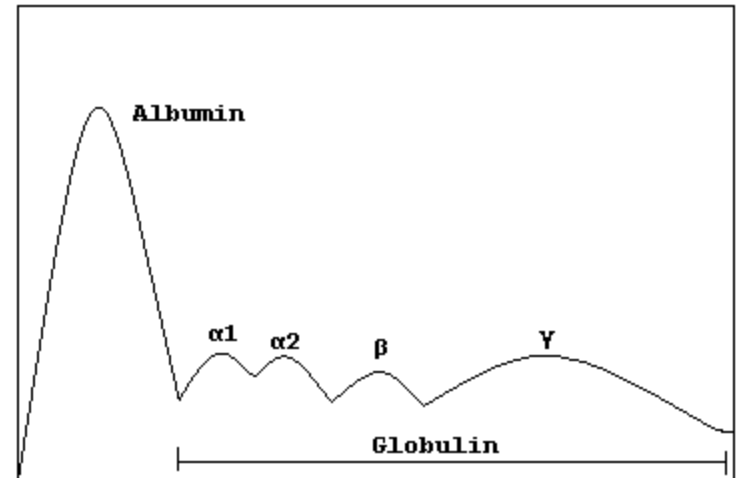


Separation of main protein in plasma and serum

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- The main plasma proteins are albumin, globulins and fibrinogen. Fibrinogen may be salted out from plasma and identified by the biuret test and by the fact that clotting occurs on addition to serum which still contains active thrombin.
- Proteins, which contain peptide linkages form a complex with copper in alkaline medium giving a violet color (Biuret reaction).
- The intensity of the color is proportional to the number of peptide bonds and thus is a measure of the concentrations of proteins



Normal values

- **Normal value**

Total protein 6.0 to 8.0 g/100 ml.

albumin, 3.5 to 5.0 g/100 ml.

globulin 2.3 to 3.6 g/ 100ml.

fibrinogen, 0.3 to 0.6 g/ 100 ml.

- *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

- **A low serum albumin may be due to:**
- i. A heavy loss of albumin in urine
- ii. Loss of protein into alimentary tract.
- iii. Malabsorption of protein from the alimentary tract
- iv. Decreased formation by the liver due to defective liver and Increase catabolism of protein or due to insufficient intake of protein in diet.

- **decrease in total protein** Generally is due to decrease in albumin fraction and increase is due to increase in globulin components.
- **increase in total protein is due to Dehydration** condition in which the increase in both albumin and globulin fractions because of increasing **haemoconcentration**.
- In this case the A / G ratio remains unaltered.

decrease in total protein

- appreciably reduced with low albumin in **severe haemorrhage, shock** whether post operative following extensive burns or traumatic as in crush injuries, malignant disease of stomach, intestine and pancreas, peptic ulcer, sprue and steatorrheas etc.
- **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.

Materials

- blood serum.
- -blood plasma.
- -0.9 saline solution.
- -2 N acetic acid.
- -Biuret reagent: dissolve 9 g of sodium potassium tartrate in
- 500 ml of 0.2 N sodium hydroxide solution .add of 3 g of cupric sulphate
- and dissolve by stirring add 5 g of potassium iodide , make up the volume
- 1 liter with 0.2 N sodium hydroxide solution.
- -saturated sodium chloride solution.
- -5% calcium chloride solution.
- -28% sodium sulphate or sulphite Na_2SO_3
- -OR Saturated ammonium sulphate solution

Method:

Part I: fibrinogen

- Add an equal volume of saturate sodium chloride solution to 5 ml of plasma.
- fibrinogen precipitates.

To fibrinogen precipitate:

- Redissolve in normal saline and divide into 3portion and carry out the following tests.

1-Biuret test	2-Clotting test	3-Heat coagulation test
add an equal volume of biuret reagent	Add an equal volume of serum and a few drops of calcium chloride solution,	add diluted acetic acid drop until the pH is between 5 and 6.
mix and allow to stand in a water path at 370c.	incubate at 370c for 10 minutes.	Heat the contents of the tube. A cloudiness confirm the presence of protein
The development of a blue color confirms the presence of protein (fibrinogen).	Clotting occurs because serum contains active thrombin which converts fibrinogen to insoluble fibrin.	

Method:

B- serum proteins (Globulin and Albumin)

Take the supernatant from part I and centrifuge at 3000 rpm for 10 min

To globulin precipitate:

- Re dissolve in normal saline and divide into 3portion and carry out the following tests

1-Biuret test	3-Heat coagulation test
add an equal volume of biuret reagent	add diluted acetic acid drop until the pH is between 5 and 6.
mix and allow to stand in a water bath at 37°C.	Heat the contents of the tube. A cloudiness confirm the presence of protein
The development of a blue color confirms the presence of protein (fibrinogen).	

Method:

To albumin filtrate:

1-Salting Out	2-Biuret test	3-Heat coagulation
Add solid ammonium sulphate until albumin is precipitated.	add an equal volume of biuret reagent	Add diluted acetic acid drop until the PH is between 5 -
	mix and allow to stand in a water bath at 37°C.	Heat the contents of the tube.
	The development of a blue color confirms the presence of protein (fibrinogen).	A cloudiness confirms the presence of protein

Both these tests confirm the presence of protein(**albumin**).

THANK YOU