

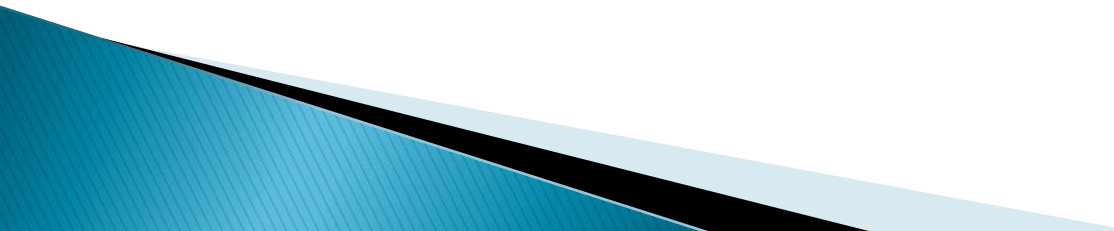
Separation of main proteins in plasma and serum

EXPERIMENT 2

The plasma proteins

The main plasma proteins are:

- ▶ albumin (36–50 g/l).
- ▶ globulins (18–32 g/l).
- ▶ fibrinogen (2–4 g/l).

- ▶ **Generally the decrease in total protein** is due to decrease in albumin fraction or 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.
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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
- v. 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.
Increase in globulin occurs most commonly in advanced liver disease, multiple myeloma and a number of chronic infections.

fibrinogen

Add an equal volume of saturate sodium chloride solution to plasma. Fibrinogen precipitates.

- ▶ **To fibrinogen precipitate:**

- ▶ Redissolve in normal saline and divide into two portions.

- ❖ **biuret test:**

add an equal volume of biuret reagent

mix and allow to stand in a water bath at 37c.

the development of a blue colour confirms the presence of protein (fibrinogen).

- ❖ **Clotting Test:**

Add an equal volum of serum+CaCL₂

Incubatie at 37c. In water bath for 10 min

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

- ❖ **Heat coagulation test:**

- ▶ Add diluted acetic acid drop until the PH is between 5 – 6.

- ▶ Heat the contents of the tube.

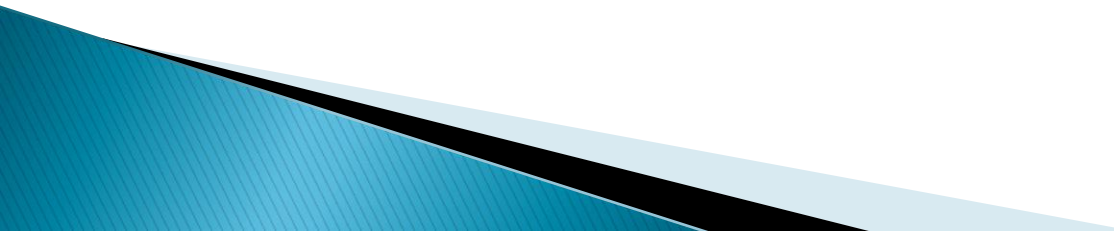
- ▶ A cloudiness confirm the presence of protein (Fibrinogen).

Serum Proteins

Add an equal volume of saturated ammonium sulphate solution to serum .

- ▶ filter off the precipitated globulin.

Globulin precipitate:

- ▶ Dissolve in saline solution .
 - ▶ divide the solution into two and carry out the following confirmatory tests.
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Globulin precipitate

❖ Biuret test:

- ▶ Add an equal volume of biuret reagent .
- ▶ mixed allow to stand in a water bath at 37c.
- ▶ the development of a blue colour confirms the presence of protein (globulin).

❖ Heat coagulation test:

- ▶ Add diluted acetic acid drop until the PH is between 5 – 6.
- ▶ Heat the contents of the tube.
- ▶ A cloudiness confirm the presence of protein (globulin).

To albumin filtrate:

❖ Salting Out:

Add solid ammonium sulphate until albumin is precipitated.

❖ Heat coagulation test

Add diluted acetic acid drop until the PH is between 5 – 6.

Heat the contents of the tube.

A cloudiness confirm the presence of protein

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

Thank you