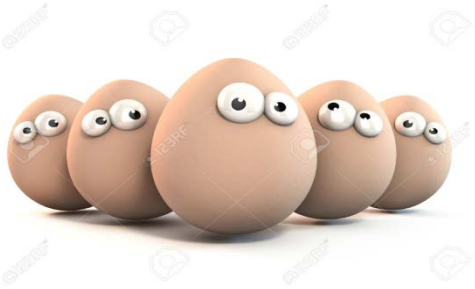
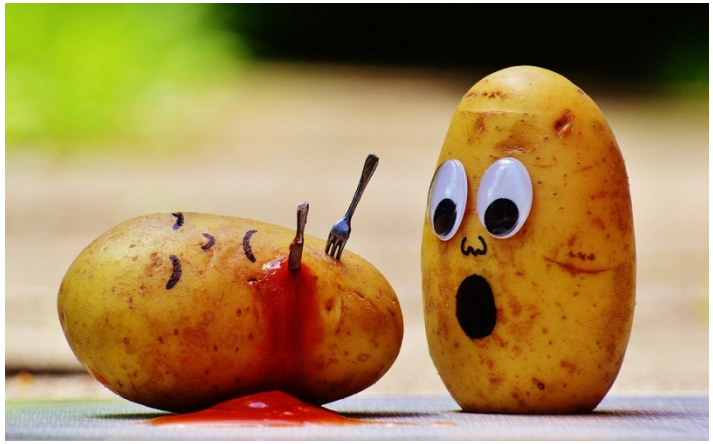


Plasma Proteins:

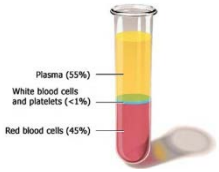
Chemistry, Functions and Clinical Significance

- **Blood composition**
  - 70 mL/kg of body weight
  - 5 L (average) in an adult
  - Suspension of *cells* in a carrier fluid (*plasma*)
    - Cells - 45% by volume
    - Plasma - 55% by volume
- **Cells**
  - Red cells (erythrocytes):
    - $5 \times 10^6/\mu L$
  - White cells (leukocytes)
    - $7 \times 10^3/\mu L$
  - Platelets (thrombocytes)
    - $3 \times 10^5/\mu L$



Plasma Proteins- Introduction

- **Plasma** consists of water, electrolytes, metabolites, nutrients, hormones, wastes and **proteins**



- Plasma – clotting factors=Serum
- The **total protein in human plasma is approximately 6.0–8.0 g/dL**
- All plasma proteins are synthesized in liver except gamma globulins which is synthesized by plasma cells.
- The proteins of the plasma includes not only simple proteins but also conjugated proteins such as **glycoproteins** and various types of **lipoproteins**.

Plasma

Water
90%

dissolved solutes
10%

blood gases
O_2 and Co_2

organic substances
9.1 %

In-organic substances
0.9 %

Plasma proteins 7.1

Others
2 %
Lipids, enzymes
Hormones,
nutrients
Waste products

Various electrolytes as Na^+
K^+
Hco_3^-
Ca^{++}
Po_4^{--}

Functions of plasma proteins

1-Osmotic function وظيفة تناضحية.

2-Buffer function.

3-Defensive function.

4-Blood clotting.

5-Blood viscosity.

6-Transport and conservation of important elements.

7-Source of amino acids for the tissues

Fractions of Plasma Proteins

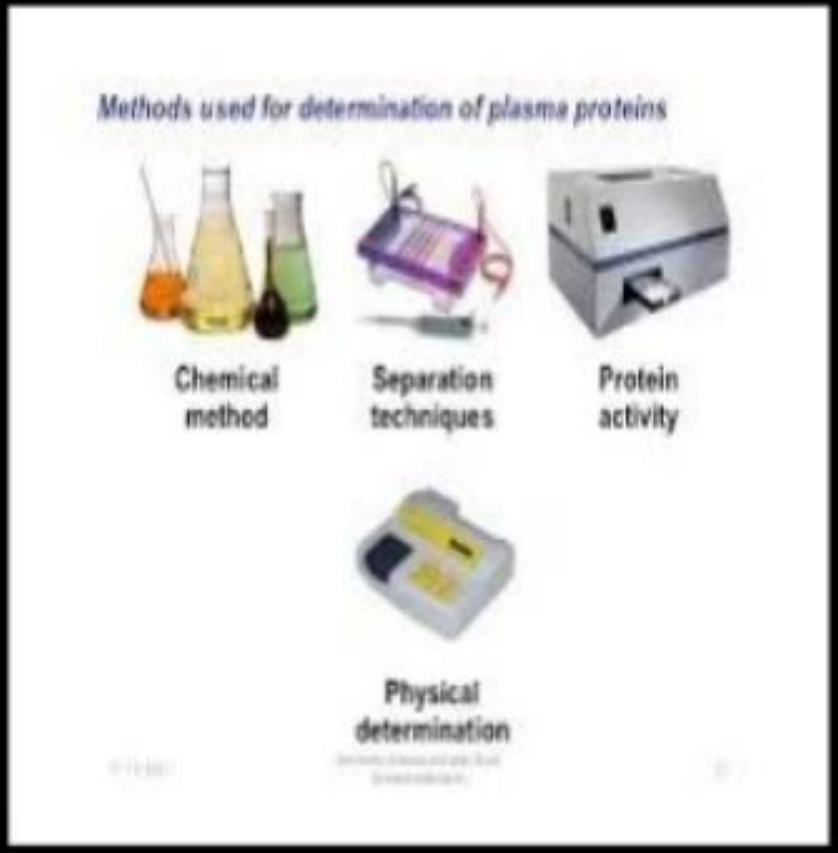
Fraction	*Rel. amount (%)*	*c (g/l)*
Albumins: albumin, pre-albumin (transthyretin),	**52 – 58**	**34 – 50**
α_1-globulins: thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein	**2,4 – 4,4**	**2-4**
α_2-globulins: haptoglobin, macroglobulin, ceruloplasmin	**6,1 – 10,1**	**5 – 9**
β-globulins: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system	**8,5 – 14,5**	**6 – 11**
γ-globulins: IgG, IgM, IgA, IgD, IgE	**10 – 21**	**8 – 15**

MAJOR PLASMA PROTIENS

ALBUMINS	58%
GLOBULINS	38%
FIBRINOGEN	4%

Methods of separation.

- Precipitation by salting out.
- Cohn's fractional precipitation method.
- Electrophoresis separation of protein fractions.
- Immunoelectrophoresis technique.
- Ultra-centrifugation technique.

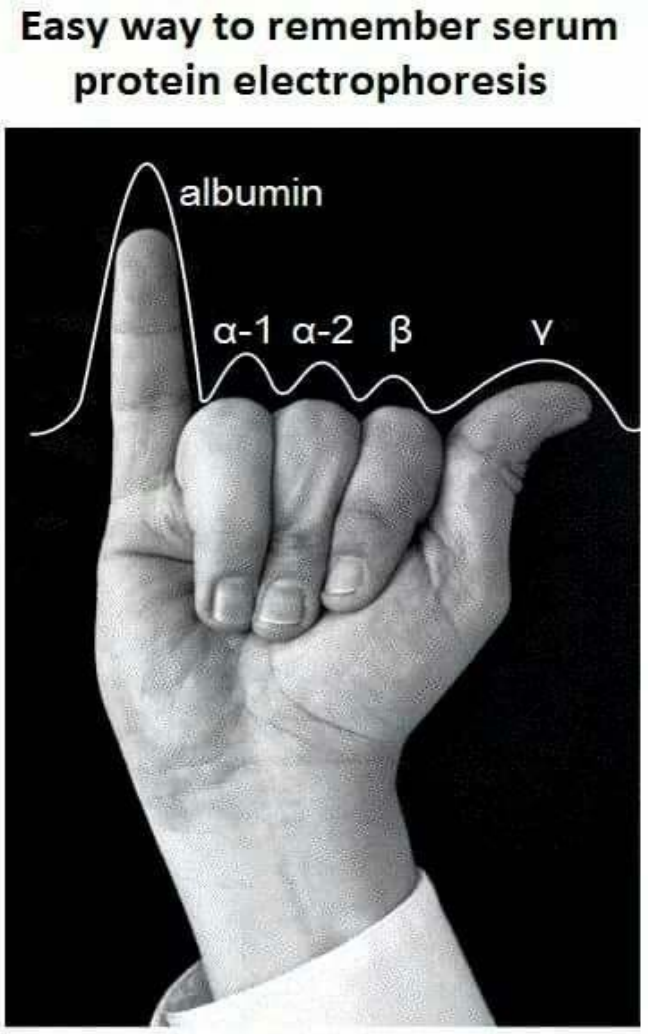


Separation of Plasma Proteins

- **Salting-out methods**-three major groups—**fibrinogen, albumin,** and **globulins**—by the use of varying concentrations of sodium or ammonium sulfate.
- **Electrophoresis**- five major fractions
- Albumin
- α1 and α2 globulins
- β globulins
- γ globulins

Electrophoresis

- Electrophoresis is the most commonly employed technique for the separation of plasma proteins.
- Electrophoresis is used for the diagnosis of certain diseases e.g multiple myeloma, acute infections, liver diseases and Neprotic syndrome etc….



Serum Protein Electrophoresis

Electrophoresis is the migration of charged molecules in an electric field. The negative charged particles (anions) moves towards the anode. Positively charged particles (cations) moves towards cathod (negatively charged electrode).

Types: Depending upon the nature of supporting medium

a. Agar gel electrophoresis (AGE).

b. PAGE, SDS PAGE,

c. Cellulose acetate electrophoresis.

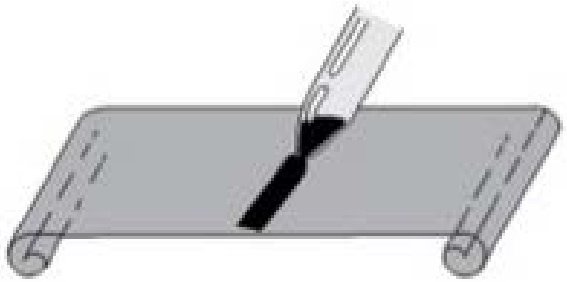
d. Capillary electrophoresis.

Most common method --
ELECTROPHORESIS

7

- Depends on the supporting medium used.
- Supporting mediums can be: **agarose gel, polyacrylamide gel, cellulose acetate membrane.**

A



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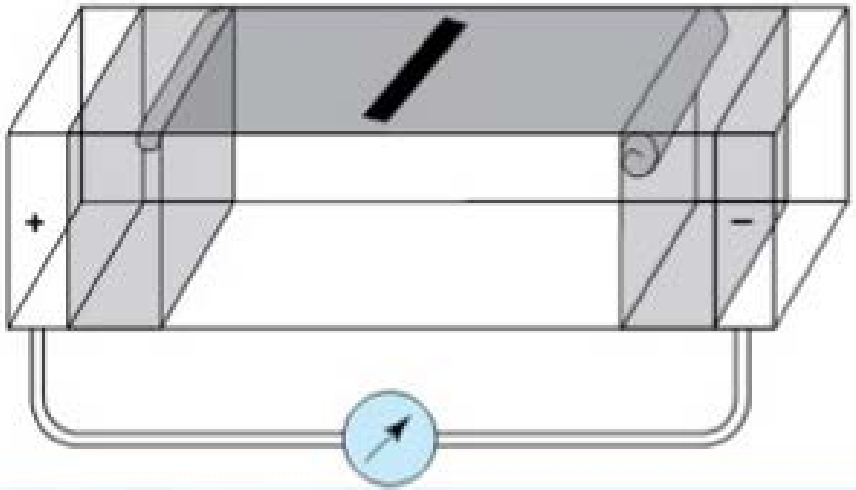
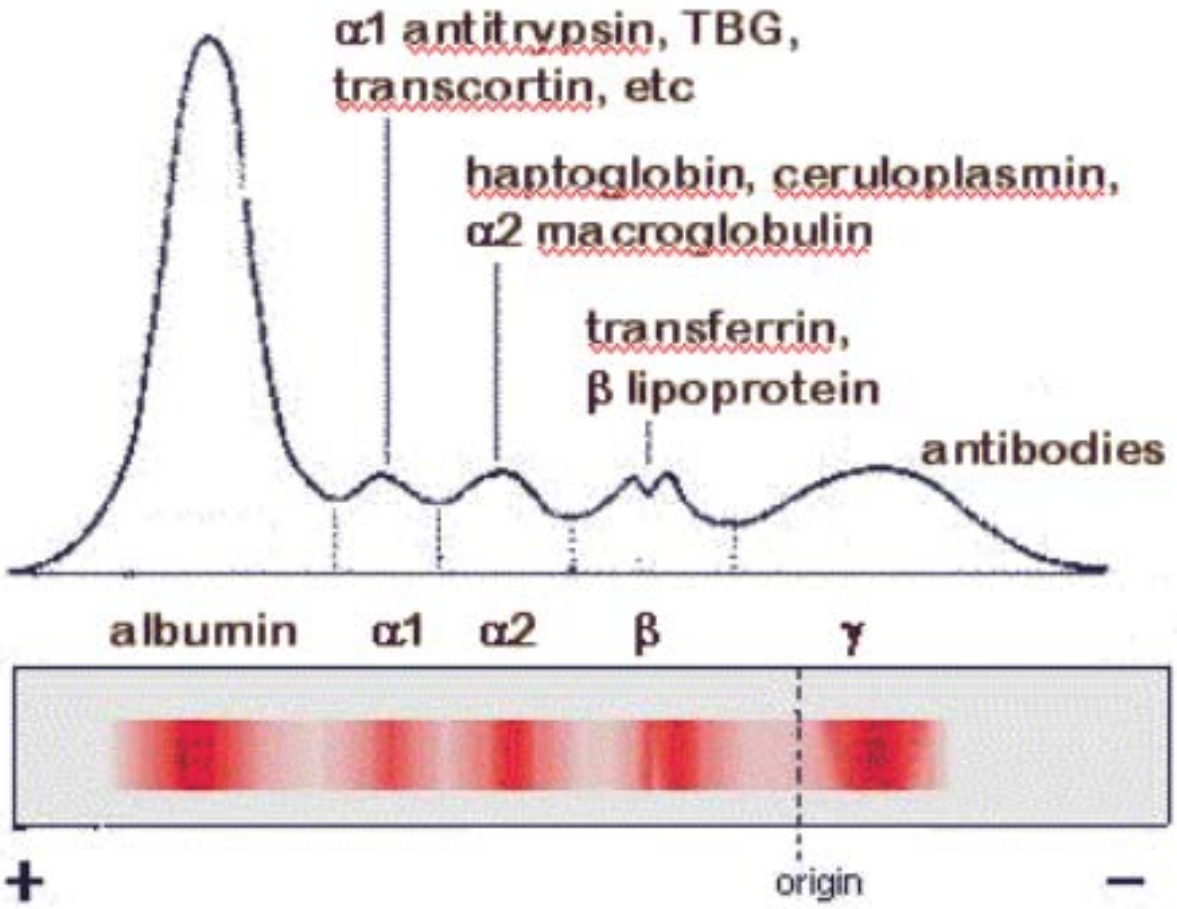


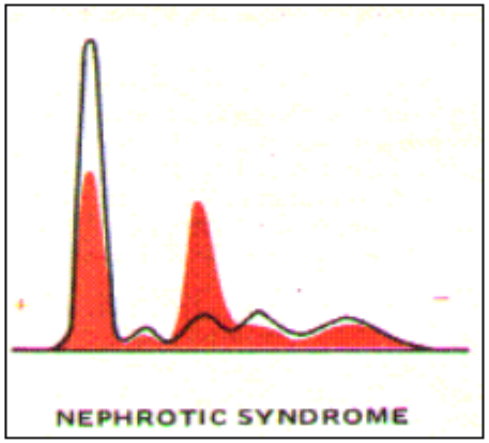
Plasma proteins are classified according to their electrophoretic mobility.

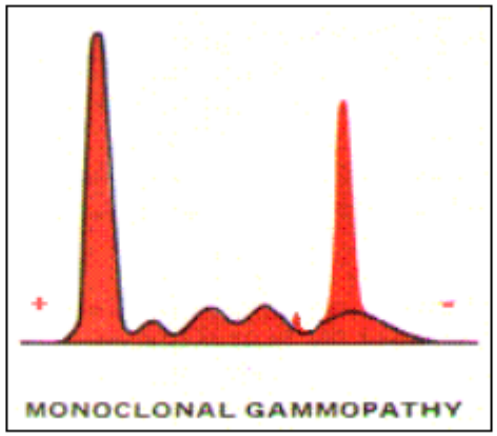
Factors affecting mobility / movement of protein in separating medium?

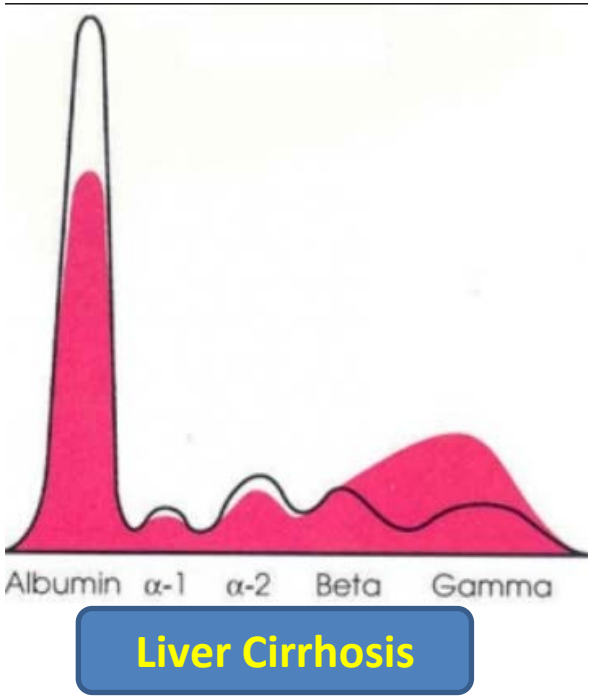
Separation of Plasma Proteins by ***Electrophoresis***

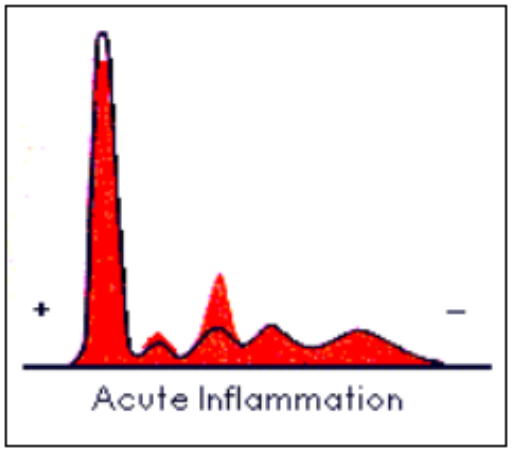


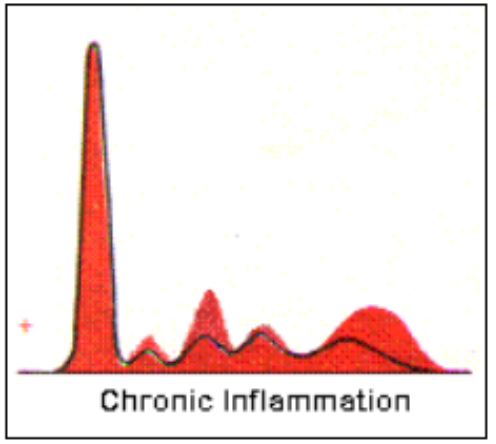
Abnormal Patterns of Serum Electrophoresis











Albumin

- Albumin (69 kDa) is the **major protein of human plasma (3.4–4.7 g/dL)**
- Makes up approximately **60% of the total plasma protein.**
- About 40% of albumin is present in the plasma, and the other 60% is present in the extracellular space.
- **Half life of albumin is about 20 days.**
- Migrates fastest in electrophoresis at alkaline pH and precipitates last in salting out methods

Synthesis of Albumin

- The **liver produces about 12 g of albumin per day**, representing about 25% of total hepatic protein synthesis .
- Albumin is initially synthesized as a **preproprotein**
- Mature human albumin consists of one polypeptide chain of **585 amino acids and contains 17 disulfide bonds**

Functions of Albumin

- **Colloidal osmotic Pressure الضغط التناضحي الغرواني-**

albumin is responsible for 75–80% of the osmotic pressure of human plasma due to its low molecular weight and large concentration

- It plays a predominant role in maintaining blood volume and body fluid distribution.
- **Hypoalbuminemia leads to retention of fluid in the tissue spaces (Edema)**

Functions of Albumin

Transport function-albumin has an ability to **bind various ligands, thus acts as a transporter for various molecules** (with low water solubility**) .** These include-

- free fatty acids (FFA),
- calcium,
- certain steroid hormones,
- bilirubin,
- copper
- A variety of drugs, including sulfonamides, penicillin G, dicoumarol, phenytoin and aspirin, are also bound to albumin

Functions of Albumin

❑Nutritive Function

Albumin serves as a source of amino acids for tissue protein synthesis to a limited extent

- ❑ **Buffering Function**-Among the plasma proteins, albumin has the maximum buffering capacity due to its high concentration and the presence of large number of histidine residues, which contribute maximally towards maintenance of acid base balance.

- ❑Viscosity- Exerts low viscosity

Clinical significance of Albumin

- **Blood brain barrier-** Albumin- free fatty acid complex can not cross the blood brain barrier, hence fatty acids can not be utilized by the brain.

Drug interactions

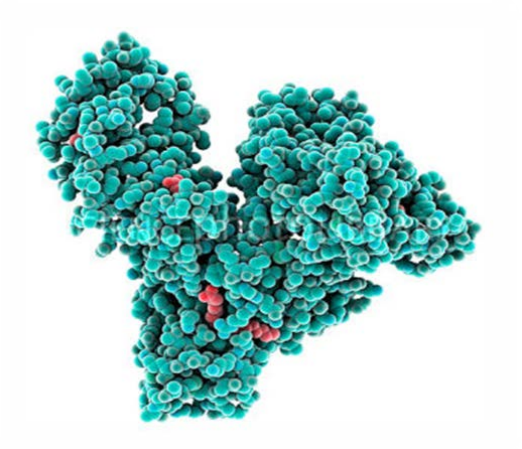
Two drugs having same affinity for albumin when administered together, can compete for available binding sites with consequent displacement of other drug, resulting in clinically significant drug interactions.

Example-Phenytoin, dicoumarol interactions

Clinical significance of Albumin

Edema (الوذمة)- Hypoalbuminemia results in fluid retention in the tissue spaces

Hypoalbuminemia- lowered level is seen in the following conditions-



- ❑Cirrhosis of liver
- ❑Malnutrition
- ❑Nephrotic syndrome
- ❑Burns
- ❑Malabsorption

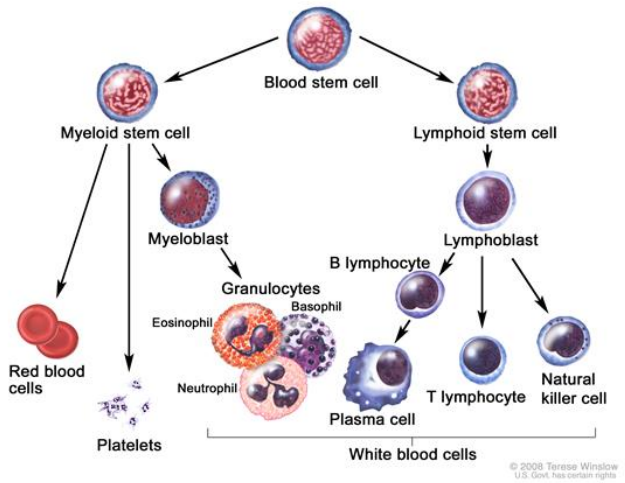
Hyperalbuminemia- In conditions of fluid depletion (Haemoconcentration)

Globulins

- Globulins are separated **by half saturation with ammonium sulphate**
- Molecular weight ranges from 90,000 to 13,00,000
- Normal concentration in blood is 2 to 3.5 g/dl
- By electrophoresis globulins can be separated into –
- α_1-globulins
- α_2-globulins
- β-globulins
- Y-globulins

Synthesis of Globulins

- α and β globulins are synthesized in the liver.
- Y globulins are synthesized in plasma cells and B-cells of lymphoid tissues
- Synthesis of Y globulins is increased in chronic infections, chronic liver diseases, auto immune diseases, leukemias, lymphomas and various other malignancies.



α- Globulins

- They are glycoproteins
- Based on electrophoretic mobility , they are sub classified in to **α_1 and α_2 globulins**
- **α_1 globulins**

Examples-

- **α_1-antitrypsin**
- **Orosomucoid** (α_1 acid glycoprotein):binds the hormone progesterone and functions as a transport protein for this hormone.
- **α_1-fetoprotein** (AFP): useful diagnostically in determining presence of hepatocellular carcinoma (tumor marker)

α1 globulins

α_1-antitrypsin

- Also called **α_1-antiprotease**
- It is a single-chain protein of 394 amino acids
- It is the major component (> 90%) of the α_{1-} fraction of human plasma.
- It is synthesized by hepatocytes and macrophages and is **the principal serine protease inhibitor of human plasma**.
- It inhibits trypsin, elastase, and certain other proteases by forming complexes with them.
- A deficiency of this protein has a role in certain cases (approximately 5%) of **emphysema.**

- **Role in lung Emphysema :**
- **Normally α1-AT protects the lung tissues from injurious effects by binding with the proteases: active elastase.**

Active elastase + α1-AT

↓

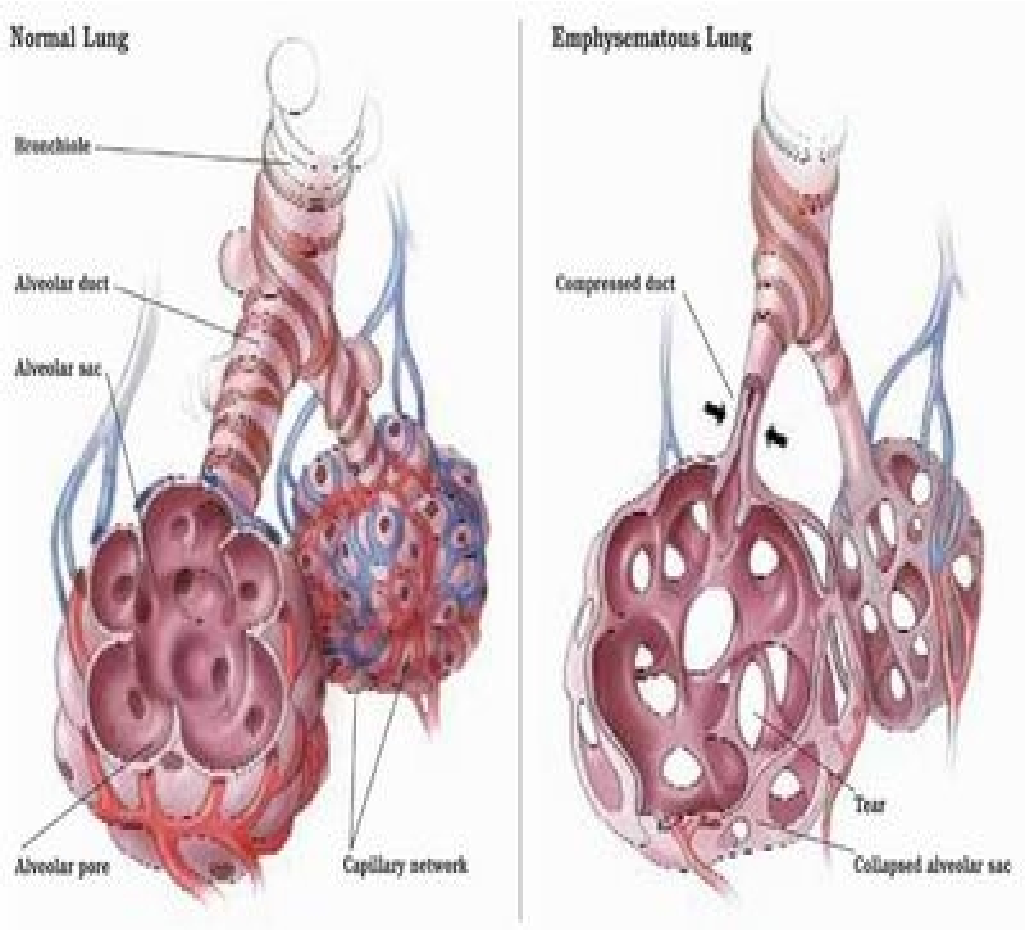
Inactive elastase: α1-AT complex

↓

No proteolysis of lung _____ No tissue damage

Clinical consequences of α_1-antitrypsin deficiency

- **Emphysema-** Normally antitrypsin protects the lung tissue from proteases (active elastase) released from macrophages
- Forms a complex with protease and inactivates it.
- In its deficiency, the active elastase destroys the lung tissue



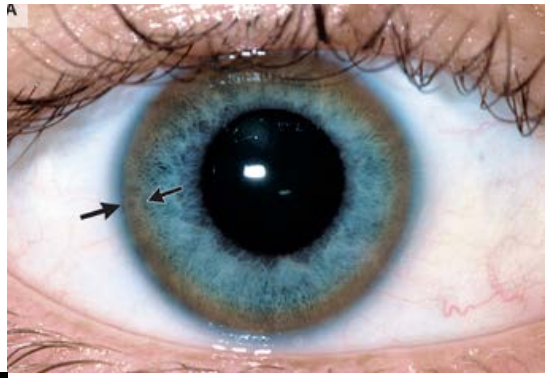
Ceruloplasmin

- Copper containing α_2-globulin (glycoprotein)
- **It has a blue color because of its high copper content**
- Carries 90% of the copper present in plasma.
- Each molecule of **ceruloplasmin binds six atoms of copper very tightly, so that the copper is not readily exchangeable.**
- **Albumin (**carries 10% of the plasma copper) **donates its copper to tissues more readily than ceruloplasmin**

Clinical Significance of Ceruloplasmin

Normal level: 25-50 mg/dl

- Low levels of ceruloplasmin are found in **Wilson disease** (hepatolenticular degeneration), a disease due to abnormal metabolism of copper.
- The amount of ceruloplasmin in plasma is also **decreased in liver diseases, mal nutrition and nephrotic syndrome.**
- Elevated levels: pregnancy, acute and chronic inflammation



Haptoglobin (Alpha 2 globulin)

- Acute phase protein.
- It binds with free hemoglobin that spills into the plasma due to hemolysis.
- The Hp-Hb complex (155,000) cannot pass through glomeruli of kidney while free Hb (65,000) can.
- Hepatoglobin therefore **prevents the loss of free hemoglobin into the kidney**, and it **helps to conserve iron**

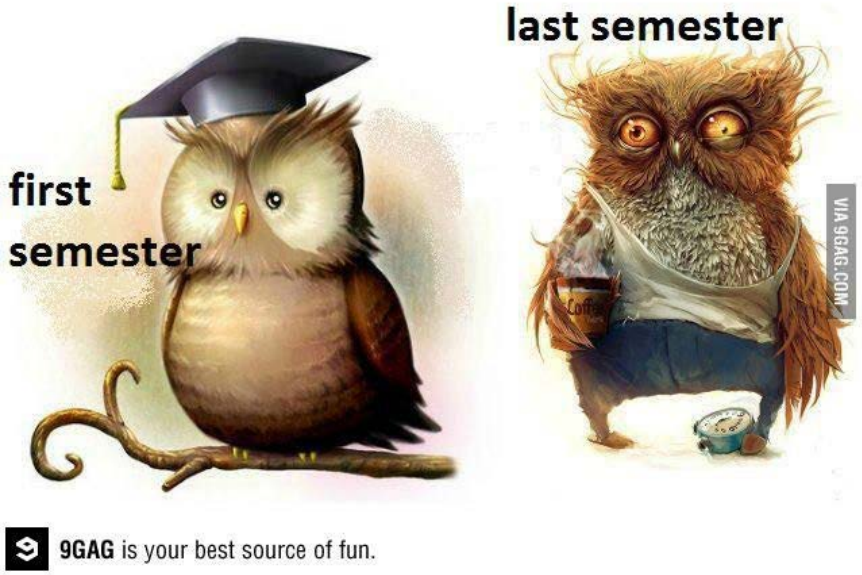
❑Concentration **rises in inflammatory conditions**

❑Concentration **decreases in hemolytic anemias**

β Globulins

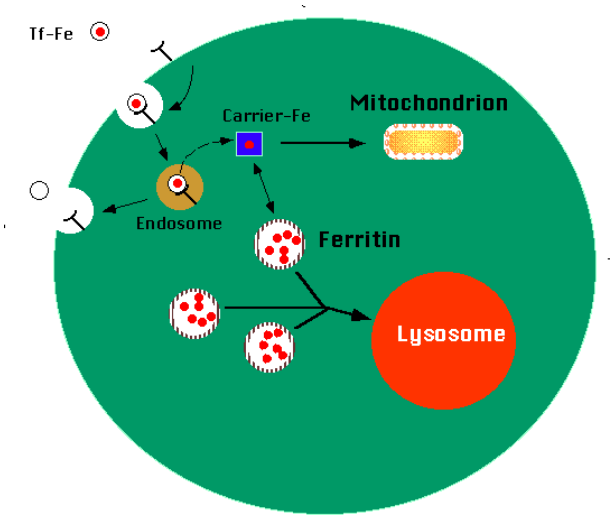
β Globulins of clinical importance are –

- ❑ Transferrin
- ❑ C-reactive protein
- ❑Haemopexin
- ❑Complement C1q
- ❑β Lipoprotein (LDL)



Transferrin

- **Transferrin (Tf)** is a β_1-globulin with a molecular mass of approximately 76 kDa.
- It is a **glycoprotein** and is synthesized in the liver.
- It **plays a central role in the body's metabolism of iron because it transports iron in the circulation to sites where iron is required,** eg, from the gut to the bone marrow and other organs.
- transferrin protein loaded with iron binds to transferrin receptor
- transported into the cell
- Each transferrin molecule has the ability to carry two iron ions in the ferric form (Fe3+).



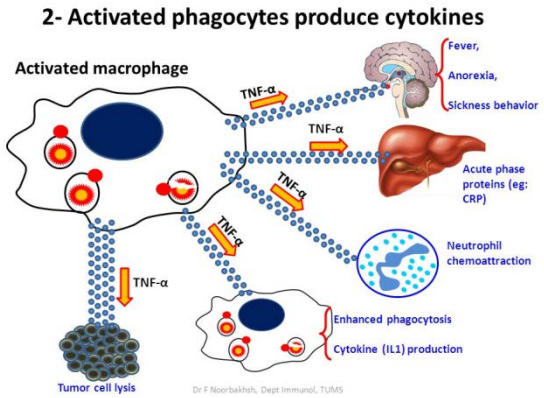
Clinical Significance of Transferrin

- **Increased levels** are seen in iron deficiency anemia and in last months of pregnancy
- **Decreased levels** are seen in-
- Protein energy malnutrition
- Cirrhosis of liver
- Nephrotic syndrome
- Acute myocardial infarction
- Malignancies

Acute phase proteins

The levels of certain proteins may increase in blood in response to inflammatory and neoplastic conditions (surgery or trauma, Infection or tumor growth), these are called **Acute phase proteins.** AP response leads to greatly increased biosynthesis of some plasma proteins: **Examples-**

- ❑ C- reactive proteins
- ❑ Ceruloplasmin
- ❑ Alpha -1 antitrypsin
- ❑ Alpha 2 macroglobulins
- ❑ Serum amyloid A



While, negative acute phase reactant are decreased (albumin and prealbumin)

Response is stimulated by release of Cytokines:**Interleukin-1**, **Interleukin-6** and **Tumor necrosis factor (TNF)**, increased plasma[**Cortisol**] and [**Glucagon**]

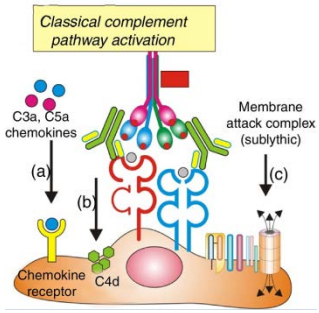
C- reactive protein (β Globulin)

- So named because it reacts with C- polysaccharide of capsule of pneumococci
- Molecular weight of 115-140 kD
- Synthesized in liver
- Can stimulate complement activity and macrophages
- Acute phase protein- concentration rises in inflammatory conditions. It is useful in differentiating bacterial from viral infections because the **level of CRP is increased in bacterial infections only**
- **Clinically important marker to predict the risk of coronary heart disease (hs-CRP)**

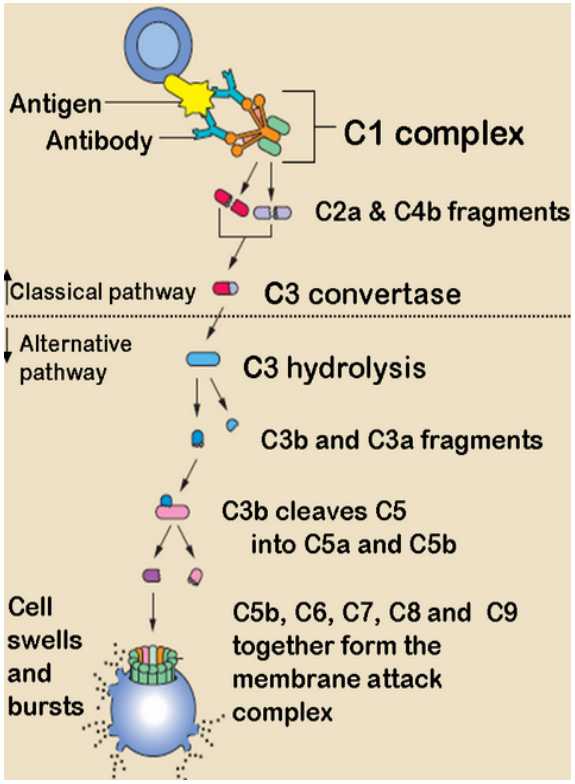
Haemopexin (β Globulin)

- Molecular weight 57,000-80,000
- **Normal level in adults-0.5 to 1.0 gm/L**
- Low level at birth, reaches adult value within first year of life
- Synthesized in liver
- Function is to bind haem formed from breakdown of Hb and other haemoproteins
- **Low level- found in hemolytic disorders, at birth and drug induced**
- **High level- pregnancy, diabetes mellitus, malignancies and Duchenne muscular dystrophy**

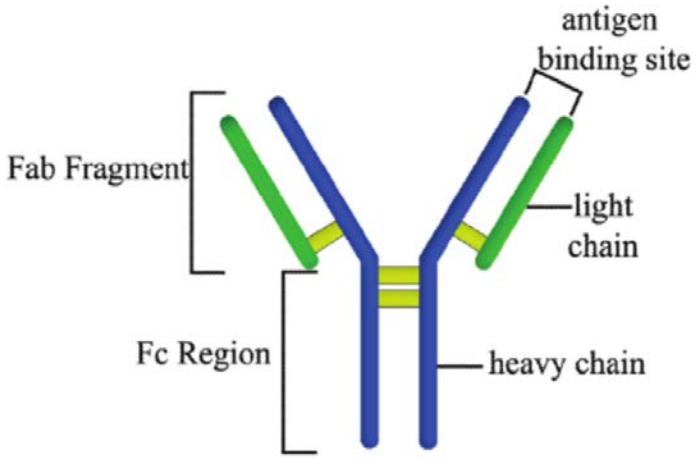
Complement C1q (β Globulin)



- **First complement factor to bind antibody**
- Binding takes place at the Fc region of IgG or Ig M
- Binding triggers the classical complement pathway
- Thermo labile, destroyed by heating
- **Normal level – 0.15 gm/L**
- Molecular weight-400,000
- Decreased level is used as an indicator of circulating Ag –Ab complex.
- High levels are found in chronic infections

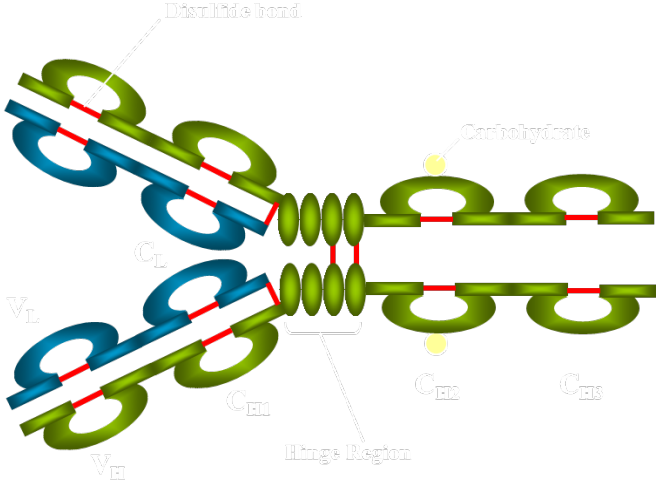


Gamma Globulins



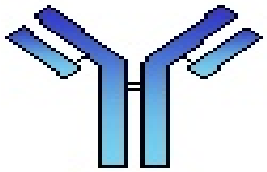
- They are immunoglobulins with antibody activity
- They occupy the gamma region on electrophoresis
- Immunoglobulins play a key role in the **defense mechanisms of the body**
- There are five types of immunoglobulins:

IgG, IgA, IgM, IgD, and **IgE.**

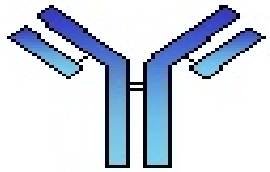


Different Classes of Immunoglobulins

IgG

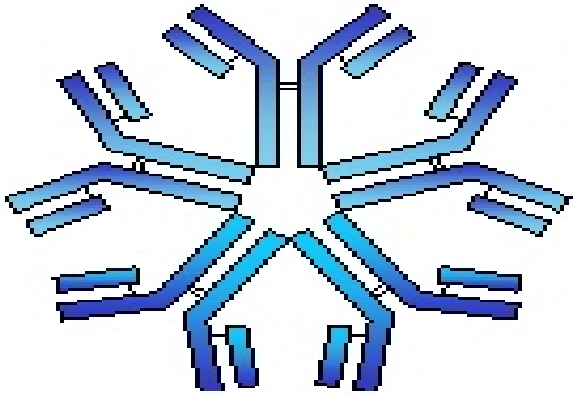


IgE

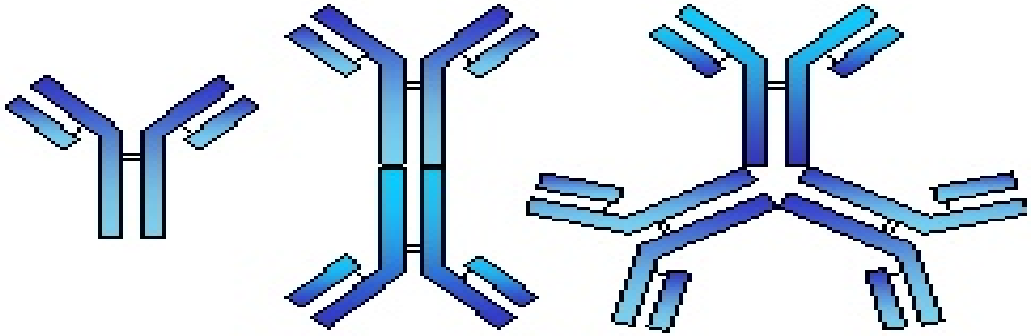


IgD

IgM



IgA



Major functions of immunoglobulins

Immunoglobulin	Major Functions
IgG	Main antibody in the secondary response, Fixes complement, neutralizes bacterial toxins and viruses and *crosses the placenta*.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membranes. Does not fix complement.
IgM	Produced in the primary response to an antigen. Fixes complement. *Does not cross the placenta*.
IgD	Uncertain. Found on the surface of many B cells as well as in serum.
IgE	Mediates immediate hypersensitivity Defends against worm infections. Does not fix

Fibrinogen

- Also called **clotting factor1**
- Constitutes 4-6% of total protein
- Synthesized in liver
- Made up of 6 polypeptide chains, linked together by S-S linkages
- Precipitated with 1/5th saturation with ammonium sulphate
- Imparts **maximum viscosity to blood**
- Amino terminal end is highly negative due to the presence of glutamic acid
- **Negative charge contributes to its solubility in plasma and prevents aggregation due to electrostatic repulsions between the fibrinogen molecules.**

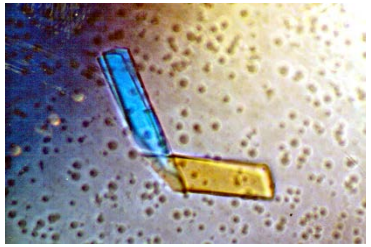
Transport proteins

Name	Compounds transported
Albumin	Fatty acids, bilirubin, hormones, calcium, heavy metals, drugs etc.
Prealbumin-(Transthyretin)	Steroid hormones thyroxin, Retinol
Retinol binding protein	Retinol (Vitamin A)
Thyroxin binding protein(TBG)	Thyroxin
Transcortin(Cortisol binding protein)	Cortisol and corticosteroids
Haptoglobin	Hemoglobin
Hemopexin	Free haem
Transferrin	Iron
HDL(High density lipoprotein)	Cholesterol (Tissues to liver)
LDL(Low density lipoprotein)	Cholesterol(Liver to tissues)

Abnormal Proteins

1) Bence – Jone's proteins

- Abnormal proteins- monoclonal light chains
- Present in the urine of a patient suffering from multiple myeloma (50% of patients)
- Molecular weight 45,000
- Identified by heat coagulation test



2)Cryoglobulins

- These proteins coagulate when serum is cooled to very low temperature
- Commonly monoclonal IgG or IgM or both
- Increased in rheumatoid arthritis, multiple myeloma, lymphocytic leukemia and in hepatitis C infection

Clinical Significance of Plasma proteins

Hyperproteinemia- Levels higher than 8.0gm/dl

Causes-

- **Hemoconcentration-** due to dehydration, albumin and globulin both are increased. A/G ratio remains same.
- **Causes-** Excessive vomiting
- Diarrhea
- Diabetes Insipidus
- Diuresis
- Intestinal obstruction

Hypoproteinemia

Decease in total protein concentration

- **Hemodilution-** Both **Albumin and globulins** are decreased, A:G ratio remains same, as in water intoxication
- **Hypoalbuminemia-** low level of Albumin in plasma

Causes-

- Nephrotic syndrome
- Protein losing enteropathy
- Severe liver diseases
- Malnutrition or malabsorption
- Extensive skin burns
- Pregnancy
- Malignancy

Clinical Significance of Transferrin

- The **concentration of transferrin in plasma is approximately 300 mg/dL.**

TIBC is a measure of the amount of iron that can be bound by transferrin.

- In the plasma, total iron averages 110 µg/dL
- Majority bound to the transferrin (capacity to bind 330 µg of iron per deciliter)
- So only one third of transferrin is saturated.

Disease	Iron	TIBC/Transferrin	UIBC	% Transferrin Saturation
Iron Deficiency	Low	High	High	Low
Hemochromatosis	High	Low	Low	High