



قسم الكيمياء الحيوية
Biochemistry Department
College of Science - King Saud University

BCH 471

BIOCHEMISTRY OF BLOOD

[Practical]

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Mark Distribution

Report [5 Marks]

Quiz [7 Marks]

Final [13 Marks, 8 for practical and 5 for theoretical]

Final exam date:

Sunday 22/2/ 1436 H - 14/12/2014

Experiment (1)

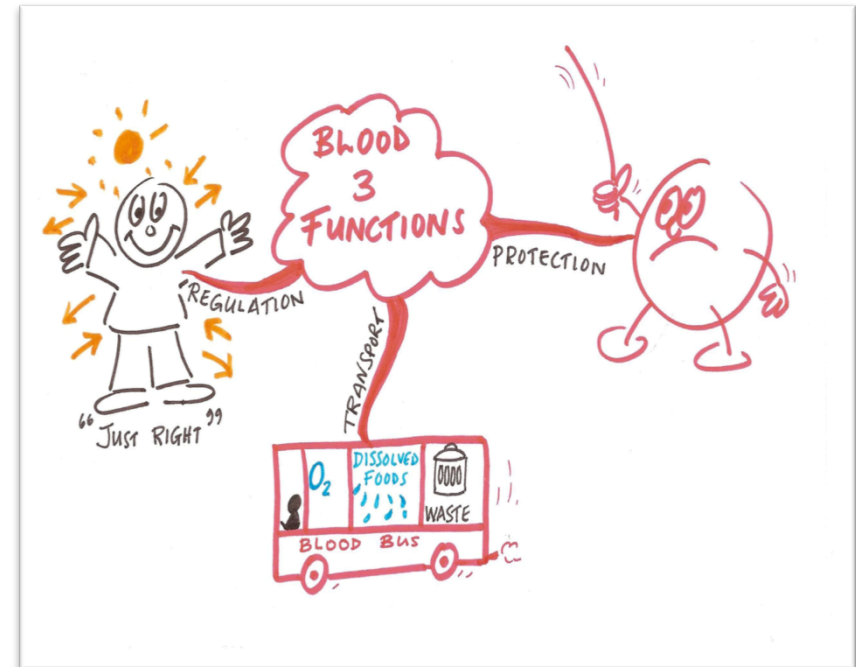
Separation of Plasma and Serum from Whole Blood

WHOLE BLOOD

- The average person circulates about 5L of blood (1/13 of body weight), of which 3L is plasma and 2L is cells.
- It is living tissue, composed of different types of cells suspended in fluid called plasma.
- The cells are produced primarily by bone marrow and account for blood “solids”.
- Plasma fluid derives from the intestines and organs, and provides a vehicle for cell measurement.

BLOOD FUNCTIONS

- Transportation of gases, nutrients, waste products, regulatory molecules (e.g. hormones) and metabolites.
- Regulation of pH and osmotic pressure.
- Maintenance of body temperature.
- Protection against infections.
- Clot formation.



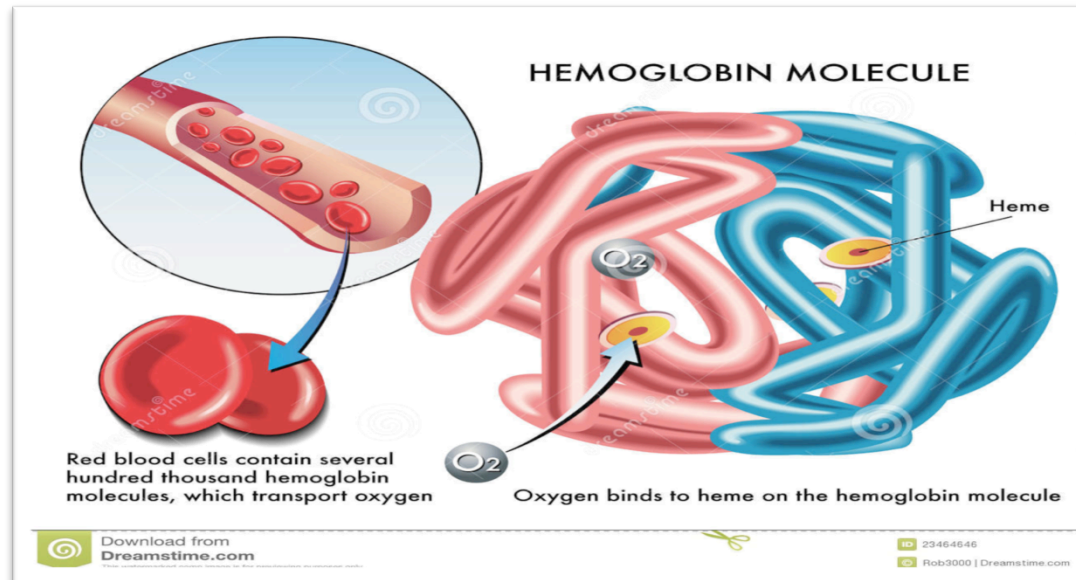
BLOOD CELLS

- Blood cells are classified as:
 - Red blood cells (erythrocytes)
 - White blood cells (leukocytes)
 - Platelets (thrombocytes)
- The size of cells differs:
 - white cells are the largest, red cells fall into the middle, and platelets are the smallest.

RED BLOOD CELLS (RBC)



- Red blood cells contain hemoglobin, a complex iron-containing protein that carries oxygen throughout the body and gives blood its red color.
- They live for approximately 120 days in the circulatory system and are eventually removed by the spleen.



WHITE BLOOD CELLS (WBC)

- They are responsible for protecting the body from invasion by foreign substances such as bacteria, fungi, and viruses.
- Types of WBCs:

- **Granulocytes**

- Neutrophils
- Eosinophils
- Basophils

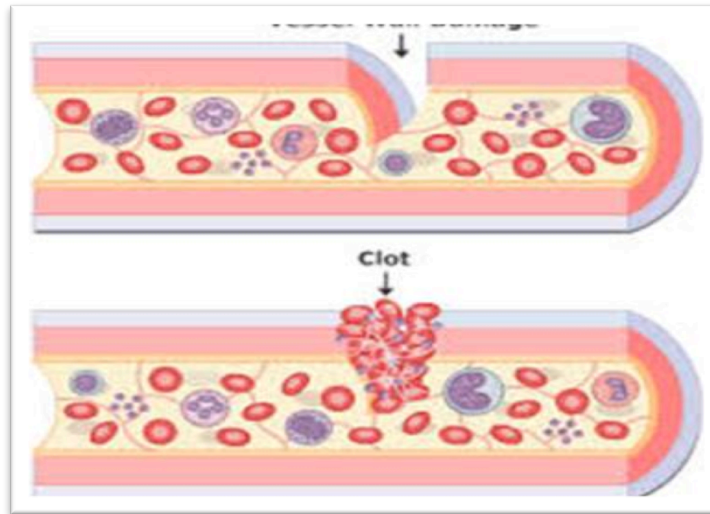


- **Agranulocytes**

- Monocytes
- Lymphocytes

PLATELETS

- They are very small cellular components of blood that help the clotting process by sticking to the lining of blood vessels.
- They survive in the circulatory system for an average of 9-10 days before being removed from the body by the spleen.
- They help prevent massive blood loss resulting from trauma, as well as blood vessel leakage.



PLASMA

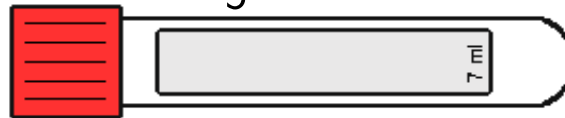
- Plasma is the liquid portion of blood
- It constitutes about 55 % of blood volume.
- 90% of plasma is water
- It contains:
 - Albumin (the chief protein constituent),
 - Fibrinogen (responsible, in part, for the clotting of blood),
 - Globulins (including antibodies).

SERUM

- It resembles plasma in composition but lacks the coagulation factors.

(Serum = Plasma – clotting factors)

- It is obtained by
 - letting a blood specimen clot prior to centrifugation usually in a red top tube with no additives or anticoagulant.



- Or by centrifugation of plasma to precipitate fibrinogen.
- Serum is preferred for many tests (e.g. determination of lactate dehydrogenase) as the anticoagulants in plasma can sometimes interfere with the results.

COLLECTION OF BLOOD SPECIMENS

- If whole blood or plasma is desired, an anticoagulant must be added to the specimen immediately after it is drawn or placed into the tube into which the blood is collected.

- Types of Anticoagulants

- Heparin

- It is the most satisfactory anticoagulant since it does not produce a change in red cell volume or interfere with subsequent determinations.
 - It inhibits the formation of thrombin from prothrombin and thus preventing the formation of fibrin from fibrinogen.





- **EDTA**

- It is a chelating agent, drives its anticoagulant activity from the fact that it binds calcium, which is essential for the clotting mechanism.



- **Potassium Oxalate**

- It inhibits blood coagulation by forming insoluble complexes with calcium ions, which is necessary for coagulation.



- **Sodium Citrate**

- It does not precipitate the calcium, but converts it into a non-ionized form, and hence prevent clotting of blood.

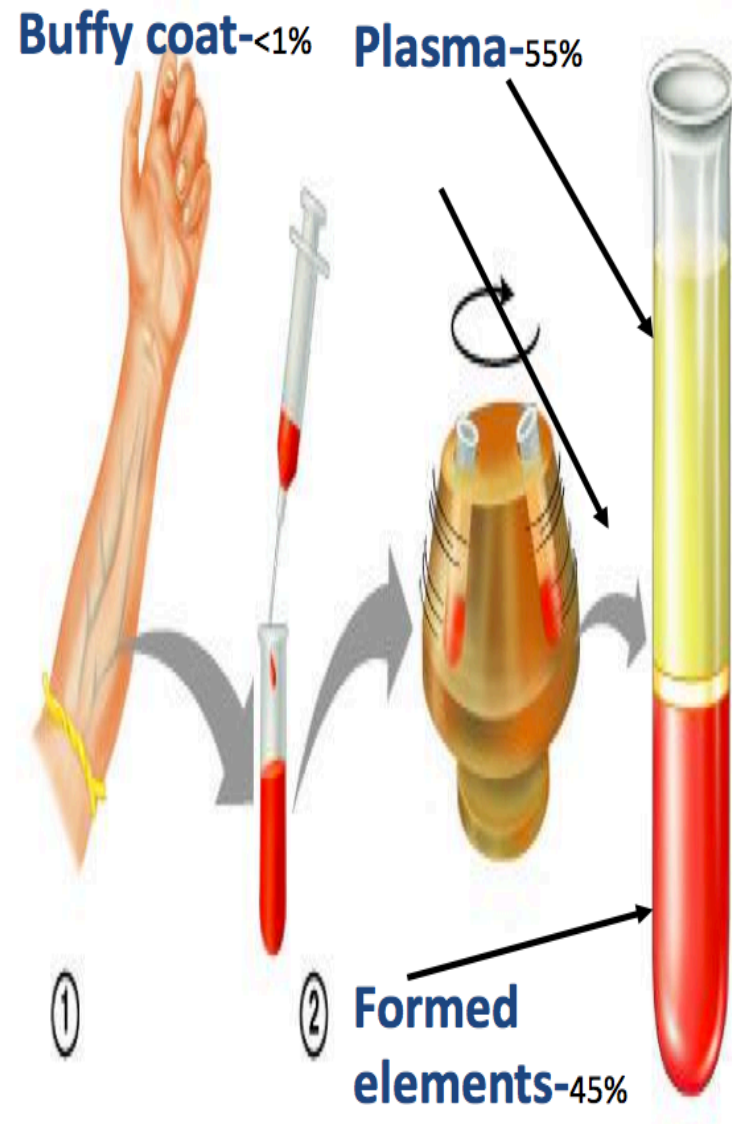


- **Sodium Fluoride**

- It acts as a weak anticoagulant.
- It has been used chiefly as a preservative since it inhibits red cell metabolism and bacterial action.

If blood is treated to prevent clotting and permitted to stand or centrifuged in a container:

- The **RBCs**, which weigh more than the other components, will settle to the bottom;
- The **plasma** will stay on top; and
- The **WBCs** and **platelets** will remain suspended between the plasma and the RBCs.



CHANGES IN BLOOD ON KEEPING

- Loss of carbon dioxide.
- Conversion of glucose to lactic acid (glycolysis).
- Increase in plasma inorganic phosphate.
- Formation of ammonia from nitrogenous substances.
- Passage of substances through the red cell envelope.
- Conversion of pyruvate into lactate.

MATERIALS

- Whole blood
- Centrifuge (up to 5000 rpm)
- Centrifuge tubes suitable for the rotor of the centrifuge (preferably plastic and capped).
- Disposable gloves
- Disposable Pasteur pipette.
- Measuring cylinder 10 ml.

METHOD

- Into dry clean centrifuge tube, pipette 15 ml of whole blood (V1).
- Place the centrifuge tube in the centrifuge machine and run it at 3000 rpm for 10 minutes. Centrifugation of whole blood separates the solid from the supernatant plasma.
- Remove the tube, withdraw the liquid layer (plasma) by pasture pipette and measure its volume using small measuring cylinder (V2). Determine the volume of blood cells too V3 (equal to $V1 - V2$).
- Transfer the supernatant (plasma) in another centrifuge tube and make further centrifugation at 3000 rpm. This will precipitate fibrinogen and the supernatant will be SERUM. Measure its Volume (V4).

RESULT

Record your results in the following table:

	Component	Total Volume	Percentage (%)
1	Whole Blood	V1=	
2	Plasma	V2=	
3	RBCs	V3=	
4	Serum	V4=	