

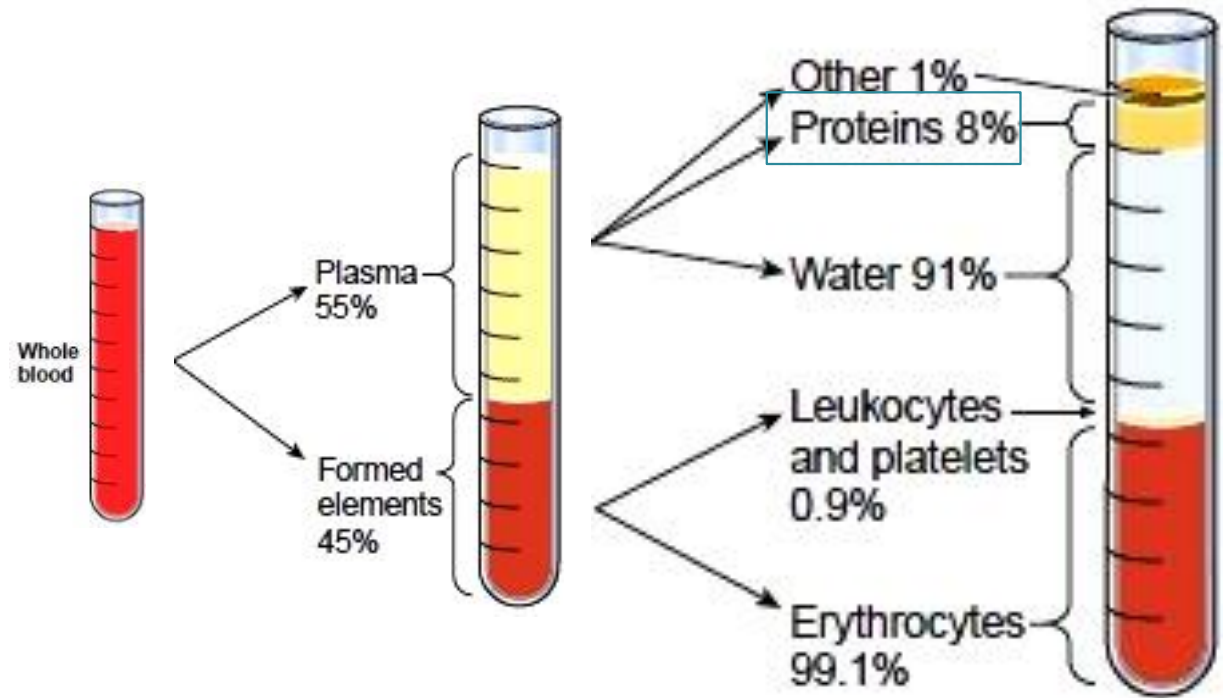


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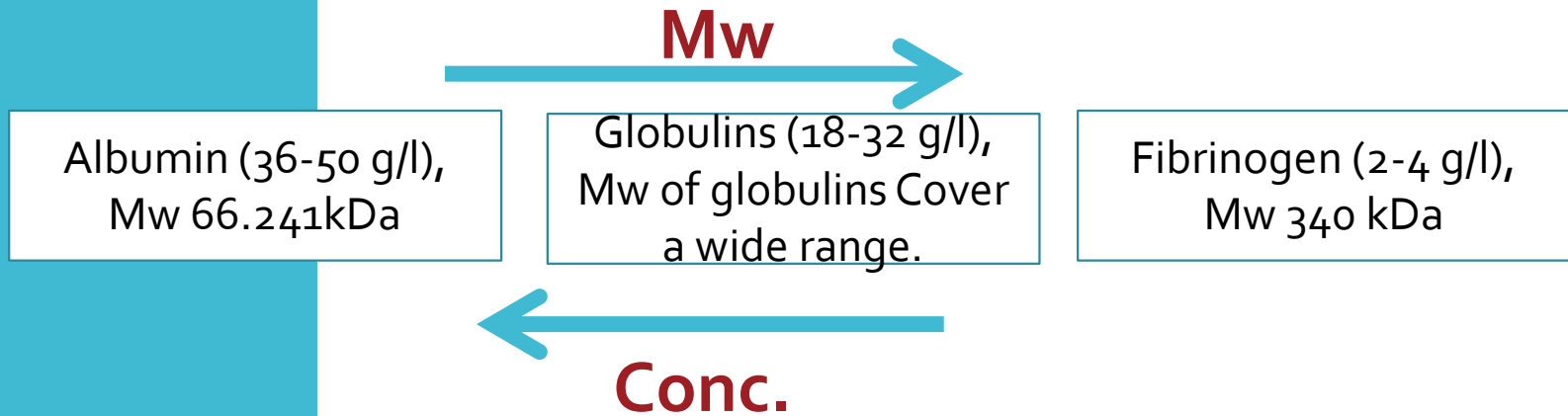
Experiment (2)

Separation of Main Proteins in Plasma and Serum

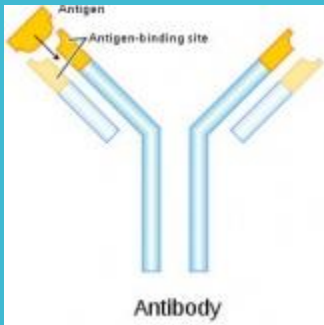
Blood contents



- The main plasma proteins are:



- All plasma proteins are synthesized in **liver** except gamma (γ) globulins (immunoglobulins), they are made by **B cells** of the immune system.
- All plasma proteins are water soluble.



immunoglobulins

Albumin

- It is the **most abundant** protein present in plasma.

- **It has many functions including:**

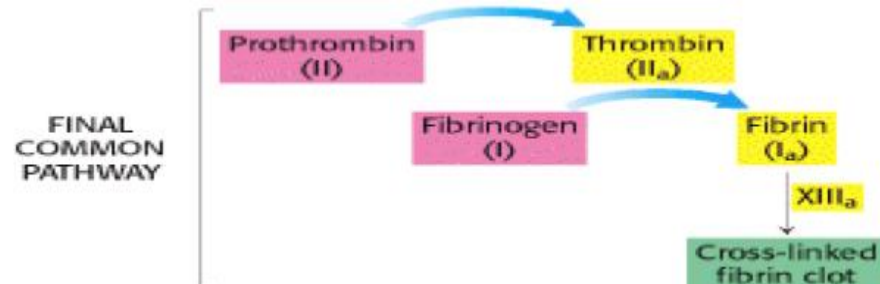
- ☑ Maintenance of the blood osmotic pressure.
- ☑ Adjusting blood pH.
- ☑ Act as a transporter, transporting free fatty acids, bilirubin, drugs, steroid hormones, calcium and copper in the blood.

Globulins

- Alpha (α) and Beta (β) globulins are transport proteins, but γ -globulins are part of the immune system.

FIBRINOGEN

- It is a glycoprotein (proteins that contain oligosaccharide chains)
- It is converted by thrombin into fibrin during blood coagulation



Serum Proteins

Total serum protein consists of two main fractions, **albumin and globulin**.

In normal people the A / G ratio is from **1.2 to 1.5** .

Generally, the decrease in total protein is due to a decrease in albumin fraction and the increase is due to an increase in globulin components.

Dehydration **is one condition** in which the increase in total protein is due to increase in both albumin and globulin fractions because of haemoconcentration → **In this case the A / G ratio remains unaltered**.

- **A low serum albumin may be due to:**
- A heavy loss of albumin in urine.
- Loss or malabsorption of protein from the digestive tract.
- Decreased formation by the liver due to defective liver.
- Increase catabolism of protein or due to insufficient intake of protein in diet.
- **Globulin:**
- Increase in globulin occurs most commonly in advanced liver disease, multiple myeloma and a number of chronic infections.

- Plasma protein can be separated from each other by :

- Salting Out**

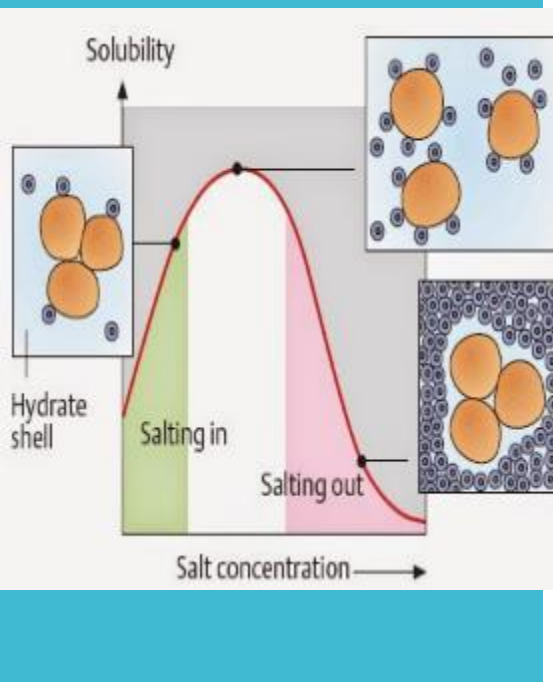
- Ultracentrifugation

- Electrophoresis

- Chromatography

Principle of salting out

- When high concentrations of salt is added to the protein solution, the solubility decreases, and the protein precipitates.



This can be explained by the following:

- The salt molecules compete with the protein molecules in binding with water, leading to **dehydration**.

The salt concentration needed for the protein to precipitate out of the solution differs from protein to protein.

Principles of identification tests

- **Biuret test**

In the presence of peptides that contain at least two peptide bonds(i.e. it is not given by **dipeptides and free amino acids**), a copper(II) ion forms violet/blue-colored complexes in an alkaline solution.

Protein + Biuret reagent → Blue Color

The intensity of the color is proportional to the number of peptide bonds and thus is a measure of the concentrations of proteins.

Heat coagulation

Protein + weak acid **heating** protein precipitate (cloudiness)

