BIOCHEMISTRY OF BLOOD

BCH 471

Separation of Plasma and Serum from Whole Blood

Experiment 1

Whole Blood

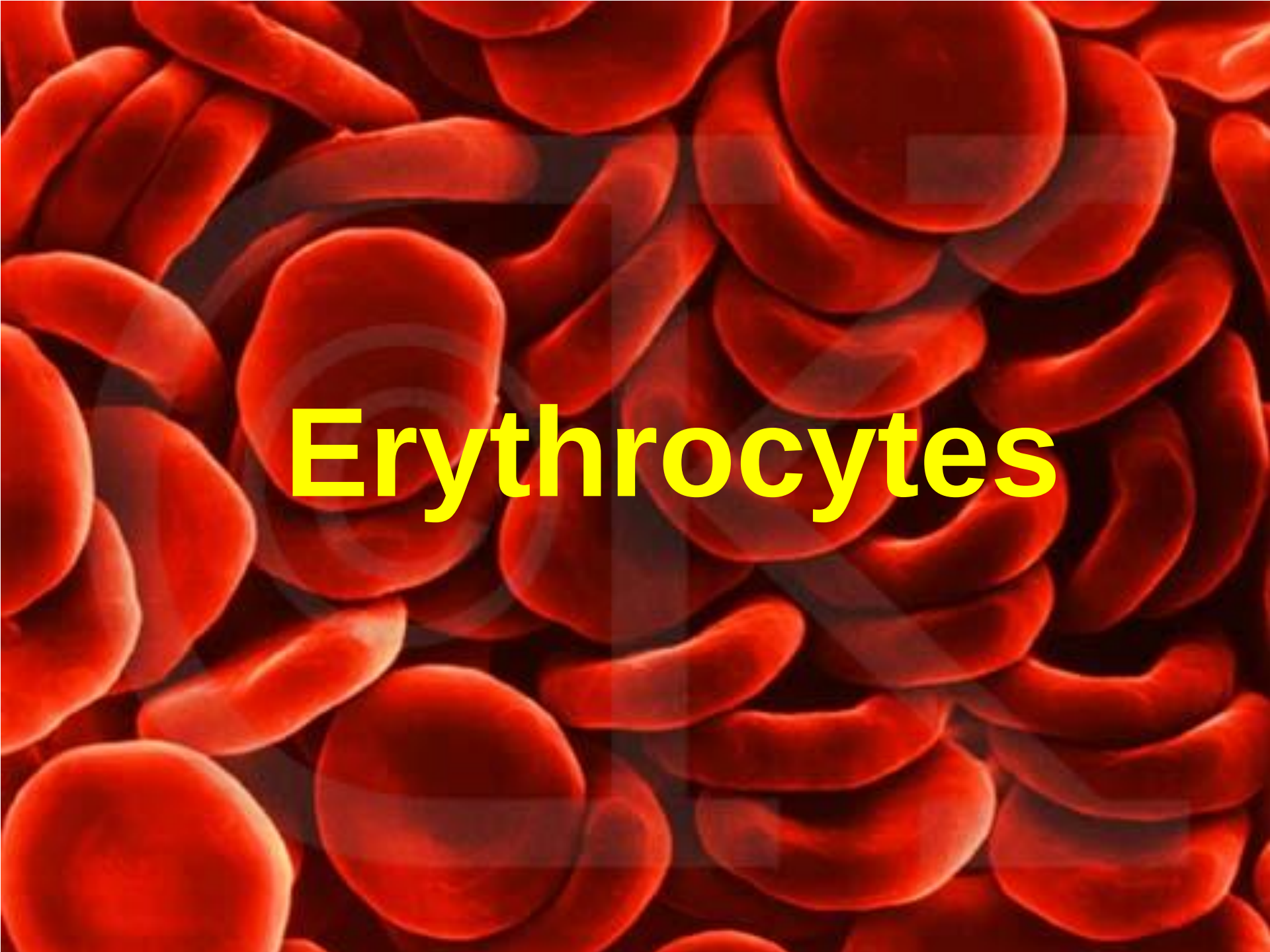
- It is living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues.
- The average person circulates about 5L of blood , of which 3L is plasma and 2L is cells.
- Plasma fluid derives from the intestines and organs, and provides a vehicle for cell measurement.
- The cells are produced primarily by bone marrow and account for blood "solids".

Blood cells

Whole blood contains cells suspended in fluid called **plasma**. :

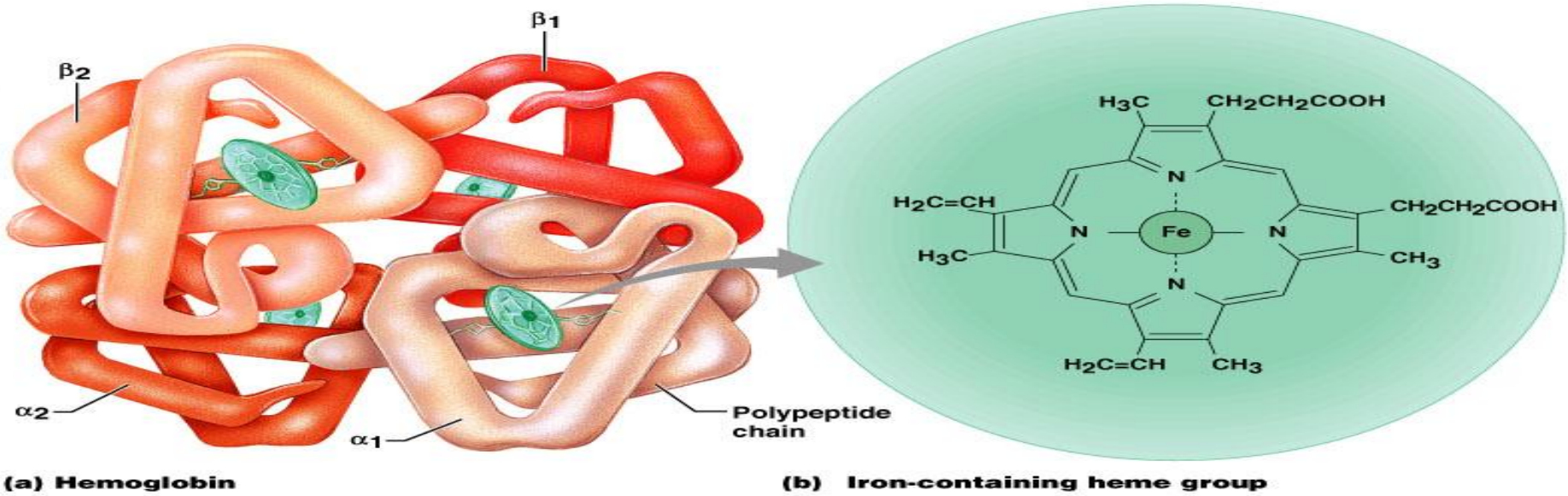
- red blood cells (erythrocytes).
- white cells (leukocytes)
- platelets (thrombocytes).

The size of cells differs:
white cells are the largest, red cells fall into the middle, and platelets are the smallest.


Erythrocytes

Red Blood Cells
(erythrocytes)

- Red blood cells contain hemoglobin, a complex iron-containing protein that carries oxygen throughout the body and gives blood its red color.



- **In 2 -3 drops of blood,** There are about one billion red blood cells.
- **For every 600 red blood cells,** there are about 40 platelets and one white cell.

White Blood Cells
(leukocytes)

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

Types of leukocytes:

Granulocytes

- Neutrophils
- Eosinophils
- Basophils

Agranulocytes

- Monocytes
- Lymphocytes

- **Granulocyes** and **macrophages** protect against infection by surrounding and destroying invading bacteria and viruses, and **lymphocytes** aid in immune defense.

Platelets
(thrombocytes)

- They are very small cellular components of blood that help the clotting process by sticking to the lining of blood vessels.
- Platelets are made in the bone marrow and survive in the circulatory system for an average of 9-10 days before being removed from the body by the spleen.
- The platelet is vital to life, because it helps prevent massive blood loss resulting from trauma, as well as blood vessel leakage.

The General Functions of Blood

- the carriage of oxygen and carbon dioxide.
- plays an important part in the body's defense mechanism(The immune response system is able to recognize foreign material within the body and a sequence of events is triggered that neutralizes and destroys the foreign material).
- So, the complex composition of blood is not constant, but changes during stress, starvation, exercise and as the result of injury or disease.

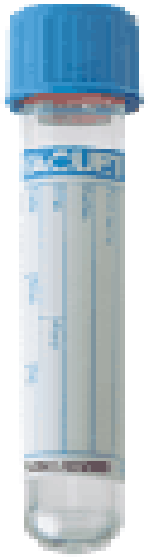

Collection of Blood Specimens

Types of Anticoagulants

- EDTA- It is a chelating agent uses as anticoagulant

  Binds to ca , which is essential for the clotting mechanism.

- 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.



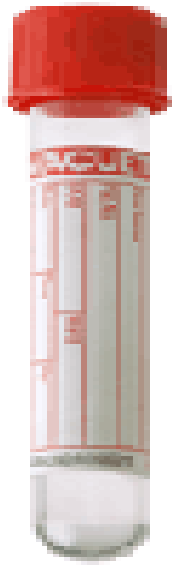
- **Sodium Citrate-** It does not precipitate the calcium, but converts it into a non-ionized form, and hence prevent clotting of blood.

- **Potassium Oxalate**

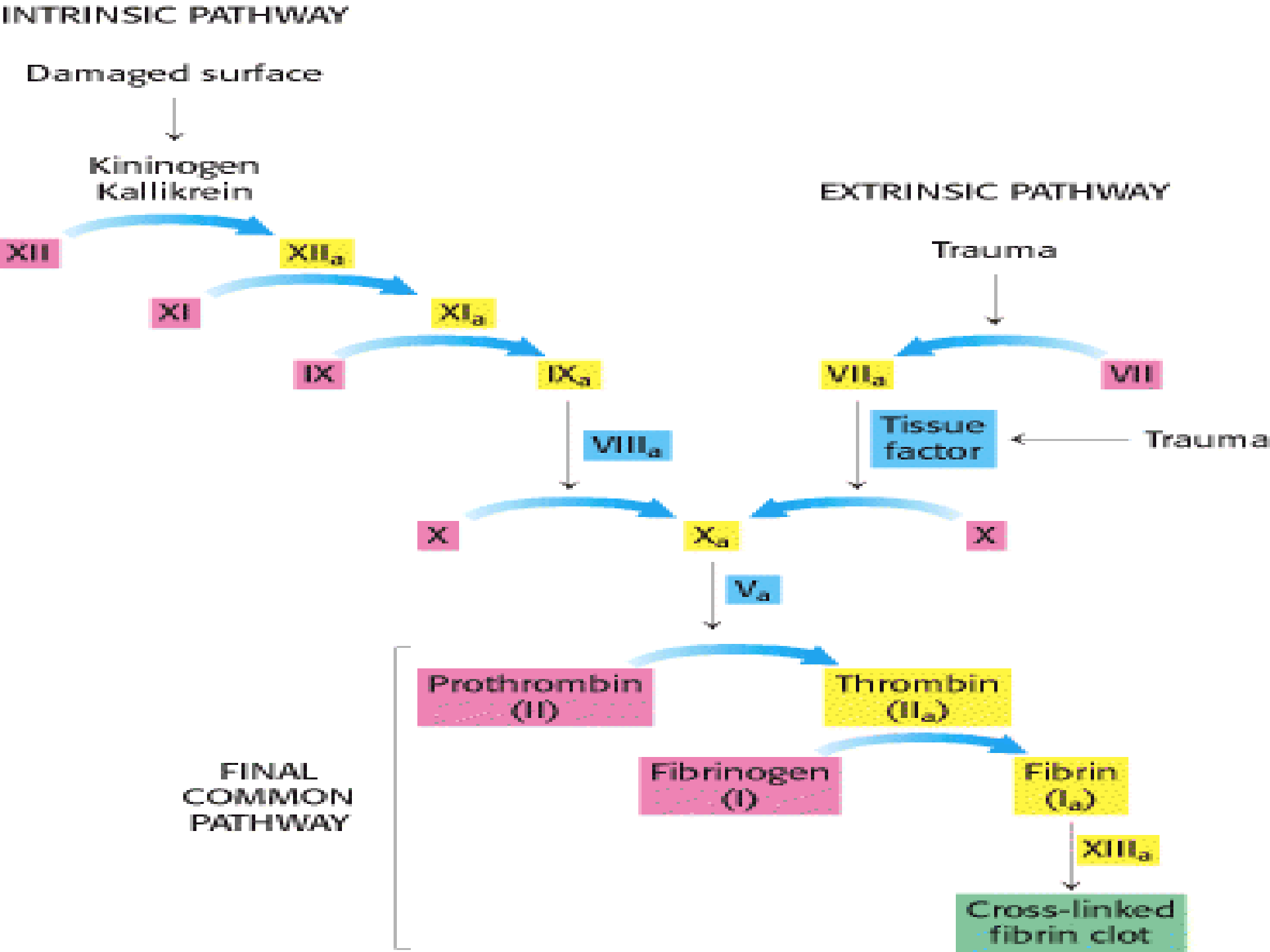
Oxalates act by precipitating the calcium, and we use potassium oxalate since it is the most soluble. It inhibits blood coagulation by forming rather insoluble complexes with calcium ions, which is necessary for coagulation.

- **Sodium Fluoride**

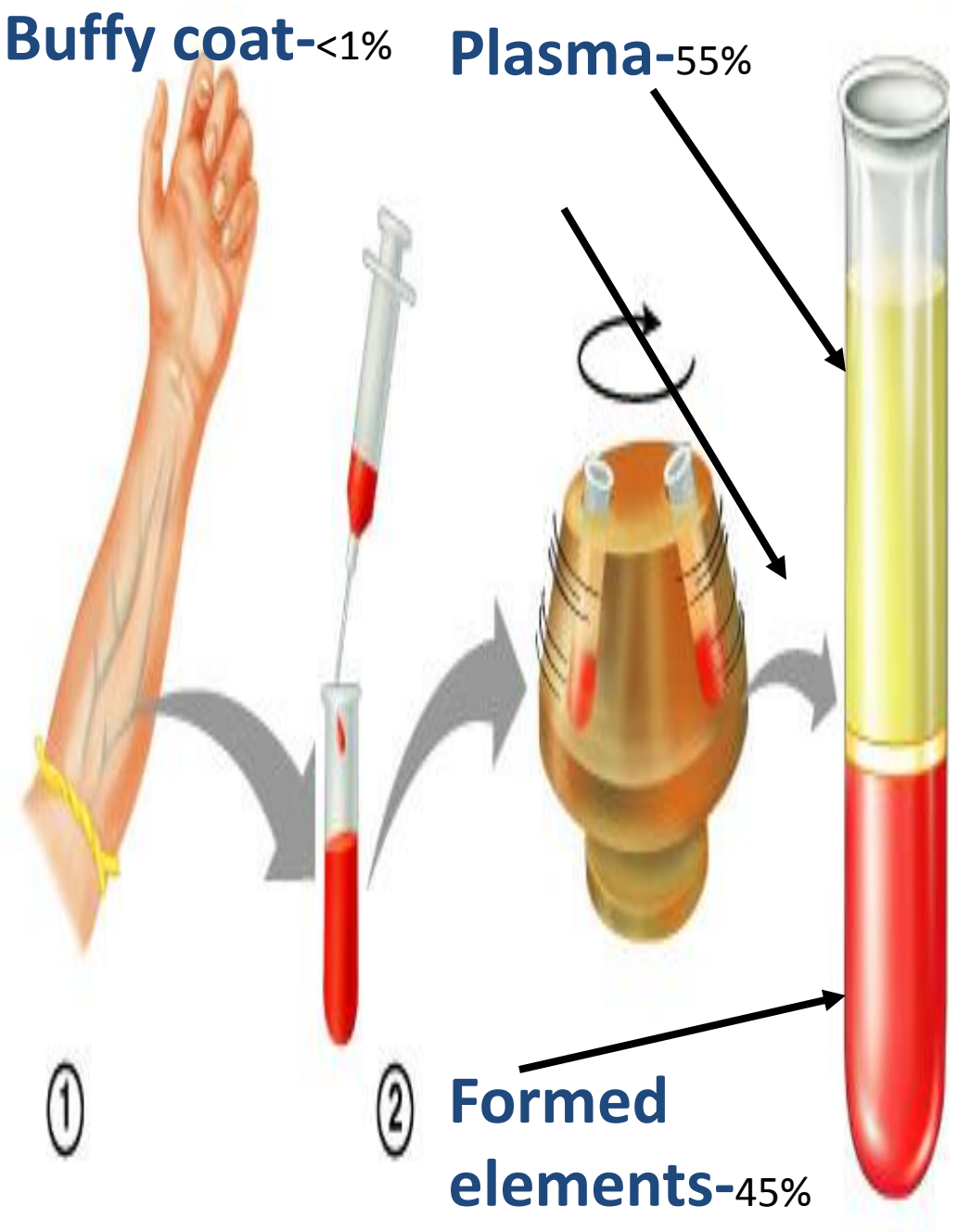
It acts as a weak anticoagulant, therefore larger amounts are required than of either oxalates or citrates. It has been used chiefly as a preservative since it inhibits red cell metabolism and bacterial action.



- No additives or no anticoagulant.
- This tube allow the blood clot and we can get serum.


INTRINSIC PATHWAY
Damaged surface
Kininogen
Kallikrein
XII
XIIa
XI
XIa
IX
IXa
VIIIa
X
Xa
Va
EXTRINSIC PATHWAY
Trauma
VIIa
VII
Tissue factor
Trauma
X
Prothrombin (II)
Thrombin (IIa)
Fibrinogen (I)
Fibrin (Ia)
XIIIa
Cross-linked fibrin clot
FINAL COMMON PATHWAY

If blood is treated to prevent clotting and permitted to stand in a container, the red blood cells, which weigh more than the other components, will settle to the bottom; the plasma will stay on top; and the white blood cells and platelets will remain suspended between the plasma and the red blood cells. A centrifuge may be used to fasten this separation process.



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.

Method of Separation of Plasma and Serum from Whole Blood

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.

Procedure

- Into dry clean Centrifuge tube, pipette 15 ml of whole blood (V_1).
- 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 (V_2). Determine the volume of blood cells too V_3 (equal to $V_1 – V_2$). Red blood cells which prepared from whole blood by removing the plasma, are kept to be used in part 2.
- **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 (V_4).

Record your results in the following table:

	Component	Total Volume	% Percentage
1	Whole Blood	V_1 =	
2	RBC's	V_3 =	
3	Plasma	V_2 =	
4	Serum	V_4 =	