

PLASMA PROTEINS: Biochemistry, and Clinical Significance

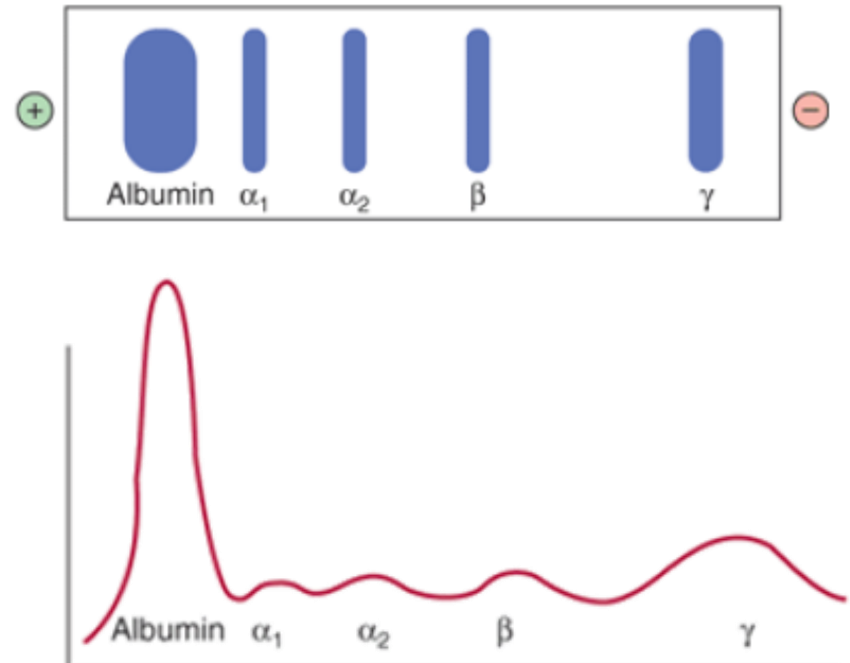
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2ND SEMESTER, 2023

Plasma

- Liquid medium where cells are suspended
- Composition: ▪ Water (92%) ▪ Solids (8%)
 - Organic:
 - **Plasma proteins: Albumin, Globulins & Fibrinogen**
 - Non-protein nitrogenous compounds: urea, free amino acids, uric acid, creatinine, creatine & NH_3
 - Lipids: Cholesterol, TG, phospholipids, free fatty acids
 - Carbohydrates: Glucose, fructose, pentose
 - Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones
 - Inorganic: Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^- , HPO_4^{2-} , SO_4^{2-}

The separation of plasma proteins

- Salting-out (ammonium sulfate): **fibrinogen, albumin, and globulins**
- Electrophoresis (most common): serum (defibrinated plasma), five bands (albumin, α_1 , α_2 , β , and γ)

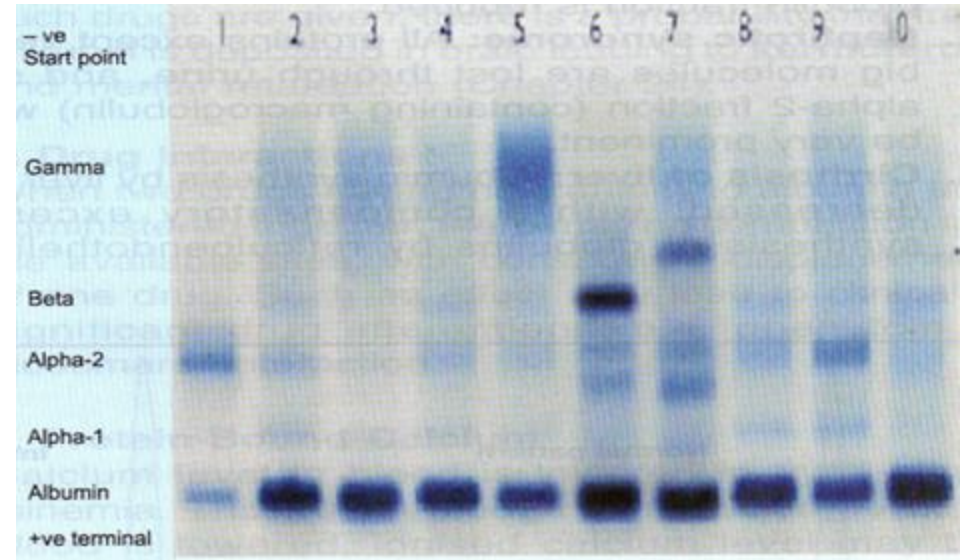


NORMAL VALUES:

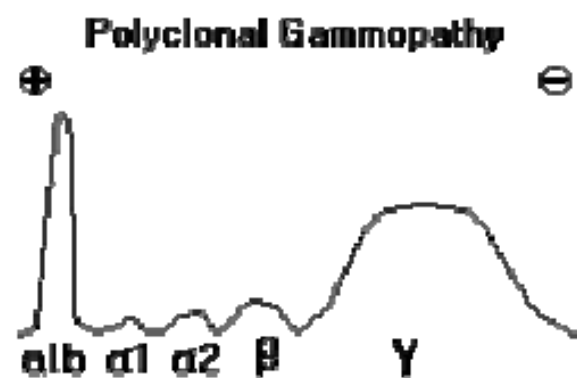
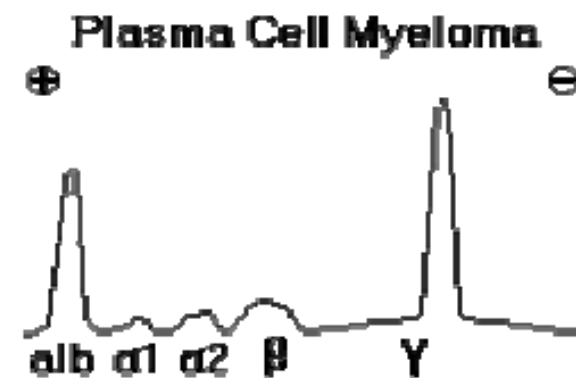
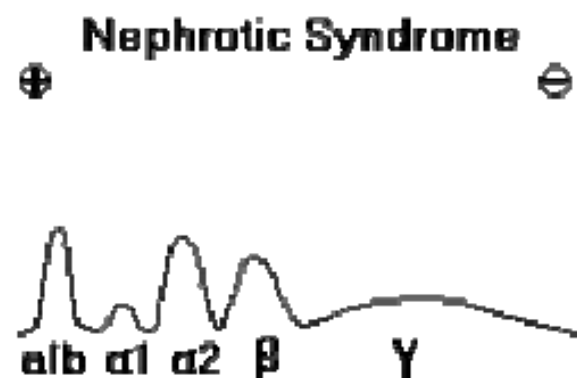
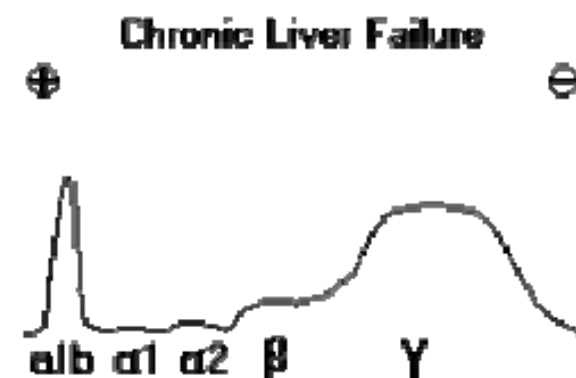
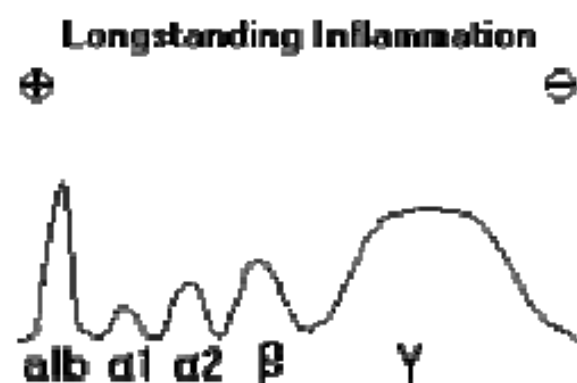
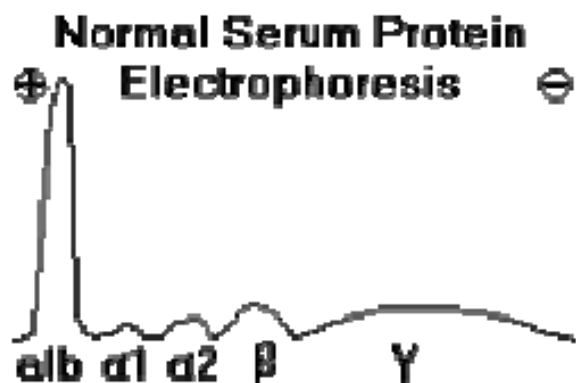
Name	Absolute values (g/l)	Relative values (%)
Albumins	35 – 55	50 – 60
α_1 -globulins	2 – 4	4.2 – 7.2
α_2 -globulins	5 – 9	6.8 – 12
β -globulins	6 – 11	9.3 – 15
γ -globulins	7 – 17	13 – 23

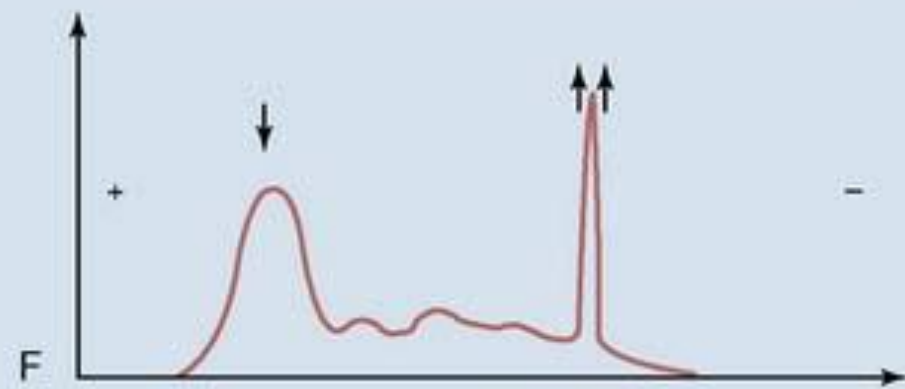
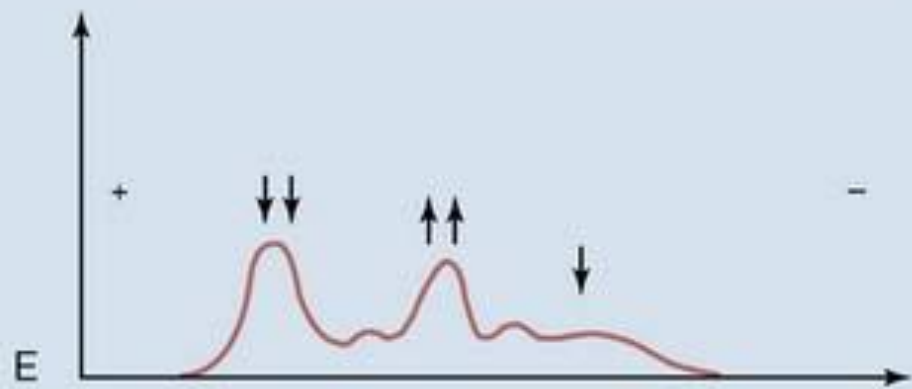
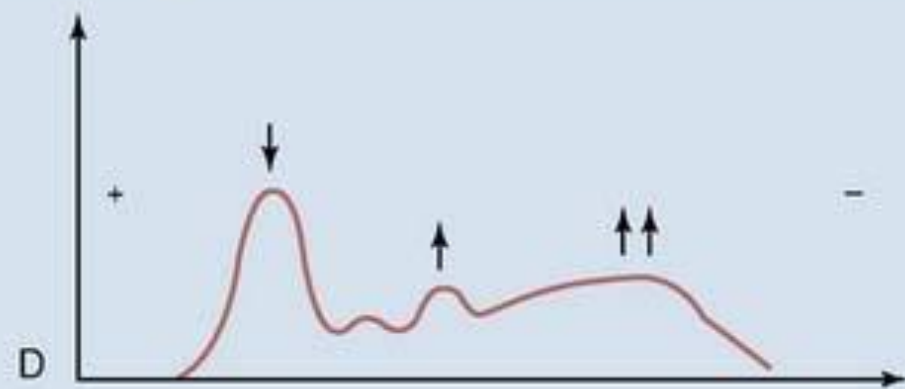
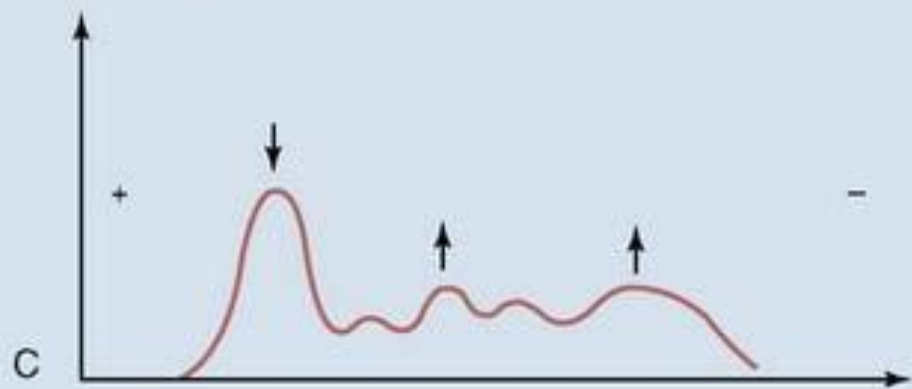
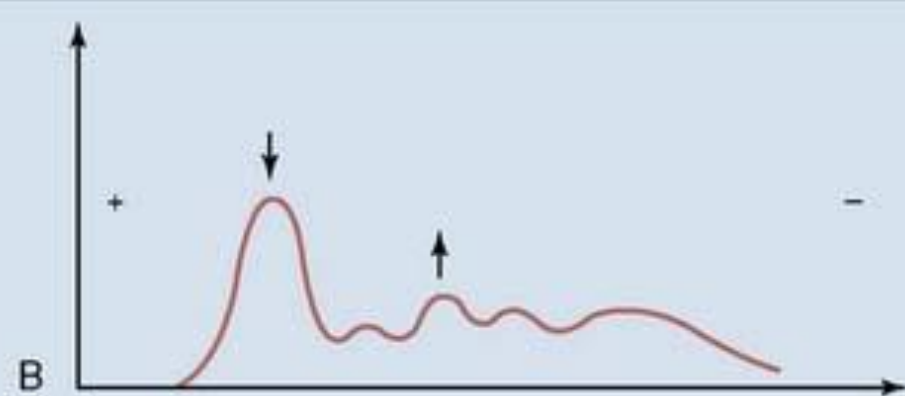
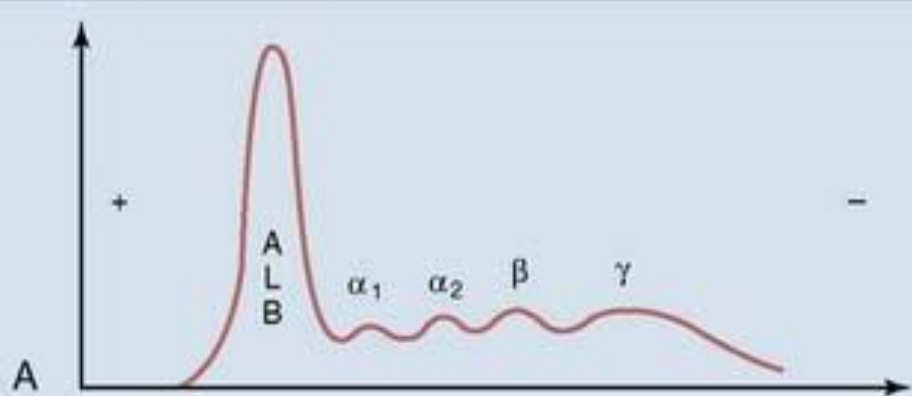
Electrophoresis of plasma proteins

- Albumin is smaller than globulin, and slightly negatively charged
- Globulins (3 bands):
- α band:
 - ✓ α_1 region consists mostly of α_1 -antitrypsin
 - ✓ α_2 region is mostly haptoglobin, α_2 -macroglobulin, & ceruloplasmin
- β band: transferrin, LDL, complement system proteins
- γ band: the immuno-globulins



ELECTROPHORESIS ASPECTS IN SEVERAL TYPES OF DYSPROTEINEMIA





Synthesis of plasma proteins

- Mostly liver (albumin, globulins), γ -globulins (plasma cells; lymph nodes, bone marrow, spleen)
- Most plasma proteins are synthesized as preproteins (signal peptide)
- Various posttranslational modifications (proteolysis, glycosylation, phosphorylation, etc.)
- Transit times (30 min to several hours)
- Most plasma proteins are Glycoproteins (N- or O-linked). Albumin is the major exception

Functions of plasma proteins

General functions

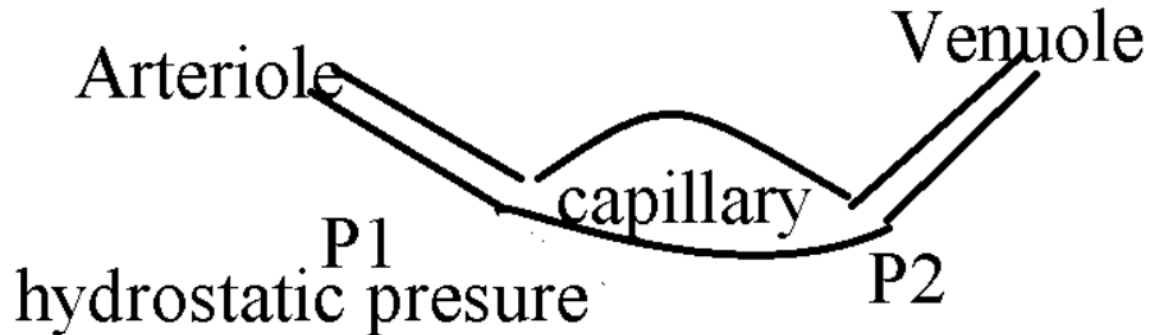
- A nutritive role
- Maintenance of blood pH (amphoteric property)
- Contributes to blood viscosity
- Maintenance of blood osmotic pressure

Specific functions

- Enzymes (e.g. rennin, coagulation factors, lipases)
- Humoral immunity (immunoglobulins)
- Blood coagulation factors
- Hormonal (Erythropoietin)
- Transport proteins (Transferrin, Thyroxin binding globulin, Apolipoprotein)

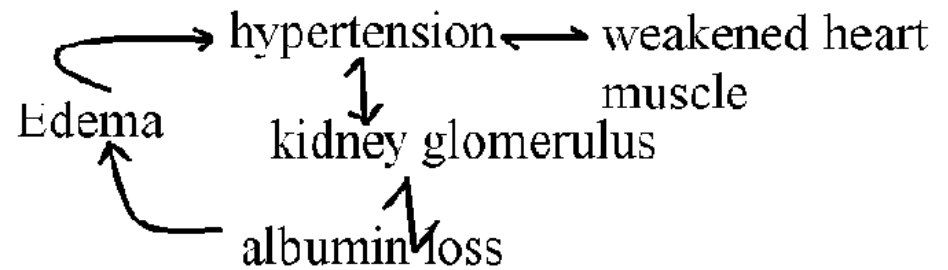
CONGESTIVE HEART FAILURE

- $P_1 > P_2$
- Water and salts diffuse out the capillary
- Albumin creates an osmotic force which results in reabsorption of water and electrolytes.



CONGESTIVE HEART FAILURE

- Congestive heart failure is cycle of hypertension, weakening of cardiac muscle, kidney damage and edema.
- Hypertension can lead to loss of albumin from the kidney
- Less plasma albumin
- Less osmotic force at capillaries gives edema
- Edema increases hypertension
- Hypertension increases weakening cardiac muscle which reciprocally increases hypertension

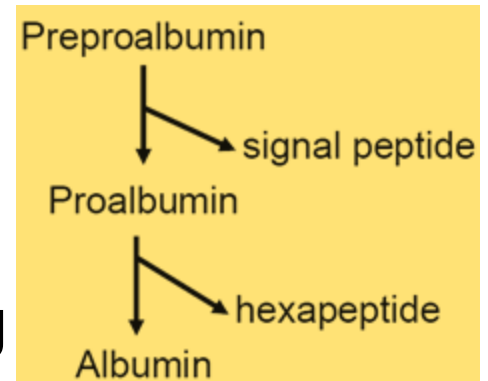


Acute-phase proteins

- Levels increase (0.5-1000 folds), acute inflammation, tissue damage, chronic inflammation & cancer. C-reactive protein (CRP), α_1 -antitrypsin, haptoglobin, & fibrinogen
- Negative acute phase proteins: prealbumin, albumin, transferrin

Albumin

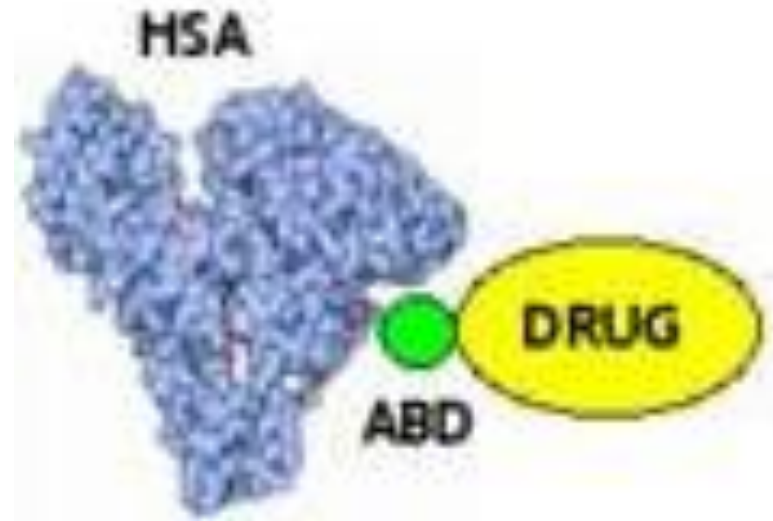
- The Major Protein in Human Plasma, 69 kDa, half-life (20 days)
- The main contributor to the osmotic pressure (75-80%)
- It plays a predominant role in maintaining blood volume and body fluid distribution.
- Liver: 12 g/day (25% of total protein synthesis) (liver function test)
- Synthesized as a preproprotein
- One polypeptide chain, 585 amino acids, 17 disulfide bonds
- Proteases subdivide albumin into 3 domains
- Ellipsoidal shape (viscosity) vs. fibrinogen



Albumin binding capacity

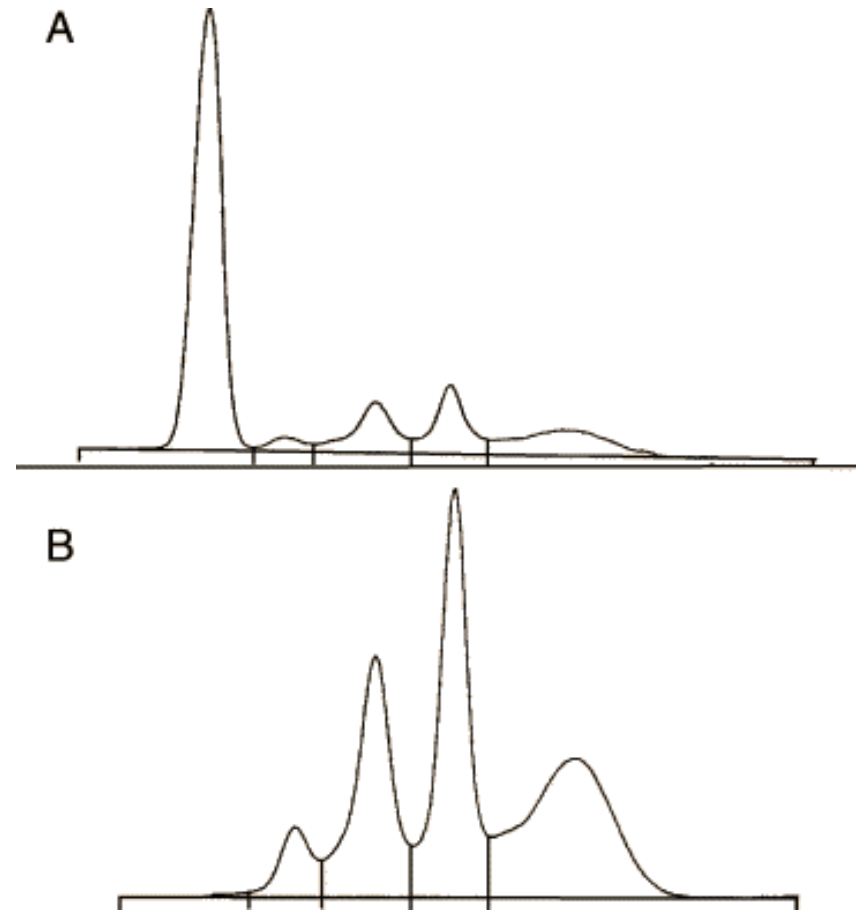
➤ binds various ligands:

- ✓ Free fatty acids (FFA)
- ✓ Certain steroid hormones
- ✓ Bilirubin
- ✓ Plasma tryptophan
- ✓ Metals: Calcium, copper and heavy metals
- ✓ Drugs: sulfonamides, penicillin G, dicumarol, aspirin (drug-drug interaction)



Analbuminemia

- There are human cases of analbuminemia (rare)
- Autosomal recessive inheritance
- One of the causes: a mutation that affects splicing
- Patients show moderate edema!!!



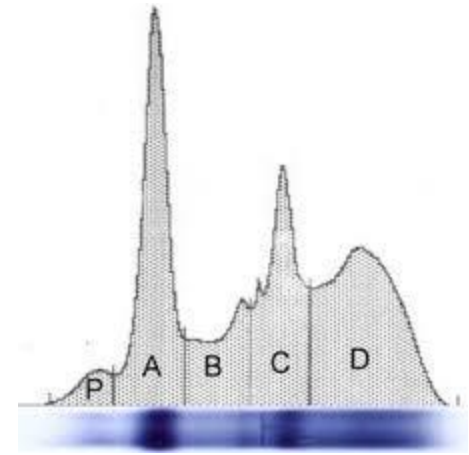
Other clinical disorders

- **Hypoalbuminemia: edema seen in conditions where albumin level in blood is less than 2 g/dl**
 - ✓ Malnutrition (generalised edema)
 - ✓ Nephrotic syndrome
 - ✓ Cirrhosis (mainly ascites)
 - ✓ Gastrointestinal loss of proteins
- **Hyperalbuminemia: dehydration (relative increase)**



Prealbumin (transthyretin)

- Migrates ahead of albumin, 62 kDa
- It is a small glycoprotein (rich in tryptophan, 0.5% carbohydrates)
- Blood level is low (0.25 g/L)
- It has short half-life (≈ 2 days): sensitive indicator of disease or poor protein nutrition
- Main function:
 - ✓ T₄ (Thyroxine) and T₃ carrier



Globulins

α 1-globulins	α 2- globulins	β - globulins	γ -globulins
<ul style="list-style-type: none">■ α1-antitrypsin■ α1-fetoprotein■ α1- acid glycoprotein■ Retinol binding protein	<ul style="list-style-type: none">■ Ceruloplasmin■ Haptoglobin■ α2-macroglobulin	<ul style="list-style-type: none">■ CRP■ Transferrin■ Hemopexin■ β2- microglobulin	<ul style="list-style-type: none">■ IGG■ IGA■ IGM■ IGD■ IGE

α_1 - antitrypsin

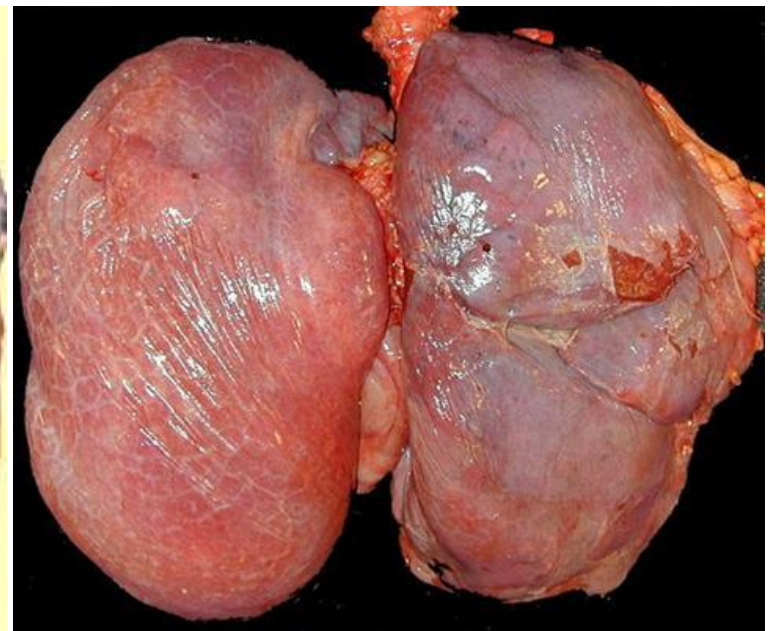
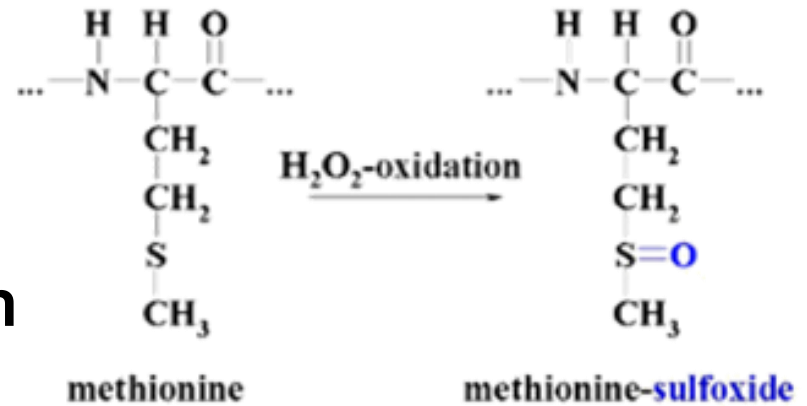
- α_1 -Antiproteinase (52 kDa)
- Neutralizes trypsin & trypsin-like enzymes (elastase)
- 90% of α_1 - globulin band
- Many polymorphic forms (at least 75)
- Alleles Pi^M, Pi^S, Pi^Z, Pi^F (MM is the most common)
- Deficiency (genetic): emphysema (ZZ, SZ). MS, MZ usually not affected
- Increased level of α_1 - antitrypsin (acute phase response)

Active elastase + α_1 -AT → Inactive elastase: α_1 -AT complex → No proteolysis of lung → No tissue damage

Active elastase + ↓ or no α_1 -AT → Active elastase → Proteolysis of lung → Tissue damage

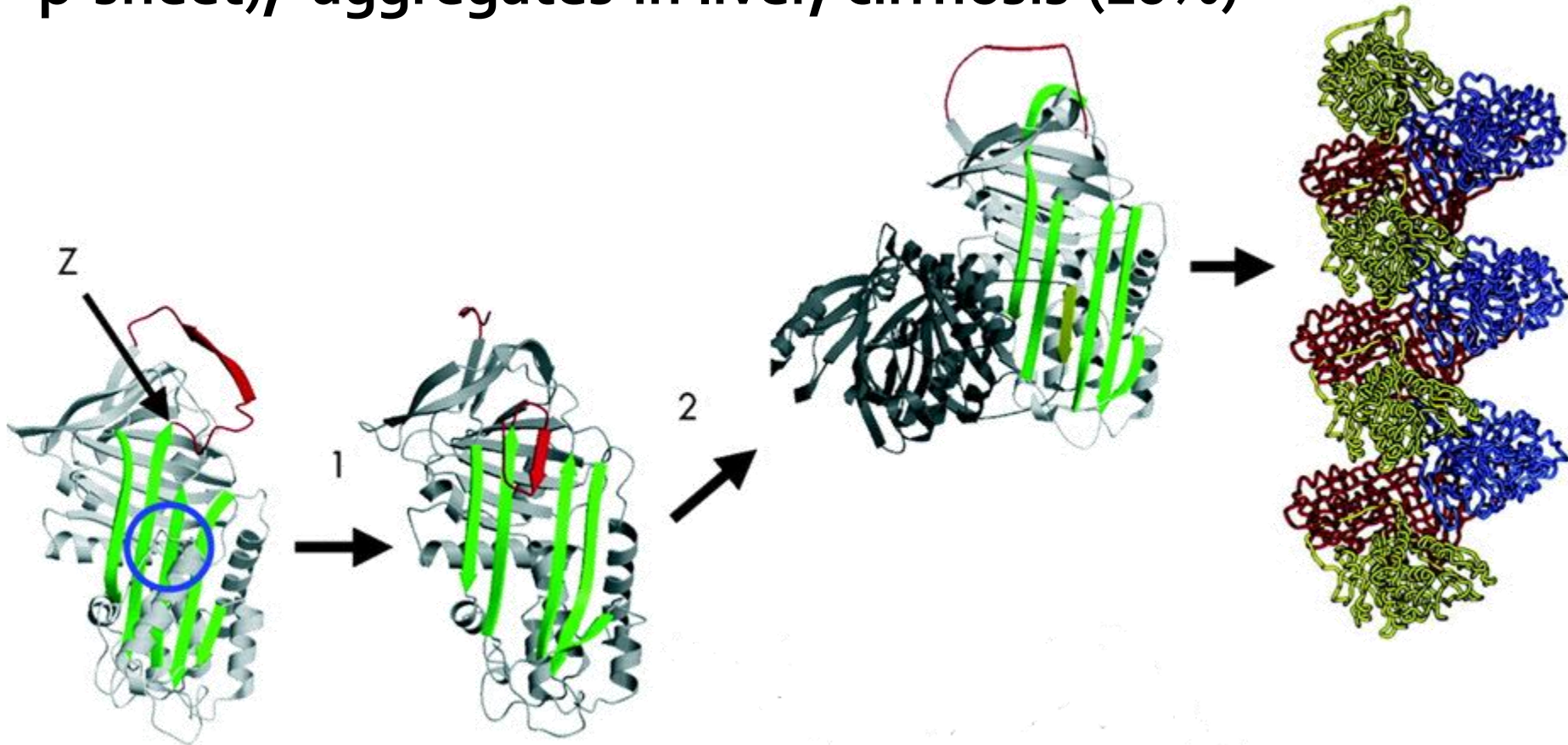
Smoking & α_1 -antitrypsin deficiency

- Chronic inflammation (neutrophil elastase)
- Oxidation of Met³⁵⁸
- devastating in patients with *PiZZ*



Liver disease & α_1 -antitrypsin deficiency

- Liver disease: ZZ phenotype polymerization (loop with β -sheet), aggregates in liver, cirrhosis (10%)



α_1 -fetoprotein

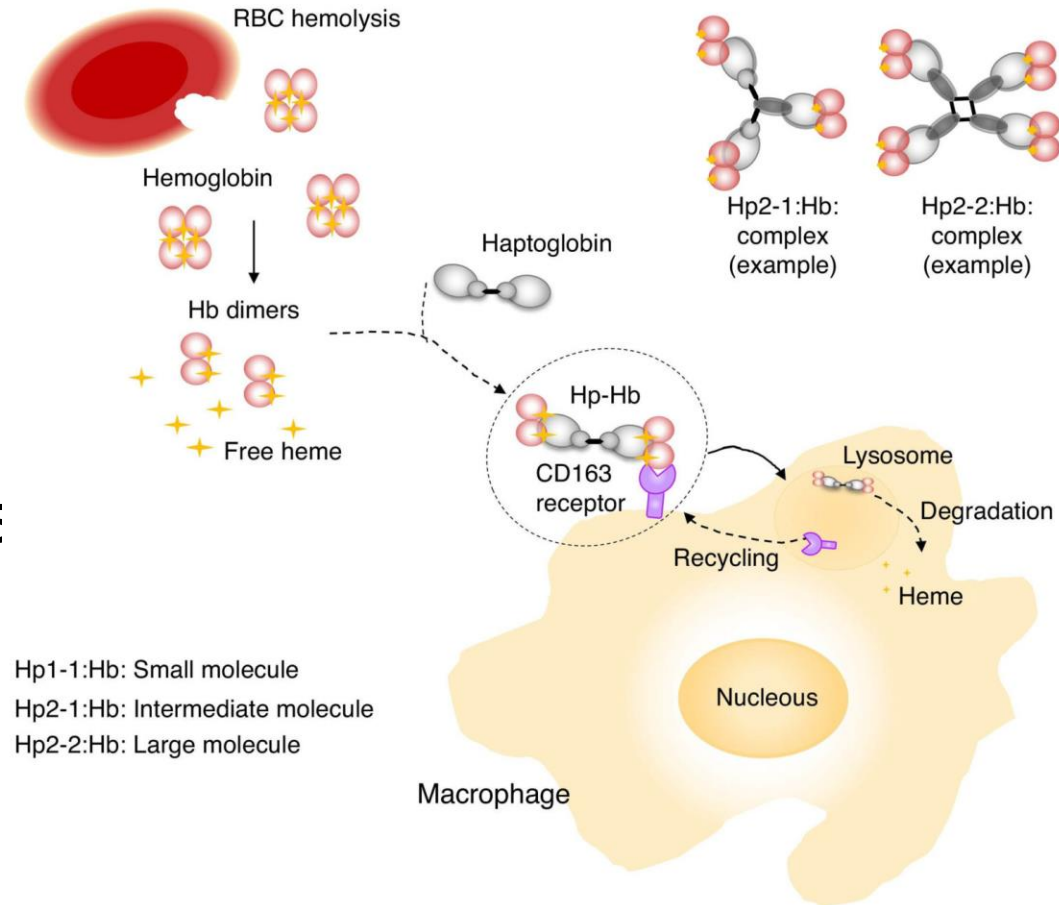
- Synthesized primarily by the fetal yolk sac and then by liver parenchymal cells
- Very low levels in adult
- Functions of α_1 -fetoprotein:
 - ✓ Protect the fetus from immunolytic attacks
 - ✓ Modulates the growth of the fetus
 - ✓ Transport compounds e.g. steroids
 - ✓ Low level: increased risk of Down's syndrome
- Level of α_1 -fetoprotein increases in:
 - ✓ Fetus and pregnant women Normally
 - ✓ Hepatoma & acute hepatitis

Orosomucoid/ alpha-1-acid glycoprotein

- ❑ Concentration in plasma– 0.6 to 1.4 G/dl
- ❑ Carbohydrate content 41%
- ❑ Marker of acute inflammation
- ❑ Acts as a transporter of progesterone
- ❑ Transports carbohydrates to the site of tissue injury
- ❑ Concentration increases in inflammatory diseases, cirrhosis of liver and in malignant conditions
- ❑ Concentration decreases in liver diseases, malnutrition and in nephrotic syndrome

Haptoglobin (HP)

- It is an acute phase reactant protein
- α_2 glycoprotein (90kDa)
- A tetramer ($2\alpha, 2\beta$)
- 3 phenotypes:
 - ✓ Hp 1-1 $\rightarrow \alpha_1, \alpha_1 + 2\beta$
 - ✓ Hp 2-1 $\rightarrow \alpha_1, \alpha_2 + 2\beta$
 - ✓ Hp 2-2 $\rightarrow \alpha_2, \alpha_2 + 2\beta$
- Binds the free hemoglobin (65 kDa); prevents loss of hemoglobin & its iron into urine
- Hb-Hp complex has shorter half-life (90 min) than that of Hp (5 days)
- Decreased level in hemolytic anemia

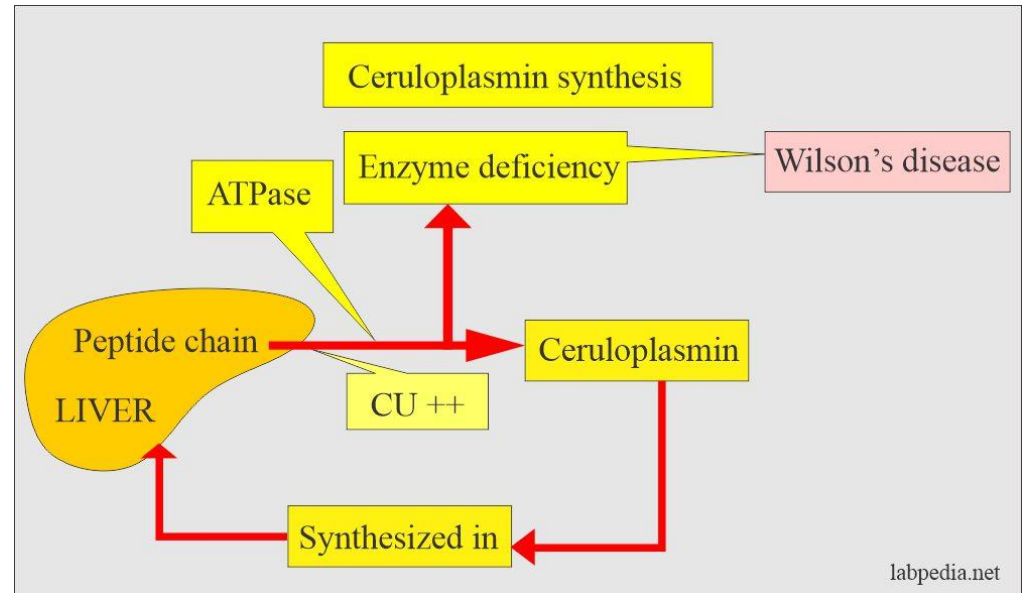


Ceruloplasmin

- A copper containing glycoprotein (160 kDa)
- It contains 6 atoms of copper
- Metallothioneins (regulate tissue level of Cu)
- Regulates copper level: contains 90% of serum Cu
- A ferroxidase: oxidizes ferrous to ferric (transferrin)
- Albumin (10%) is more important in transport
- Decreased levels in liver disease (ex. Wilson's, autosomal recessive genetic disease)

Cu-containing enzymes

- Amine oxidase
- Copper-dependent superoxide dismutase
- Cytochrome oxidase
- Tyrosinase



Alpha- 2 Macroglobulin

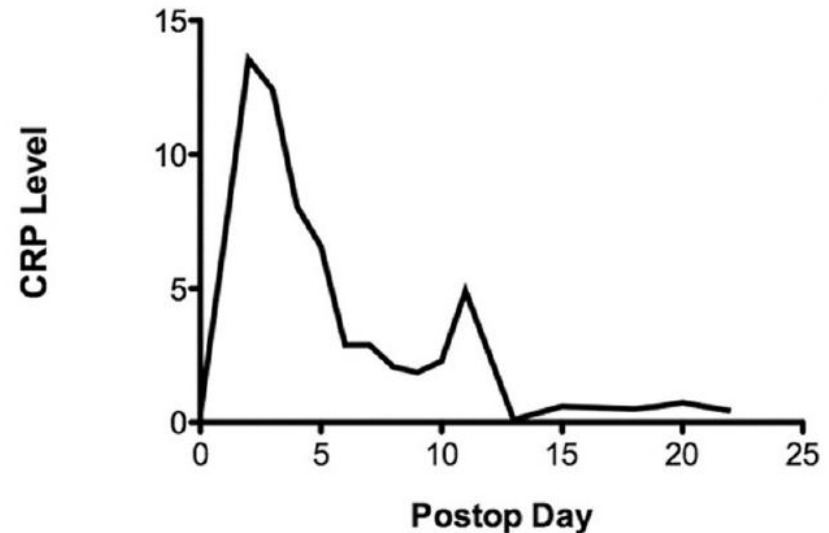
- Comprises **8-10% of the total plasma protein** in humans.
- Synthesized by **hepatocytes and macrophages**
- Inactivates all the proteases and thus is an important in vivo anticoagulant.
- Carrier of many growth factors
- **Normal serum level-130-300 mg/dl**
- **Concentration is markedly increased in nephrotic syndrome, since other proteins are lost through urine in this condition.**

Haemopexin(β -globulin)

- 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

C- reactive protein (CRP)

- Able to bind to a polysaccharide (fraction C) in the cell wall of pneumococci
- Help in the defense against bacteria and foreign substances



- Undetectable in healthy individuals, detectable in many inflammatory diseases (Acute rheumatic fever, bacterial infection, gout, etc.) & Tissue damage
- Its level reaches a peak after 48 hours of incident (monitoring marker)

Complement C1 q (β -globulin)

- **First complement factor to bind antibody**
- Binding triggers the classical complement pathway
- Thermo labile, destroyed by heating
- **Normal level – 0.15 gm/L**
- Decreased level is used as an indicator of circulating Ag – Ab complex.
- High levels are found in chronic infections

γ -Gamma-globulin

- 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

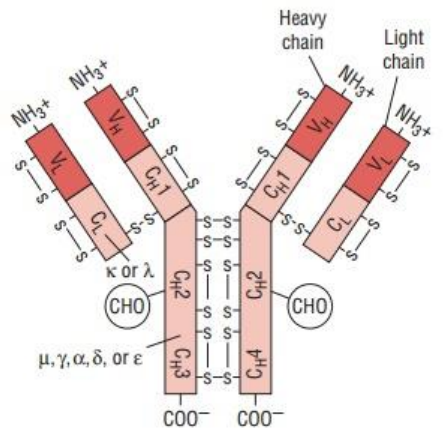
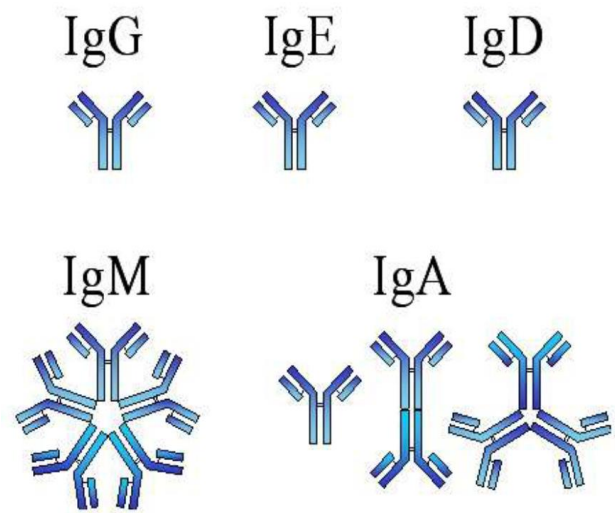


FIG. 13-1. Schematic diagram of monomer of the immunoglobulin.



Immunoglobulin Major Functions

Immunoglobulin

Major Functions

IgG `

Main antibody in the secondary response. Opsonizes bacteria, 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
Does

Produced in the primary response to an antigen. Fixes complement. not cross the placenta. Antigen receptor on the surface of B cells.

IgD

Uncertain. Found on the surface of many B cells as well as in serum.

IgE

Mediates immediate hypersensitivity Defends against worm infections.

Fibrinogen

- ❑ Also called **clotting factor 1**
- ❑ Constitutes 4–6% of total protein
- ❑ Highly elongated with axial ratio of 20:1
- ❑ Imparts **maximum viscosity to blood**
- ❑ Synthesized in liver
- ❑ Made up of 6 polypeptide chains
- ❑ Chains are linked together by S–S linkages
- ❑ 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

<u>Name</u>	<u>Compounds transported</u>
Albumin heavy	Fatty acids, bilirubin, hormones, calcium, 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)

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

Negative Acute Phase Proteins

The levels of certain proteins are decreased in blood in response to certain inflammatory processes.

Examples–

- ❑ Albumin
- ❑ Transthyretin
- ❑ Retinol binding protein
- ❑ Transferrin

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
- 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

Hypogammaglobulinemia

- **Losses from body**– same as albumin– through urine, GIT or skin
- Decreased synthesis
- Primary genetic deficiency
- **Secondary** – drug induced (Corticosteroid therapy), uremia, hematological disorders
- AIDS(Acquired Immuno deficiency syndrome)

Hypergammaglobulinemia

1) Polyclonal-

- ❑ Chronic infections
- ❑ Chronic liver diseases
- ❑ Sarcoidosis
- ❑ Auto immune diseases

2) Monoclonal

- ❑ Multiple myeloma
- ❑ Macroglobulinaemia
- ❑ Lymphosarcoma
- ❑ Leukemia
- ❑ Hodgkin's disease