) **(WBC)** and **Patelets** are collectively called formed elements.

I. RED BLOOD CELLS

- Red blood cells are abundant than the other blood cells. There are about 5 million to 5.5 millions of RBC mm^{-3} of blood in a healthy man and 4.5-5.0 millions of RBC mm^{-3} in healthy women.

- **Erythropoietin** is a hormone secreted by the kidneys in response to low oxygen and helps in differentiation of stem cells of the bone marrow to erythrocytes (erythropoiesis) in adults. The ratio of red blood cells to blood plasma is expressed as **Haematocrit** (packed cell volume).

WHITE BLOOD CELLS

- **White blood cells** (leucocytes) are colourless, amoeboid, nucleated cells devoid of haemoglobin and other pigments. Approximately 6000 to 8000 per cubic mm of WBCs are seen in the blood of an average healthy individual.
- The different types of WBCs are shown in Figure 7.3.
- Depending on the presence or absence of granules, WBCs are divided into two types,
    - Granulocytes
    - Agranulocytes.

COLLECTION OF BLOOD

INTRODUCTION

- Blood is one of the most common specimens used in laboratory determinations.
- Venous blood is preferred for most hematological examinations.
- Peripheral samples (capillary blood) can be used satisfactorily for many purposes if a free flow of blood is obtained, but this procedure should be avoided in patients who may be possible carriers of transmissible diseases.
- Capillary blood is used commonly for hemoglobin estimation, cell counts, blood grouping, bleeding and clotting time determination, and other investigations that use less blood, whereas venous blood is preferred for a comprehensive hematological investigation.

What are Arteries?

Arteries are blood vessels that carry blood from the heart to the entire body. The blood is 'mostly' oxygenated in arteries. Arteries are also responsible for carrying nutrients to the cells.
Two exceptions where arteries carry deoxygenated blood.

i. Pulmonary artery where it carries blood from the heart to lungs and
ii. second is the Umbilical artery which carries the blood from fetus to the placenta.

What are Veins?

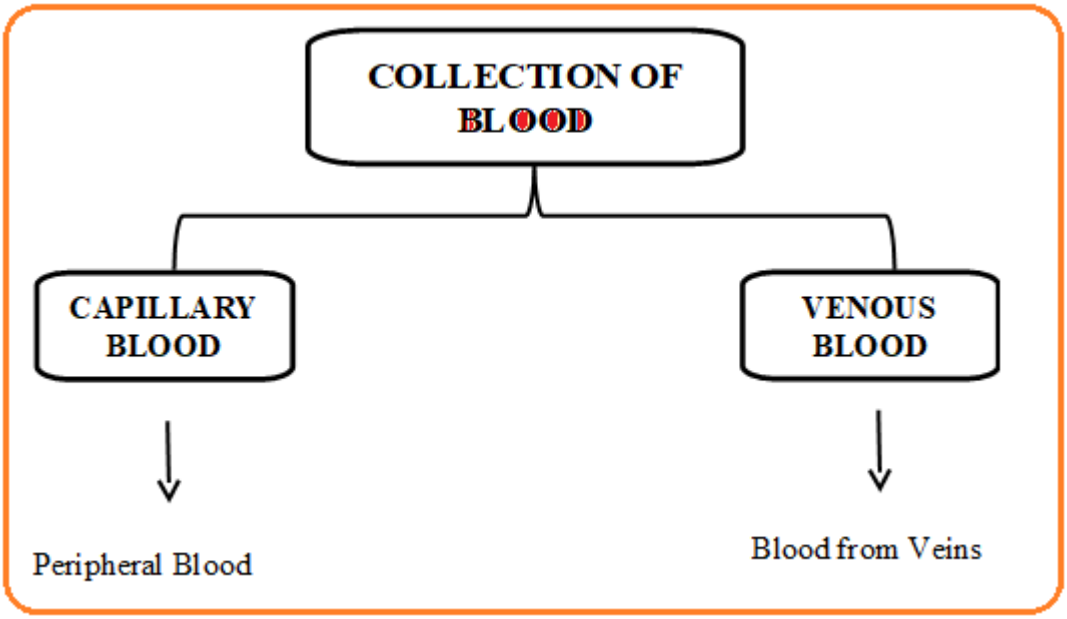
Veins are blood vessels that carry blood from the body to the heart. And again, it 'mostly' carries deoxygenated blood. The pulmonary and umbilical veins carry oxygenated blood to the heart. The veins have valves present in them. These valves prevent backflow (Flow in the reverse direction). Veins are also less muscular in comparison to arteries.

METHODS OF COLLECTION

For physiology practicals, blood is often collected by pricking the tip of the finger. Therefore, collection of peripheral blood by finger puncture method is described clearly.

Principle

- There are two general sources of blood for clinical laboratory tests: peripheral (capillary) blood and venous blood. For small quantities of blood for hematologic investigations, the specimen is obtained from the capillary bed by puncturing the skin. The tip of the finger is the most common site for puncture.
- For larger quantities of blood, a puncture is made directly into a vein (phlebotomy) using a sterile syringe-and-needle collection system.



COLLECTION OF CAPILLARY (Peripheral) BLOOD

- Capillary blood is often used for bedside investigations. But this blood is likely to give erroneous results if not collected properly; therefore, it should only be used when it is not possible to obtain venous blood.
- Free flow of blood is essential and only the very gentlest squeezing is permissible. Ideally, large drops of blood should exude slowly but spontaneously.
- Capillary blood sampling, which refers to sampling blood from a puncture on the finger, heel or an earlobe, is increasingly common in medicine.

Capillary blood collection from Adults

Skin puncture blood sampling is also recommended for adult patients with

- severe burns,
- those who are obese or older or anxious about sampling,

Advantages

Capillary blood sampling has several advantages over venous blood sampling:

→ it is less invasive,

→ it requires smaller amounts of blood volume and

→ it can be performed quickly and easily.

Disadvantages

If carried out incorrectly, capillary blood sampling can cause

→ inaccurate test results, pain and tissue damage.

COLLECTION OF BLOOD FROM VEINS (VENOUS BLOOD)

The process followed to draw blood is called Phlebotomy.

For blood sampling, blood is taken from veins and not arteries because:

- Veins are close to the surface of the skin. This makes the process easier by avoiding a deep needle plunge just to draw a bit of blood. Arteries, on the other hand, are a tad bit deeper.

PROCEDURE

- The first step is to identify the vein. The most common vein for adults is the median cubital vein. It's a large vessel which makes drawing blood easier. It also gives excellent results if the blood is drawn properly.
  - The next step is to actually draw the blood.
  - We begin by hyperextending the arm of the patient and putting the tourniquet around 3-4 inches from the site. Tell the patient to form a fist and keep it tight.
  - Next is to cleanse the site with alcohol swabs.
  - Now grab the lower arm which helps to anchor the vein and stop it from rolling. Then insert the needle at 15-30 degrees into the vein.
  - If done correctly, you can see blood in the catheter!
  - Use the right tubes to remove the appropriate amount of blood.
  - Now remove the tourniquet and remove the needle.
  - Then press down on the site with a gauze.
  - Label the tubes correctly and then provide it to the labs within time.

Venipuncture:

- The blood that is collected, called venous blood, can then be used for a variety of purposes, such as intravenous therapy, blood sampling, diagnosis and so on.

EXAMINATIONS

A **blood test** is a laboratory analysis performed on a blood sample

For detailed examinations, blood is subjected to the following tests

1. Ions and Trace metals
2. Acid base and blood gases
3. Liver function tests
4. Cardiac tests
5. Lipids
6. Tumour markers
7. Endocrinology
    a. Thyroid hormones
    b. Sex hormones

→ Blood test are often used in healthcare to determine physiological and biochemical status such as

- disease, mineral content, pharmaceutical drug effectiveness, and organ function.
- glucose test or a cholesterol test (LDL, HDL & Triglycerides)
- Basic metabolic test done to measure sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), magnesium, creatinine, glucose and calcium
- Arterial blood gas test- to monitor CO2, O2 levels, blood pH and bicarbonate levels
- Blood tests are also used in drug tests to detect drug abuse.

→ Blood tests are also used in drug tests to detect drug abuse.

→ A blood test is required before marriage.

Normal ranges

- Blood tests results should always be interpreted using the ranges provided by the laboratory that performed the test. Example ranges are shown below.

TEST	LOW	HIGH	Unit
Sodium	134	145	mmol/L
Potassium	3.5	5.0	mmol/L
Urea (Blood Urea Nitrogen)	2.5	6.4	mmol/L
Urea	15	40	mg/dL
Creatinine - Male	0.7	1.3	mg/dL
Creatinine – Female	0.6	1.2	mg/dL
Glucose (Fasting)	3.9	5.8	mmol/L
Glucose (Fasting)	70	120	mg/dL

ANTICOAGULANTS

Whole blood

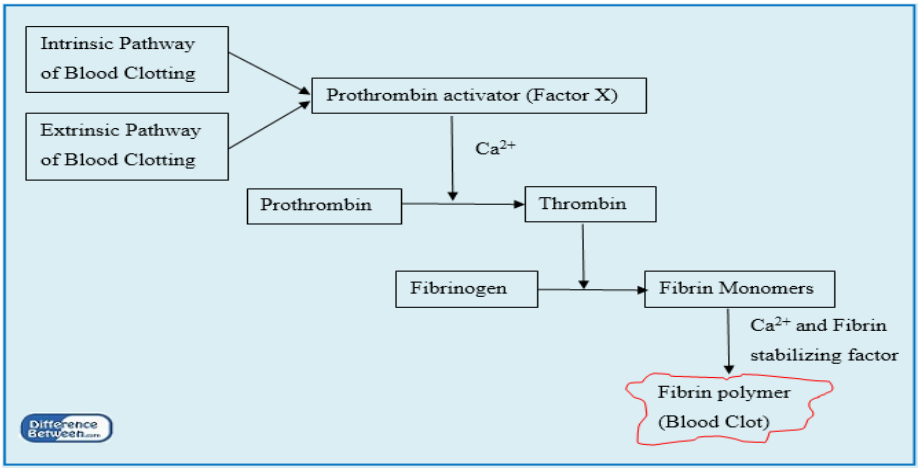
- A venous, arterial or capillary blood sample in which the concentrations and properties of cellular and extra-cellular constituents remain relatively unaltered when compared with their *in-vivo* state.
- Anticoagulation *in-vitro* stabilizes the constituents in a whole blood sample for a certain period of time.

Plasma

- The virtually cell-free supernatant of blood containing anticoagulant obtained after centrifugation.

Serum

- The undiluted, extracellular portion of blood after adequate coagulation is complete.



CLOTTING MECHANISM

ANTICOAGULANTS

- Anticoagulants prevent blood from clotting. They are added to the blood sample, especially when blood is collected by venipuncture, and sent to the laboratories for investigation.
- They are additives that inhibit blood and/or plasma from clotting (ensuring that the constituent to be measured is non-significantly changed prior to the analytical process).

- Anticoagulation occurs by binding calcium ions (EDTA, citrate) or by inhibiting thrombin activity (heparinates, hirudin).

– ***EDTA*** is usually used in 'hematology' while ***citrated blood*** is used for 'coagulation studies' and in blood banks.

- The following solid or liquid anticoagulants are mixed with blood immediately after sample collection:
    A. EDTA,
    B. Trisodium citrate,
    C. Double oxalate,
    D. Sodium fluoride and
    E. Heparin.

A. *EDTA* (Ethylenediamine Tetra-acetic Acid)

- This is also known as Sequestrene or Versene. The sodium and potassium salts of EDTA are powerful anticoagulants.
- **Preparation**
  - Prepare a 10 % solution of dipotassium salts of EDTA.
  - Dissolve 10 g of salt in about 80 ml of water in a 100 ml volumetric flask and then make the volume of the solution to 100 ml.
- **Mechanism of Action**
  - EDTA acts by its chelating effect on the calcium molecules of the blood. Calcium is one of the important factors required in the coagulation process.
- **Effective Concentration**
  - To achieve the chelating effect, a concentration of 1.2 mg of the anhydrous salt per ml of blood (1.2 mg/mL) is required (4.1 mMol/L).
- **Uses**
  - EDTA is suitable for all routine hematological investigations except coagulation studies.

B. *Citrate*

- Trisodium citrate (32 g/l, Na,C,H,O,-2H,O) is the anticoagulant of choice in coagulation studies.

Uses

- It is used for coagulation studies, including prothrombin times and partial thromboplastin tests, in blood banks, in the estimation of ESR, especially by the Westergren method.

C. *Double Oxalate*

- This is an anticoagulant containing **ammonium oxalate** and **potassium oxalate**. Therefore, it is called double oxalate.

- Potassium oxalate alone causes shrinkage of red cells whereas ammonium oxalate increases their volume. So, double oxalate is also called **balanced oxalate** as it preserves cell morphology.
- **Uses**
  - This is used for estimation of ESR, PCV or investigations in which the volume of the cells should not be affected.

D. *Heparin*

- **Heparin** is, theoretically, the best anticoagulant because it is a natural constituent of blood and introduces no foreign contaminants into the blood specimen.
- **Uses**
  - It is used for blood gas determination and pH assays.
  - It is the best anticoagulant for the osmotic fragility test.

E. *Hirudin*

- Hirudin is an antithrombin extracted from leeches or prepared by a genetic engineering process.
- Hirudin inhibits thrombin by forming a 1:1 hirudin-thrombin complex.
- Hirudin is used at a concentration of 10 mg/L.

The *colour codes* of anticoagulants described in ISO/DIS 6710 are:

EDTA	= lavender/red;
citrate 9 + 1	= light blue/green;
citrate 4 + 1	= black/mauve;
heparinate	= green/orange;
no additives (for serum)	= red/white.