Plasma Proteins

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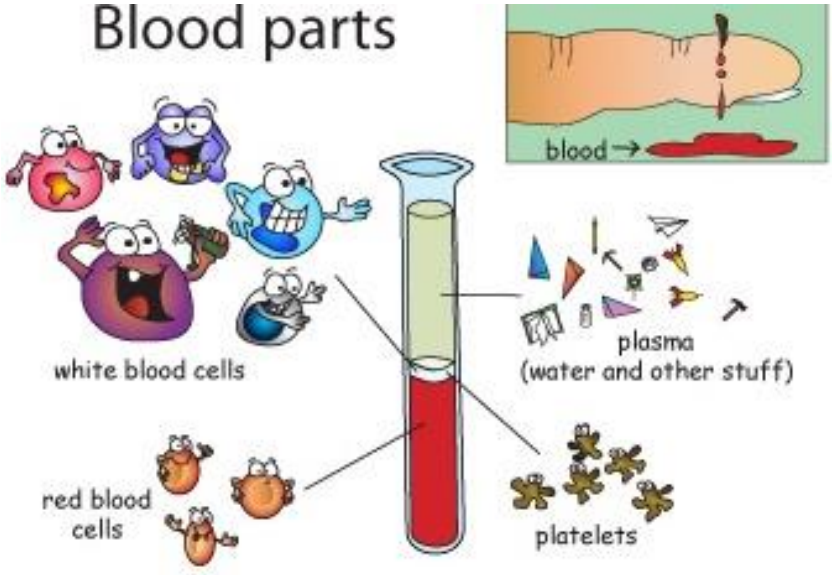
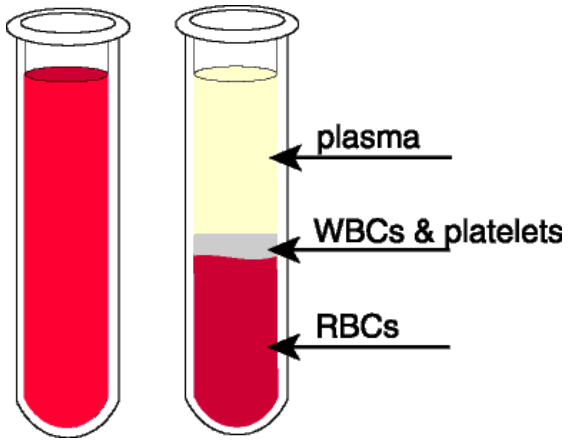
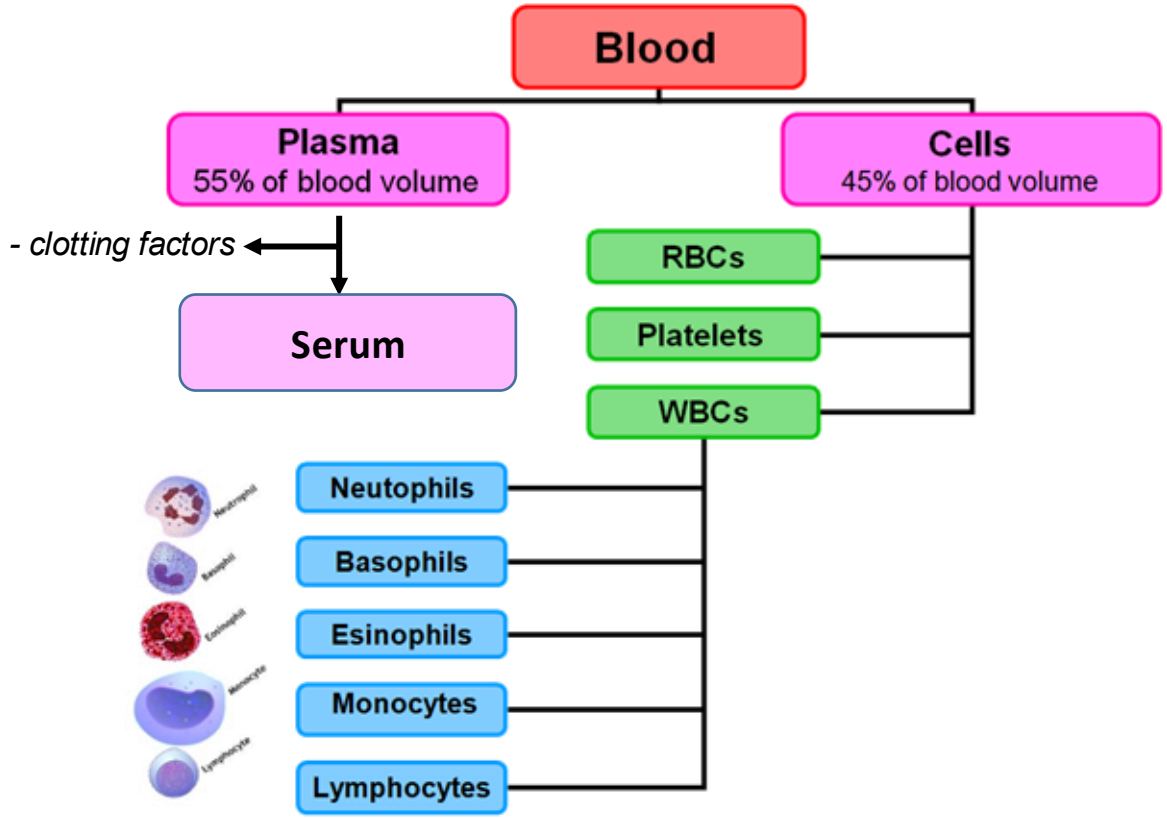
Blood

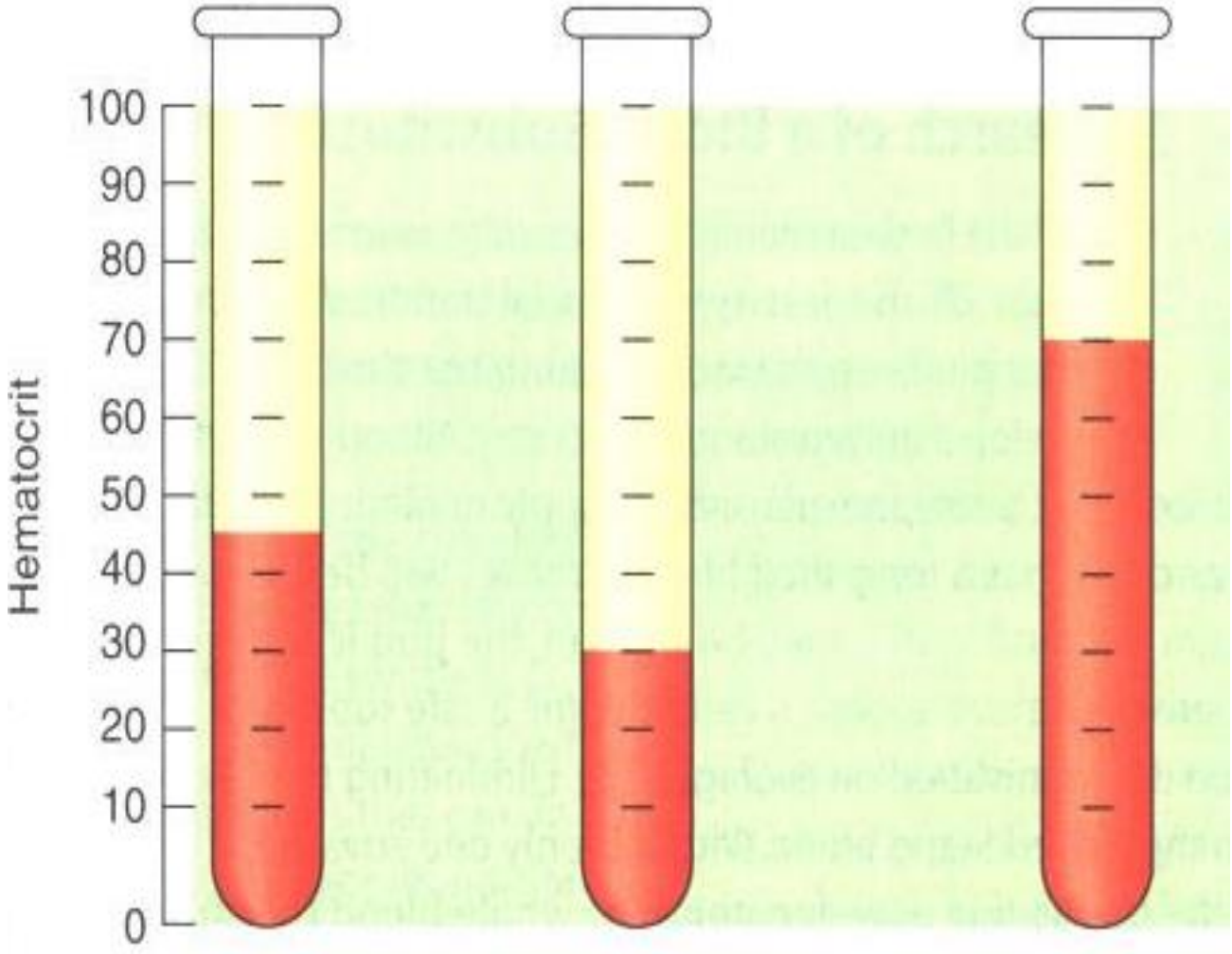

Blood parts
blood
white blood cells
plasma
(water and other stuff)
red blood
cells
platelets


plasma
WBCs & platelets
RBCs

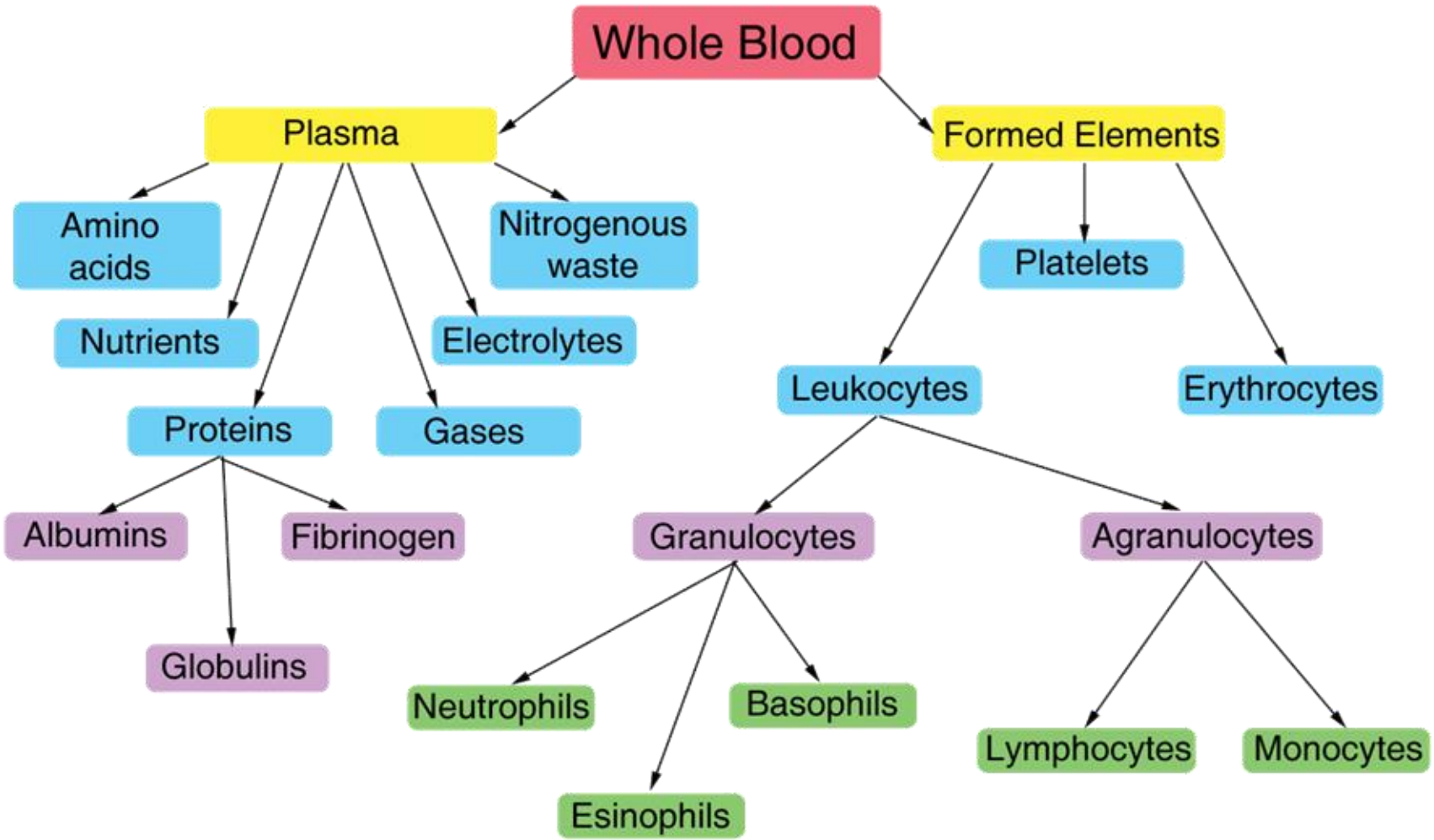

Blood
Plasma
55% of blood volume
Cells
45% of blood volume
- clotting factors
Serum
RBCs
Platelets
WBCs
Neutophils
Basophils
Esinophils
Monocytes
Lymphocytes

Blood: plasma vs. hematocrit

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



Blood: plasma vs. cells

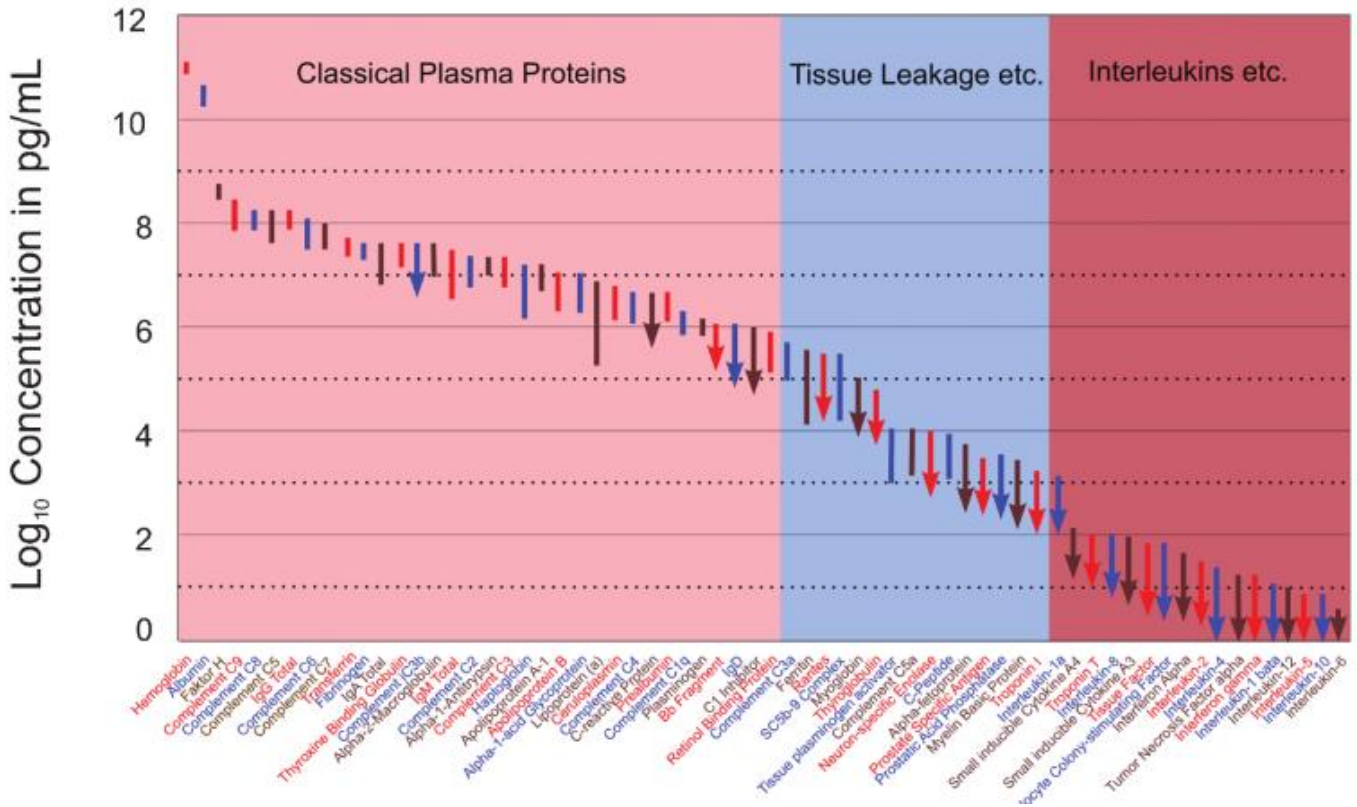


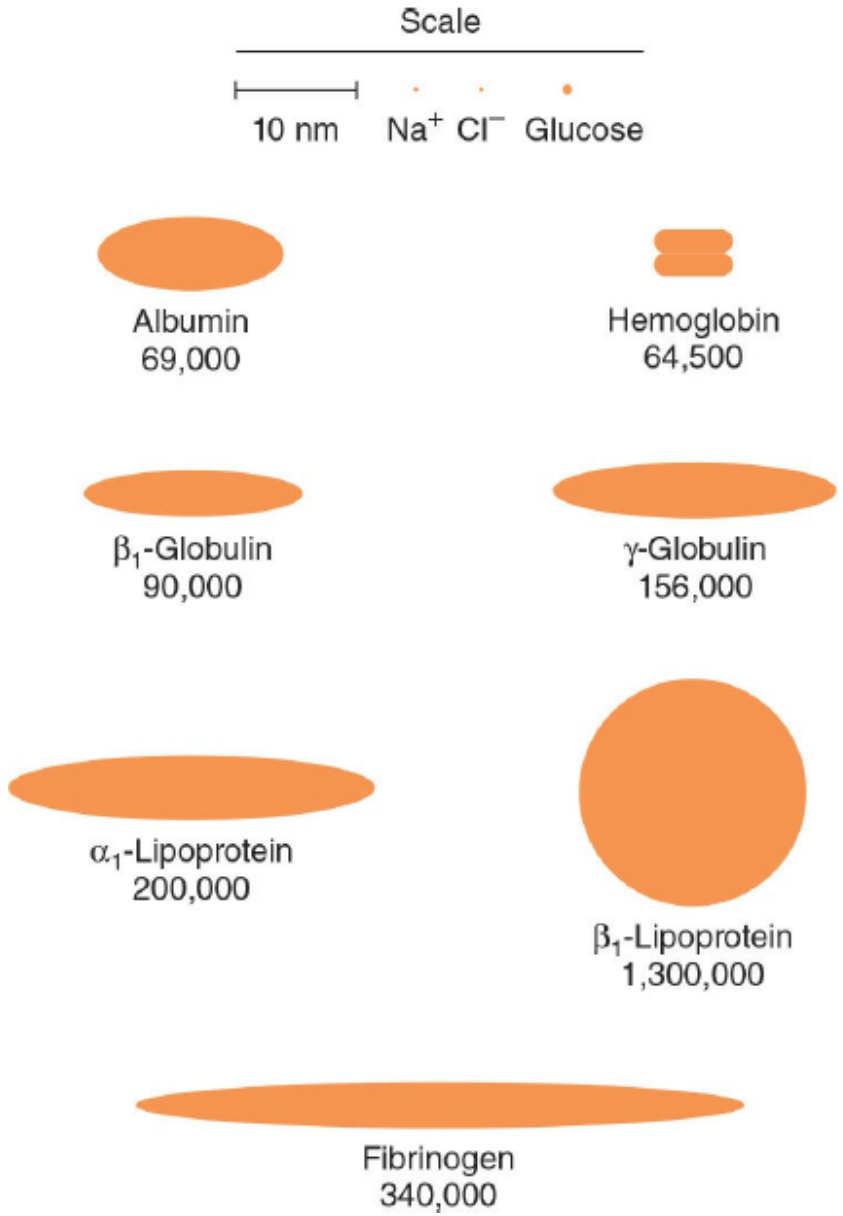
Plasma

- It 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 & NH3
    - Lipids: Cholesterol, TG, phospholipids, free fatty acids
    - Carbohydrates: Glucose, fructose, pentoses
    - 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 diverse

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



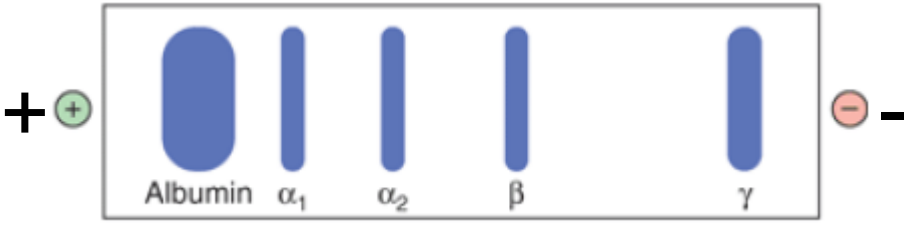


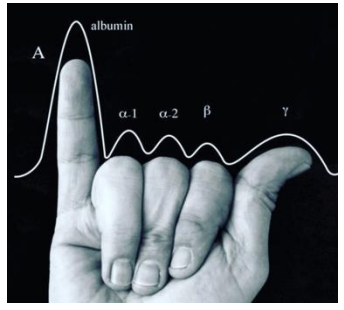
The separation of plasma proteins

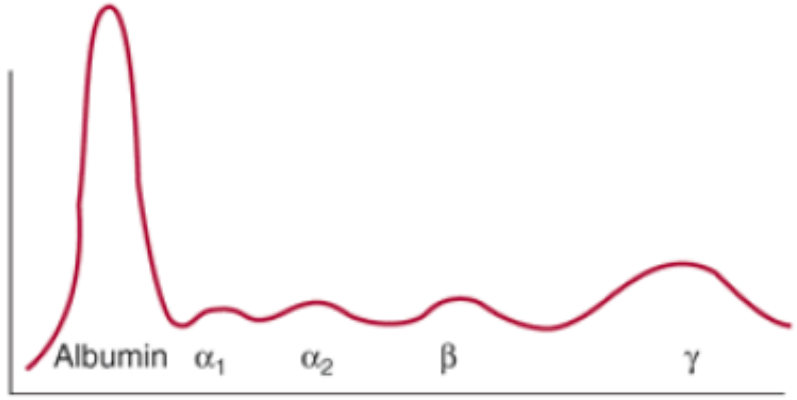
- Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins
- Electrophoresis (most common): serum (defibrinated plasma), five bands (albumin, α1 , α2 , β, and γ)

NORMAL VALUES:

Name	Absolute values (g/l)	Relative values (%)	
Albumins	35 – 55	50 – 60	
α1-globulins	2 – 4	4.2 – 7.2	5 %
α2-globulins	5 – 9	6.8 – 12	9 %
β-globulins	6 – 11	9.3 – 15	12 %
γ-globulins	7 – 17	13 – 23	18 %



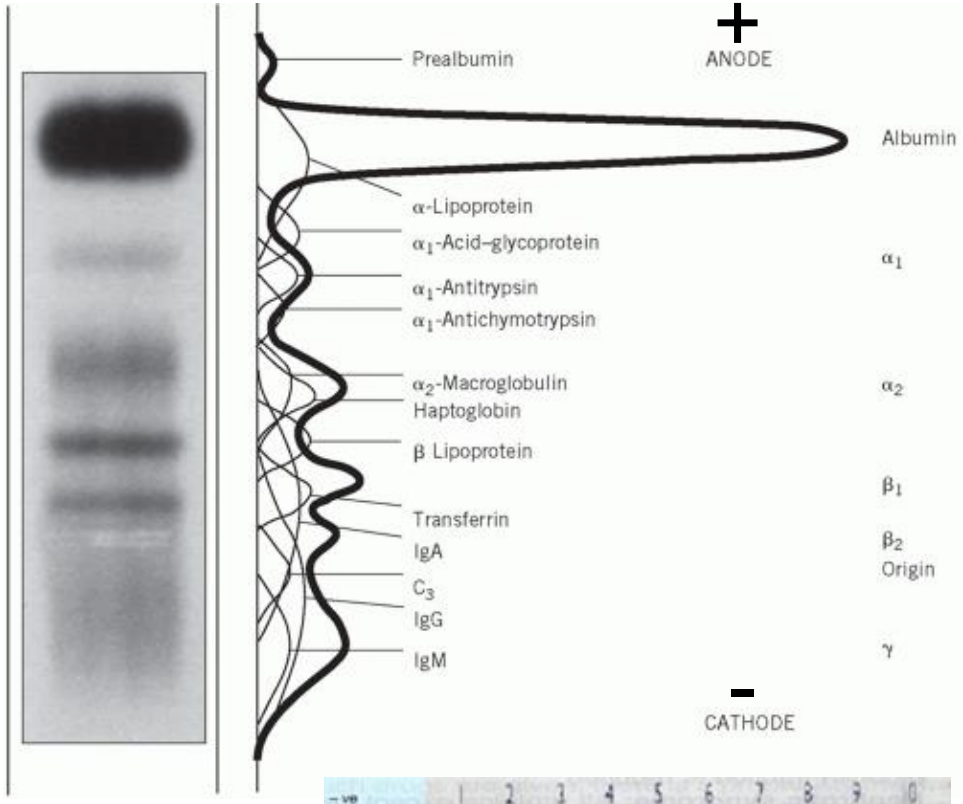


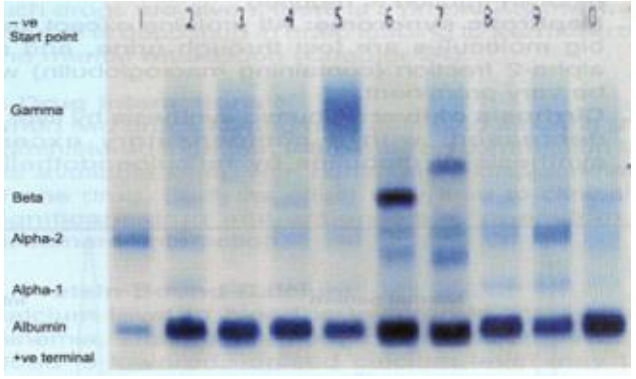


Electrophoresis of plasma proteins

Electrophoresis is movement of proteins through a medium like a gel after applying an electrical current resulting in their separation based on their charge, size, or both

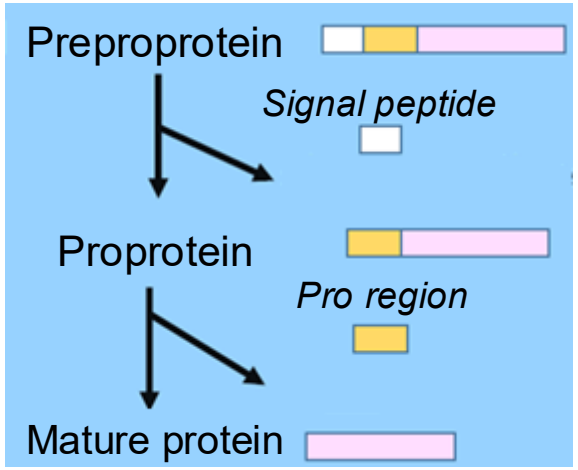
- Albumin is smaller than globulin, and slightly negatively charged
- Globulins (3 bands):
  - α band:
    - α1 region consists mostly of α1-antitrypsin
    - α2 region is mostly haptoglobin, α2-macroglobulin, & ceruloplasmin
  - β band: transferrin, LDL, complement system proteins
  - γ band: the immunoglobulins

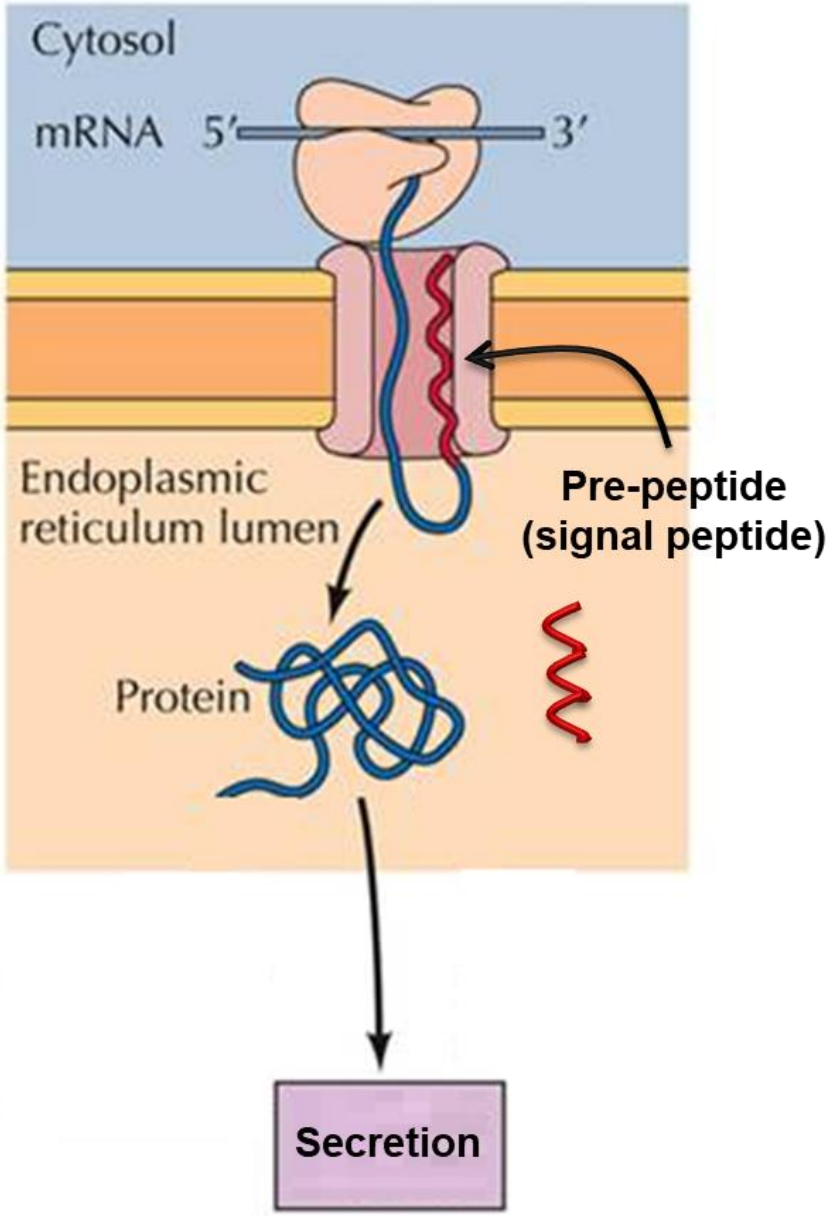




Synthesis of plasma proteins

- 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 & genetic variation (polymorphism)

- The follow a Mendelian or monogenic trait
  - A trait controlled by one gene with two alleles
  - A person can be a homozygote having two identical alleles (form of a gene) or a heterozygote with two dissimilar alleles
  - A dominant/recessive pattern of inheritance
  - Inheritance can be autosomal (chromosomes 1-22) or X-linked
  - The ABO blood groups are the best-known examples

Plasma Proteins Half-Lives

- Albumin & haptoglobin (20 & 5 days, respectively)
- Diseases can affect half-lives
- In protein-losing gastroenteropathy such as Crohn's disease, albumin may be reduced (1 day).

General and specific functions of plasma proteins

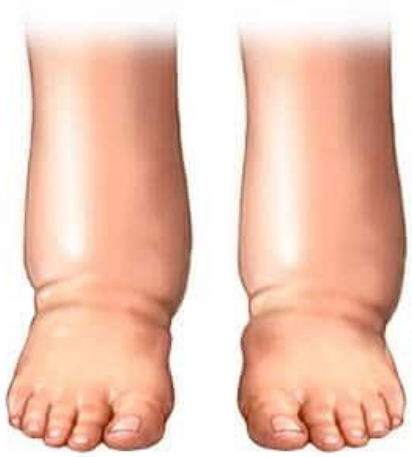
- A nutritive role
- Maintenance of blood pH (amphoteric property)
- Contributes to blood viscosity
- Maintenance of blood osmotic pressure

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

Starling forces

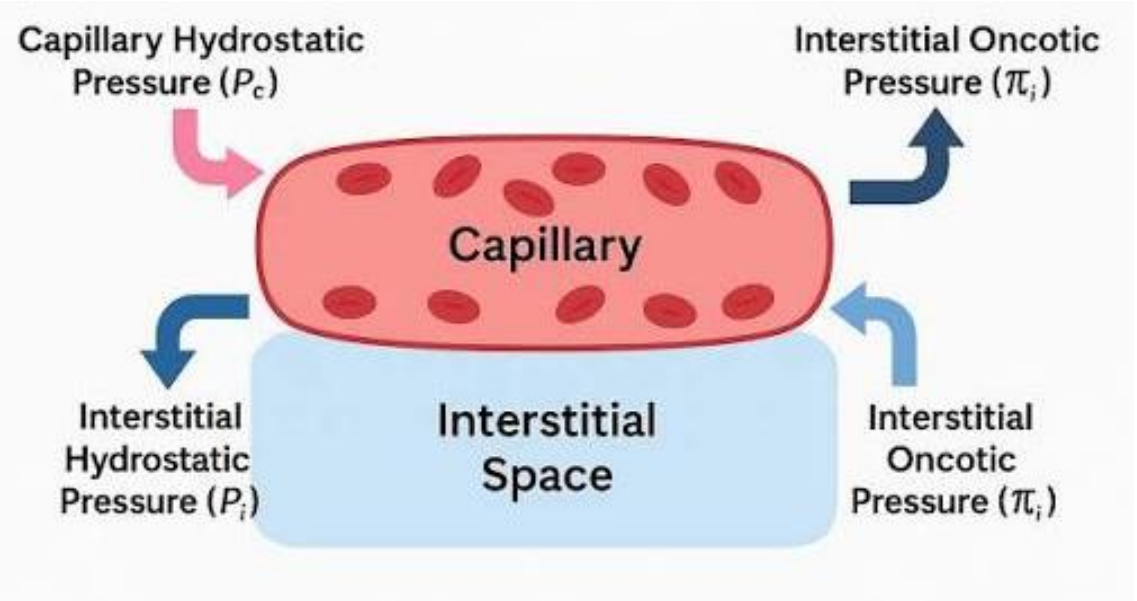
The four physical pressures that control how fluid moves in and out of blood capillaries

- **Capillary Hydrostatic Pressure:** Blood pressure inside the capillary. It pushes fluid **out** of the vessel.
- **Interstitial Hydrostatic Pressure:** Pressure of fluid in the tissue outside. It pushes fluid **into** the vessel.
- **Capillary Oncotic Pressure:** Osmotic pull from large blood proteins like albumin. It pulls fluid **into** the vessel.
- **Interstitial Oncotic Pressure:** Osmotic pull from proteins in the tissue. It pulls fluid **out** of the vessel.



Edema (swelling) of the ankles and feet

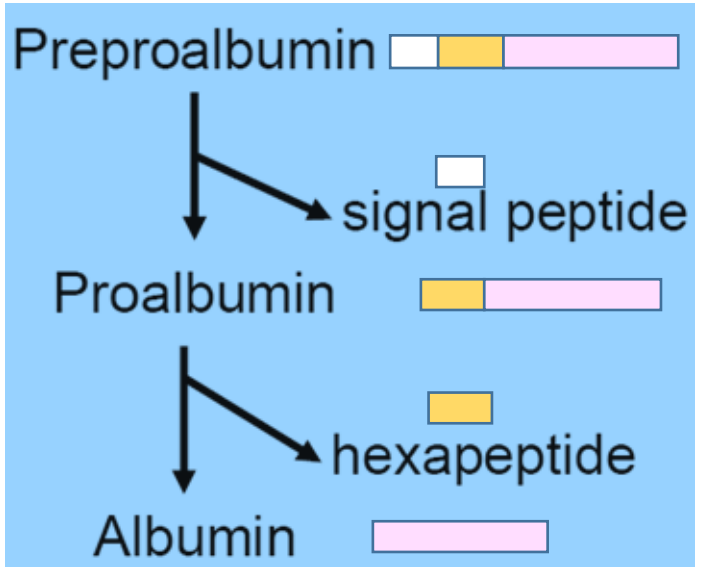
- Edema can be a result of protein deficiency

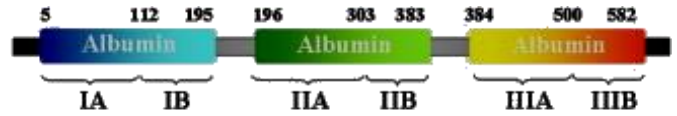


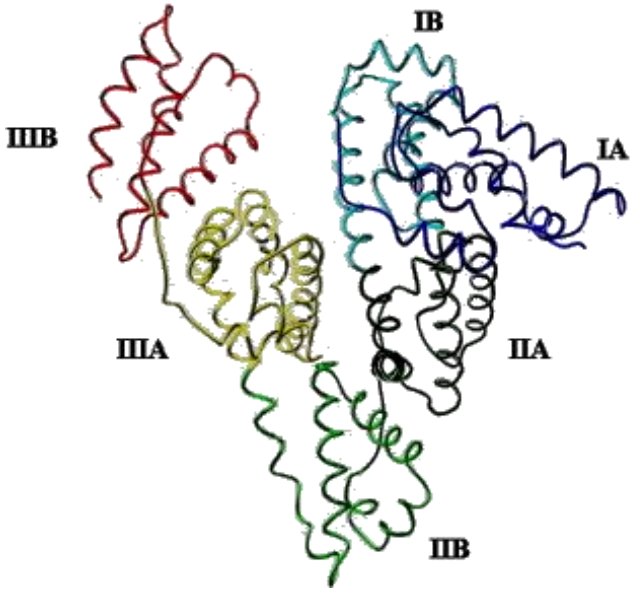
Albumin

- 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)
- 3/5 total plasma proteins (3.4-4.7g/dL)
- Synthesized as a preproprotein
  - Pre sequence takes the protein to the endoplasmic region
  - Pro sequence makes the protein immature
- Monomeric
- Ellipsoidal shape (does not increase viscosity like fibrinogen)

- Anionic at pH 7.4 with 20 negative charges

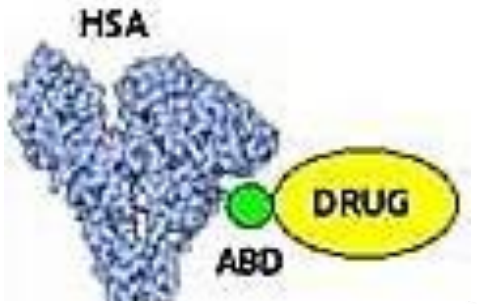


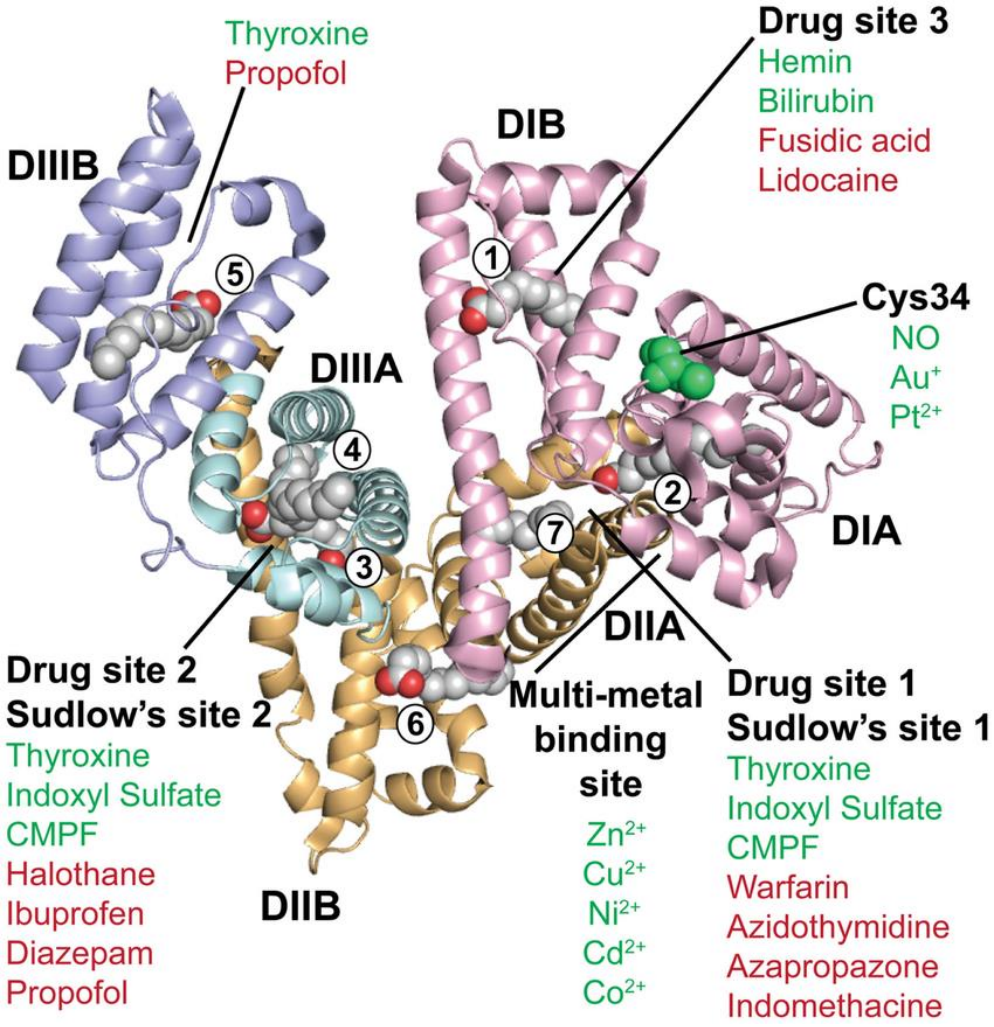




Albumin's binding capacity

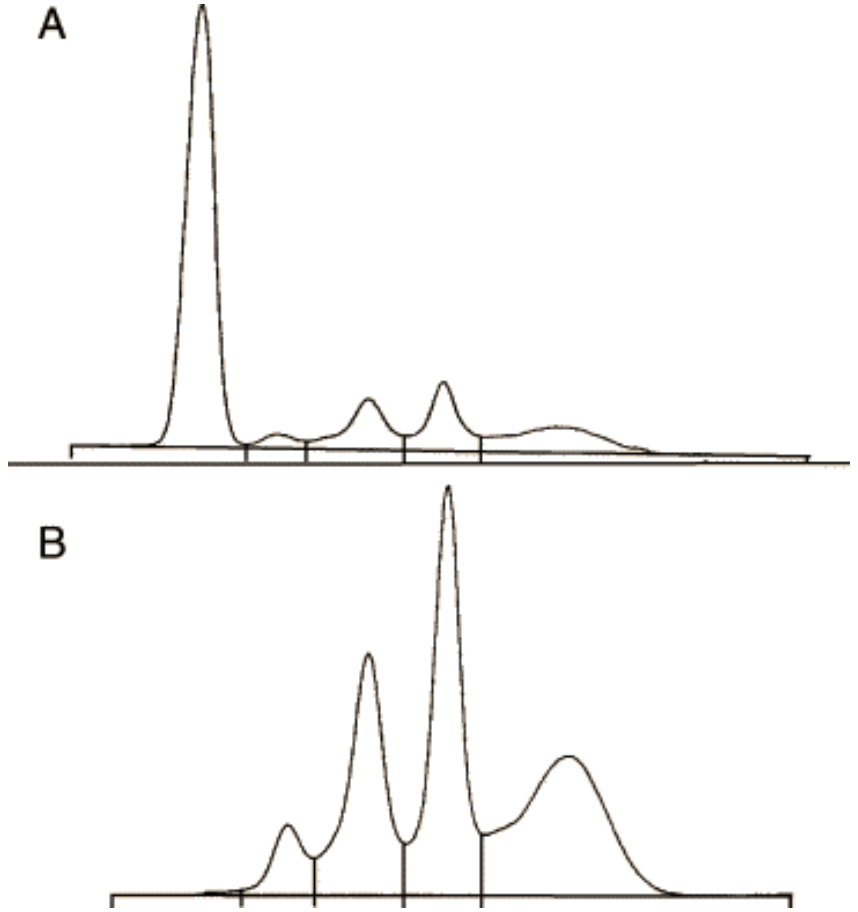
- It binds various ligands:
  - Free fatty acids (FFA)
  - Certain steroid hormones
  - Bilirubin
  - Plasma tryptophan
  - Metals: Calcium, copper and heavy metals
  - Drugs: sulfonamides, penicillin G, dicumarol, aspirin (drug-drug interaction)





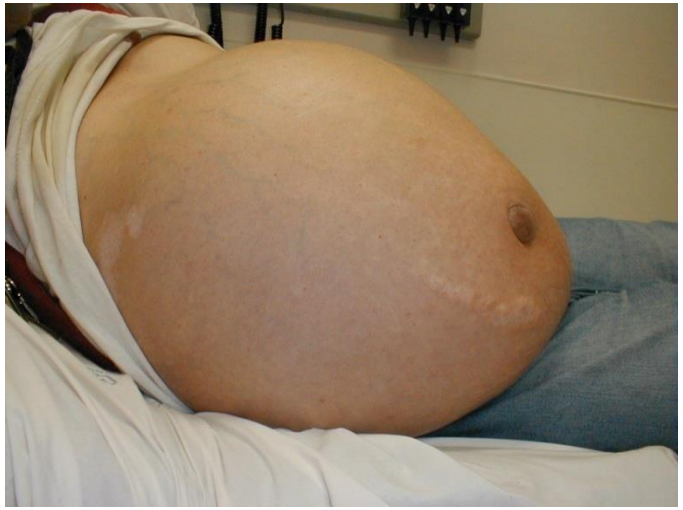
Analbuminemia

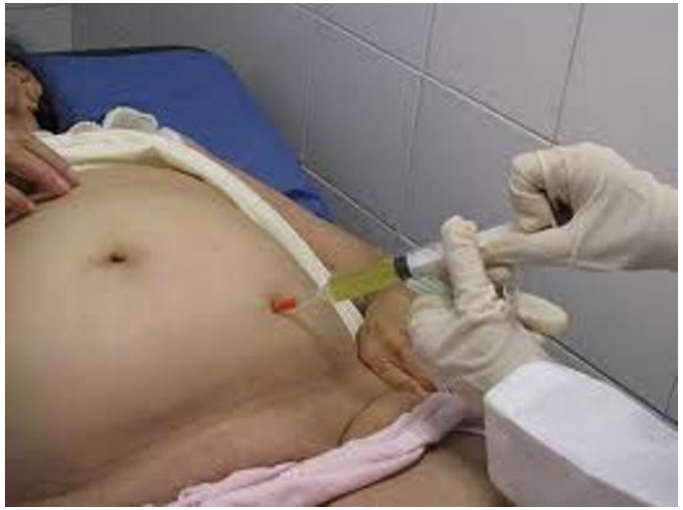
- There are human cases of analbuminemia (rare)
- Autosomal recessive inheritance
  - Meaning that both alleles must be defective for the symptoms to appear
- Patients show moderate edema!!!



Other clinical disorders

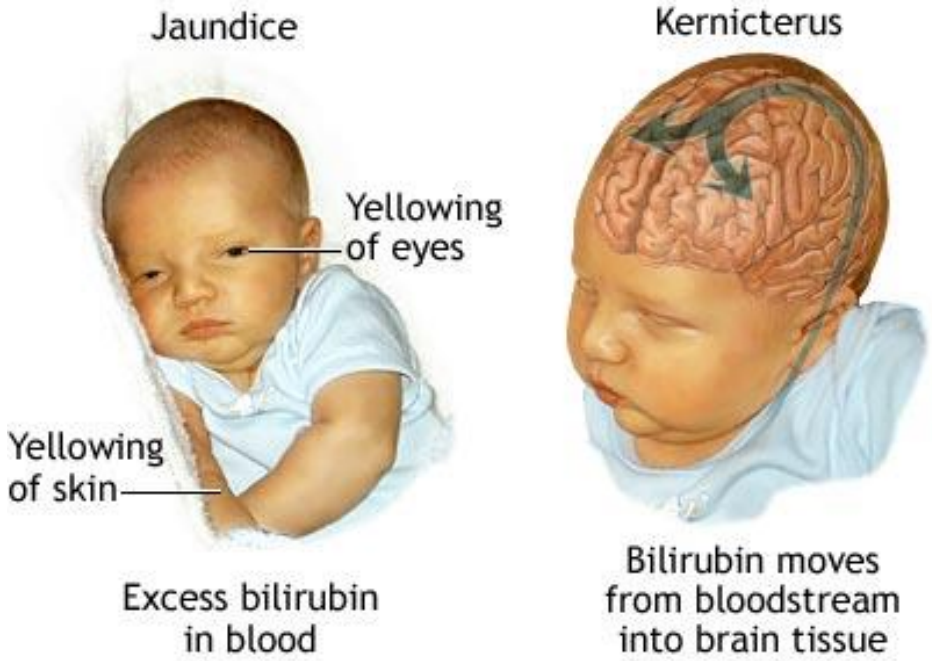
- Hypoalbiminemia: edema seen in conditions where albumin level in blood is less than 2 g/dl
  - Malnutrition (generalized edema)
  - Nephrotic syndrome
  - Cirrhosis (mainly ascites- abnormal buildup of fluid inside the abdomen)
  - Gastrointestinal loss of proteins
- Hyperalbuminemia: dehydration (relative increase)





Other clinical disorders

- Drug-drug interaction:
  - Bilirubin toxicity (aspirin is a competitive ligand): kernicterus (a rare and severe form of brain damage in newborns) and mental retardation

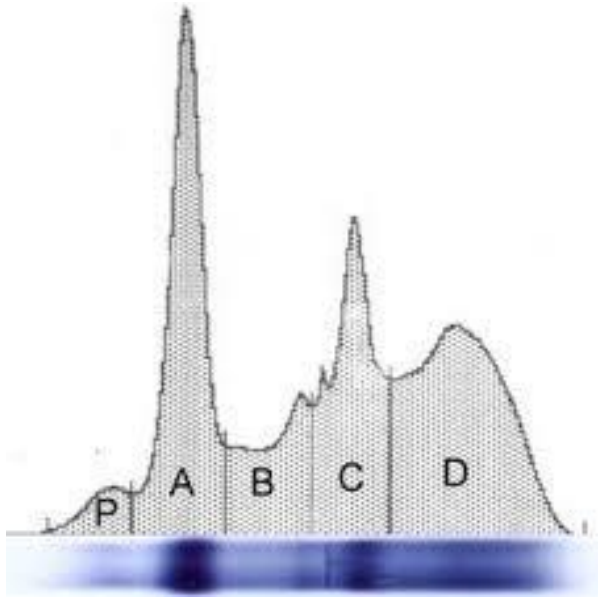


Anti-epileptic phenytoin-anti-coagulant dicoumarol interaction

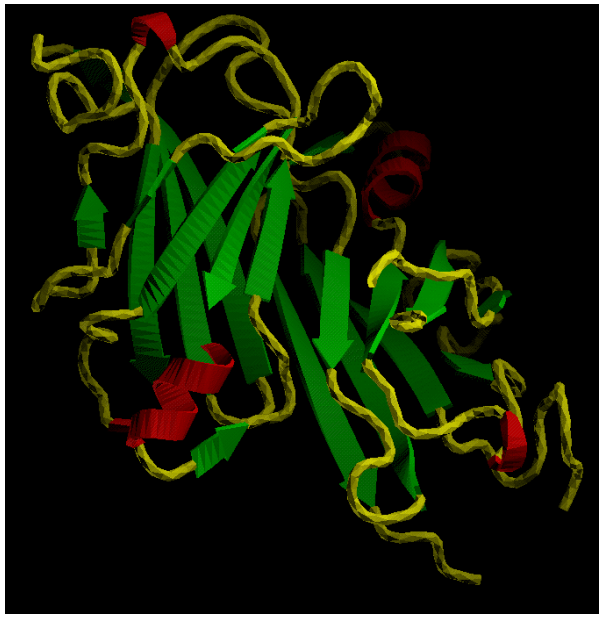
Phenytoin induces liver enzymes to decrease dicoumarol's blood-thinning effect

Dicoumarol inhibits phenytoin metabolism, potentially raising phenytoin levels and causing toxicity.

Prealbumin (transthyretin)



- It migrates ahead of albumin, 62 kDa
- Its blood level is low (0.25 g/L)
- It exists as a 62-kDa glycoprotein.
- It has short half-life (≈2 days).
- It is a sensitive indicator of poor protein nutrition.
- Main function:
  - T4 (Thyroxine) and T3 carrier



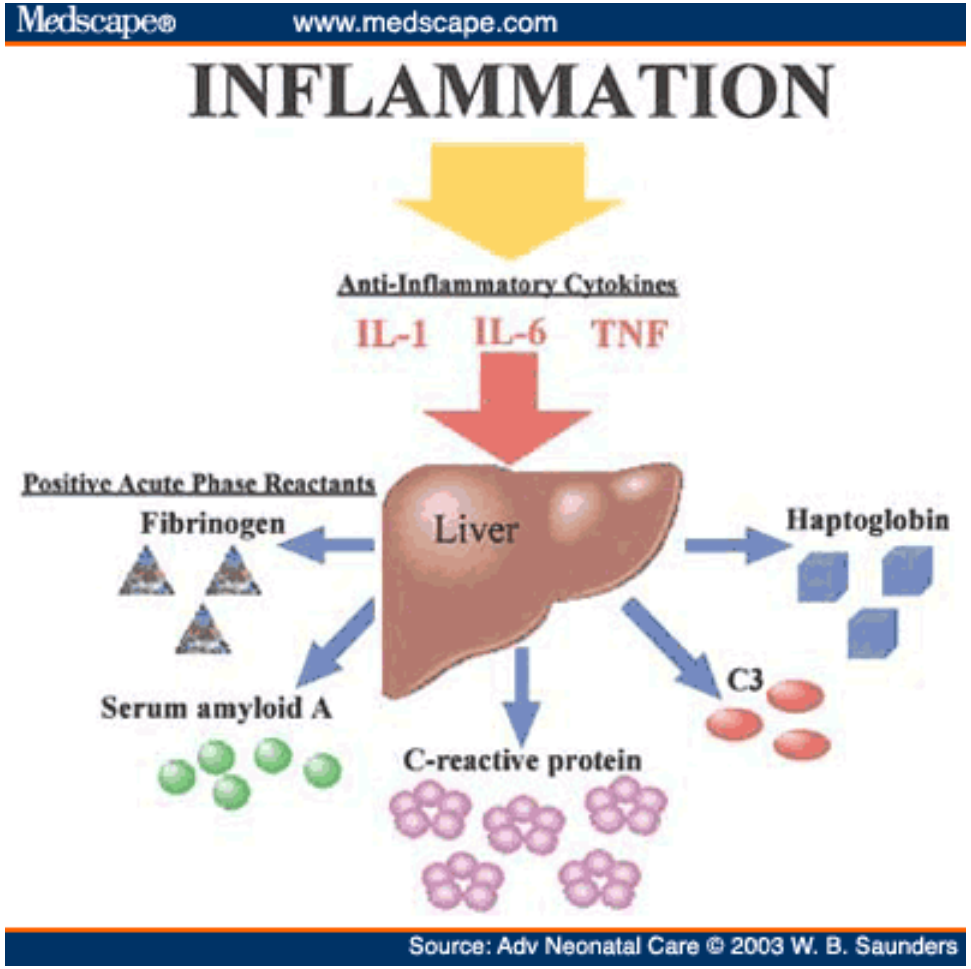
Globulins

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

Acute-phase proteins

- Plasma proteins whose Levels increase (up to 1000 folds) upon acute inflammation, tissue damage, chronic inflammation, and cancer.
- C-reactive protein (CRP), α1 - antitrypsin, haptoglobin, and fibrinogen
- Interleukin-1 (IL-1) and IL-6 are the main stimulators.

- Negative acute phase proteins: prealbumin, albumin, transferrin

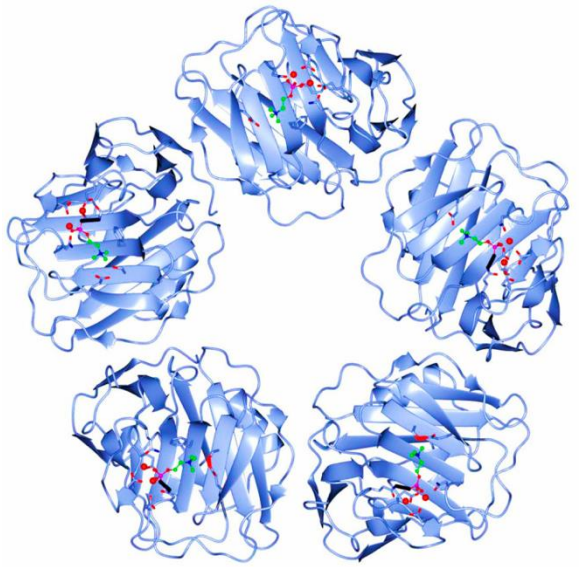


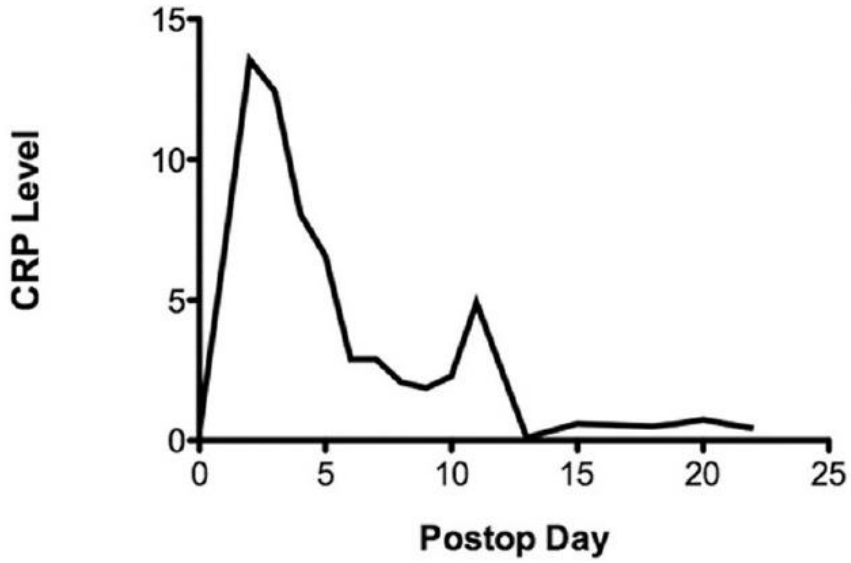
Purpose of acute of phase proteins

Protein	Function
C-reactive protein	Stimulates the complement pathway (an immune response)
α1-antitrypsin	neutralizes certain proteases released during acute inflammation
Fibrinogen	Coagulation factor
Transferrin	Iron binding (preventing microbe uptake of iron)
Haptoglobin	Hemoglobin binding (iron protection)
Ceruloplasmin	Iron oxidation (iron binding by ferritin)

C-reactive protein (CRP)

- It is called CRP because it is able to bind to a polysaccharide called fraction C in the cell wall of a bacterial species called pneumococci.
- It is a homo-pentameric acute-phase inflammatory protein where the subunits are connected non-covalently.
- It is undetectable in healthy individuals.
- It helps in the defense against bacteria and damaged cells by binding phosphocholine residues on damaged cell membranes and foreign pathogens (bacteria, fungi) allowing for immune cell recognition and clearing.
- It is detectable in many inflammatory diseases (Acute rheumatic fever, bacterial infection, gout, etc.) & Tissue damage
- Its level reaches a peak after 48 hours of the incident (monitoring marker).





α1-antitrypsin

- Also known as (AKA) α1-antiproteinase (52 kDa)
- It neutralizes the digestive trypsin & trypsin-like enzymes (such as elastase).
- It constitutes 90% of α1- 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 is found in people with ZZ or SZ.
  - MS and MZ are usually not affected
- It is increased during an acute phase response.

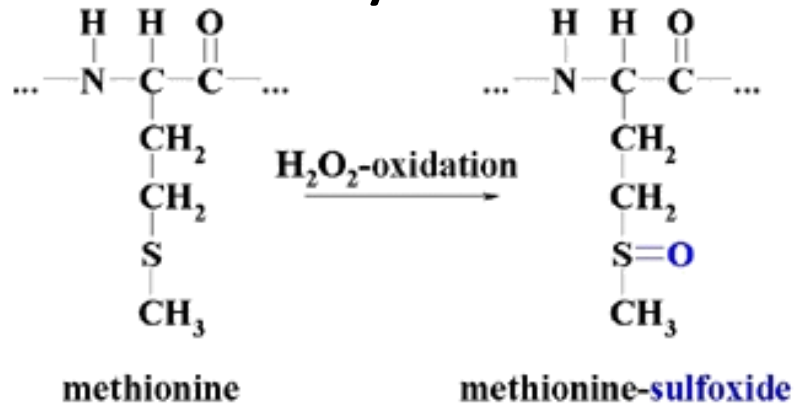
Emphysema is a chronic lung disease where the tiny air sacs (alveoli) in the lungs are damaged and rupture, creating larger air spaces, reducing lung surface area, and trapping air, making it hard to breathe. It is a major type of chronic obstructive pulmonary disease (COPD).

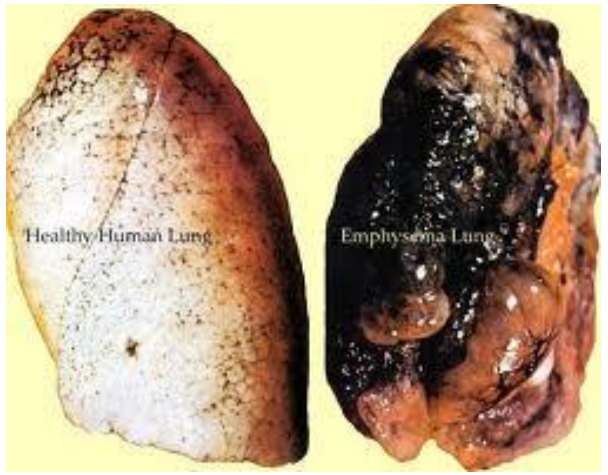
Active elastase + α_1–AT → Inactive elastase: α_1–AT complex → No proteolysis of lung → No tissue damage

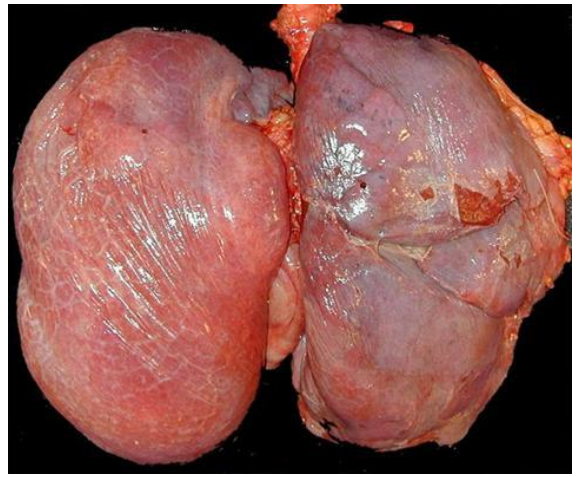
Active elastase + ↓ or no α_1–AT → Active elastase → Proteolysis of lung → Tissue damage

Smoking & α1- antitrypsin deficiency

- Smoking causes chronic inflammation. Why?
- Oxidation of Met358:
  - Oxidation of methionine 358 (Met358) in alpha-1 antitrypsin (AAT) changes the amino acid into methionine sulfoxide.
  - This change blocks the enzyme's ability to inhibit neutrophil elastase from damaging healthy lung tissue during bad inflammation.
- It is even more devastating in patients with PiZZ.

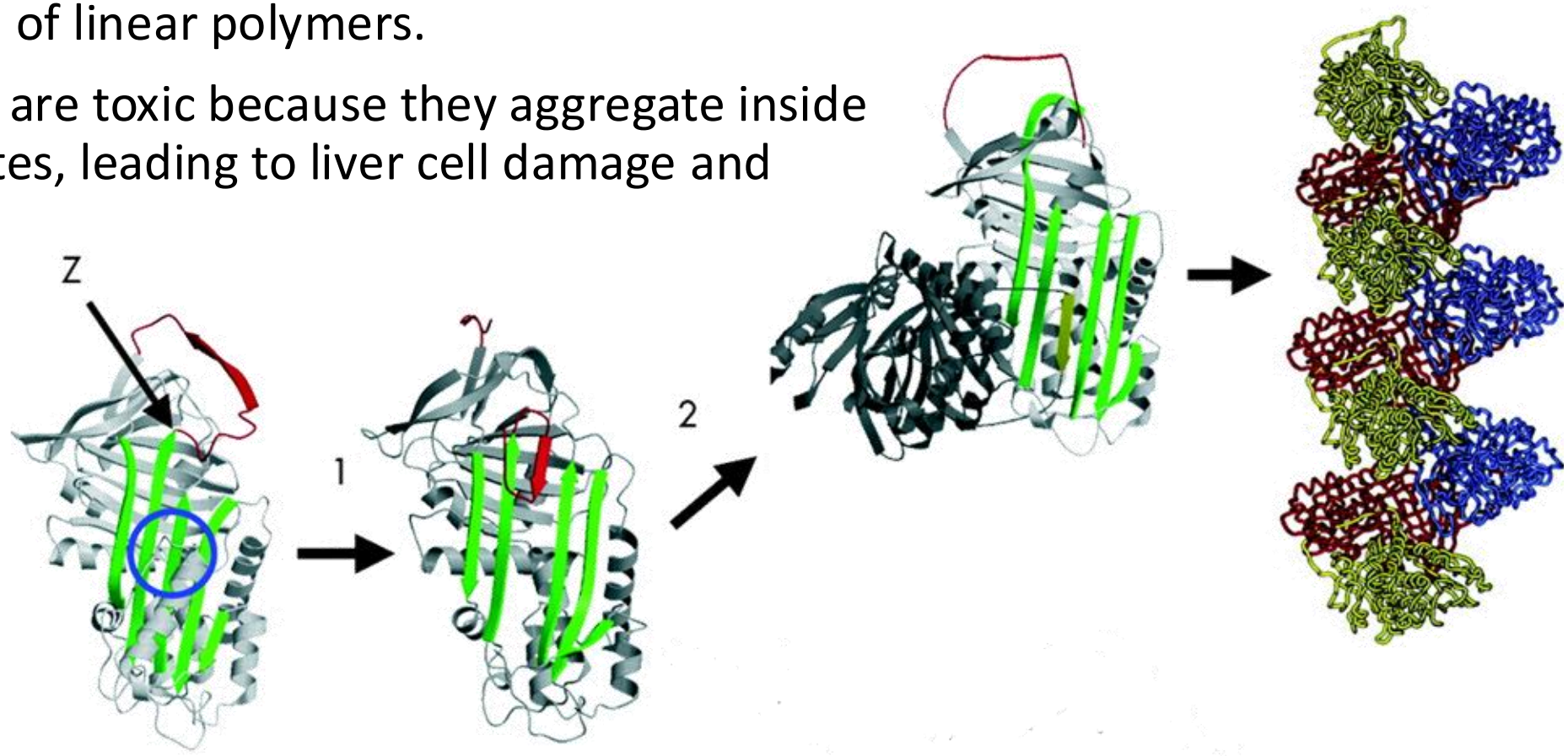






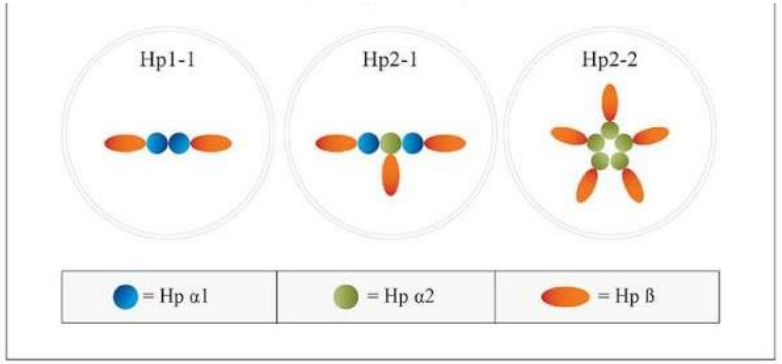
Liver disease & α1- antitrypsin deficiency

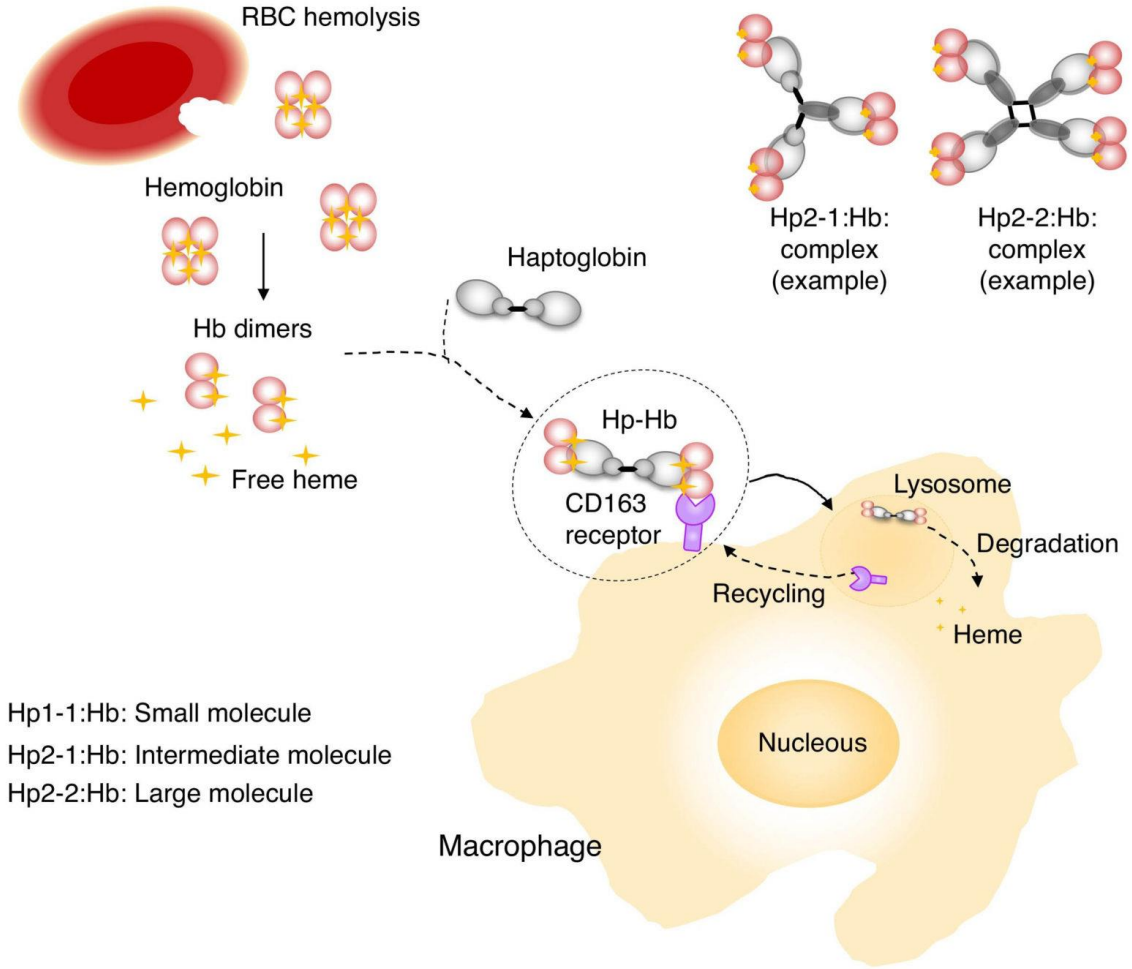
- The ZZ genotype causes the mutant Z protein to misfold inserting a loop into a beta-sheet resulting in the formation of linear polymers.
- The polymers are toxic because they aggregate inside the hepatocytes, leading to liver cell damage and cirrhosis.



Haptoglobin (Hp)

- It is an acute phase protein.
- α2 glycoprotein (90kDa)
- A tetramer (2α, 2β)
- Two genes, designated Hp1 and Hp2, producing 3 phenotypes:
  - Hp 1-1→ α1, α1 + 2β
  - Hp 2-1→ α1, α2 + 2β
  - Hp 2-2 → α2, α2 + 2β (weakest binder)
- It 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 free Hp (5 days)
- Decreased level in hemolytic anemia

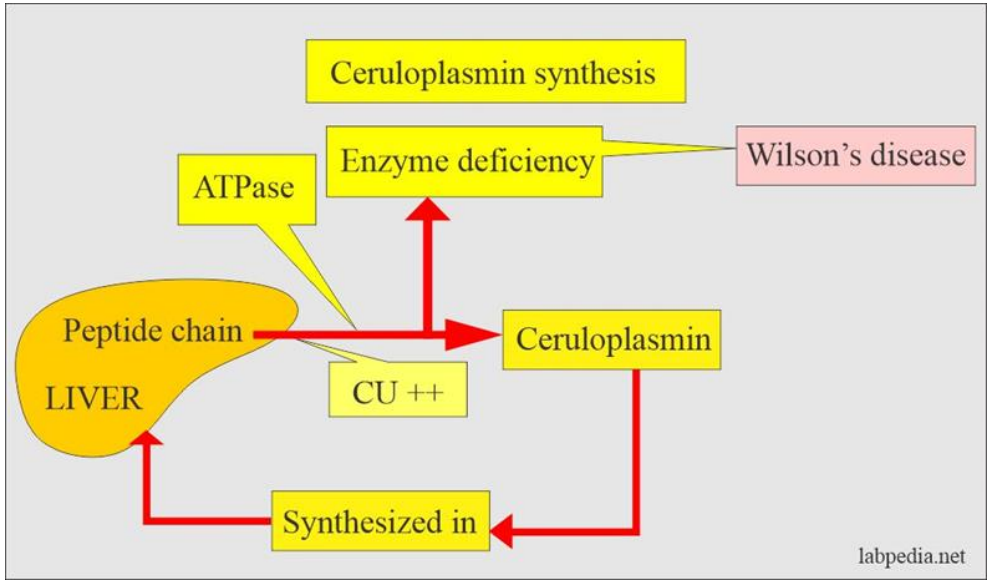




Ceruloplasmin

Cu-containing enzymes
Amine oxidase
Copper-dependent superoxide dismutase
Cytochrome oxidase
Tyrosinase

- A copper-containing glycoprotein (160 kDa)
  - It contains 6 atoms of copper.
- It belongs to the metallothionein family that regulates tissue level of Cu.
- It carries 90% of serum Cu.
- It has a ferroxidase activity that oxidizes ferrous to ferric forms of iron.
- Albumin is more important in transporting 10% of copper.

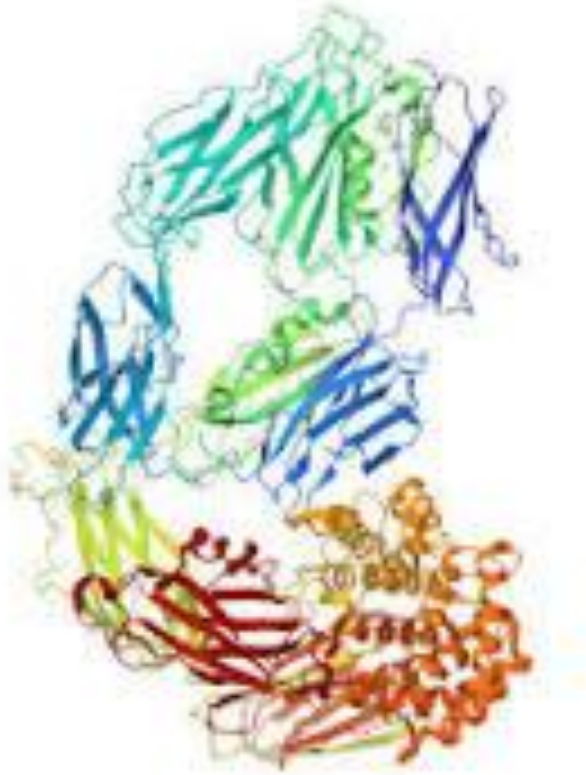


Pathological conditions related to ceruloplasmin

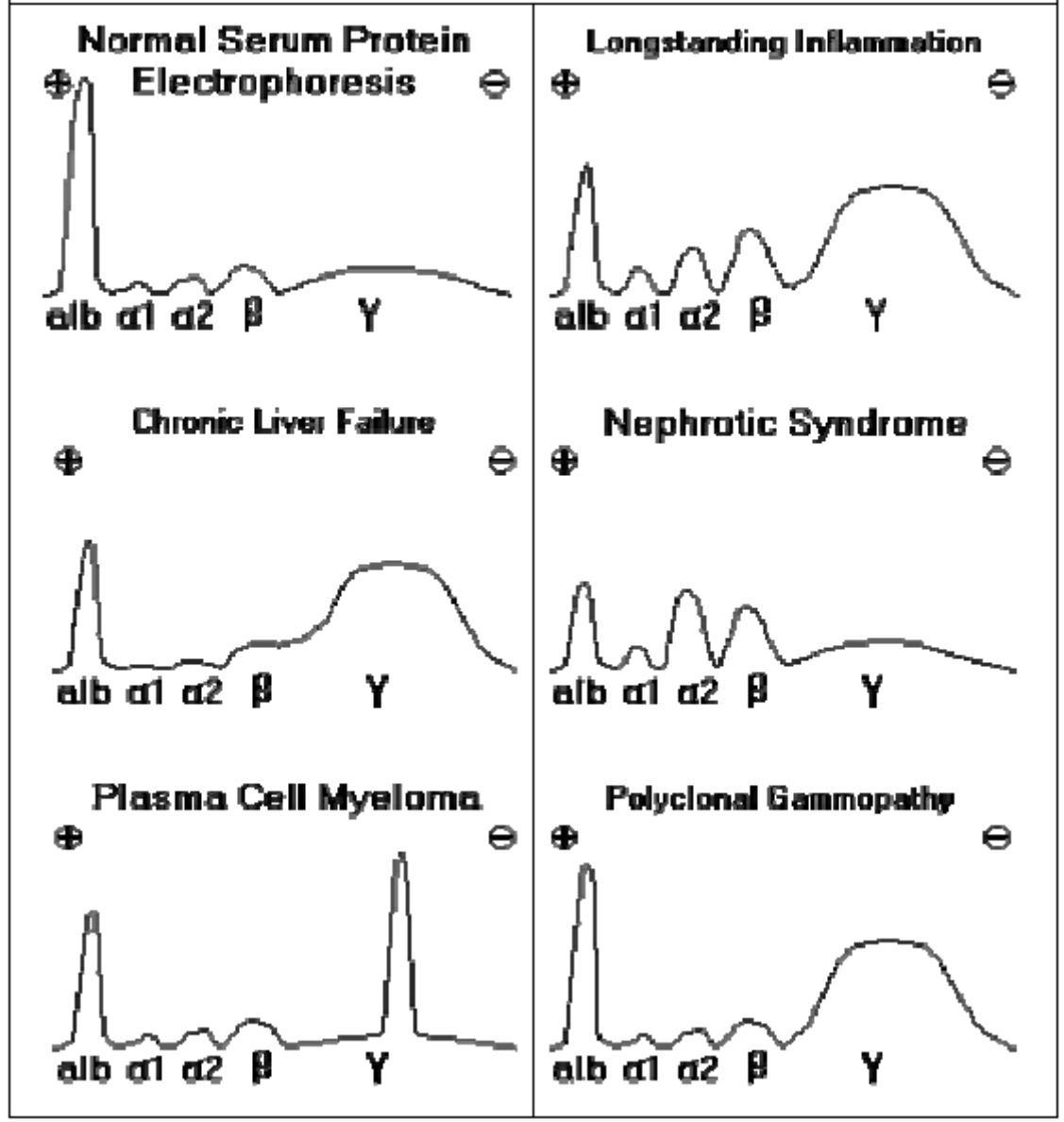
- Ceruloplasmin deficiency can arise from genetic causes or lack of dietary copper.
- Hypoceruloplasmenia
  - Ceruloplasmin levels are ~50% of normal
  - No clinical abnormalities
- Aceruloplasminemia
  - No ferroxidase activity of ceruloplasmin
  - If left untreated, accumulation of iron in tissues and organ failure
- Wilson’s disease
  - Defective transporter (copper-binding P-type ATPase or ATP7B protein) leading to excess liver copper, increased apoceruloplasmin, and copper toxicosis.

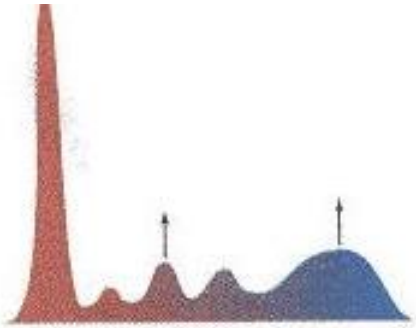
Alpha-2 macroglobulin

- It is a large plasma protein.
- It is responsible for the transport of 10% of zinc and cytokines in blood.
- α2-macroglobulin binds to and inactivates diverse types of proteases.
  - Blood coagulation
- Its levels often rise significantly in nephrotic syndrome or drop during acute pancreatitis and severe liver disease.

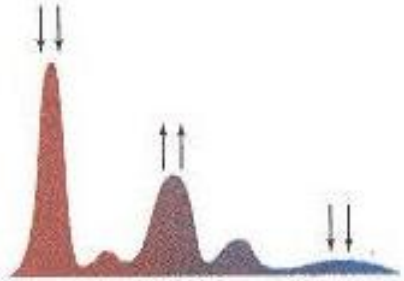


ELECTROPHORESIS ASPECTS IN SEVERAL TYPES OF DYSPROTEINEMIA

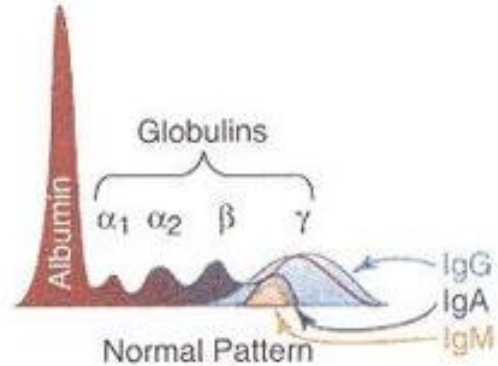




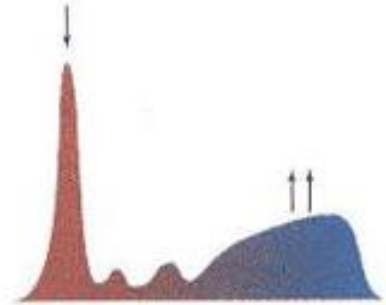
"Delayed Response Pattern"
(c)



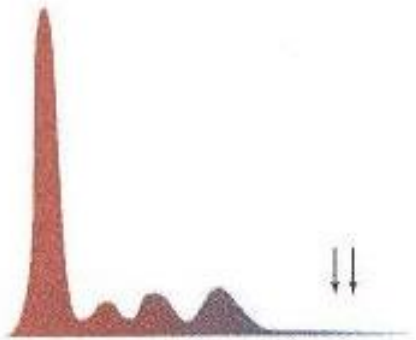
Nephrotic Syndrome
(g)



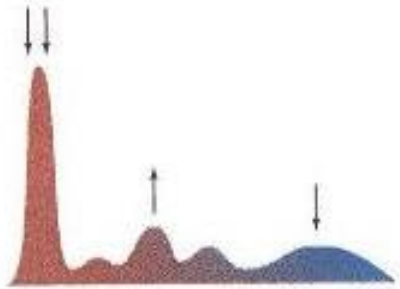
Normal Pattern
(a)



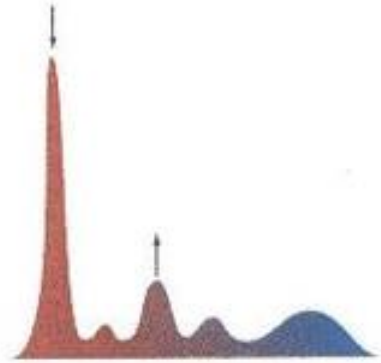
Hepatic Cirrhosis
("Polyclonal Gammopathy")
(e)



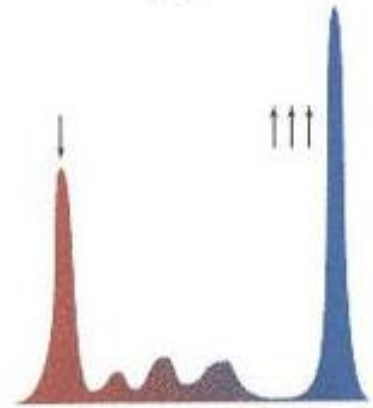
Hypogammaglobulinemia
(d)



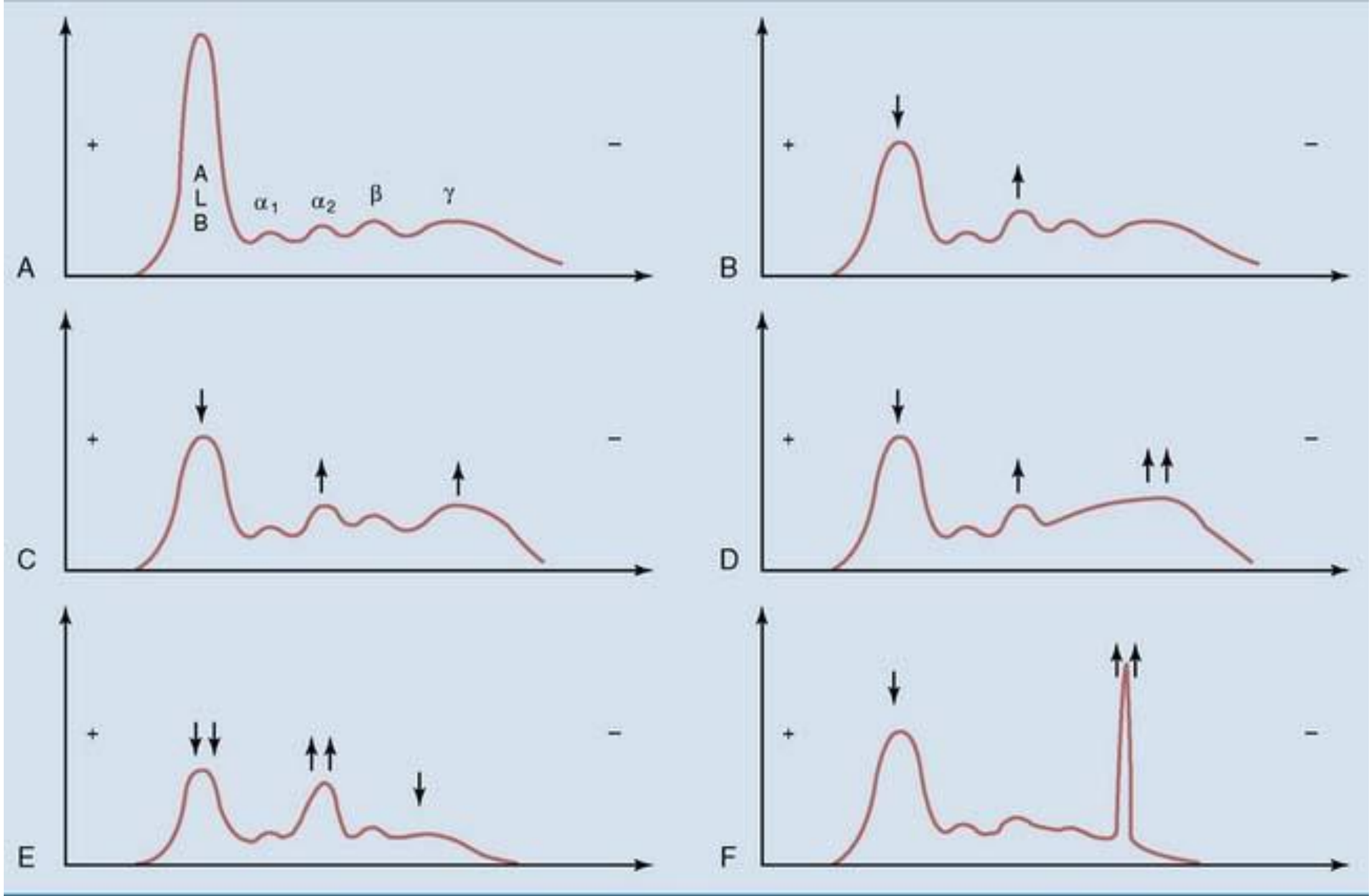
Protein-losing Enteropathy
(h)



"Immediate Response Pattern"
(b)



Paraprotein
("Monoclonal Gammopathy")
(f)


A
+
−
A
L
B
α1
α2
β
γ
B
C
D
E
F