Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

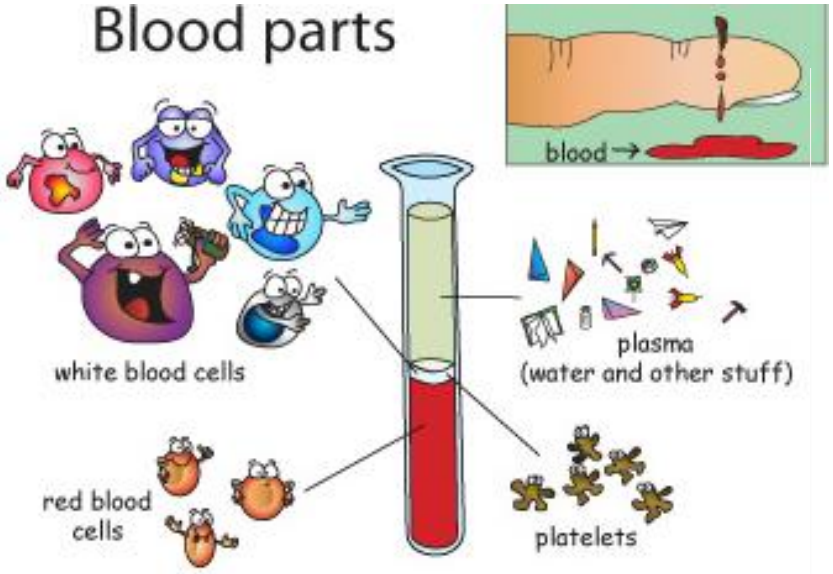
Plasma Proteins (1,2)

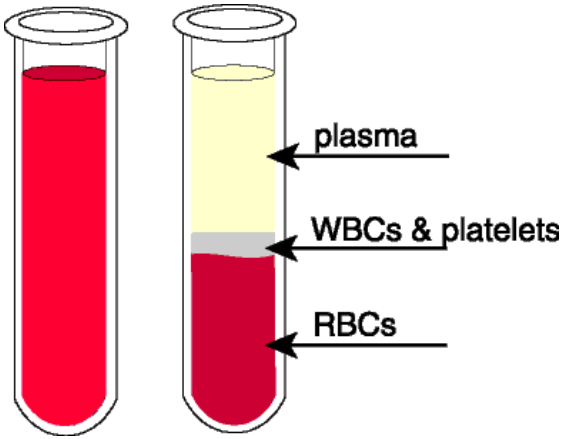
What should we know?

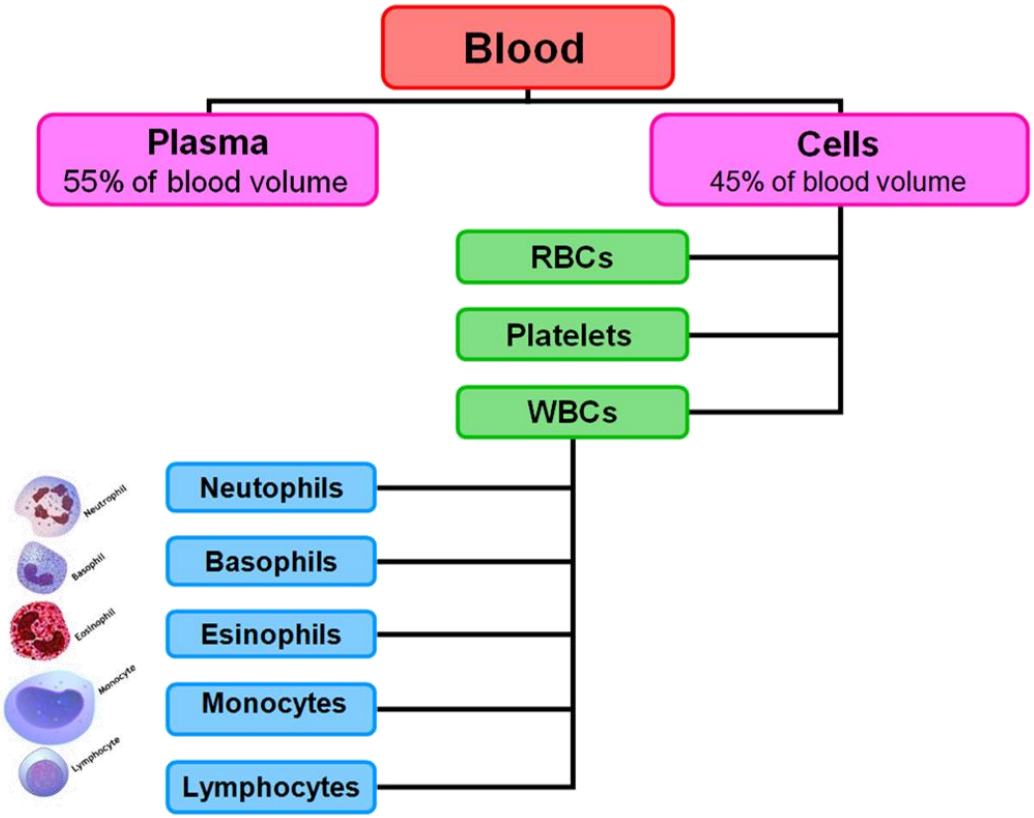
1. What is plasma, and how can we get it?
2. What are the different components of plasma?
3. Plasma proteins (general functions, basis of classification, associated processes and molecules)
4. Different types:
   - Albumin & prealbumin
   - α1-antitrypsin
   - Haptoglobin (Hp)
   - α1-fetoprotein (AFP)
   - α2-Macroglobulin
   - Ceruloplasmin
   - C-Reacrive Protein

(structure, synthesis, function & diseases associated)

Blood

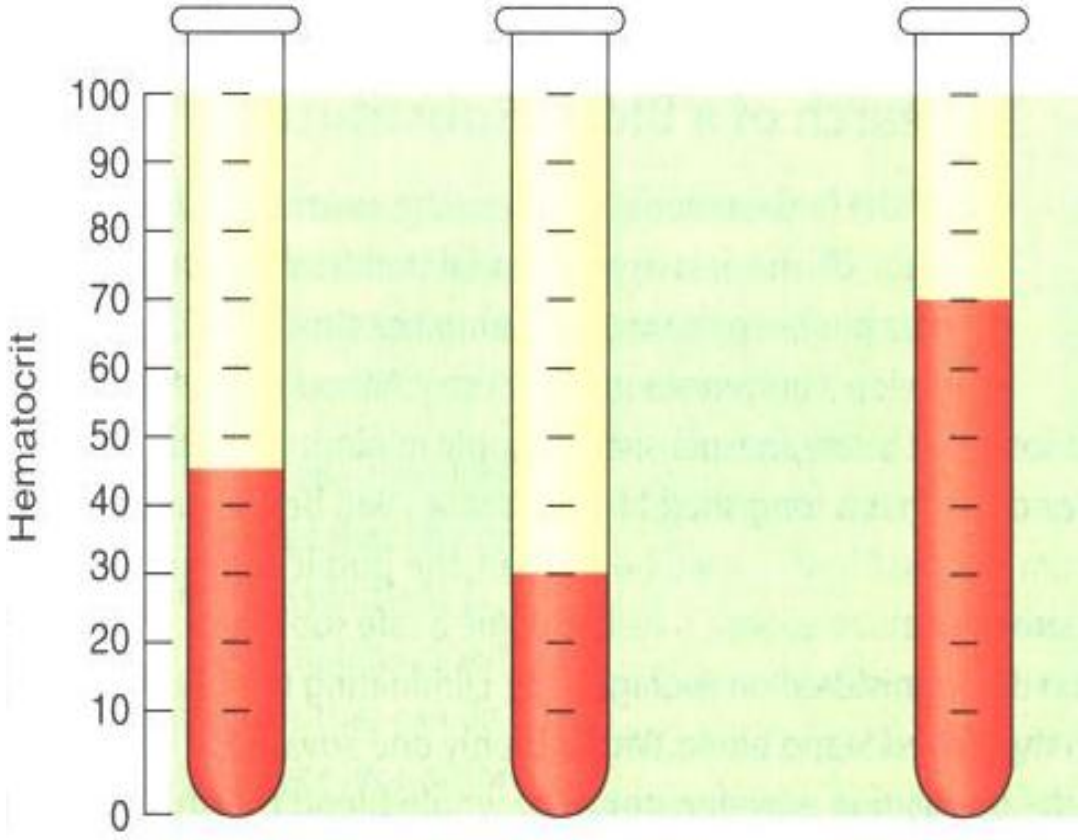

Blood parts
blood
white blood cells
plasma
(water and other stuff)
red blood cells
platelets


plasma
WBCs & platelets
RBCs

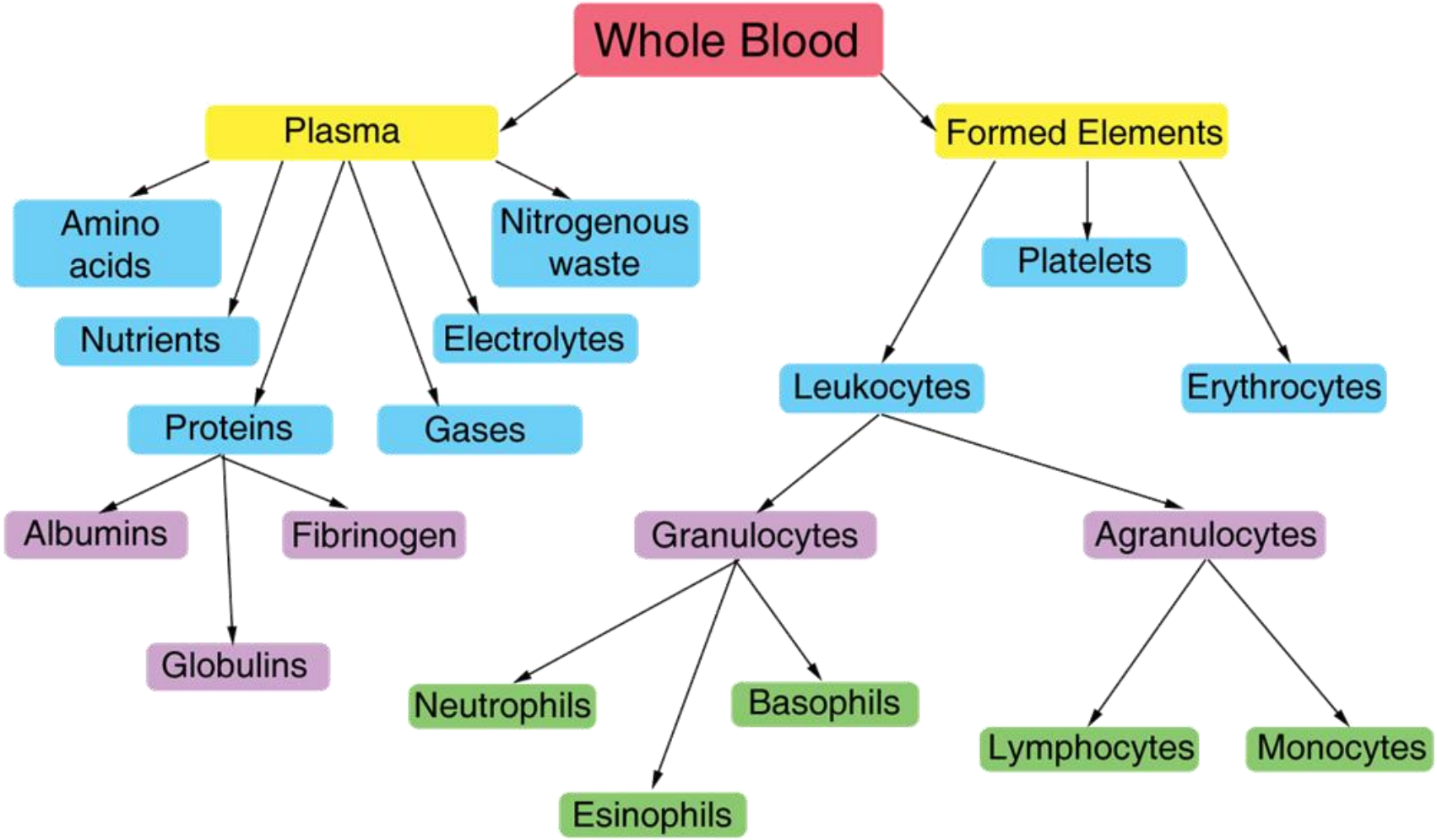

Blood
Plasma
55% of blood volume
Cells
45% of blood volume
RBCs
Platelets
WBCs
Neutophils
Basophils
Esinophils
Monocytes
Lymphocytes

Blood: plasma vs. hematocrit

- Hematocrit or packed cell volume (Adult male: 47 %, Adult females: 42 %)



Blood: plasma vs. cells

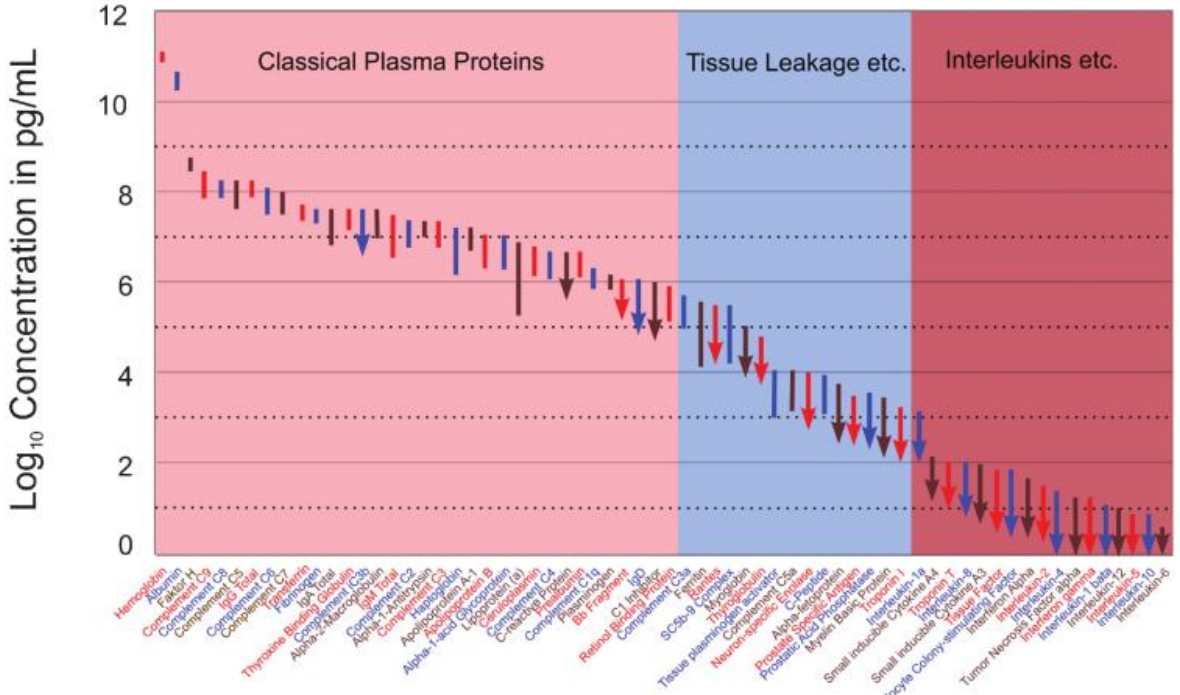


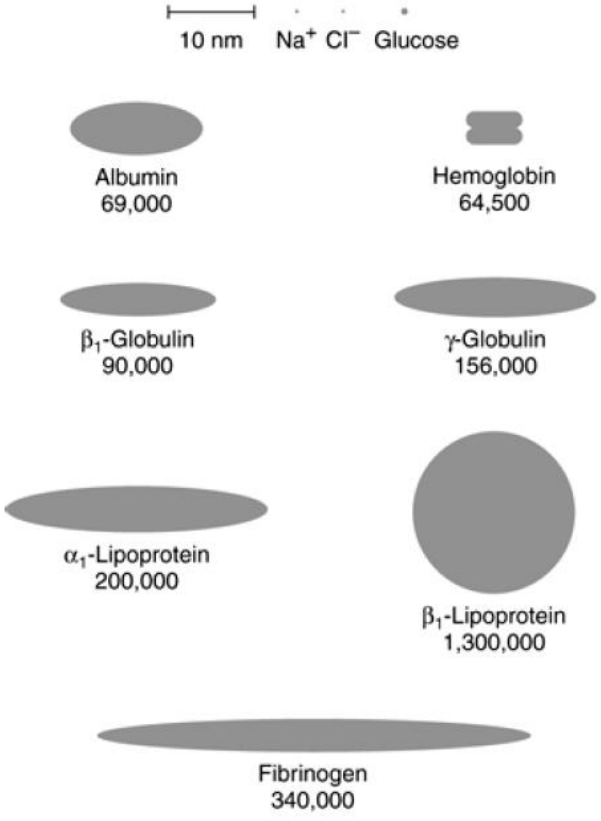
Plasma

- Is the liquid medium in which blood 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-}

Plasma proteins are a mixture

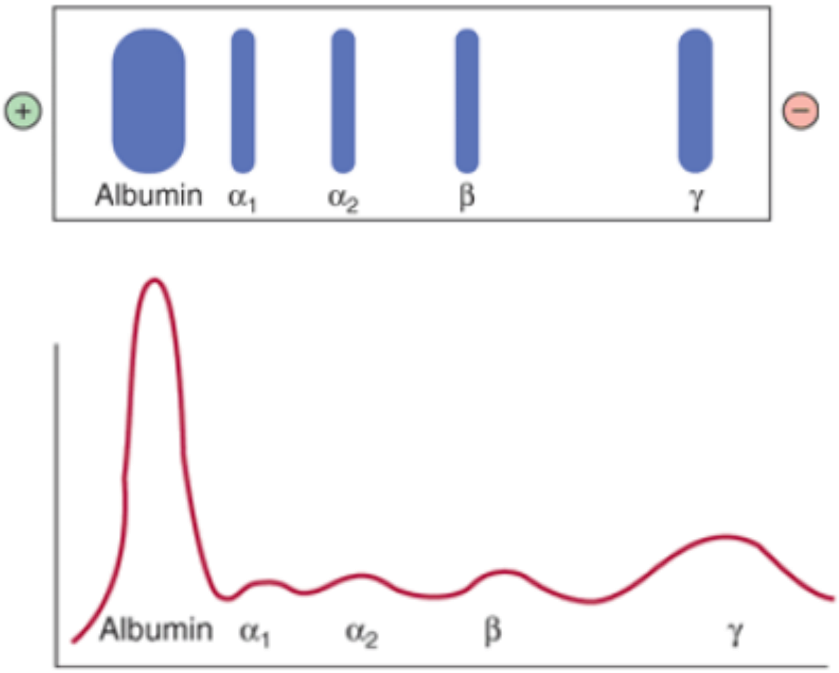
- More than 500 plasma proteins have been identified
- Normal range 6-8 g/dl (the major of the solids)
- Simple & conjugated proteins (glycoproteins & lipoproteins)





The separation of plasma proteins

- Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins
- Electrophoresis (most common): serum (defebinated plasma), five bands (albumin, α1 , α2 , β, and γ)

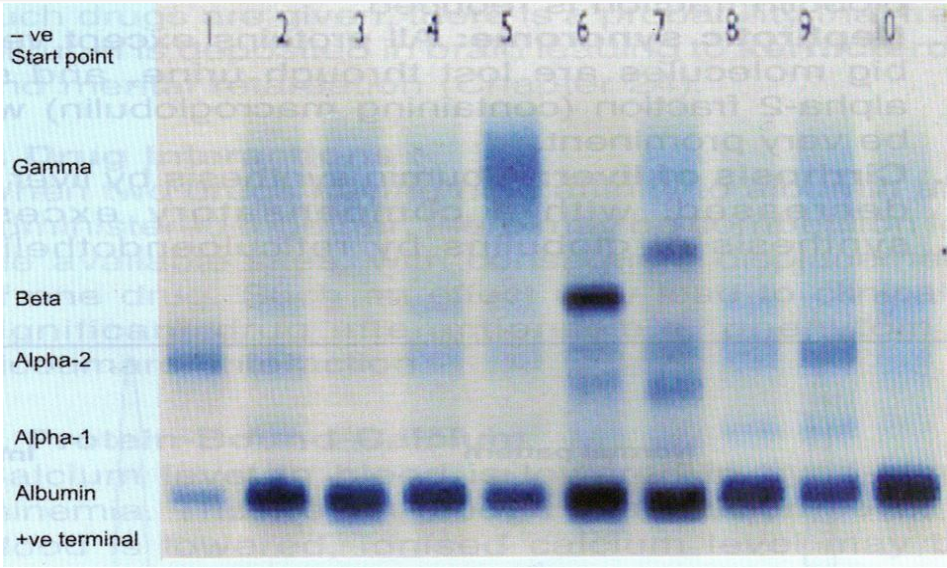


NORMAL VALUES:

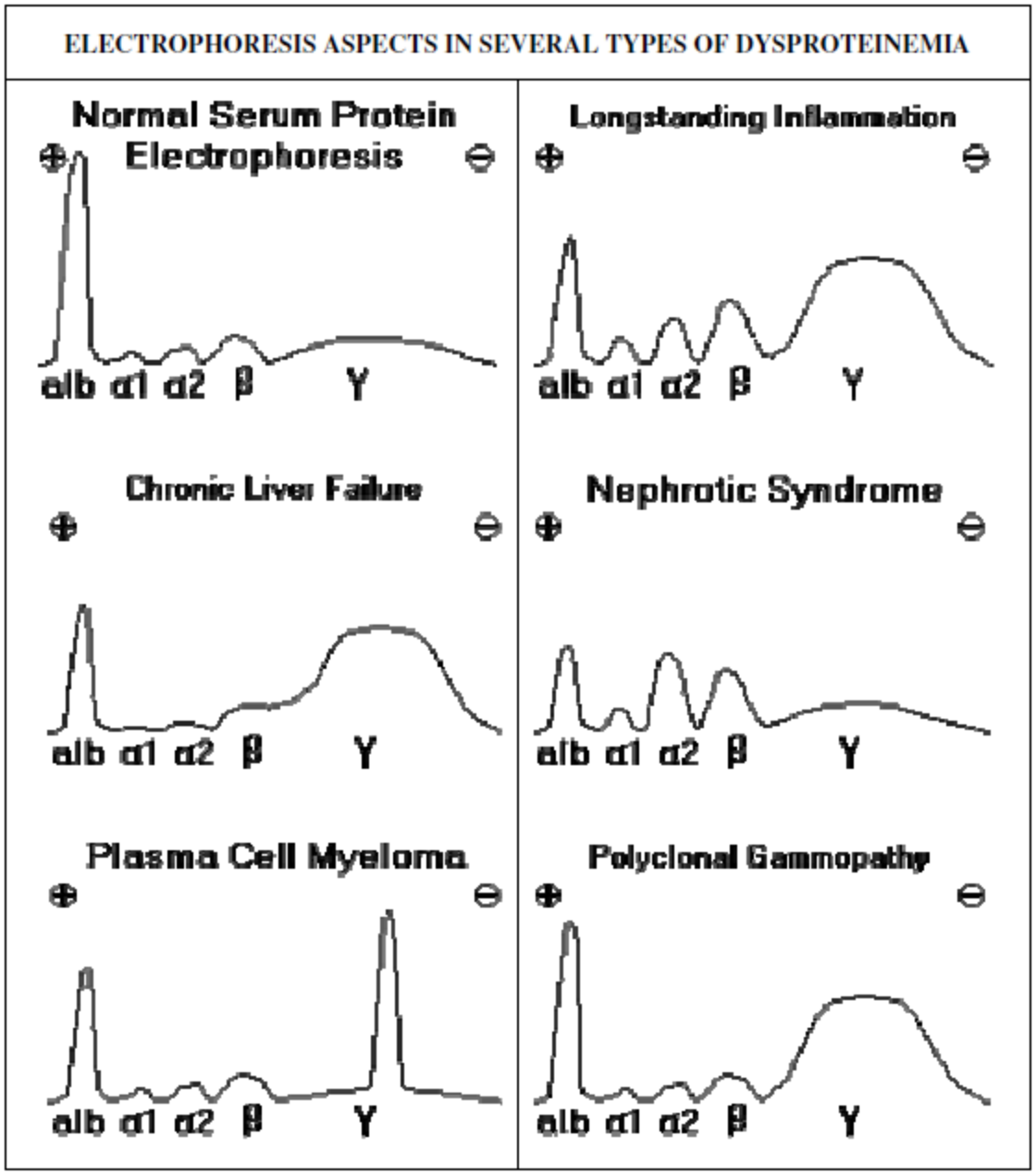
Name	Absolute values (g/l)	Relative values (%)
Albumins	35 – 55	50 – 60
α1-globulins	2 – 4	4.2 – 7.2
α2-globulins	5 – 9	6.8 – 12
β-globulins	6 – 11	9.3 – 15
γ-globulins	7 – 17	13 – 23

Electrophoresis of plasma proteins

- Albumin is smaller than globulin, and slightly negatively charged
- Globulins (3 bands):
- α band:
  - ✓ α1 region consists mostly of α1-antitrypsin
  - ✓ α2 region is mostly haptoglobin, α2-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

Plasma Proteins & Polymorphism

- A mendelian or monogenic trait
- Exists in population in at least two phenotypes, neither is rare
- The ABO blood groups are the best-known examples
- α1-antitrypsin, haptoglobin, transferrin, ceruloplasmin, and immunoglobulins
- Electrophoresis or isoelectric focusing

Plasma Proteins Half-Lives

- Determined through isotope labeling studies (I^{131})
- Albumin & haptoglobin (20 & 5 days)
- Diseases can affect half-lives (ex. Crohn's disease), albumin may be reduced (1 day)
- Protein-losing gastroenteropathy

Functions of plasma proteins

General functions

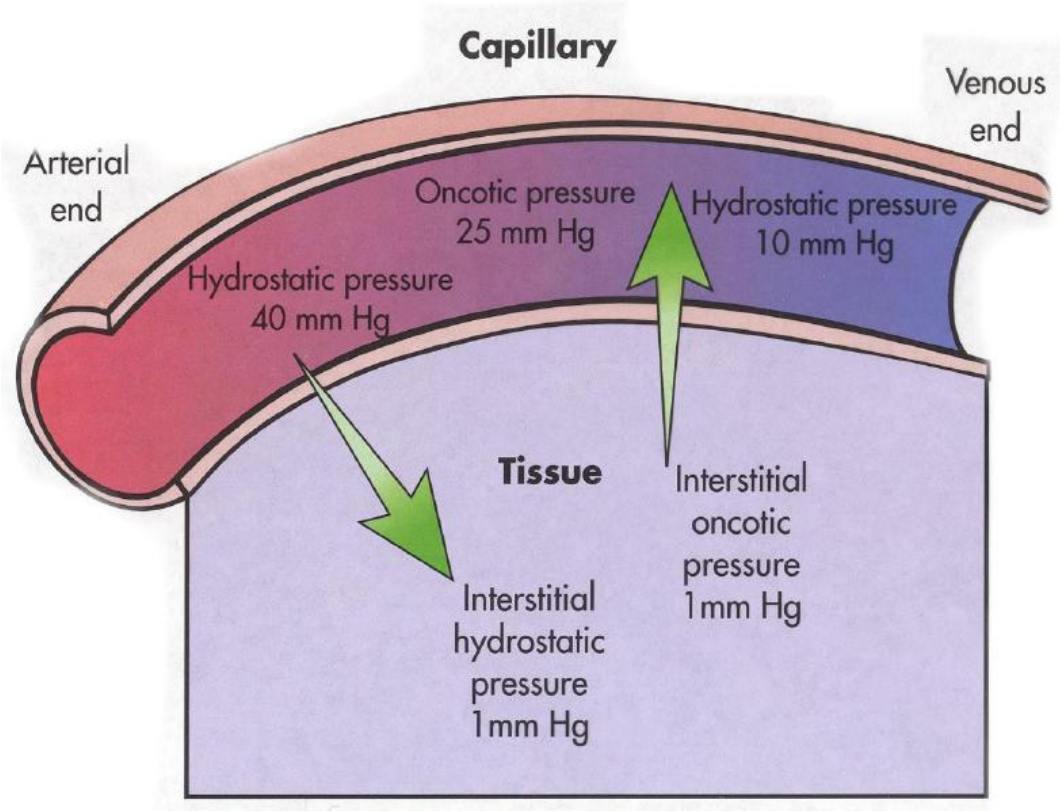
- A nutritive role
- Maintenance of blood pH (amphoteric property)
- Contributes to blood viscosity
- Maintenance of blood osmotic pressure

Specific functions

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

Starling forces

- ✓ Arterioles, venules vs. tissue hydrostatic pressure (37 & 17 vs. 1 mm Hg)
- ✓ Plasma proteins oncotic pressure is 25 mm Hg
- ✓ Edema can be a result of protein deficiency

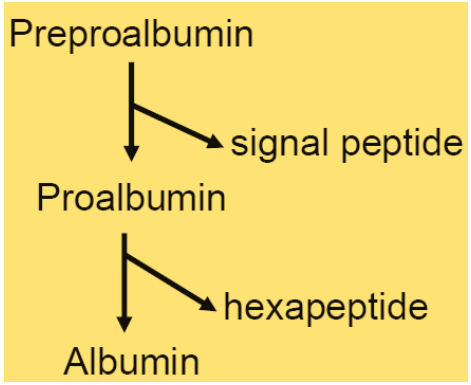


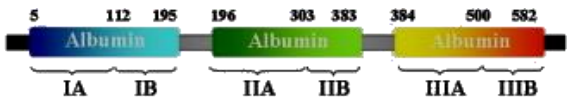
Acute-phase proteins

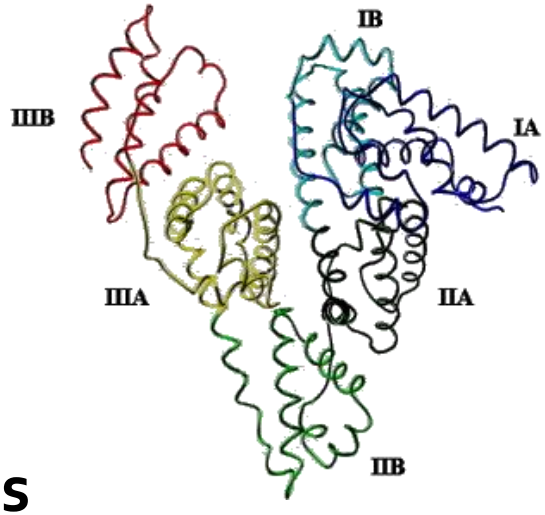
- Levels increase (0.5-1000 folds), acute inflammation, tissue damage, chronic inflammation & cancer. C-reactive protein (CRP), α1 -antitrypsin, haptoglobin, & fibrinogen
- Role in inflammation: CRP (classic complement pathway); CRP (marker of tissue injury, following atherosclerosis); α1-antitrypsin (certain proteases)
- Interleukin-1 (IL-1), main stimulator (gene transcription)
- Nuclear factor kappa-B (NFkB): Exist in an inactive form in cytosol, activated and translocated to nucleus (interleukin-1)
- 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%)
- 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
- Anionic at pH 7.4 with 20 negative charges

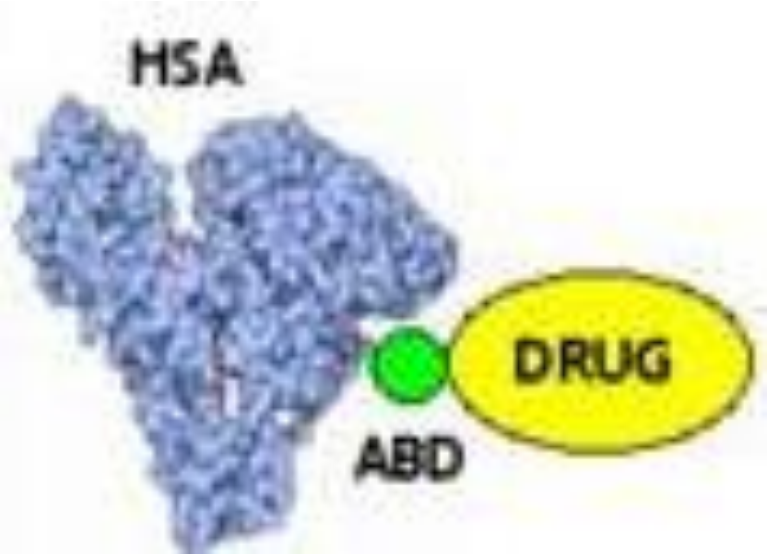






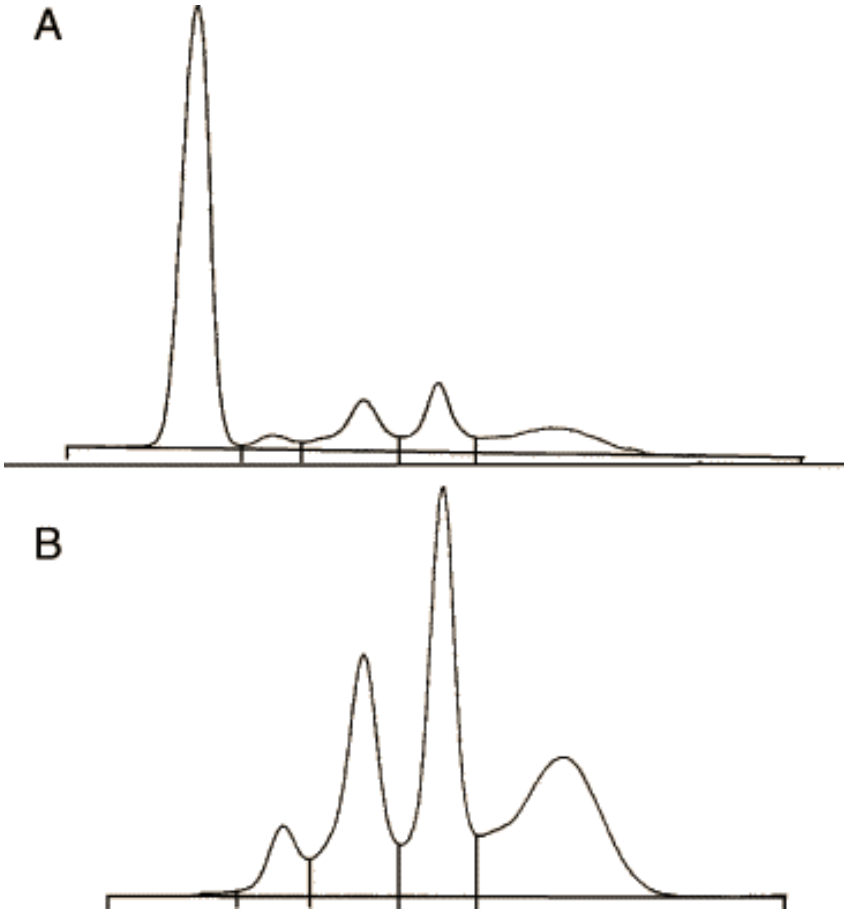
Albumin binding capacity

- 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 inherittance
- One of the causes: a mutation that affects splicing
- Patients show moderate edema!!!

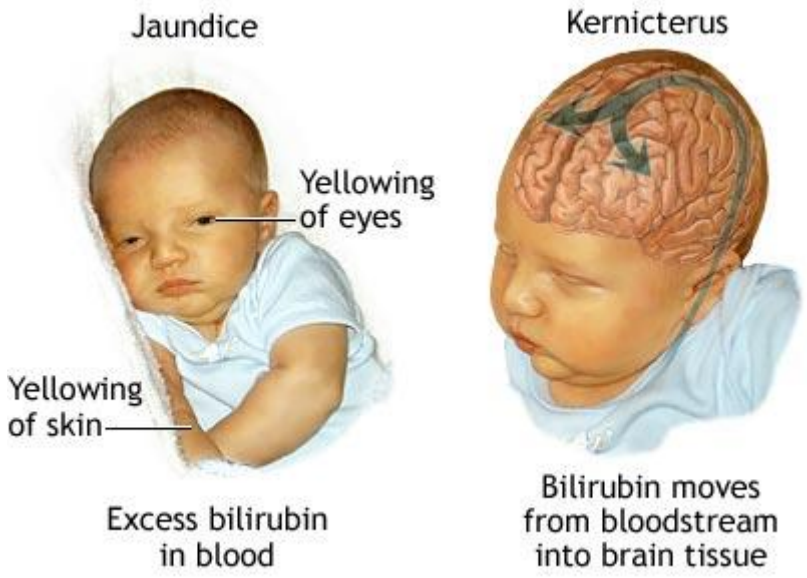


Other clinical disorders

- Hypoalbiminemia: 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
- Hyperalbuminemia: dehydration (relative increase)

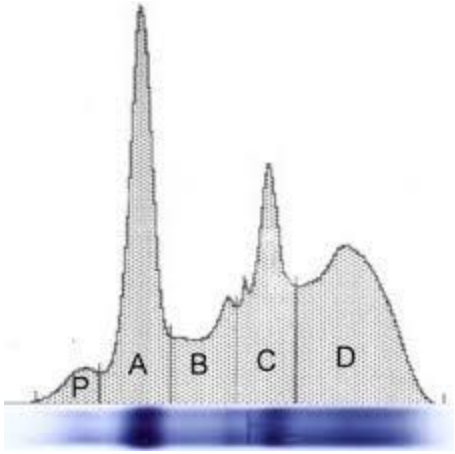
Other clinical disorders

- Drug-drug interaction:
  - ✓ Bilirubin toxicity (aspirin is a competitive ligand): kernicterus and mental retardation
  - ✓ Phenytoin-dicoumarol interaction



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:
  - ✓ T_4 (Thyroxine) and T_3 carrier



Globulins

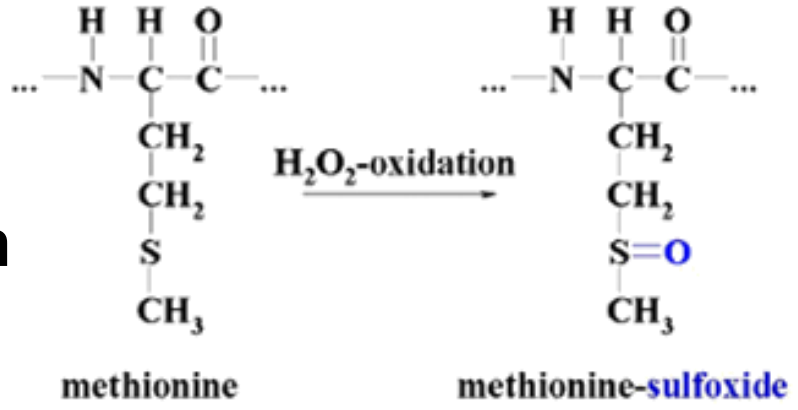
α1-globulins	α2- globulins	β- globulins	γ-globulins
▪ **α1-antitrypsin** ▪ **α1-fetoprotein** ▪ α1- acid glycoprotein ▪ Retinol binding protein	▪ **Ceruloplasmin** ▪ **Haptoglobin** ▪ **α2-macroglobulin**	▪ **CRP** ▪ Transferrin ▪ Hemopexin ▪ β2-microglobulin	▪ IGG ▪ IGA ▪ IGM ▪ IGD ▪ IGE

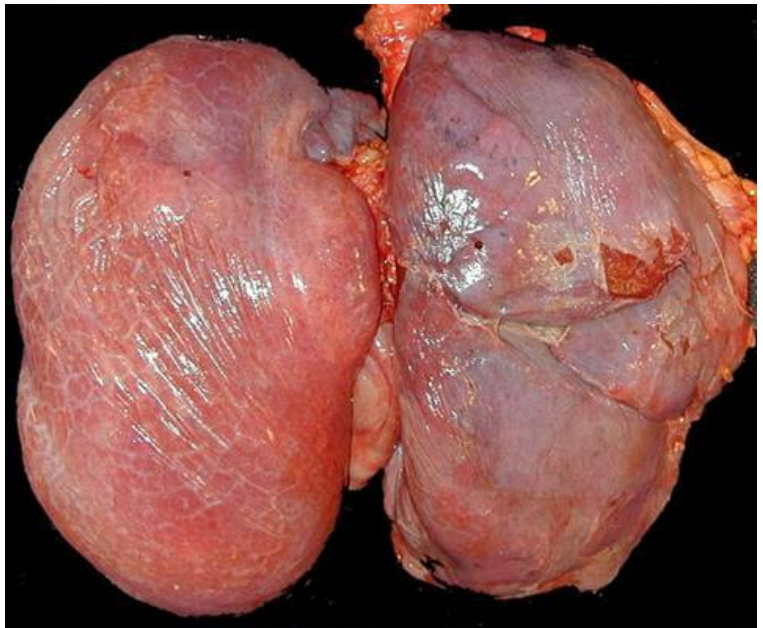
α1- antitrypsin

- α1-Antiproteinase
- Neutralizes trypsin & trypsin-like enzymes (elastase)
- 90% of α1- globulin band
- The principal serine protease inhibitor (Pi)
- 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 α1- antitrypsin (acute phase response)

Smoking & α1- antitrypsin deficiency

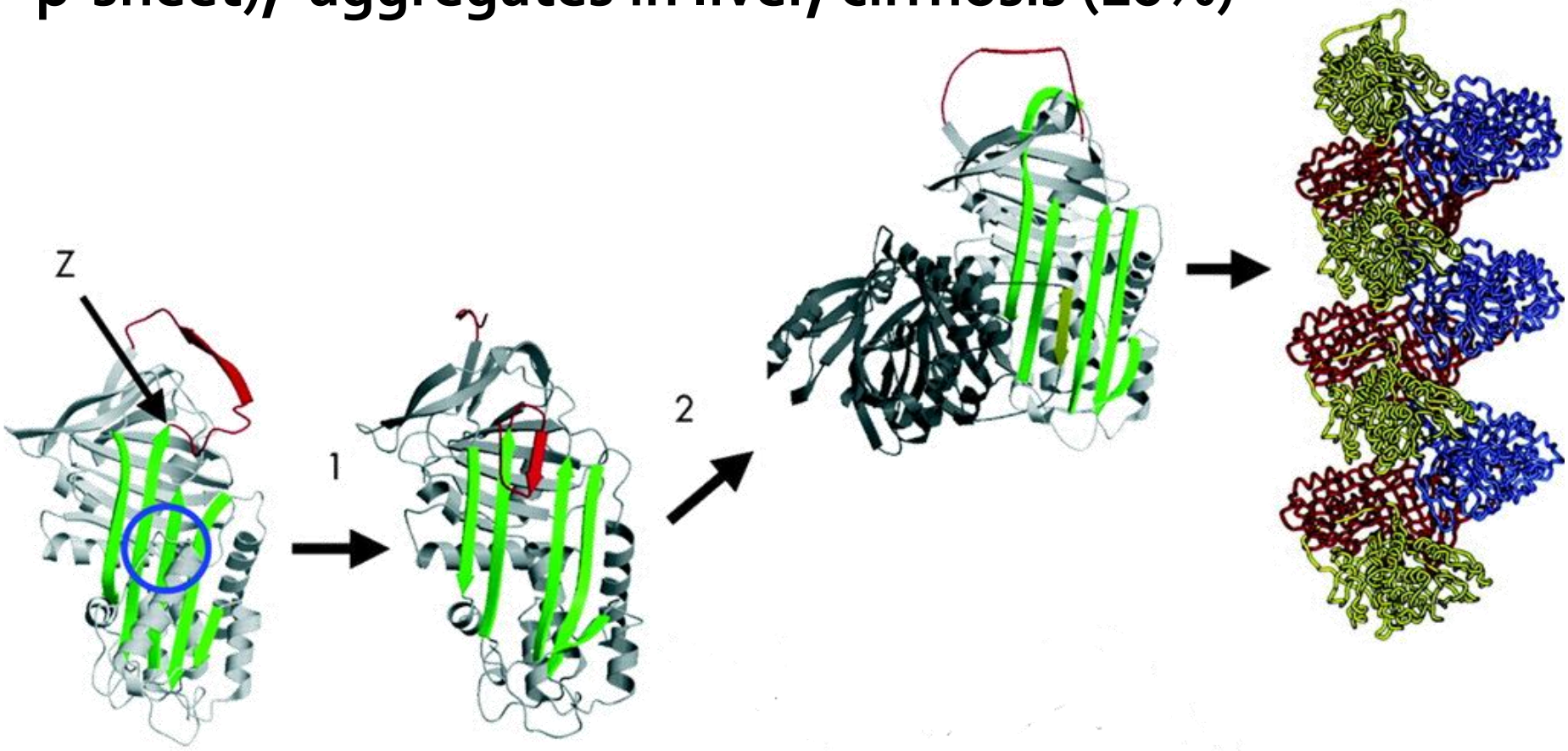
- Chronic inflammation
- Oxidation of Met^{358}
- devastating in patients with *Pi^{ZZ}*





Liver disease & α1- antitrypsin deficiency

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



Haptoglobin (HP)

- It is an acute phase reactant protein
- α2 glycoprotein (90kDa)
- A tetramer (2α, 2β)
- 3 phenotypes:
  - ✓ Hp 1-1→ α1, α1 + 2β
  - ✓ Hp 2-1→ α1, α2 + 2β
  - ✓ Hp 2-2 → α2, α2 + 2β
- 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

α1- fetoprotein

- Synthesized primarily by the fetal yolk sac and then by liver parenchymal cells
- Very low levels in adult
- Functions of α1-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-fetoprotein increases in:
  - ✓ Fetus and pregnant women <u>Normally</u>
  - ✓ Hepatoma & acute hepatitis

Ceruloplasmin

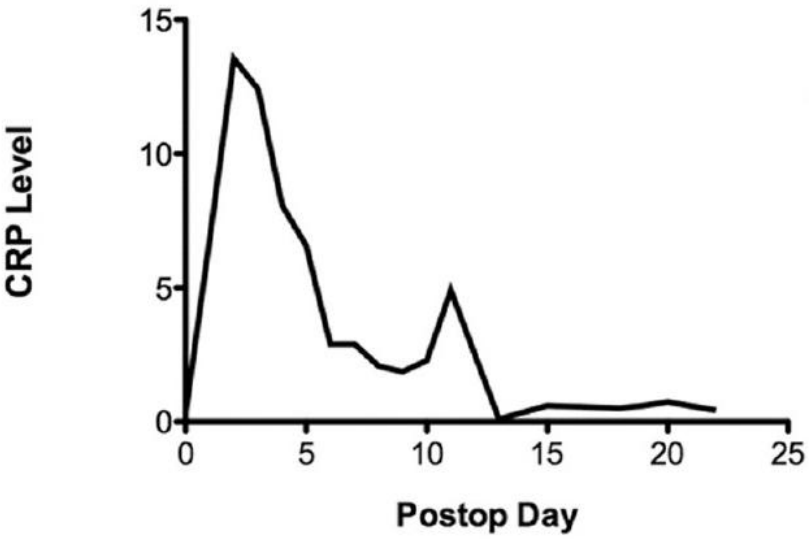
- A copper containing glycoprotein (160 kDa)
- It contains 6 atoms of copper (3 cuprous & 3 cupric)
- Metallothioneins (regulate tissue level of Cu)
- Regulates copper level: contains 90% of serum Cu
- A ferroxidase: oxidizes ferrous to ferric (transferrin)
- Albumin (10%) is more important in transport
- Decreased levels in liver disease (ex. Wilson's, autosomal recessive genetic disease)

α2- macroglobulin

- It is a tetrameric (identical) large protein (720 kDa)
- 8-10 % of the total plasma proteins
- Synthesized by: Monocytes, Hepatocytes, Astrocytes
- Inhibits proteases (trypsin, pepsin, plasmin, thrombin)
- inhibitor of (Coagulation & Fibrinolysis)
- Its level increases in cases of:
  - ✓ Nephrosis, diabetes and liver disease
  - ✓ Pregnancy and contraceptive medication

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)