

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

In the name of Allah, Most Gracious, Most Merciful.

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PROTEIN CHEMISTRY (Plasma Proteins)

By Dr. Kalsoom Tariq

Learning Objectives

- » Denaturation of protein
- » Separation techniques for Protein
- » Plasma proteins, including
 - » Albumin
 - » Globulin
 - » Fibrinogen

Denaturation of proteins

- » Defined as disruption of secondary , tertiary and whenever applicable quaternary organization of protein molecule due to cleavage of non-covalent bond
- » Primary structure i-e peptide bond remains intact

Alteration in protein after denaturation:

(a) Chemical alterations: Greatly decreased solubility specially at PI of the protein.

- Many chemical groups which were rather inactive become exposed, e.g. -SH group.
- Denaturation can be reversible.

(b) Physical alterations:

- Confers increased viscosity of the solution.
- Rate of diffusion of the protein molecules decreases

(c) Biological alterations:

- Increased digestibility by proteolytic enzymes.
- Denaturation destroys enzymal and hormonal activity.
- Biologically becomes inactive.

Protein Separation

Protein separation is based on

- 1) Protein solubility**
- 2) Size of protein molecule**
- 3) Charge of the molecule**

Methods of protein separation

- Chromatography.**
- Salting out**
- Electrophoresis.**
- Dialysis.**
- Ultracentrifugation.**
- Cohn's Fractionation**

Chromatography

Chromatography is a group of separation techniques, where a mixture of molecules is separated.

- **The separated molecules are divided between a stationary solid phase and liquid mobile phase.**
- **The separation process depends on the tendency of one type of molecules in the mixture to **associate more strongly with one phase than the other.****

Salting out:

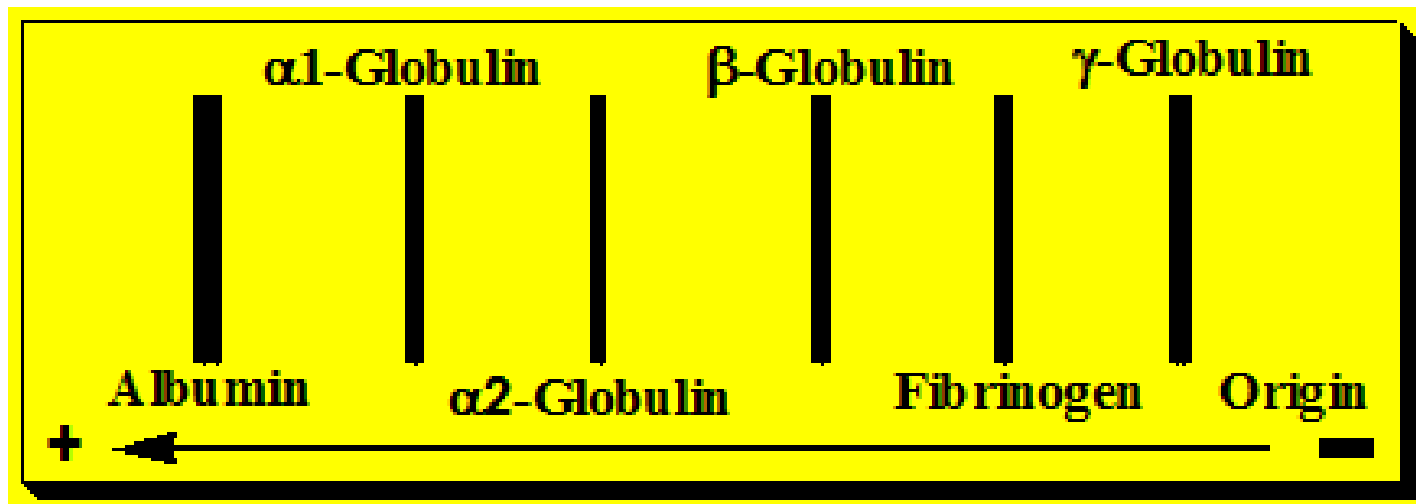
- » Based on solubility of protein in solutions of high salt concentrations
- » Addition of salt ions shield proteins with multi-ion charges helping protein molecules to aggregate
- » Conc. is different for different proteins eg albumin by full saturation with ammonium sulphate and globulin with half saturation

Cohn's Fractionation:

- » Cohn used varying concentrations of ethanol at low temperature to separate out fractions of proteins which are called fraction I, II, etc.
- » Fraction I is rich in fibrinogen
- » Fraction II is γ -globulins
- » Fraction III contains α and β -globulins including isoagglutinins and Prothrombin
- » Fraction IV contains α and β -globulins
- » Fraction V contains predominantly albumin

Electrophoresis

- It is movement of charged particles in an electric field towards the oppositely charged electrode.
- By electrophoresis a mixture of amino acids, polypeptides or proteins can be separated into distinct bands by using electric current.



Paper electrophoresis:

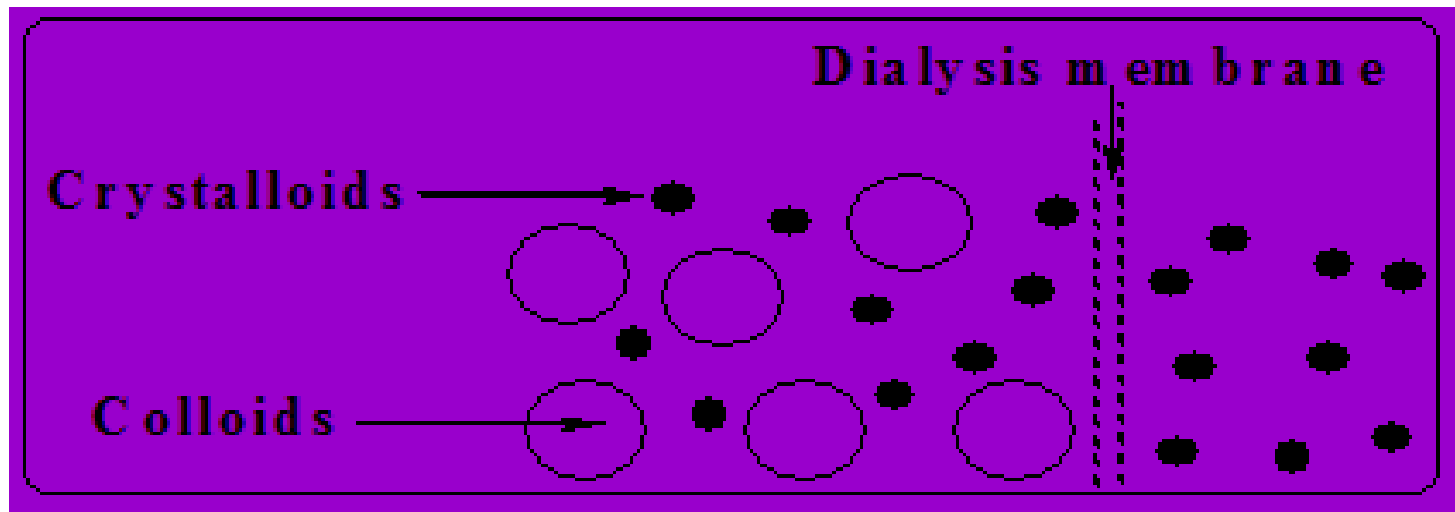
- » Paper electrophoresis uses filter paper, ends of filter paper strip dipped into buffer solution of known PH usually 8.6 with sample of protein at one end
- » Current is passed, strip dried and stained with suitable dye

Gel electrophoresis:

- » Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)
- » Proteins move based on molecular weight & charge.

Dialysis

- Dialysis means separation of colloids from crystalloids. Proteins have a high molecular weight that forms a colloidal solution.
- If there is a mixture of proteins (**colloids**) and salts (**crystalloids**) they can be separated by dialysis, i.e., by using a semi-permeable membrane. Crystalloids with very small molecular weight can pass through this membrane, while colloids can not due to the large size of their particals



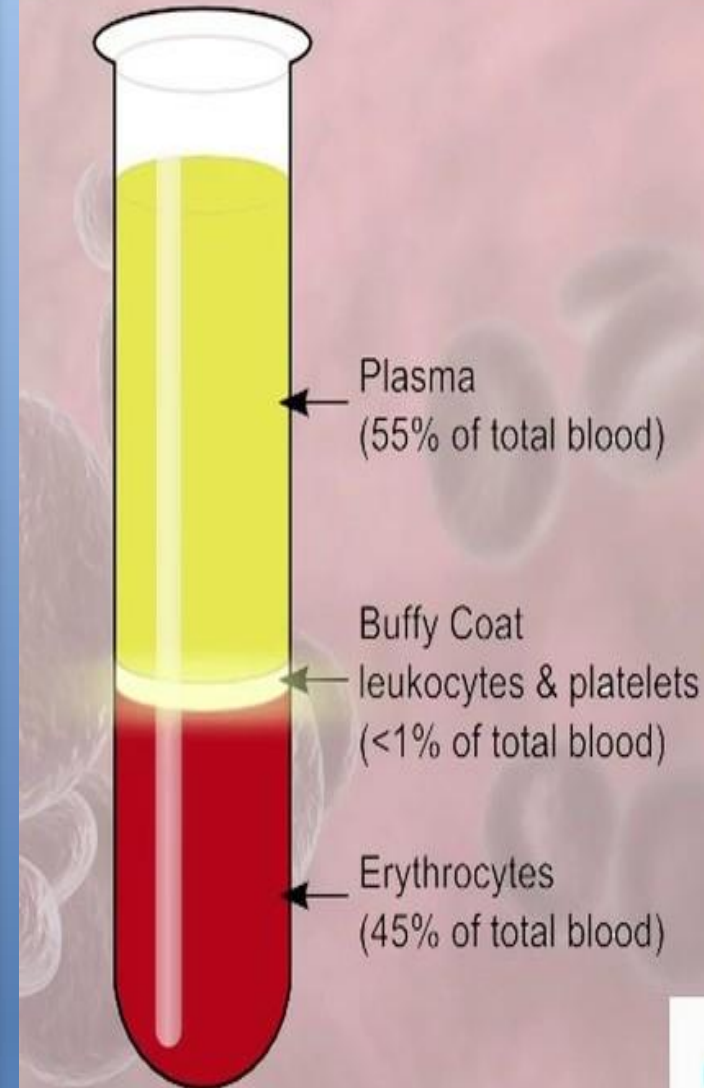
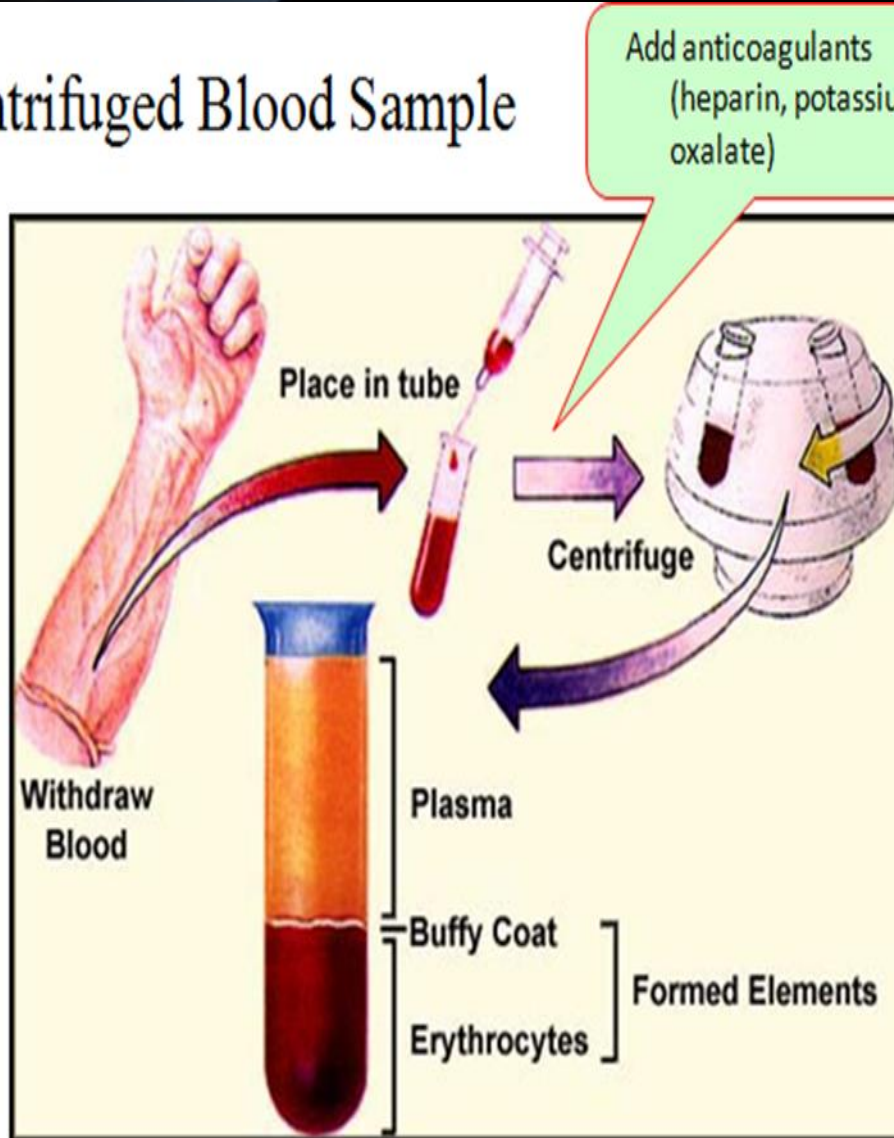
Ultracentrifugation

- Using high speed centrifuge, a mixture of proteins is separated into different fractions according to their densities.

Lipoproteins can be separated by ultracentrifugation to chylomicrons, VLDL, LDL, and HDL.

PLASMA PROTEINS

Centrifuged Blood Sample



Components of Plasma

Blood plasma Consists of:

Water 90%

Plasma Proteins 6-8 %

Electrolytes (Na^+ & Cl^-) 1%

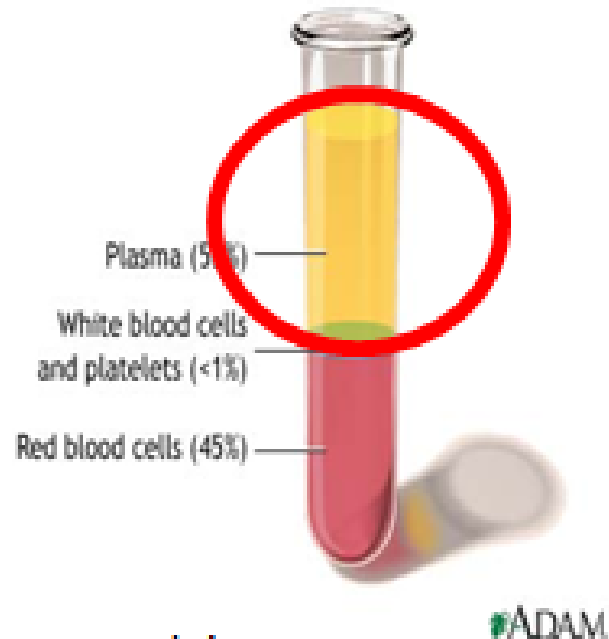
Other components:

Nutrients (e.g. Glucose and amino acids)

Hormones (e.g. Cortisol, thyroxine)

Wastes (e.g. Urea)

Blood gases (e.g. CO_2 , O_2)



TYPES OF PLASMA PROTEINS

» Three major types:

A. Albumin

B. Globulin

C. Fibrinogen

General characteristics of plasma proteins

1. They are **synthesized in liver** except immunoglobulin.
2. Almost all plasma proteins are **glycoproteins**.
3. Many plasma proteins exhibit **polymorphism** such as α_1 -antitrypsin, transferrin, haptoglobin.
4. Each plasma protein has a **characteristic half-life** in the circulation.
5. **Acute Phase Proteins, APP**

Types of plasma proteins

1. Albumin

2. Globulins

α -globulins : α_1 a α_2 -globulins

β -globulins: β_1 a β_2 -globulins

γ -globulins

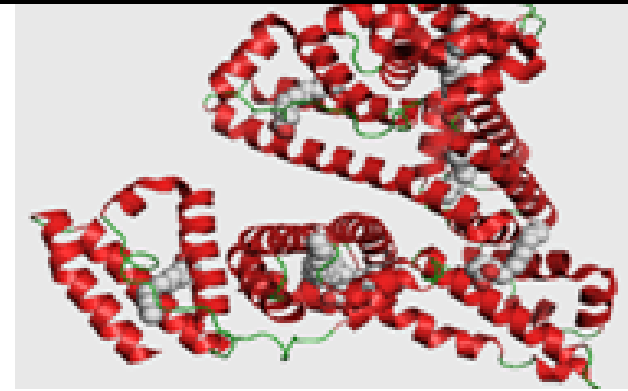
3. Fibrinogen

Under different pathological conditions the protein levels depart from the normal range.

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

Albumin



- Highest concentration plasma protein
- 585 aa and 17 disulphide bonds
- Two primary functions
 - Colloidal osmotic pressure (80%)
 - Bind and transport of numerous substances
 - Bilirubin, steroids, fatty acids, Ca^{++} , Mg^{++} ,
 - salicylic acid & other medications

Functions of Albumin

- **Colloidal osmotic Pressure**—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)**

EDEMA

- **Colloid osmotic pressure**, is a form of osmotic pressure exerted by **proteins** in blood plasma that usually **tends to pull water into the circulatory system**.
 - Because **large plasma proteins** cannot easily cross through the **capillary walls**.
- In conditions where **plasma proteins are reduced**,
 - e.g. from being lost in the urine (**proteinuria**) or from malnutrition,
 - there will be a reduction in osmotic pressure, leading to enhanced fluid retention in tissue spaces (**edema**).

Functions of Albumin

Transport function—albumin has an ability to bind various ligands, thus acts as a transporter for various molecules. 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, particularly in nutritional deprivation of amino acids.

□ **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 aspects

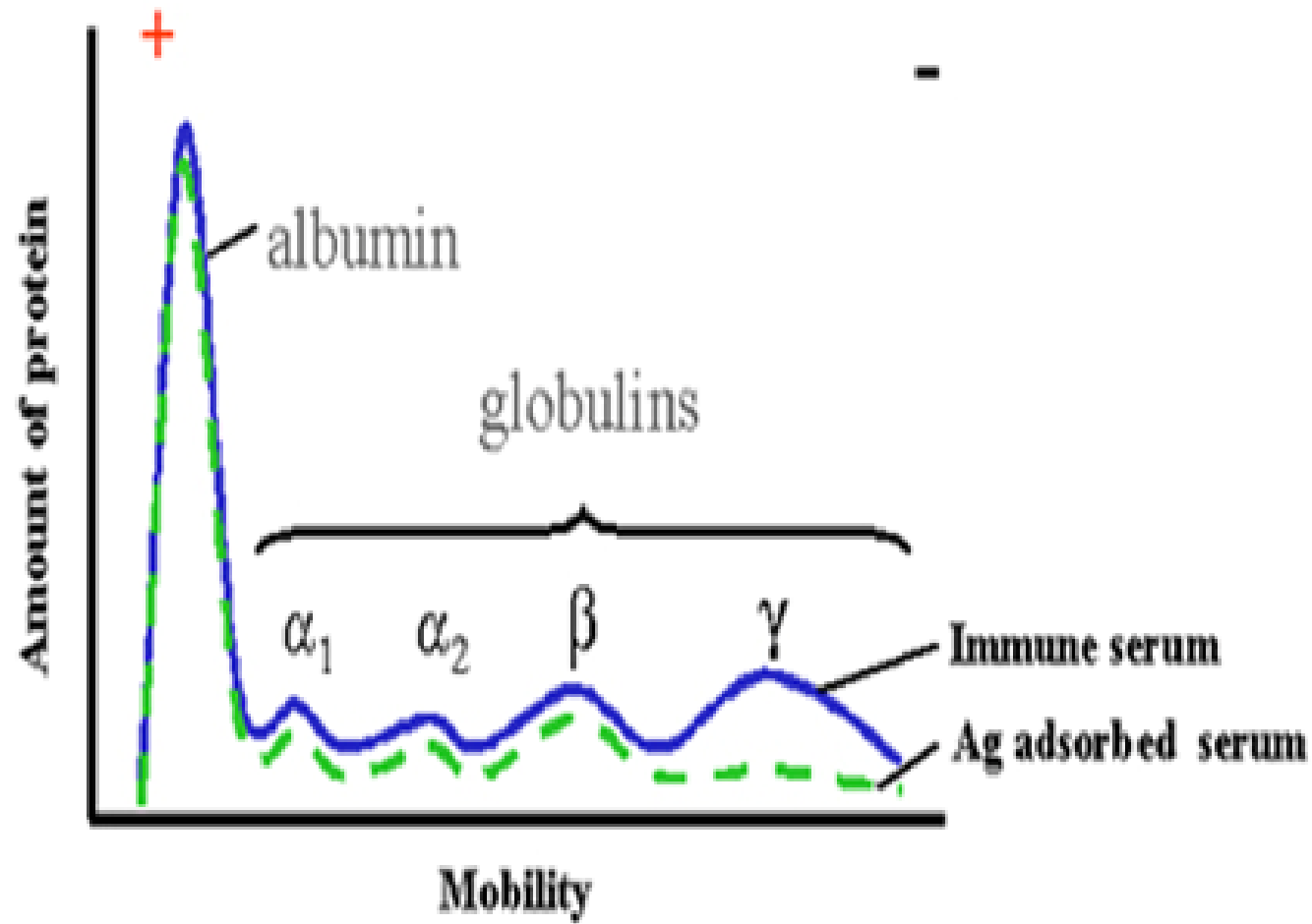
Hypoalbuminemia

- lowered plasma albumin
- in malnutrition, nephrotic syndrome and cirrhosis of liver.

Albuminuria

- albumin is excreted into urine
- in nephrotic syndrome and certain inflammatory conditions of urinary tract.

Globulins



Globulins

- Globulins are separated **by half saturation with ammonium sulphate**
- Molecular weight ranges from 90,000 to 13,00,000
- By electrophoresis globulins can be separated in to –
 - α_1 -globulins
 - α_2 -globulins
 - β -globulins
 - γ -globulins

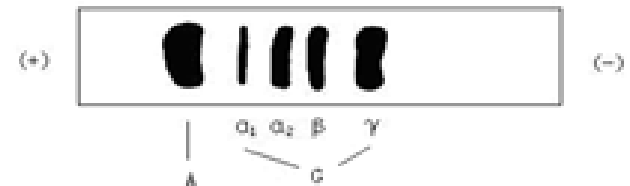
Synthesis

- » Alpha and beta synthesized in liver
- » Gamma by B-cells of lymphoid tissue

High mol wt(90k to 1300k), separated by half saturation

Alpha 1 globulins

α_1 -Antitrypsin

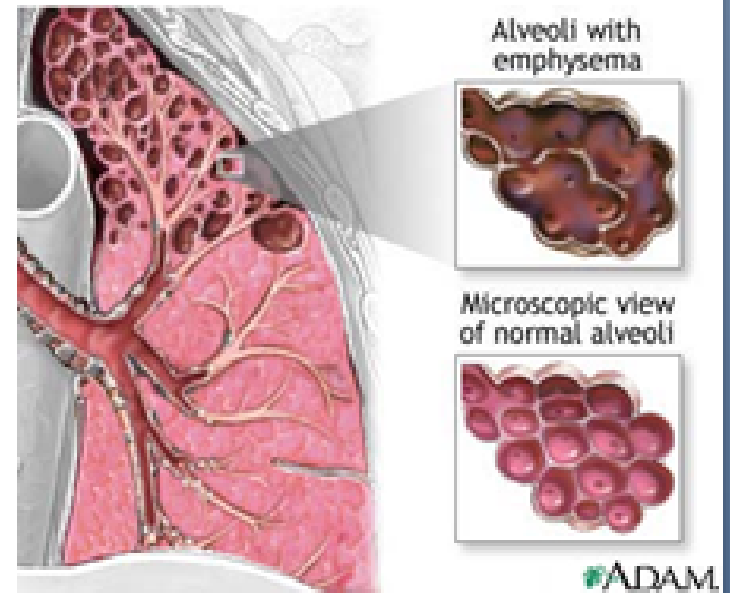


- It is a glycoprotein with 394 aa.
- It is a major constituent of α_1 globulin fraction of plasma protein, normal concentration about 200mg/dl.
- It is a ***serine protease inhibitor*** and can combine with trypsin, elastase and other protease and inhibits them.



α_1 -Antitrypsin

1. Emphysema: used to represent the abnormal distension of lungs.
 - About 5% is due to the deficiency of α_1 -AT.
 - This is associated with *lung infection and increase the activity of macrophage* that damage lungs tissue.



☀ Smoking can cause oxidation of Met₃₅₈ to methionine sulfoxide and inactivate α_1 -AT.

1. *Role in Emphysema Lung*: A deficiency of α_1 -AT has a role in certain cases, approx. 5 per cent, of emphysema of lung. This occurs mainly with ZZ phenotype who synthesise Pi^Z .

Biochemical mechanism

- Normally α_1 -AT protects the lung tissues from injurious effects by binding with the proteases, viz. *active elastase*. A particular methionine (358 residue) is involved in binding with the protease.

Thus,

Active elastase + α_1 -AT



Inactive elastase: α_1 -AT complex



No proteolysis of lung → No tissue damage

- When α_1 -AT is deficient or absent the above complex with active elastase does not take place and active elastase brings about proteolysis of lung and tissue damage.

Active elastase + No or ↓ α_1 -AT



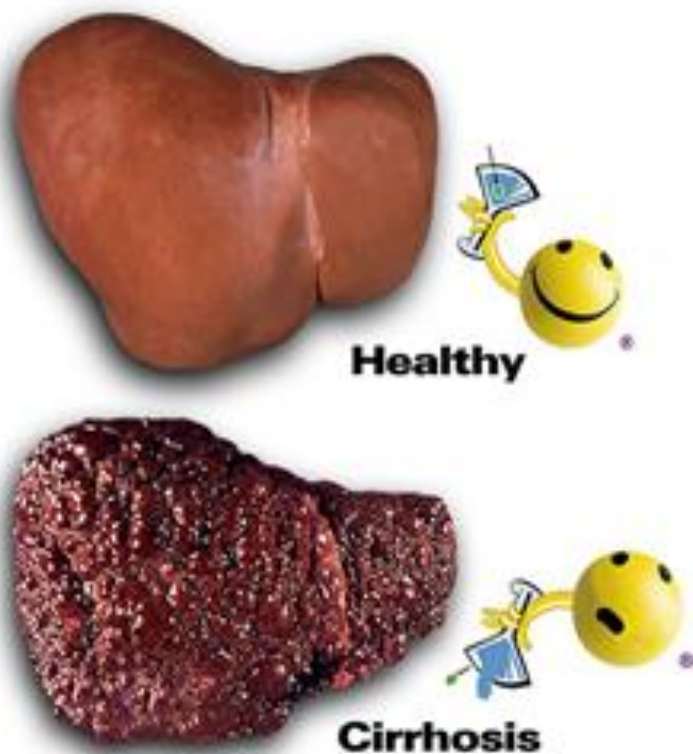
Active elastase → proteolysis of lung
(complex does not form)



Tissue damage

2. α_1 -antitrypsin deficiency liver disease

due to mutant α_1 -antitrypsin accumulates and aggregates to form polymers, by unknown mechanism, cause **liver damage** followed by accumulation of collagen resulting in **fibrosis (cirrhosis)**.



Alpha 2 globulin:

Hepatoglobulin (Hp)

- It can bind with the free hemoglobin (extra-corpuseular Hb) in a tight noncovalent complex *Hp-Hb* during hemolysis.
- Hp-Hb cannot pass through glomeruli of kidney while free Hb can, and Hp *prevent the loss of free Hb into urine*.
- ✘ Low levels of plasma concentration of Hp can diagnose hemolytic anemia.

Ceruloplasmin(CER)

- It is a blue-coloured, copper-containing α_2 fraction.
- It can carry 90% of plasma copper tightly so that copper is not readily exchangeable. It processes copper-dependent oxidase activity.
- Wilson disease (Excess Copper),

- » Not involved in Cu transport
- » Main function is as ferroxidase(oxidises iron which can then be incorporated into transferrin)
- » Increased in inflammation, pregnancy, Oral Contraceptive use
- » Decreased in Wilsons disease

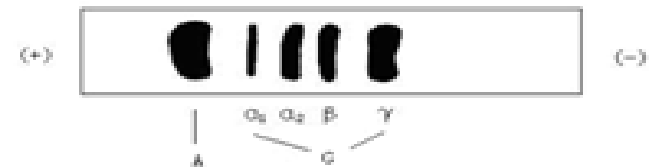
β Globulins

β Globulins of clinical importance are –

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

Beta globulin

Transferrin (Tf)



- It is a glycoprotein, part of β fraction.
- It can transport iron in plasma as ferric ions (Fe^{3+}) and protect the body against the toxic effects of free iron.

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
 - Trauma
 - Acute myocardial infarction
 - Malignancies
 - Wasting diseases

iii) C-reactive protein

- » Normally less than 1 mg/100ml
- » Sensitive indicator of inflammation, high in leukemias and tumors, cancers.
- » High CRP levels can also indicate that there is inflammation in the arteries of the heart, meaning a high risk of heart attack.

iv) Haemopexin:

» Mol.wt.: 57000 – 80,000

» Synthesized in the parenchymal cells of liver.

Function: is to bind & remove circulating heme which is formed in the body from breakdown of Hb., myoglobin or catalases.

It binds heme & other porphyrin in 1:1 ratio.

This complex is removed by parenchymal cells of liver.

v) Complement C1q:

- » It is a thermo labile, destroyed by heat.
- » Normal value: 0.15gm/l
- » Mol. Wt.:400,000
- » It can bind heparin & bivalent ions such as Ca^{++} .
- » It participate in immune reactions,
- » Is the 1st complement factor that binds after the formation of immune complexes.

3. Fibrinogen(clotting factor I)

- » A glycoprotein constitute 4 to 6 percent of plasma protein
- » It takes part in coagulation of blood & is the precursor of fibrin .
- » synthesized in liver.
- » Being large & asymmetrical with an axial ratio 20:1, is important for viscosity of blood.

Other proteins of clinical importance:

1. Bence-Jones' Protein

An abnormal protein occurs in blood and urine of people suffering from a disease called *multiple myeloma* (a plasma cell tumour). Defined as *monoclonal light chains* present in the urine of patients with paraproteinaemic states. Either monoclonal 'κ' or 'λ' light chains are excreted in significant amounts in about 50 per cent cases of multiple myeloma. It has a molecular weight 45000, and has sedimentation coefficient of 3.5 S. Sometimes the chains excreted may be a 'dimer' of L-chains.

Identification—Heat Test

- The protein is identified easily in urine by a simple **Heat Test**. On heating the urine 50 to 60°C,

Bence-Jones' proteins are precipitated, but when heated further it dissolves again. Reverse occurs on cooling.

- Best detected by zone electrophoresis and immunoelectrophoresis of concentrated urine.

Note

Normally only very small quantities of 'κ' and 'λ' immunoglobulin L-chains are excreted in urine. They pass through glomerular filter but are reabsorbed by kidney tubules and broken down in the lining cells and thus not found in urine.

2. Cryoglobulins

These are proteins which are coagulated when plasma or serum is cooled to very low temperature (2 to 4°C). Most commonly they are monoclonal IgG or IgM or a mixture of two. Traces are present even in normal individuals. Their molecular weights vary from 1,65,000 to 6,00,000.

Increase: They are increased in rheumatoid arthritis, lymphocytic leukaemia, multiple myeloma, lymphosarcomas and systemic lupus erythematosus (SLE).

Acute phase proteins

The levels of certain proteins may increase in blood in response inflammatory and neoplastic conditions, these are called Acute phase proteins.

Examples–

- ❑ C– reactive proteins
- ❑ Ceruloplasmin
- ❑ Alpha –1 antitrypsin
- ❑ Alpha 2 macroglobulins
- ❑ Alpha–1 acid glycoprotein

Functions of plasma proteins

- Nutritive
- Fluid exchange
- Buffering
- Binding and transport
- Enzymes
- Hormones
- Blood coagulation
- Viscosity
- Defense
- Reserve proteins
- Tumor markers

Clinical Significance of Plasma proteins

Hyperproteinemia– Levels higher than 8.0gm/dl

Causes–

□ **Hemoconcentration**– due to dehydration, albumin and globulin both are increased
Albumin to Globulin ratio remains same.

□ **Causes**– Excessive vomiting

□ Diarrhea

□ Diabetes Insipidus

□ Pyloric stenosis or obstruction

□ Diuresis

□ Intestinal obstruction

Hypoproteinemia

Decrease 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
- Mal nutrition or malabsorption
- Extensive skin burns
- Pregnancy
- Malignancy

Proteinuria:

Types of proteinurias: Classified under two major heads:

A. Functional proteinurias

B. Organic proteinurias

A. Functional Proteinurias: Those conditions that are not related to a diseased organ. The amount of protein excreted is usually small, majority of cases show < than 0.2 per cent and the condition is usually temporary.

Causes

- **Violent exercise:** Soldiers after long marches, athletes after strenuous contests can have such temporary proteinurias. Here, there may be slight kidney damage to account for it, but the condition almost always clear up.
- **Cold bathing:** Leading to constriction of renal blood vessels and producing temporary anoxia.
- **Alimentary proteinuria:** Occasionally proteinuria may occur after excessive protein ingestion.
- **Pregnancy:** Proteinuria is frequently, associated with pregnancy, probably as a result of pressure interfering with the return of blood in renal veins.
- **Orthostatic/or postural proteinuria:** This occurs chiefly in children or in adolescents, usually in age group of 14 to 18 years. In these young individuals, the urine contains protein when they are in upright position only. When they are lying down it is free from proteins.

B. Organic Proteinurias: There are many pathologic conditions that cause organic proteinuria, which may be classified in **three major groups**.

- (a) *Pre-renal*,
- (b) *Renal*, and
- (c) *Post-renal*.

(a) Pre-renal

Conditions causing proteinuria of this group are those that are primarily not related to kidney. In most cases, they affect the kidneys in such a way as to render it more permeable to the protein molecule.

Causes

- **Cardiac diseases:** By affecting the circulation of kidneys leads to proteinuria.
- Any **abdominal tumors**, or mass of fluid in the abdomen does the same by exerting pressure on the renal veins.
- **Fevers**, convulsions, anaemias and other blood diseases, liver diseases and many other pathologic states can affect in similar manners as stated above.
- **Cancers:** An increased amount of urinary mucoproteins generally accompanies elevated serum mucoprotein levels. Such has been observed in patients with cancers, with highest values when carcinomatous invasion is widespread.
- **Collagen diseases** and inflammatory conditions also have high mucoprotein levels.

(b) Renal

Proteinurias are found in various types of kidney diseases and are called as **Renal proteinurias**.

Causes

- **Acute glomerulonephritis:** Always associated with proteinuria.
- **Chronic glomerulonephritis:** Proteinuria is seen in early stages, but may disappear later as the kidney becomes more and more impaired.
- **In nephrosclerosis, TB of kidney and in carcinoma of kidney:** Proteinuria is frequently found but it is not always.
- **In nephrotic syndrome (Type II): Large quantities of albumin is lost in the urine** and there may be gross hypoalbuminaemia in blood.
- Polypeptides, the so-called, proteoses and peptones, sometimes are excreted in urine. This may happen in pneumonias, diphtheria, carcinoma and other conditions and is due to some protein containing materials, e.g. an exudate or a tissue mass/pus undergoing autolysis.

(c) Post-renal

These are sometimes called as **false proteinurias**, whereas the above two are *true*, because in these conditions (postrenal) **proteins do not pass through the kidneys**.

Causes

- May be due to inflammatory, degenerative or traumatic lesions of the pelvis of the kidney, ureter, bladder, prostate or urethra.
- Bleeding in genitourinary tract also will account for proteinuria.



THANK YOU