بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture #9

Plasma Proteins

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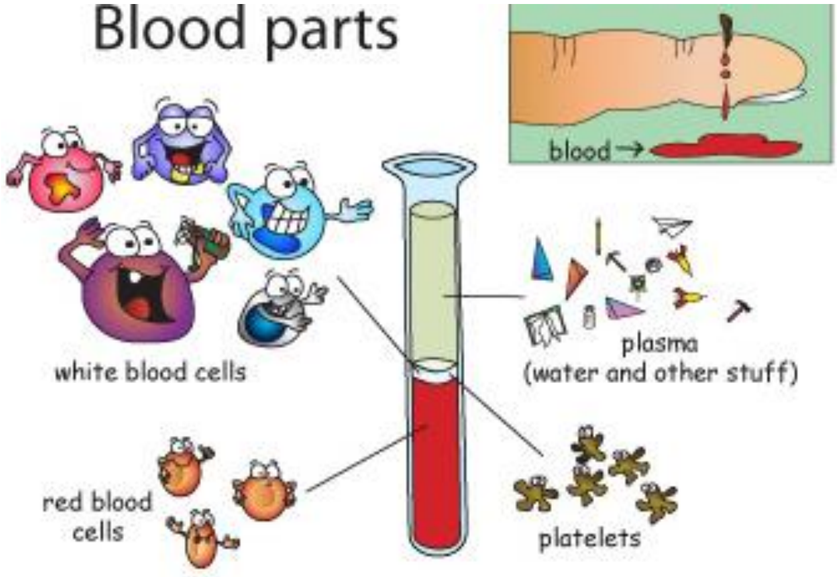
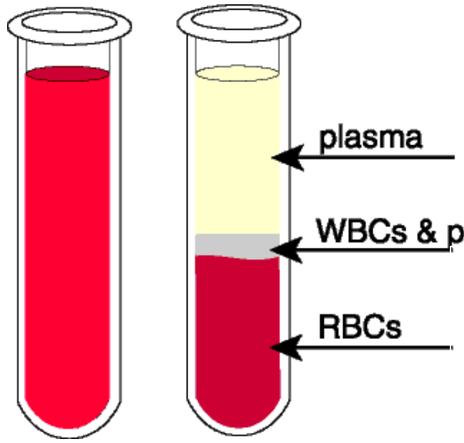
Yaman Khalil

Color coding used in the modified:

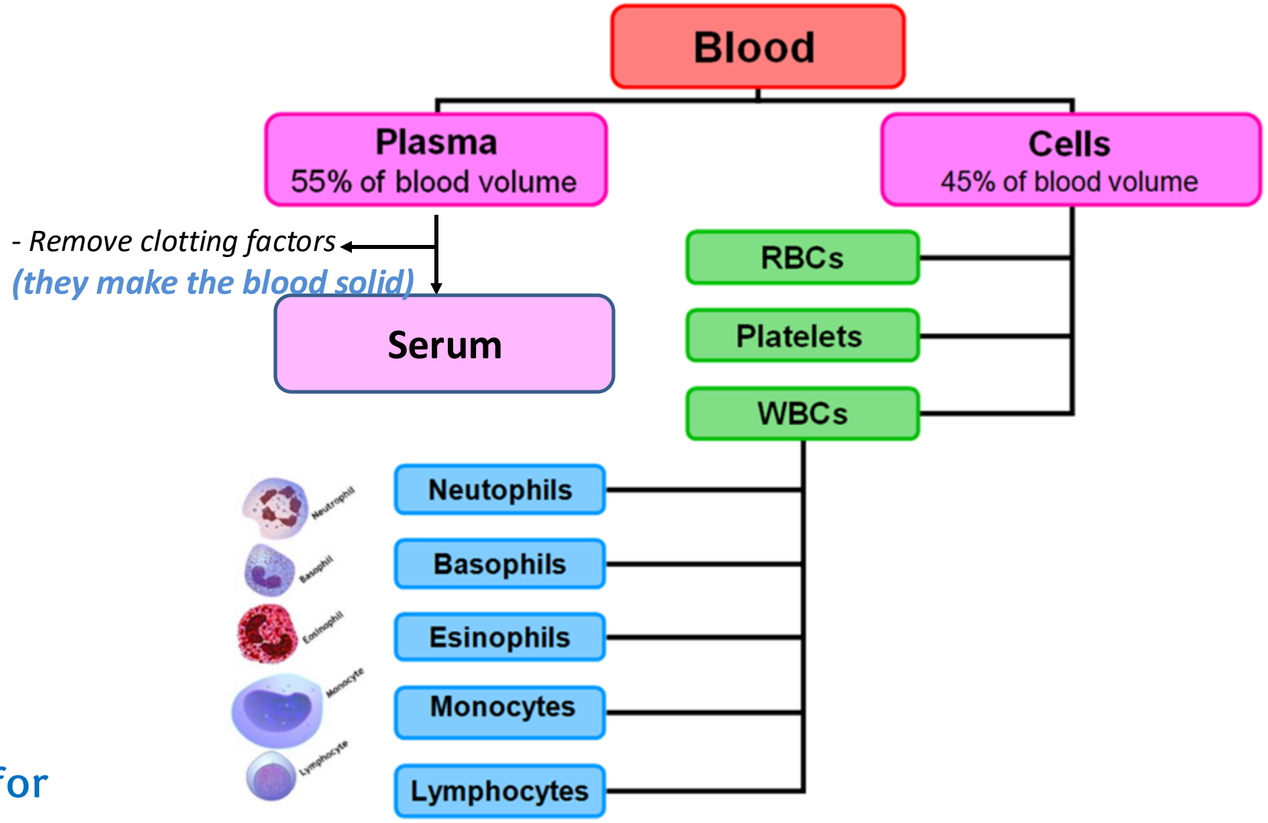
- *Black: the original slides*
- *Blue: the doctor’s explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Blood





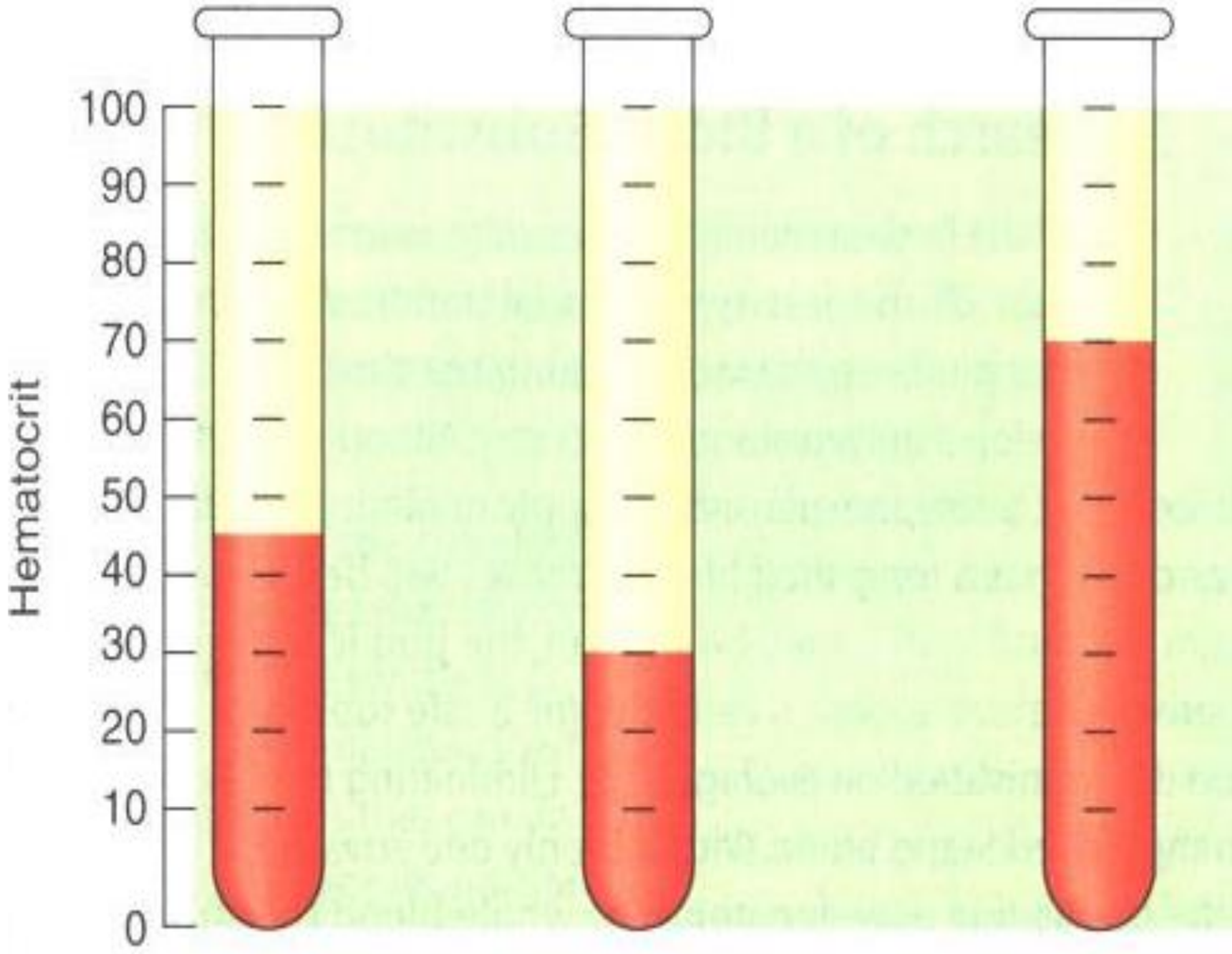
If you leave a blood sample for 5 minutes, RBCs WBCs and platelets will precipitate



Blood: plasma vs. hematocrit

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

Hematocrit (the percentage of your total blood volume made up of red blood cells) is measured in case of doing CBC test (complete blood count).

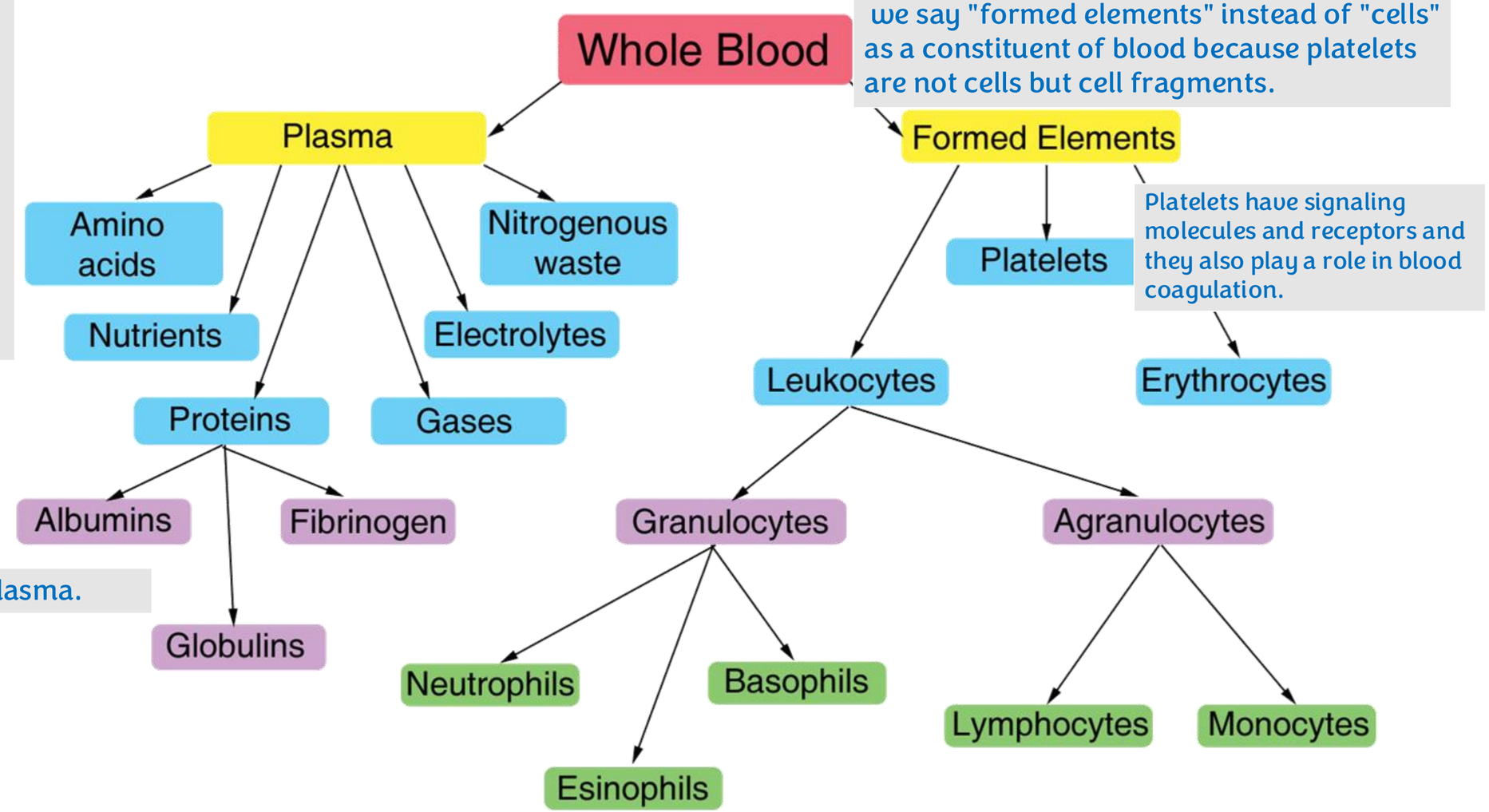


Blood: plasma vs. cells

Proteins present in plasma could be either plasma proteins or cellular proteins that got to plasma as a result of cell damage.

Note: An increase in cellular proteins in blood could be a measure to indicate cases. For example, troponin levels in blood could be an indicator for myocardial infarction and liver enzymes could indicate several cases aswell. The increase of cellular proteins usually mean there's a tissue damage, viral or bacterial infection in the body that led the protein to leak in the plasma.

we say "formed elements" instead of "cells" as a constituent of blood because platelets are not cells but cell fragments.



Platelets have signaling molecules and receptors and they also play a role in blood coagulation.

Albumin is predominant in plasma.

Plasma

- It is the liquid medium in which blood cells are suspended.
- Composition: ▪ Water (92%) ▪ Solids (8%) Could be proteins, electrolytes , etc.

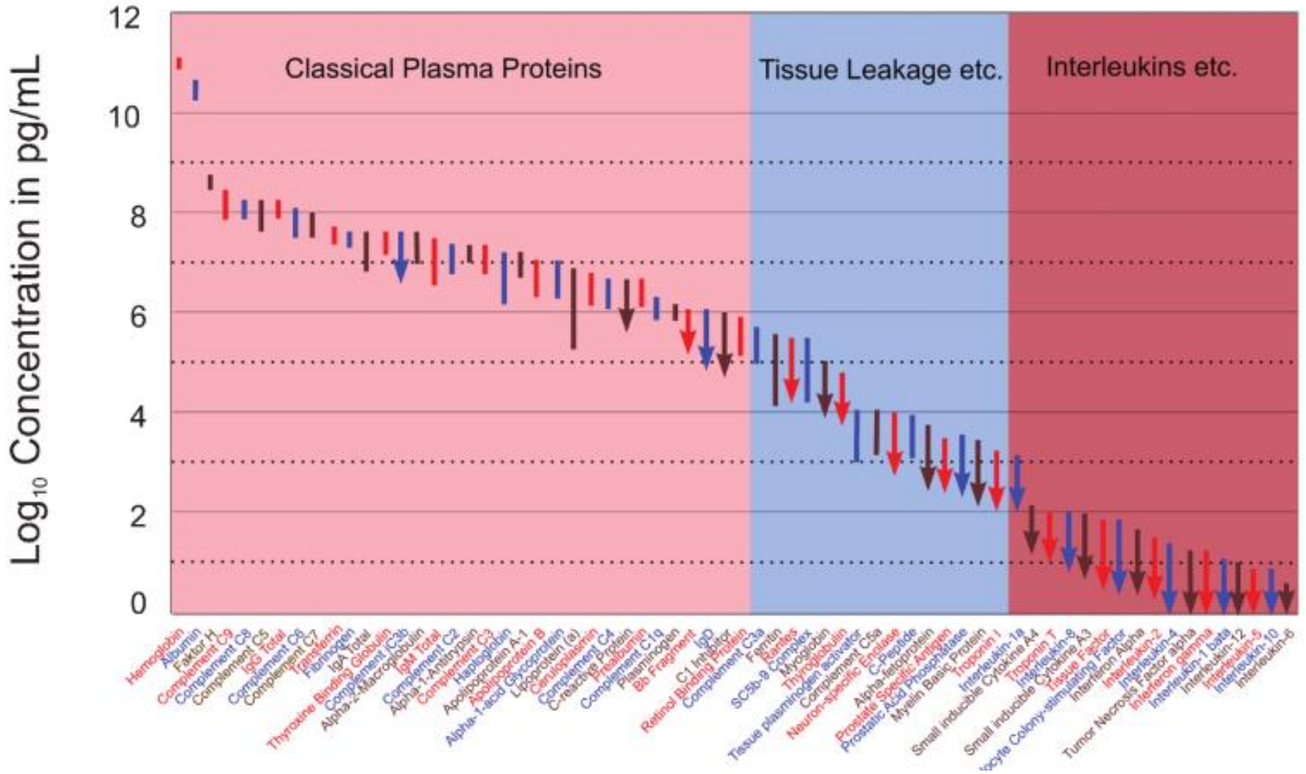
  –Organic:
  - Plasma proteins: Albumin, Globulins & Fibrinogen
  - Non-protein nitrogenous compounds: urea, free amino acids, uric acid, creatinine, creatine & NH3
  - Lipids: Cholesterol, TG, phospholipids, free fatty acids
  - Carbohydrates: Glucose, fructose, pentoses
  - Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones

  ▪ Inorganic: Na^{+},K^{+},Ca^{2+},Mg^{2+},Cl^{-},HCO_3^{-},HPO_4^{2-}, SO_4^{2-}

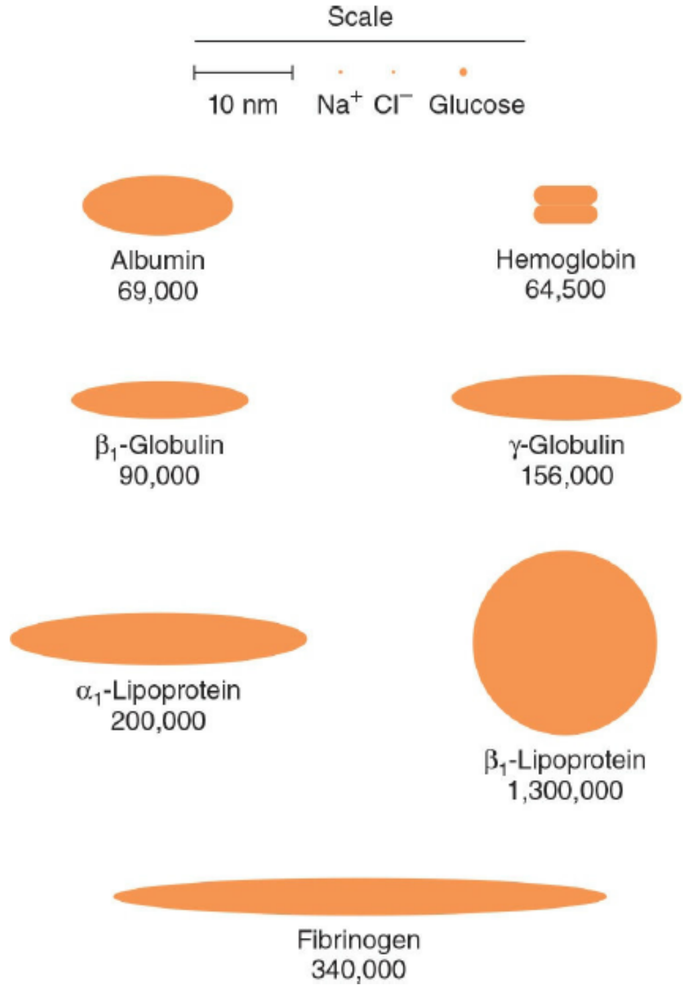
Plasma proteins are diverse in terms of both concentration and function, as well as structure.
Plasma proteins are usually measured to confirm whether there is a disease or not, often called biomarkers.

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)



Do not memorize the names here.



Keep in mind that structure fits function, so the structure of fibrinogen (highly elongated) fits its function which is forming networks for clotting.

The separation of plasma proteins

- Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins.

(Salting-out is the process of adding salts until the protein is precipitated)

- Electrophoresis (most common): serum (defibrinated plasma), five bands (albumin, α1 , α2 , β, and γ)

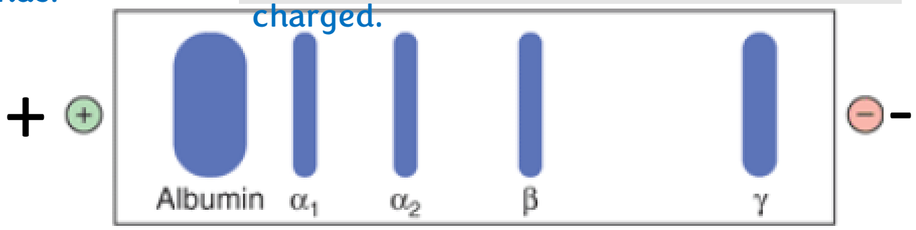
Electrophoresis is movement of proteins through a medium like a gel after applying an electrical current resulting in their separation based on their charge, size, or both

Once we stop the current (in the process of electrophoresis) and stain the proteins they form bands.

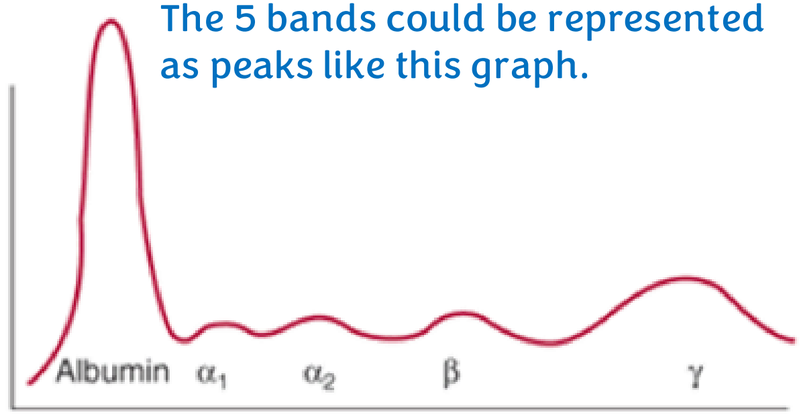
NORMAL VALUES:

Name	Absolute values (g/l)	Relative values (%)
Albumins Most abundant in plasma	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 %**

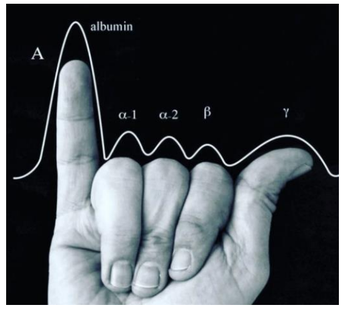
Albumin is close to the Anode which means that the protein is negatively charged.



The 5 bands could be represented as peaks like this graph.

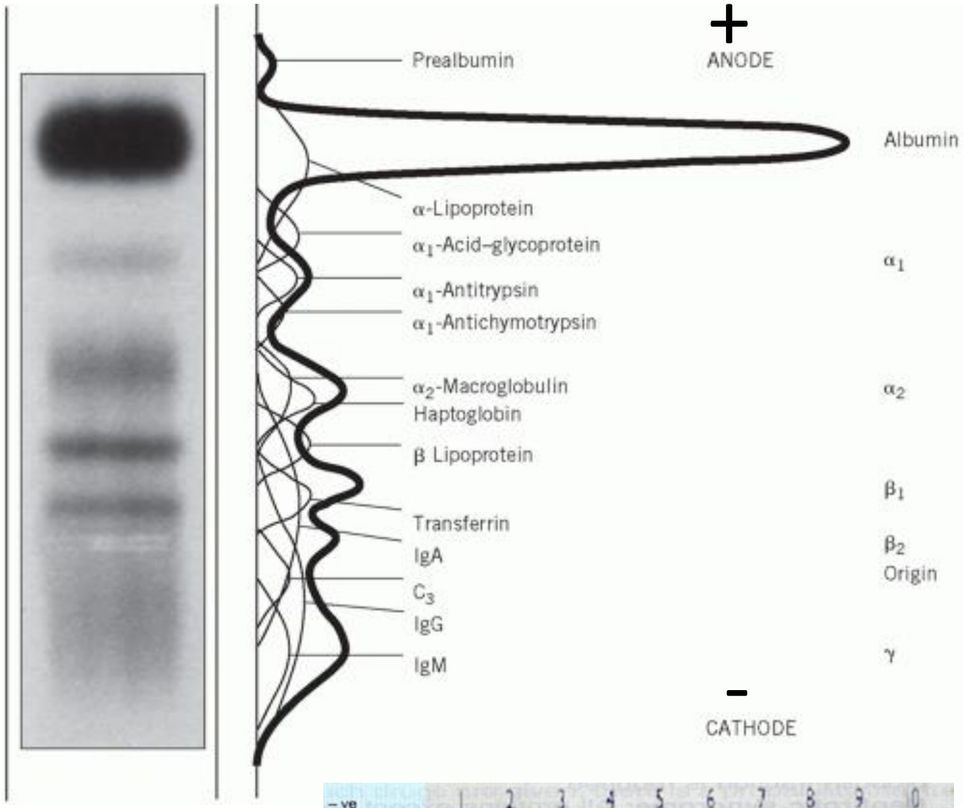


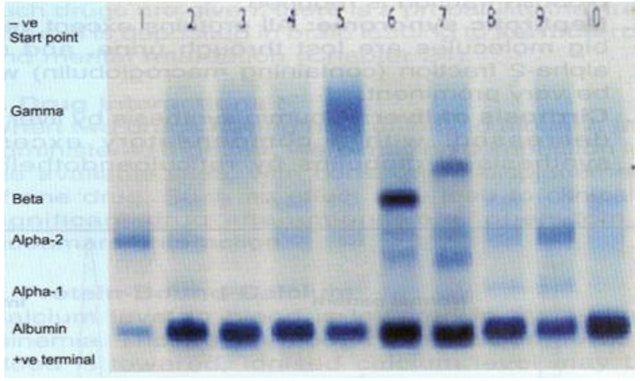
Scientists decided to name plasma proteins depending on which band are they found in electrophoresis .



Electrophoresis of plasma proteins

- Albumin is smaller than globulin, and slightly negatively charged
- Globulins (3 bands): Globulins are named so as they are globular.
  - **α band:**
- α1 region consists mostly of α1-antitrypsin
  - α2 region is mostly haptoglobin, α2-macroglobulin, & ceruloplasmin
  - β band: transferrin, LDL, *complement system proteins* (immune system proteins)
  - γ band: the immunoglobulins (antibodies)

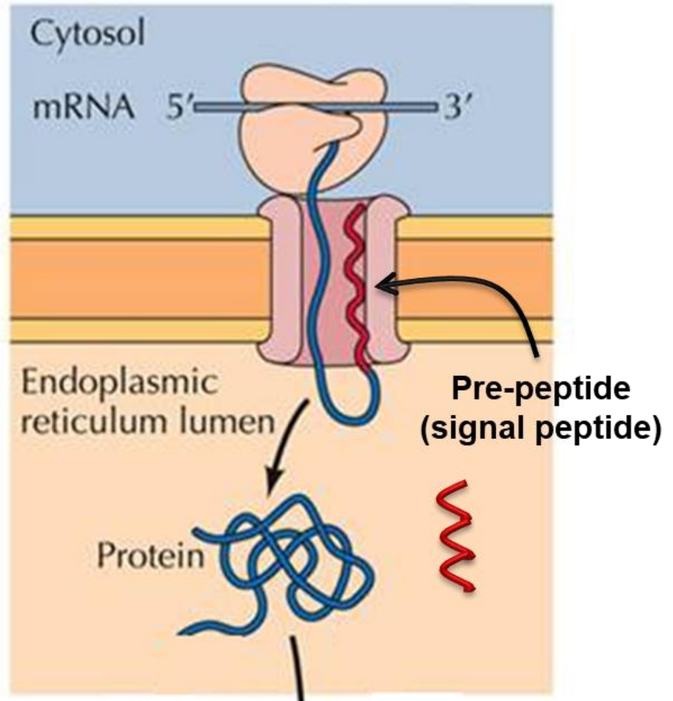


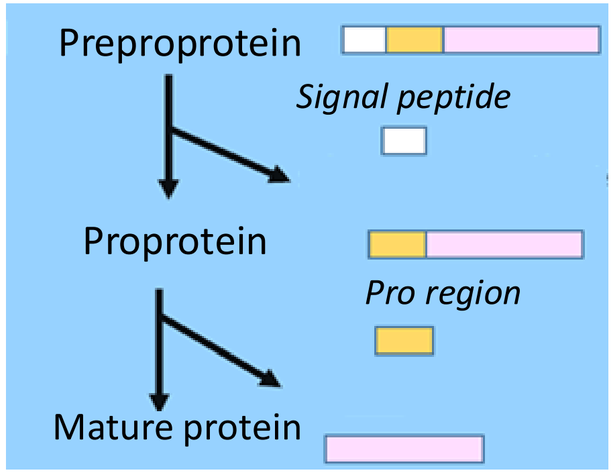


Synthesis of plasma proteins

Plasma proteins are mostly synthesized in liver, except for immunoglobulins

- Mostly liver (albumin, globulins), γ-globulins (plasma cells/B cells; lymph nodes, bone marrow, spleen).
- Most plasma proteins are synthesized as preproproteins (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.





- Pre (signal peptide) = a small sequence of amino acids that signals the protein to get into the endoplasmic reticulum (for modification). Once the protein enters the ER the peptide region (pre region) is cleaved.
- Pro = immature protein(inactive). A protein is mature once the pro sequence of peptides is cleaved.
- Note: Most plasma proteins are synthesized as preproproteins but some are synthesized as preproteins.

Plasma Proteins & genetic variation (polymorphism)

- Plasma proteins follow a Mendelian or monogenic trait:
  - A trait controlled by one gene with two alleles. (one gene makes one plasma protein)
  - 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

X-linked means that the gene in on the X chromosome (Females are going to have 2 copies of the gene , while males would have only one copy).

Plasma Proteins Half-Lives

- Albumin & haptoglobin (20 & 5 days, respectively)
- Diseases can affect half-lives
- In protein-losing gastroenteropathy such as Crohn's disease (inflammatory disease which causes severe diarrhea so the body loses plasma proteins), albumin may be reduced (1 day).

Some diseases change the levels of plasma proteins because they change their half lives (such as liver malfunction, kidney failure, myocardial infarction), So plasma proteins can be a biomarker to indicate these diseases.

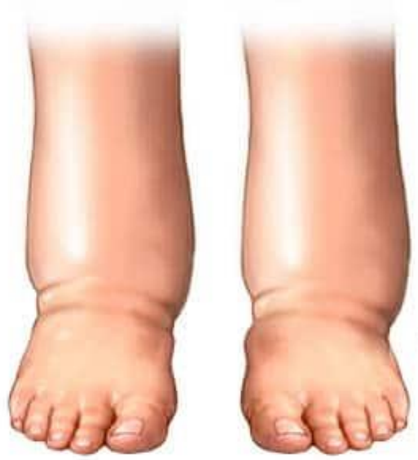
General and specific functions of plasma proteins

- 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

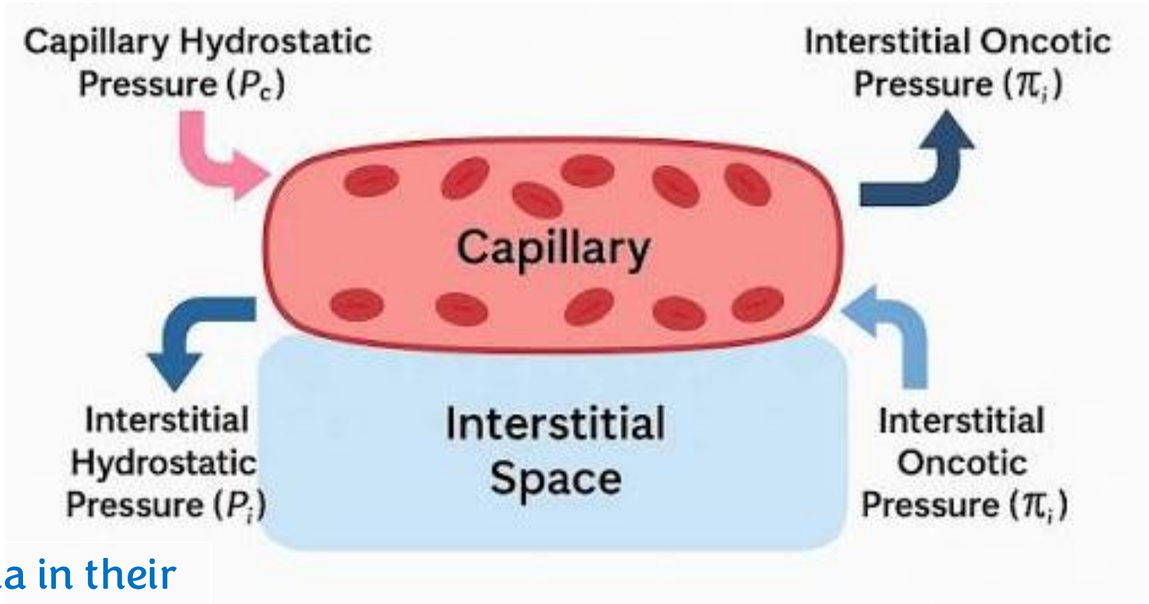
The four physical pressures that control how fluid moves in and out of blood capillaries. (the balance between plasma proteins and the fluid portion determines the viscosity, this balance is stabilized by starling forces).

- **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 (a disturbance in the fluid transfer)

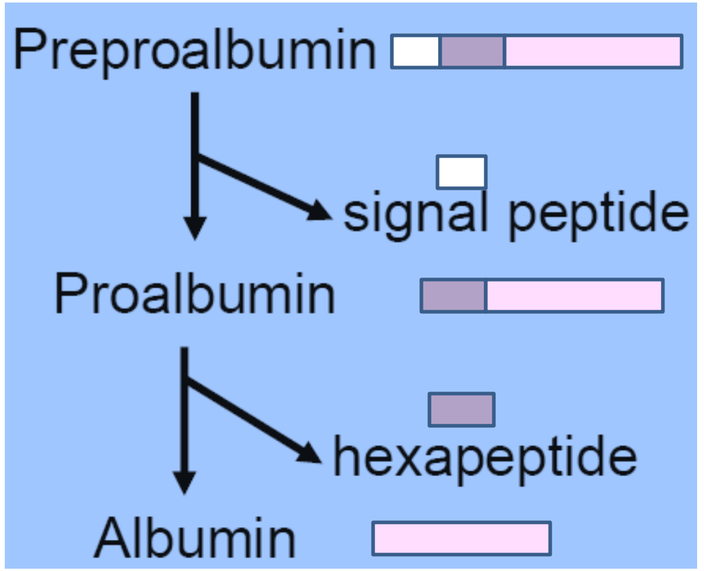


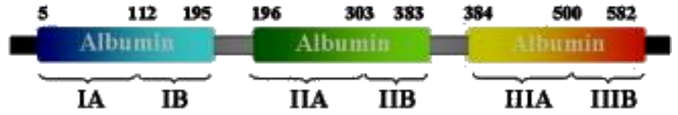
Heart isn't pumping the blood efficiently so they develop edema in their limbs because of pressure disturbance, so fluids flow to the tissues more than the vessels which causes edema.

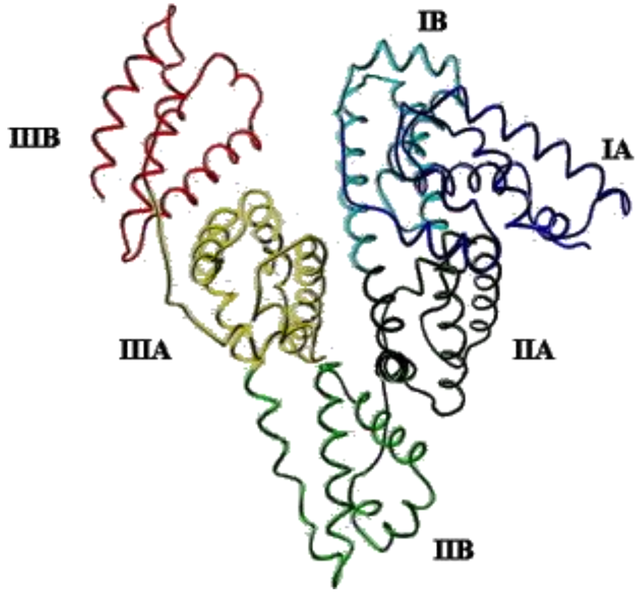
Albumin

- 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** divided into 3 domains
- Ellipsoidal shape (**does not increase viscosity like fibrinogen**)

- Anionic (which explains why it moves towards the anode) at pH 7.4 with 20 negative charges



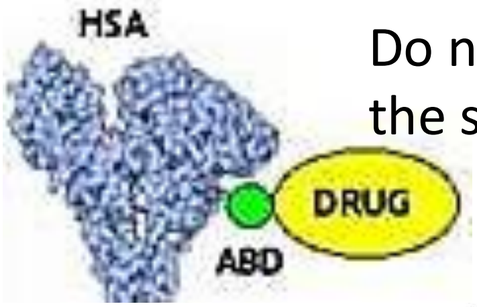




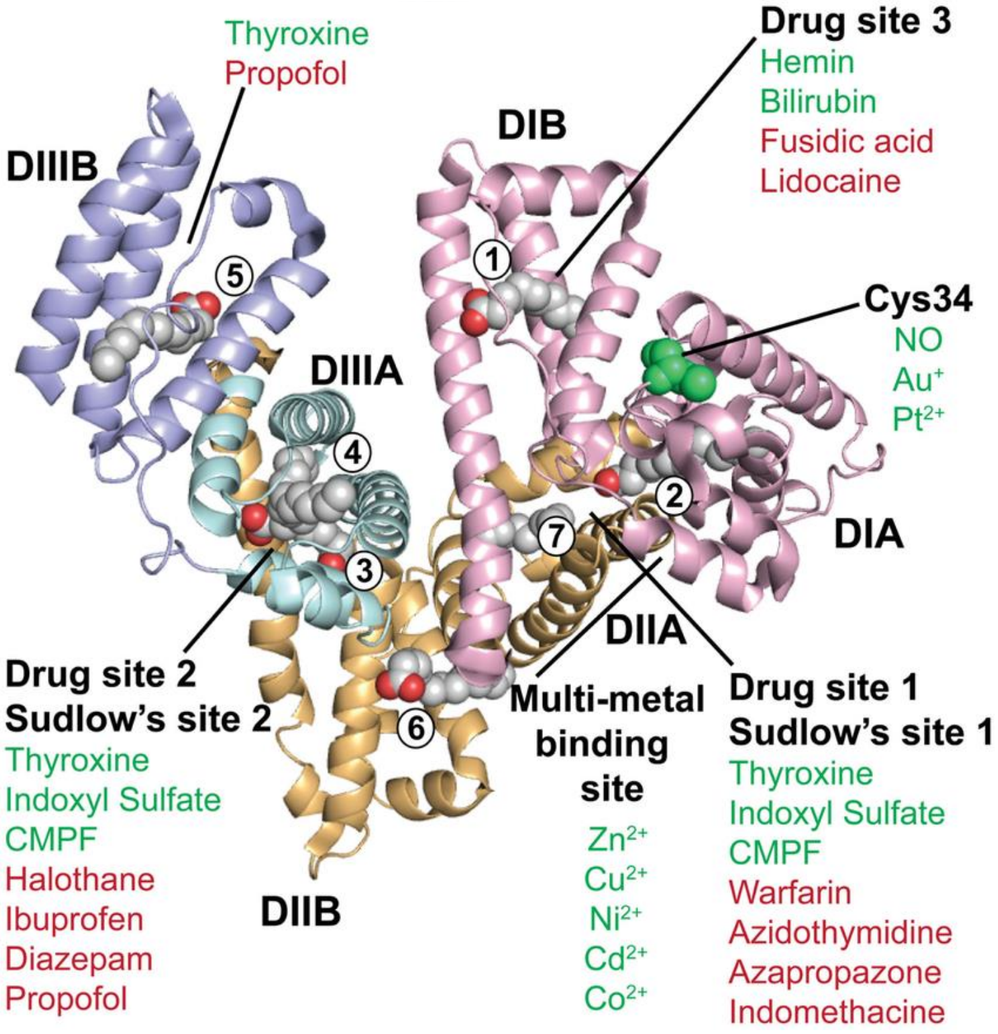
Albumin's binding capacity
binds nonspecifically

- It binds various **ligands** (small molecule that binds to a protein):
  - Free fatty acids (FFA) (albumin transports lipids from adipocytes to peripheral tissues)
  - Certain steroid hormones
  - Bilirubin (as a result of degradation of hemoglobin (heme))
  - Plasma tryptophan
  - Metals: Calcium, copper and heavy metals
  - Drugs: sulfonamides, penicillin G, dicumarol, aspirin (drug-drug interaction)

When a person is hit, the bruised area turns yellow due to bilirubin.
Newborns are yellowish in color because of bilirubin.
This substance is toxic and the body must dispose it.



Do not memorize the structure.



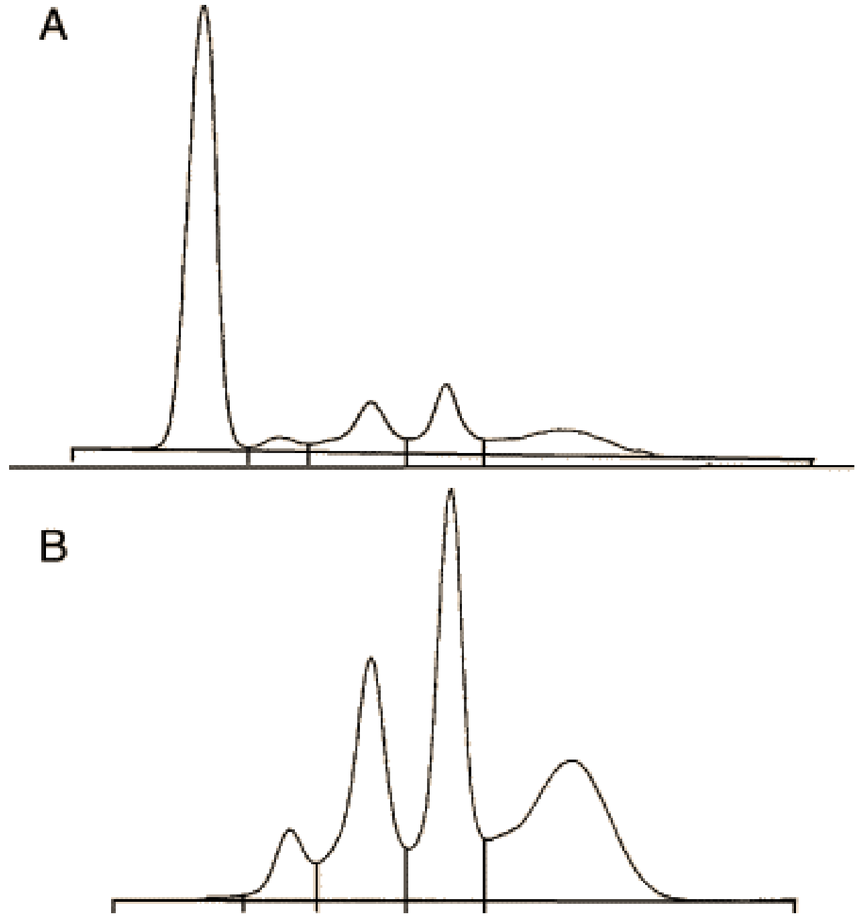
Analbuminemia

absence of albumin

- 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!!!

The levels of other plasma proteins will increase to compensate for the absence of albumin.

Normal profile



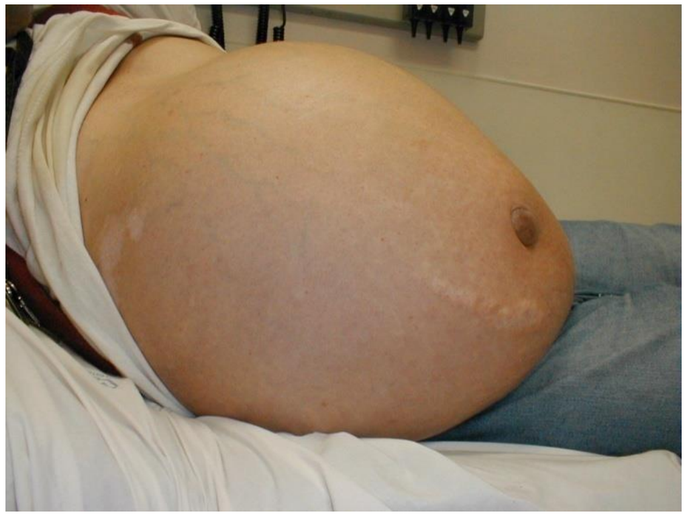
Abnormal profile

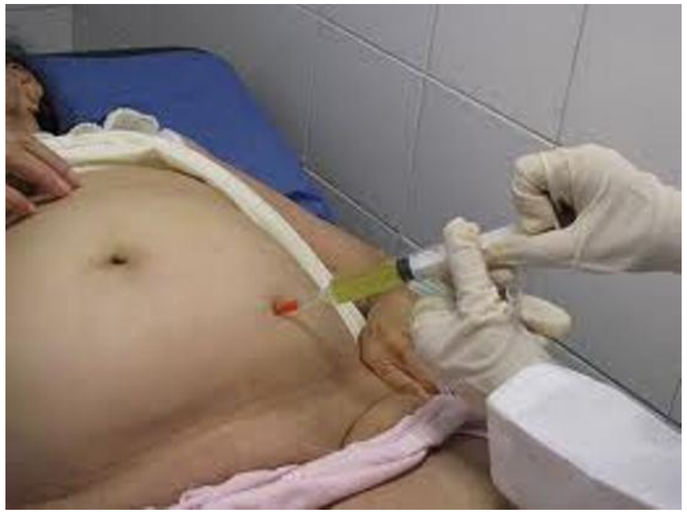
Other clinical disorders

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

- Hyperalbuminemia: dehydration (relative increase)

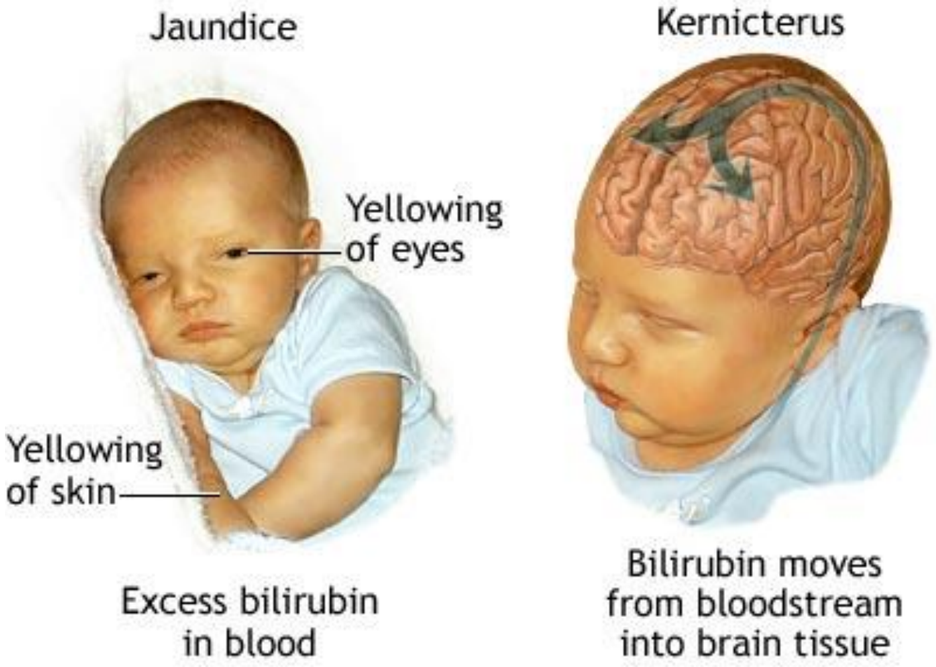
In the case of hypoalbuminemia, edema develops as there is no compensation of albumin's low level, because the gene (the one responsible for the production of albumin) is active





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** and 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.

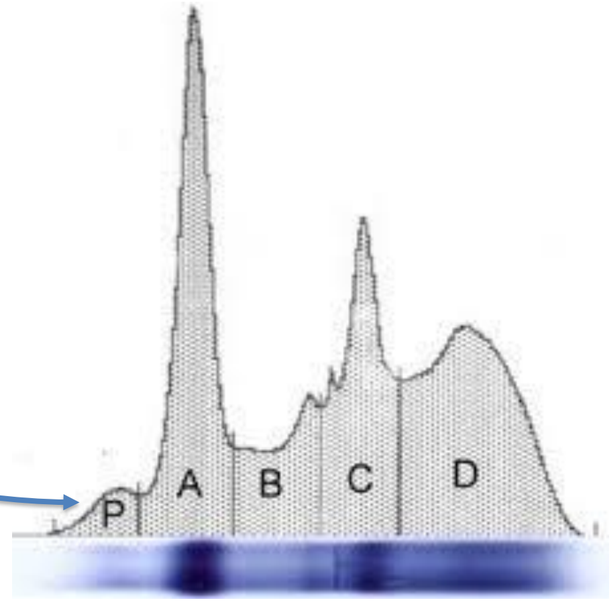
Both drugs bind to albumin.

If infants take aspirin (which is a competitive ligand), it will compete with bilirubin to bind to albumin.

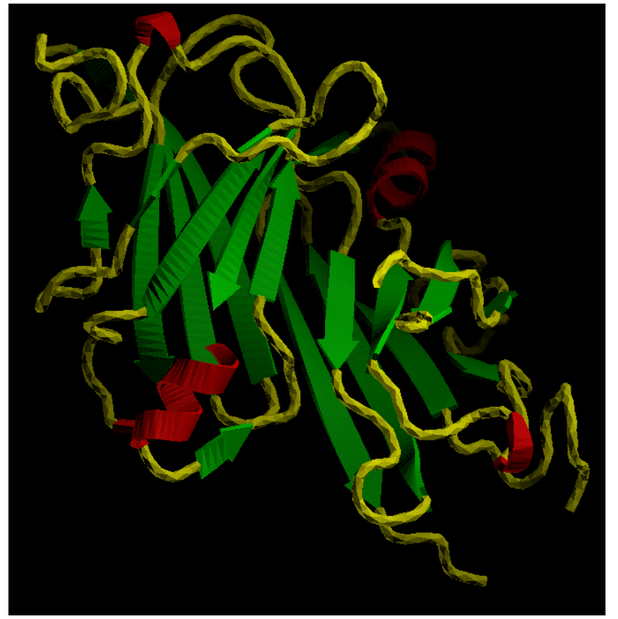
Then free bilirubin levels in blood will rise and might reach the brain (because blood-brain barrier isn't well developed yet) and cause mental retardation and brain damage which is known as **kernicterus.**

Prealbumin (transthyretin)

The pre here is part of the name and doesn't refer to the peptide sequence that gets the protein into the ER.



- 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:
  - Binding to T4 (Thyroxine) and T3 (It's a carrier)



Globulins

Do not memorize the names from this table

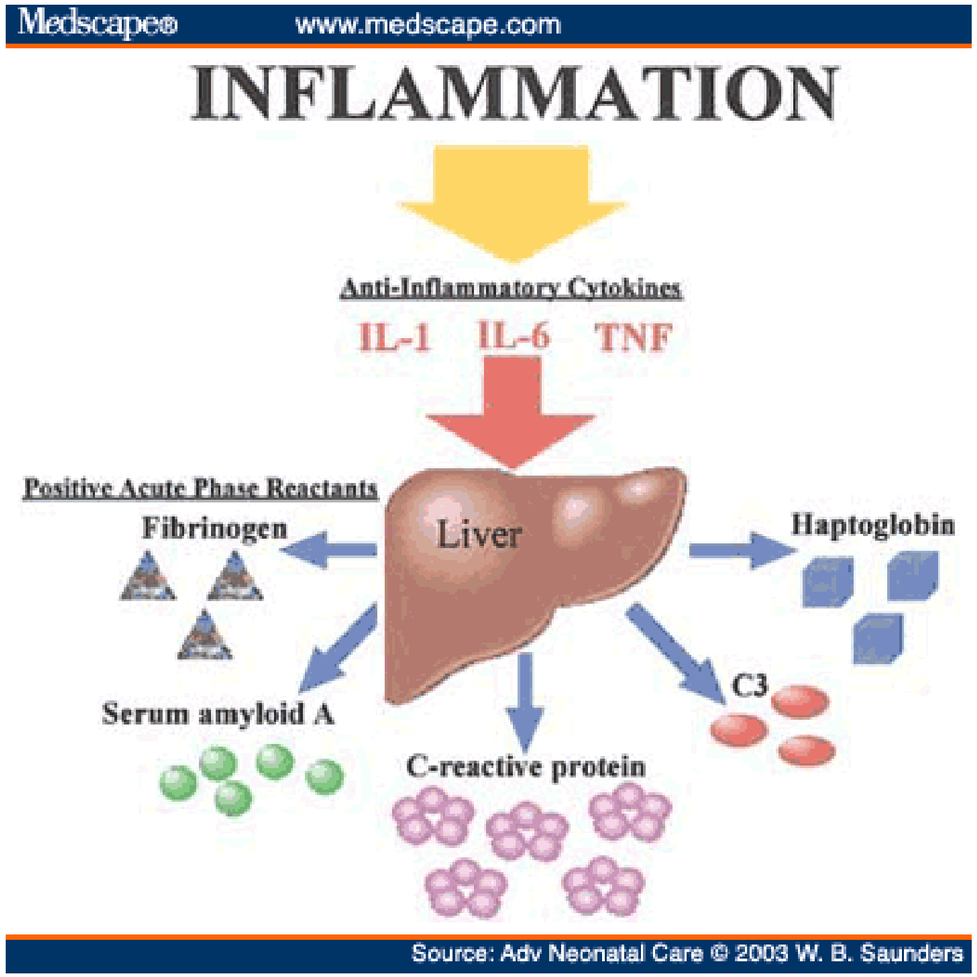
α1-globulins	α2- globulins	β- globulins	γ-globulins
▪α1-antitrypsin ▪α1-fetoprotein ▪α1- acid glycoprotein ▪Retinol binding protein	▪Ceruloplasmin ▪Haptoglobin ▪α2-macroglobulin	▪CRP ▪Transferrin ▪Hemopexin ▪β2-microglobulin	▪IgG ▪IgA ▪IgM ▪IgD ▪IgE

Acute-phase proteins

- **Positive 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**. (they enter the liver and cause either positive or negative acute-phase)
- **Negative acute** phase proteins: prealbumin, albumin, transferrin (their levels drop during inflammation)



Purpose of acute of phase proteins

When there is a tissue damage, the blood flow is reduced in this region by coagulation factors to repair the tissue

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)

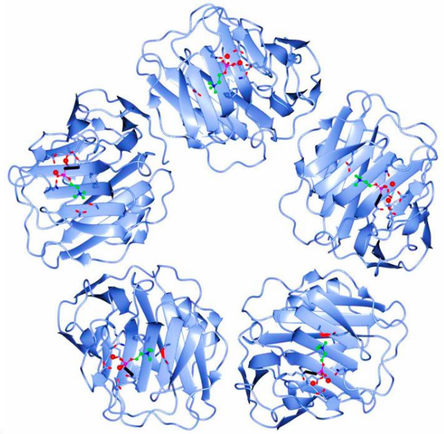
Bacteria love iron.
Transferrin levels drop during an inflammation thus it won't bind iron and carry it in the blood, so bacteria won't bind to it.

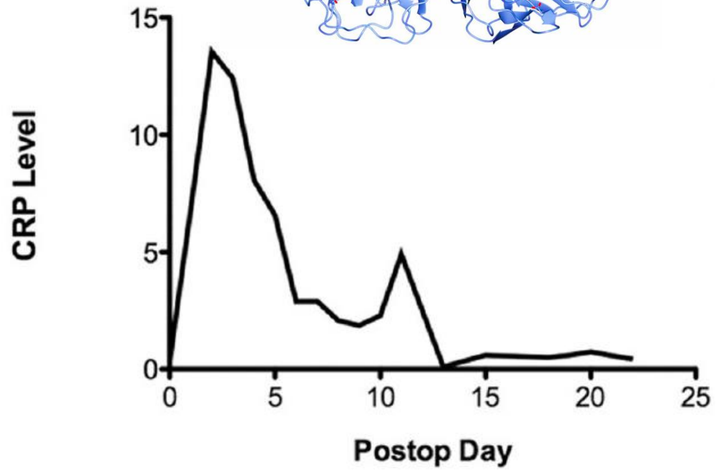
C-reactive protein (CRP)

- 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. (its levels are very low)
- 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).

So high levels mean there is an infection/inflammation

And low levels after the peak mean there is tissue repair





CRP induces immune response and at the same time it binds to pathogens on damaged cells and stimulates the immune system.
So the immune system either fights the antigens or clears the area (phagocytosis)

α1-antitrypsin

- Also known as (AKA) *α1-antiproteinase* (52 kDa)
- It neutralizes (inhibits) the digestive trypsin & trypsin-like enzymes (such as elastase).
- It constitutes 90% of α1- globulin band
- Many polymorphic forms (at least 75 alleles)
  - 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).

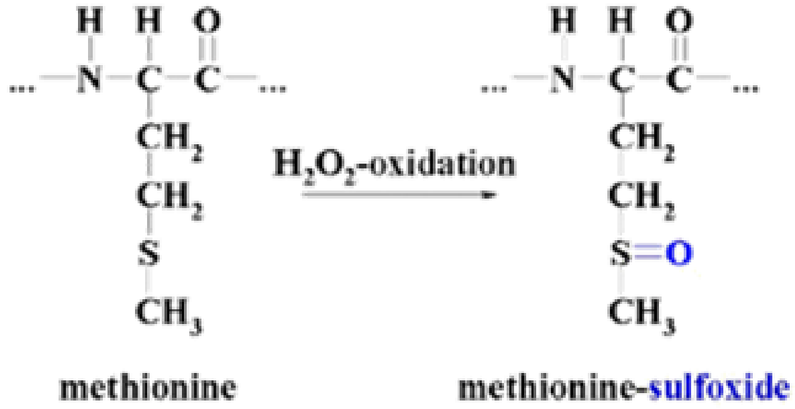
Active elastase + α_1–AT → Inactive elastase: α_1–AT complex → No proteolysis of lung → No tissue damage

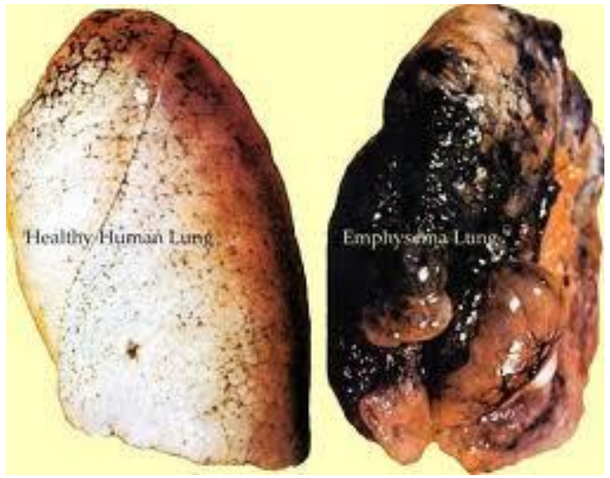
Active elastase + ↓ or no α_1–AT → Active elastase → Proteolysis of lung → Tissue damage

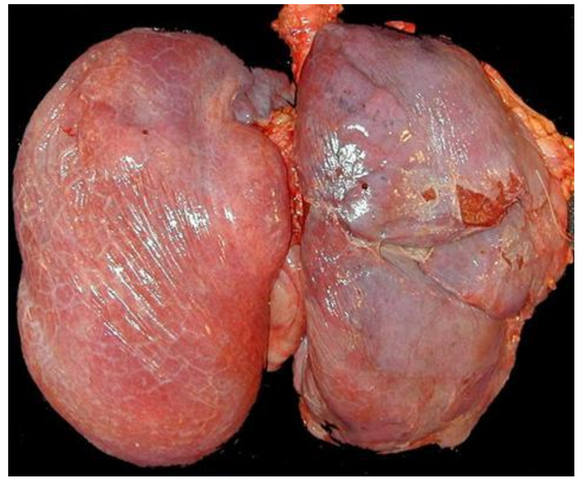
Smoking & α1- antitrypsin deficiency

Protease is an enzyme that breaks down other proteins.

- Smoking causes chronic inflammation. Why?
- Oxidation of Met358: (makes α1- antitrypsin non-functional)
  - 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.

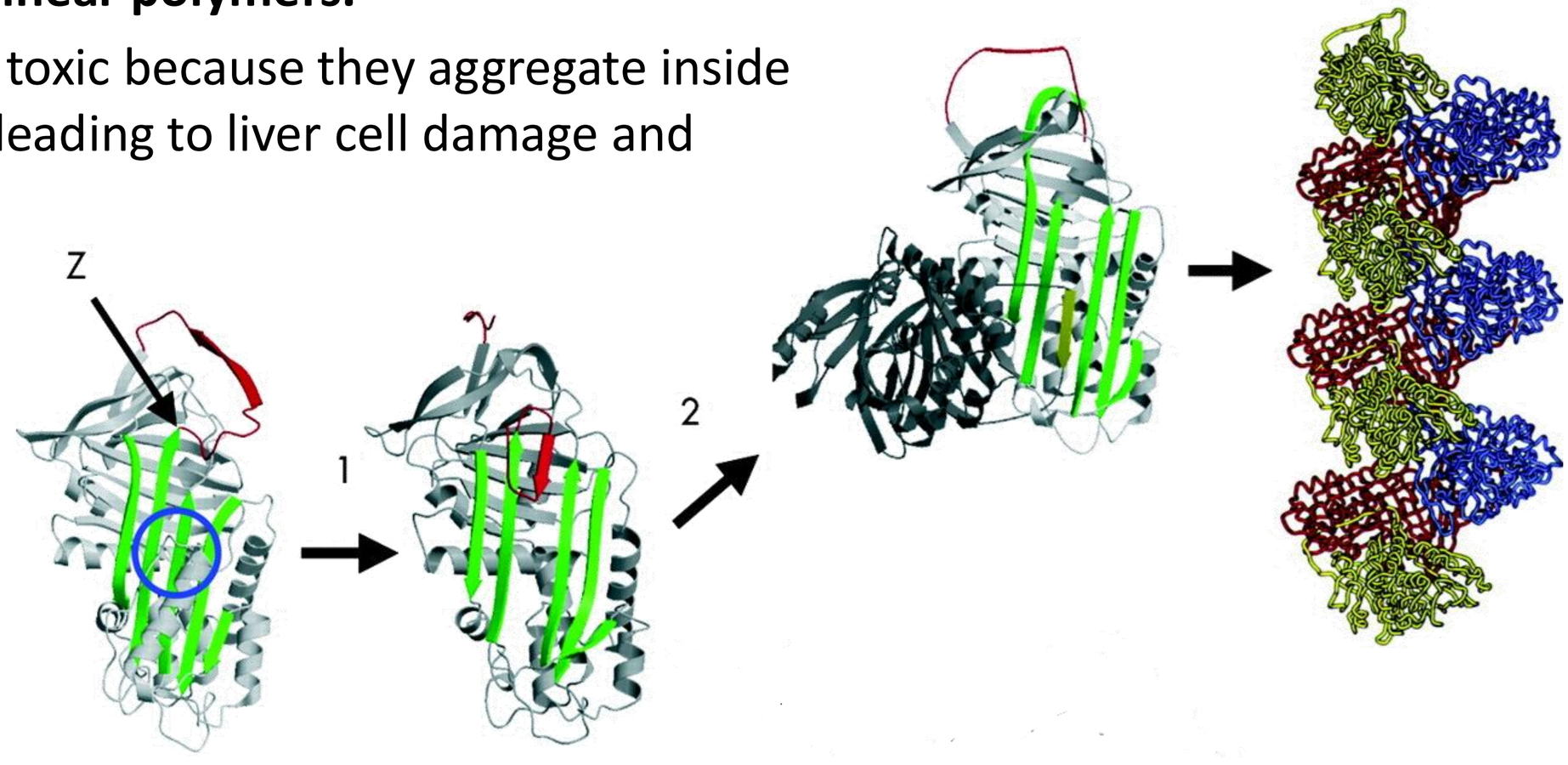






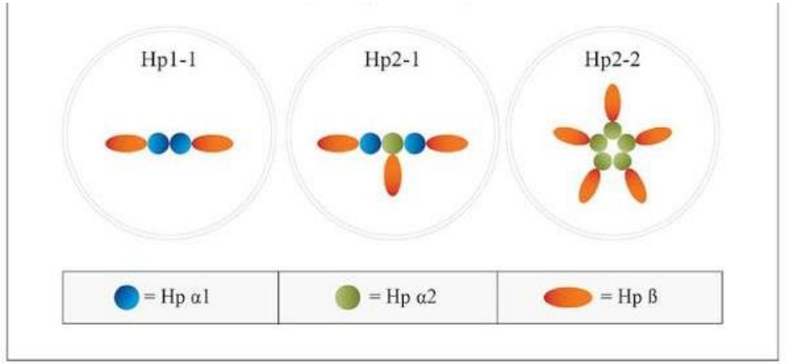
Liver disease & α1- antitrypsin deficiency

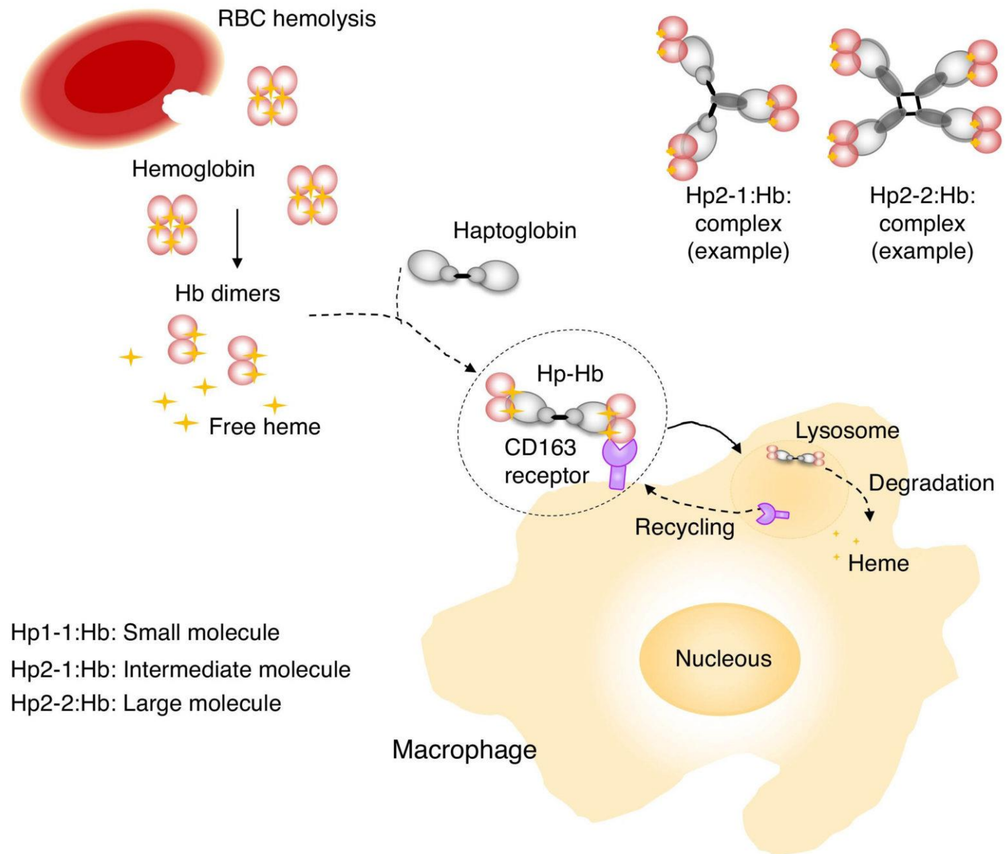
- The ZZ genotype causes the **mutant Z protein** to misfold **inserting a loop** into a beta-sheet resulting in the formation of **linear polymers.**
- The polymers are toxic because they aggregate inside the hepatocytes, leading to liver cell damage and cirrhosis.



Haptoglobin (Hp)

- It is an acute phase protein.
- α2 glycoprotein (90kDa)
- A **tetramer** (2α, 2β)
- Two genes, designated Hp1 and Hp2, producing **3 phenotypes**: (memorize the ones in red only)
  - Hp 1-1 homozygote→ α1, α1 + 2β
  - Hp 2-1 heterozygote→ α1, α2 + 2β
  - Hp 2-2 homozygote→ 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 (RBCs break rapidly, Hp binds to a lot of Hb, Hp's half live becomes 90mins).





Iron is important for cells for two reasons:

1- it makes hemoglobin

2- it is toxic when it’s left free in our bodies

When RBCs die, they release hemoglobin molecules.

Haptoglobin's half-life is 5 days, but it drops to 90 minutes when bound to hemoglobin.

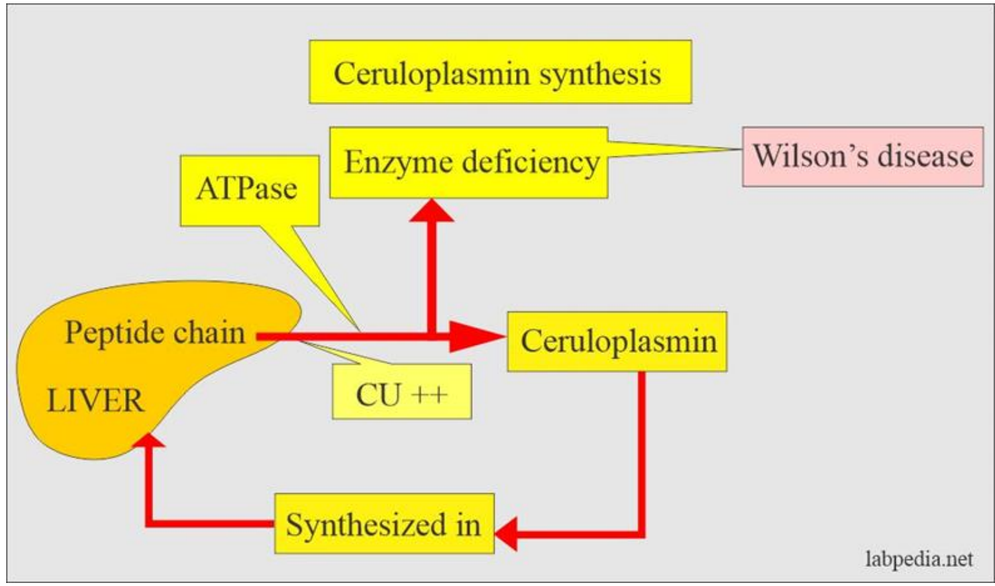
Ceruloplasmin

- 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 (enzymatic activity) that oxidizes ferrous to ferric forms of iron. (aids in absorption in intestines)
- Albumin is more important in transporting 10% of copper.

Copper's high levels in blood could lead to tissue damage like iron.

Cu-containing enzymes

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



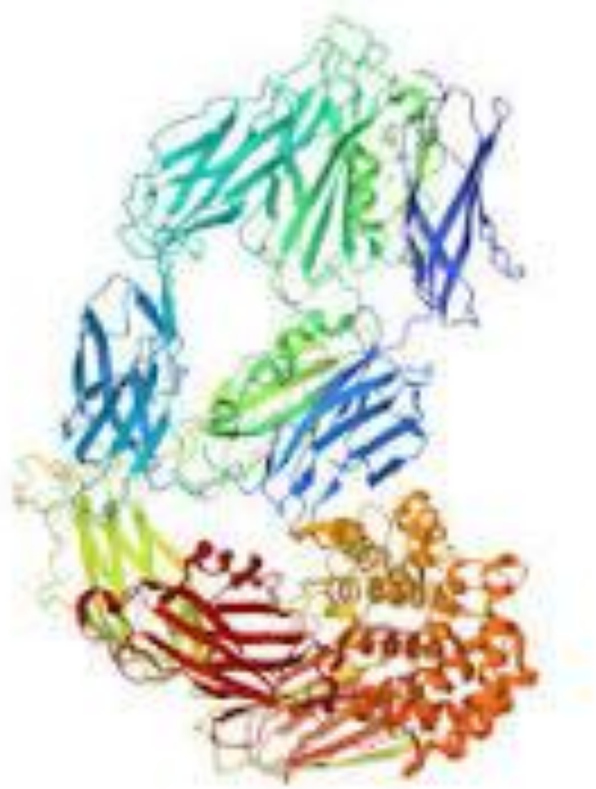
In fact, the copper atoms in ceruloplasmins aids the ferroxidase activity, so the bounding of copper to ceruloplasmins is irreversible. Therefore, albumin is more important is transporting copper to tissues, because it bounds reversibly to it.

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.

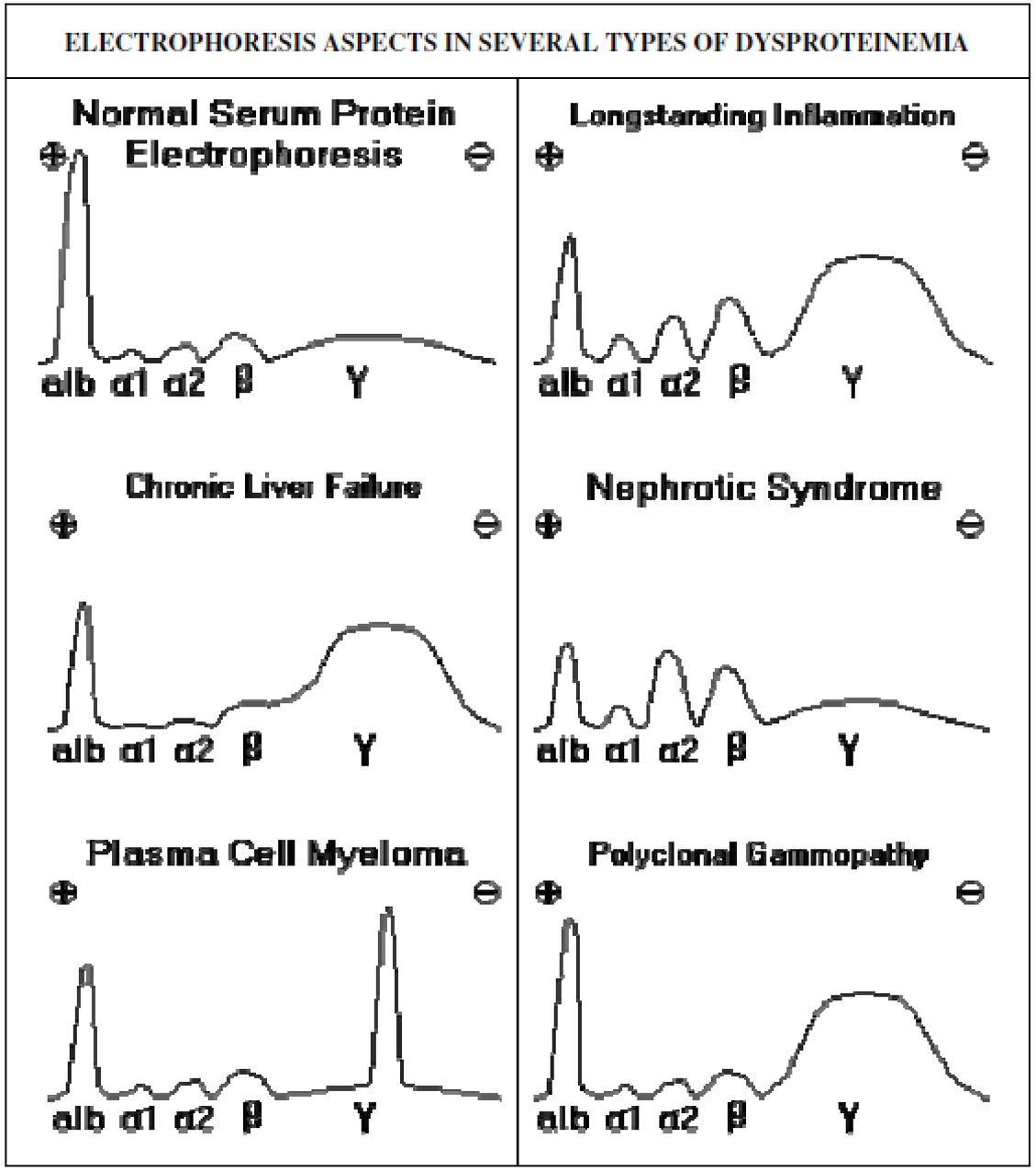
Alpha-2 macroglobulin

- 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 (because it's too large, it will stay in blood, but other molecules will be lost) or **drop** during acute pancreatitis and severe liver disease.



Gamma globulin levels will increase relatively as other globulin levels decrease.

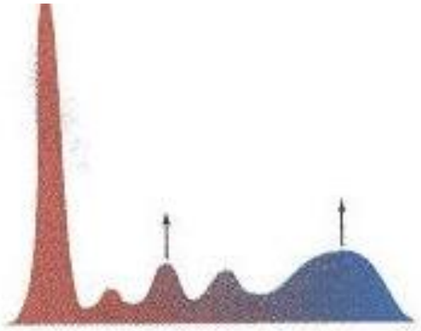
Plasma cell myeloma: an immune cell became a cancerous one, so certain type of gamma globulins increase in levels **(sharp peak)**



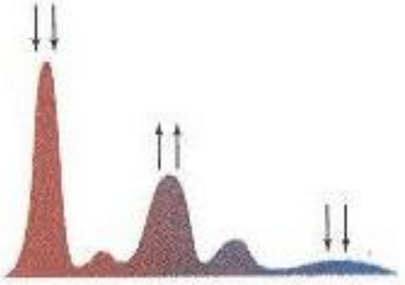
During longstanding inflammation, immunoglobulin levels increase alongside with positive acute-phase plasma proteins, while negative acute-phase protein levels decrease.

Alpha 2 globulin (macroglobulin) levels will increase relatively as small globulins and albumin will be excreted.

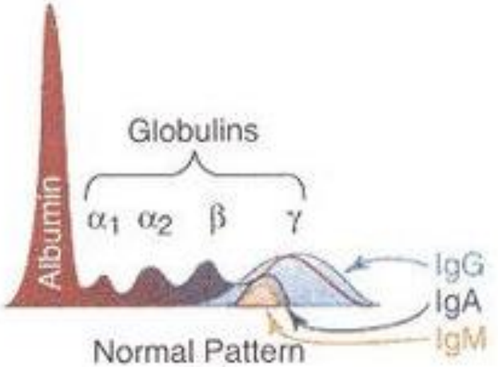
Different B cells making different antibodies, so gamma peak rises and **widens**



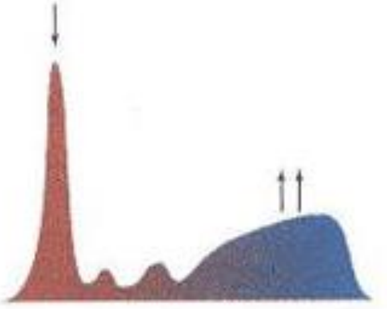
"Delayed Response Pattern"
(c)



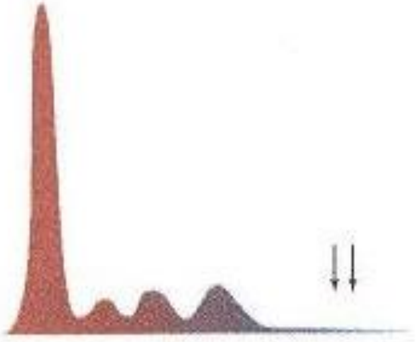
Nephrotic Syndrome
(g)



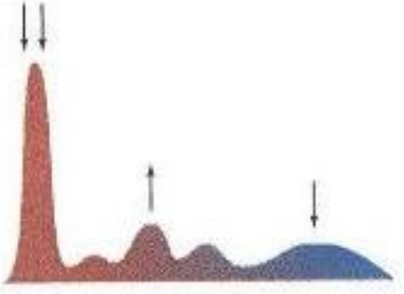
Normal Pattern
(a)



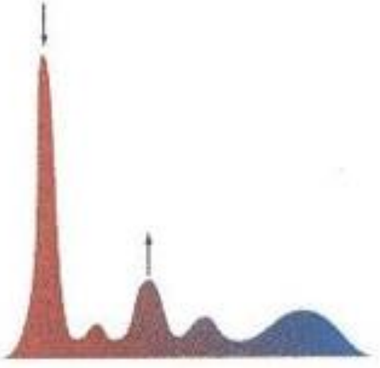
Hepatic Cirrhosis
("Polyclonal Gammopathy")
(e)



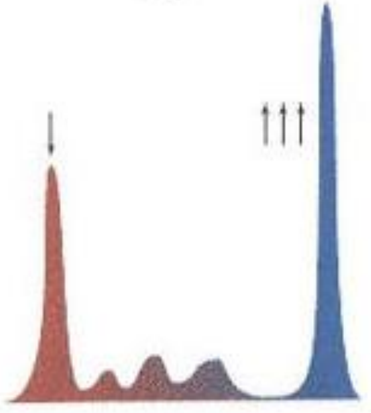
Hypogammaglobulinemia
(d)



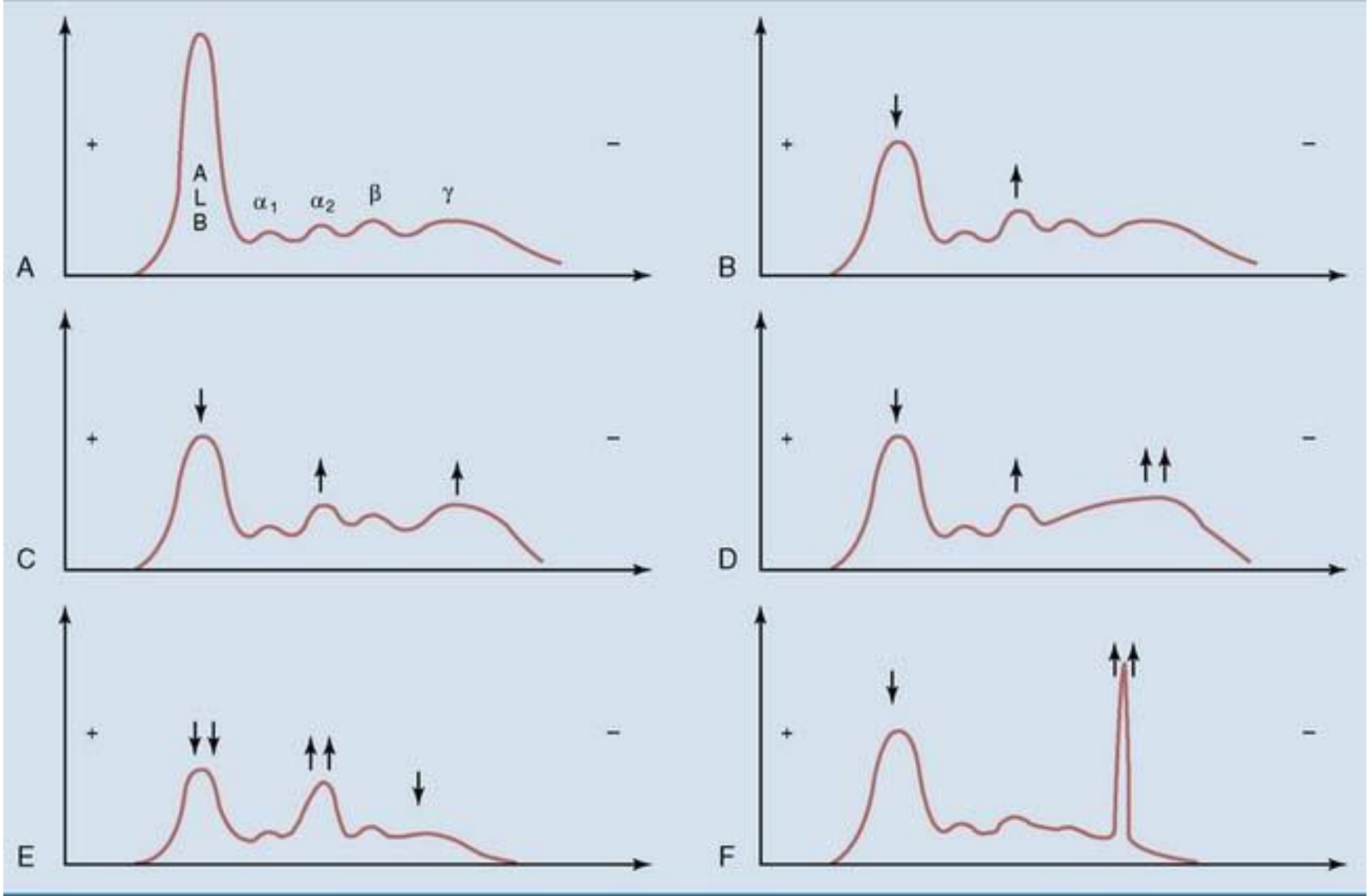
Protein-losing Enteropathy
(h)



"Immediate Response Pattern"
(b)



Paraprotein
("Monoclonal Gammopathy")
(f)


A
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Quiz!

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks’ Basic Medical Biochemistry (6th edition)