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BCH 471

Experiment (2)

Separation of Main Proteins in Plasma and Serum

PLASMA PROTEINS

- **The main plasma proteins are:**
  - ☑ Albumin (36-50 g/l), Mw 66.241kDa .
  - ☑ Globulins (18-32 g/l), Mw of globulins Cover a wide range.
  - ☑ Fibrinogen (2-4 g/l), Mw 340 kDa .

Mw ↓

Conc. ↑

- 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.

ALBUMIN

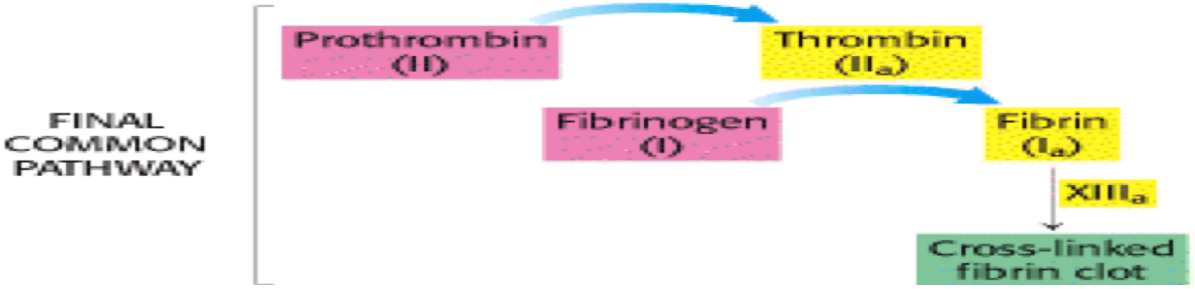
- It is the **most abundant** protein present in plasma.
- **It has many functions including:**
  - ☑ Maintenance of the blood osmotic pressure.
  - ☑ Adjusting blood pH.
  - ☑ Act as a transporter, transporting free fatty acids, bilirubin, drugs, steroid hormones, calcium and copper in the blood.

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.



SEPARATION OF PLASMA PROTEINS

- **Plasma protein can be separated from each other by :**

  ☑ **Salting Out**

  ☑ Altracenterifuge

  ☑ Electrophoresis

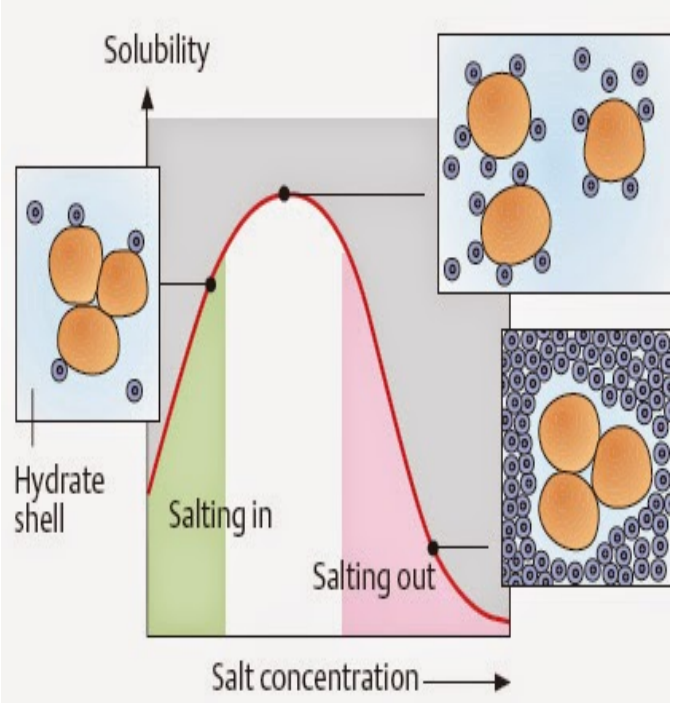
  ☑Chromatography

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.



IDENTIFICATION OF PLASMA PROTEINS

- **Fibrinogen :**
  1. Biuret test.
  2. Clotting test, by appearance of clotting after adding equal volume of serum and $CaCl_2$→ because serum contains active thrombin which converts fibrinogen to insoluble fibrin.
  3. Heat Coagulation.

- **Globulins :**
  1. Biuret test.
  2. Clotting test.
  3. Heat Coagulation.

- **Albumin :**
  1. Heat Coagulation.

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 $\xrightarrow{\text{heating}}$ protein precipitate (cloudiness)

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 decrease in albumin fraction and increase is due to 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.

- **Total serum protein is appreciably reduced with low albumin in:**
  - Severe haemorrhage both acute and chronic,
  - Shock whether post operative following extensive burns or traumatic as in crush injuries,
  - Malignant disease of stomach, intestine and pancreas,
  - Peptic ulcer.
- **In liver disease, particularly severe ones:**
  - Albumin is reduced and A/G ratio altered.
  - Total protein may be reduced but more commonly it is found within normal limits or even may be increased because globulin is increased in liver disease.
  - Increase in globulin occurs most commonly in advanced liver disease, multiple myeloma and a number of chronic infections.