



Experiment 2

Separation of Main Proteins in Plasma and Serum

Introduction:

PLASMA PROTEINS

The main plasma proteins are:

Mwt ↓	▪ Albumin (36-50 g/l), Mw 66.241kDa .	↑ Conc.
	▪ Globulins (18-32 g/l), Mw of globulins Cover a wide range.	
	▪ Fibrinogen (2-4 g/l), Mw 340 kDa .	

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

SEPARATION OF PLASMA PROTEINS

Plasma protein can be separated from each other by :

- Salting Out
- Ultracentrifuge
- Electrophoresis
- Chromatography

ALBUMIN

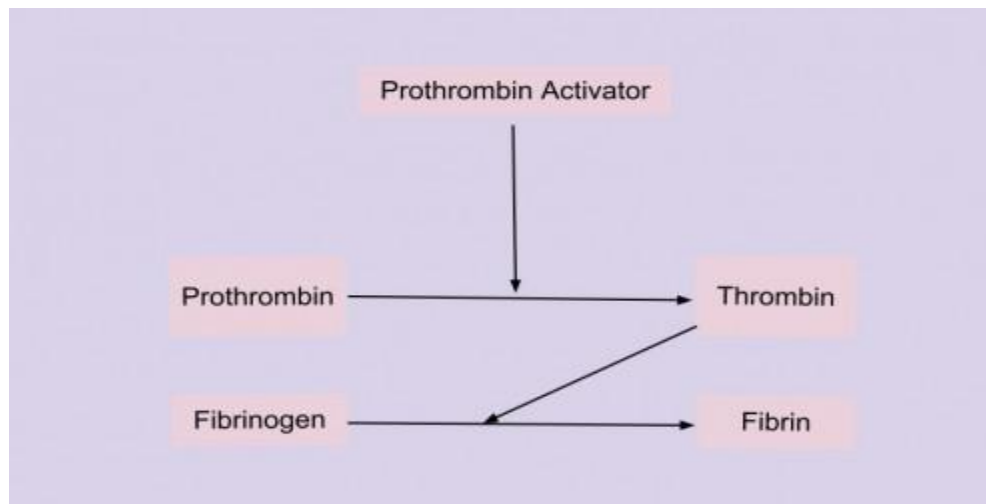
- ❑ It is the **most abundant protein present in plasma.**
- ❑ **It has many functions including:**
 - Maintenance of the blood osmotic pressure.
 - Adjusting blood pH.
 - Act as a transporter, transporting free fatty acids, bilirubin, drugs, steroid hormones, calcium and copper in the blood.

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.



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 and albumin:**

1. Biuret test.

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.



- 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** .
- This test provides information about the amount of albumin you have compared with globulin, a comparison called the A/G ratio. This test is useful when your doctor suspects you have liver disease.
- Generally, the decrease in total protein is due to decrease in **albumin** fraction and increase is due to increase in **globulin** components.

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 hemorrhage both acute and chronic,
- Shock whether post operative following extensive burns
- 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



- In high A/G ratio that indicate for

There are abnormality increasing in albumin and that occur in genetic deficiencies or leukemia

Objective:

To separate the principle proteins in serum and plasma and determination albumin/globulin ratio .

Method:

Part I (plasma protein)

(1) Fibrinogen precipitate From plasma:

- In centrifuge tube ;Add an 2 ml saturate sodium chloride solution to of 2ml plasma.and put in centrifugation for 3000rpm, 5min.
- To fibrinogen precipitates: Redissolve the fibrinogen precipitates in normal saline (about 2ml saline) and carrying the clotting test.

(2) Clotting test:

Add 0.5 ml of serum + 0.5 ml Fibrinogen solution and 3 drop drops of calcium chloride solution, incubate at 37°C for 5 minutes.

Result : Clotting occurs because serum contains active thrombin which converts fibrinogen to insoluble fibrin.

Part II(serum protein)

serum proteins and calculate A/G ratio

- Two dry test tubes labeled P (total protein) and G (globulin)

	P	G
Saline	3.8 ml	-
saturated ammonium sulphate	-	3.8 ml
Serum	0.2 ml	0.2 ml

- Mix G by inverting the tube a couple of times then centrifugation at 3000rpm, 5 min.

Note: the filtrate is containing albumin and precipitate is globulin

Label 4 test tubes as T (test), A (albumin), B (blank), and S (standard)

	T	A	B	S
Saline	-	-	1 ml	-
BSA (5mg/dl)	-	-	-	1 ml
Solution from P	1 ml	-	-	-
Filtrate of G	-	1 ml	-	-
Biuret reagent	5 ml	5 ml	5 ml	5 ml

- Mix and keep standing for 1 minutes, read the absorbance using a spectrophotometer at 540 nm

Result:

1- Concentration of proteins in serum:

$$\frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times \text{conc. Of Standard}$$

2- Concentration of albumin in serum:

$$\frac{\text{Absorbance of A}}{\text{Absorbance of S}} \times \text{conc. Of Standard}$$

3- Concentration of globulin = Total protein – Albumin

4. Calculate the A/G ratio

Note: BSA (mg/dl) = \div 1000 (g/dl)
5mg/dl = 0.005g/dl