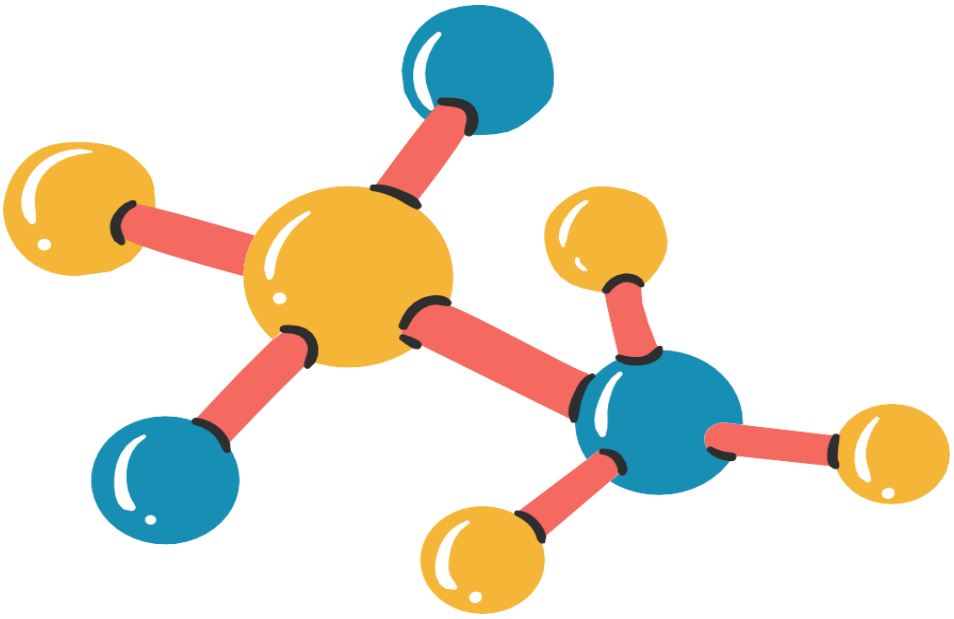
Sheet no.24

Biochemistry



Summer 2022

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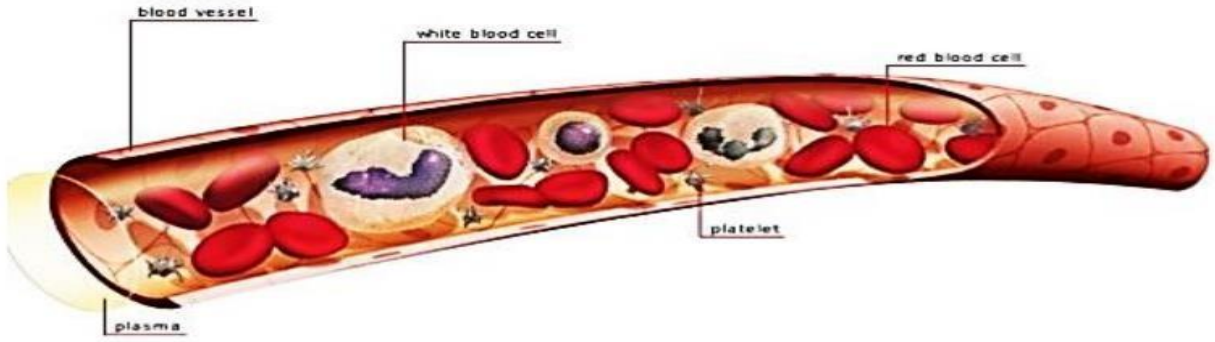
Plasma Protein



Plasma Proteins

Firstly , we discuss the plasma proteins from its structure and some functions of it . Also, we know that the liver is the major factory of these proteins , they are synthesized in inactive form for two purposes :

1* Because we need them in other places to function not in the liver

2* To regulate the effect of (we don't needthem active all the time or inactive as well)

We shall go in a small journey for some of these proteins (nice trip)

What should we know?

1. What is plasma, and how can we extract it?
2. What are the different components of plasma?
3. Plasma proteins (general functions, basis of classification, associated processes and molecules)
4. Plasma proteins: (structure, synthesis, function & diseases associated)

Albumin & pre-albumin	α1-antitrypsin	Haptoglobin (Hp)
α1-fetoprotein (AFP)	Ceruloplasmin	C-Reactive Protein

Plasma→ a liquid part of the blood

Types of Plasma Protein:

-Pre-albumin.

-Albumin

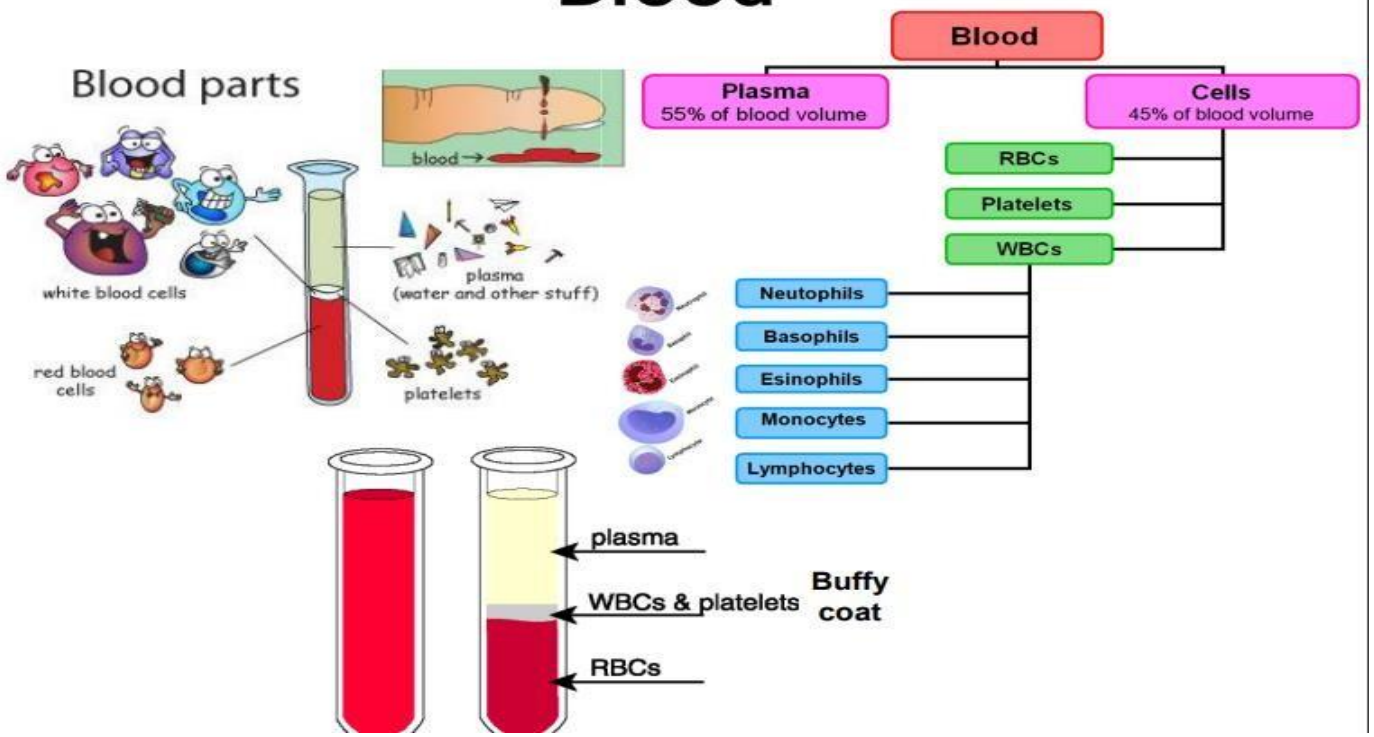
-α1 -Globulins: α1 -Antitrypsin, α-fetoprotein

-α2 -Globulins: Ceruloplasmin, haptoglobin

-β-Globulins: CRP, transferrin, β2-microglobulin

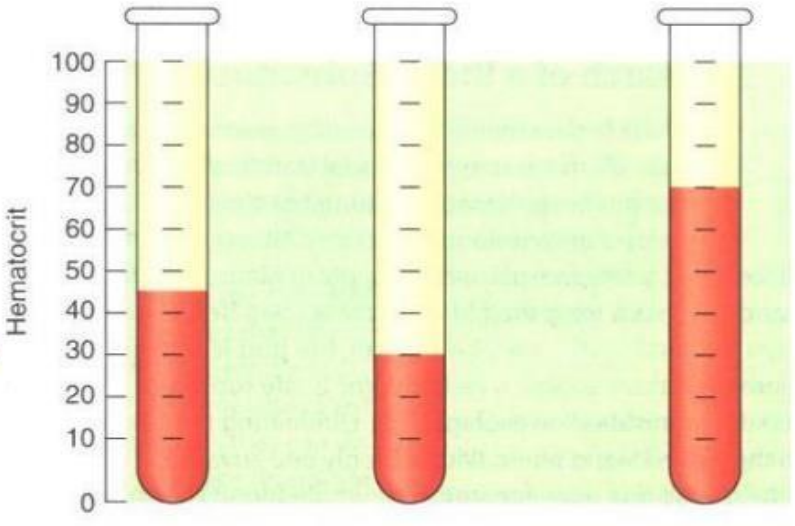
-γ- Globulins T: immunoglobins

Blood

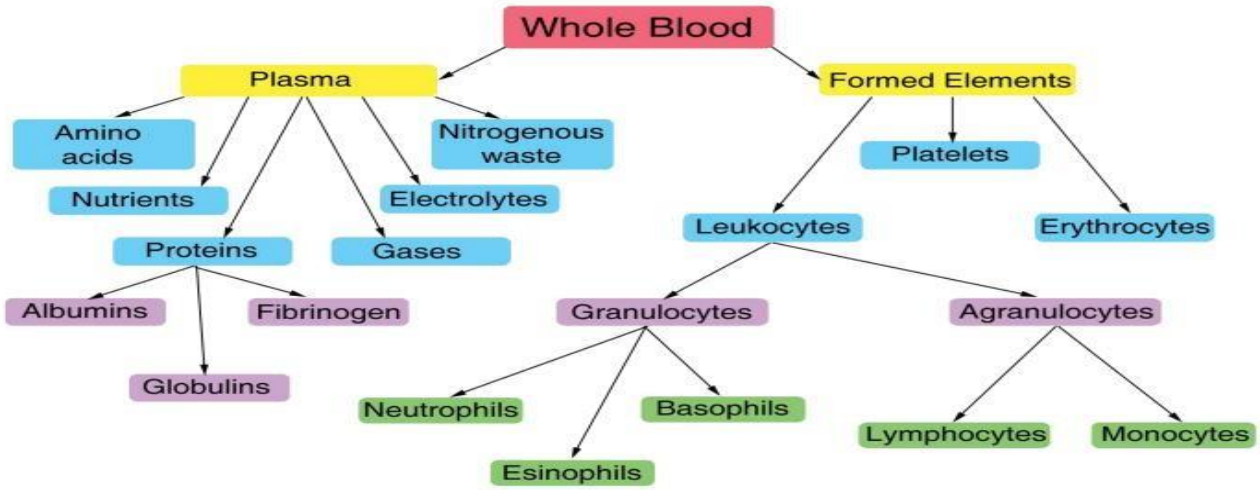


Blood: plasma vs. hematocrit

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



Blood: what is inside plasma

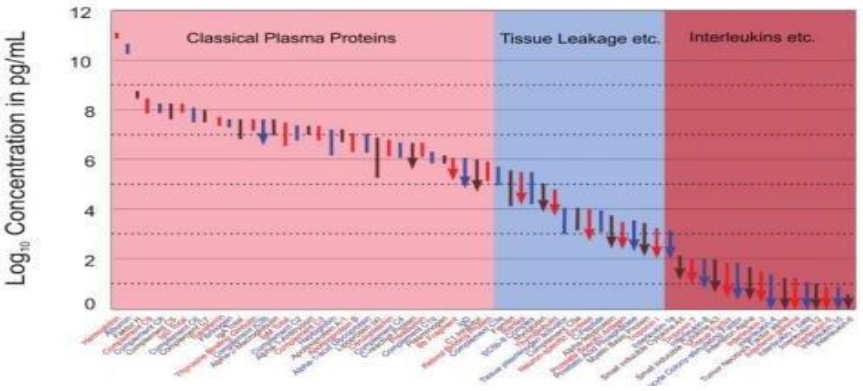


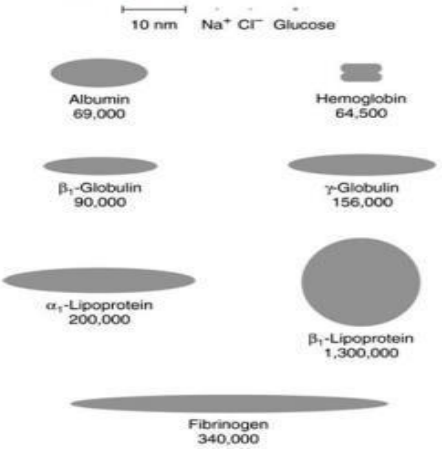
Plasma

- 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-}

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)





There are many plasma proteins varying in size , like :

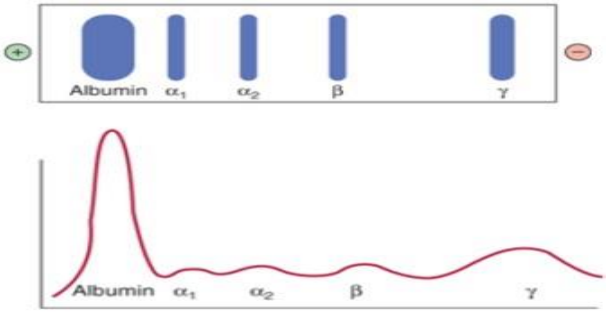
*** Albumin → ellipsoidal in shape**

***Fibrinogen→ elongated shape**

***Although the atomic number of hemoglobin is approximately equal to the atomic number of albumin , it has a completely different shape .**

The separation of plasma proteins

- Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins
- Electrophoresis (most common): serum (defebrinated 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

If we take a blood sample and we want to extract the plasma , we have two ways:

1* TIME: by putting the sample for a time to precipitate and separate the components(فصل بالراحة) .

2*CENTRIFUGE MACHINE: by putting the sample in the machine so the components will be separated by MW in every cycle. (In BIO 101, we saw this technique in separating the cell's organelles , just to visualize it)

We take the plasma, now we want the proteins in this sample.

Using two methods:

-Salting in, Salting out.

Firstly, we all know that the salts when we adding it to aq solution, it get ionization into (+ve and -ve) ions forming hydrogenation shell on each of them. The capacity of salts to ionize in -aq- solutions is more than the other molecules.

In this scenario:

1* Adding salts to the solution (actually we add +ve and – ve charges) makes the whole charge of the solution appears clearly – not increasing in the amount – because we add equal amounts of ions . by more and more increasing of salts, the electronegativity of the solution appears more and more.

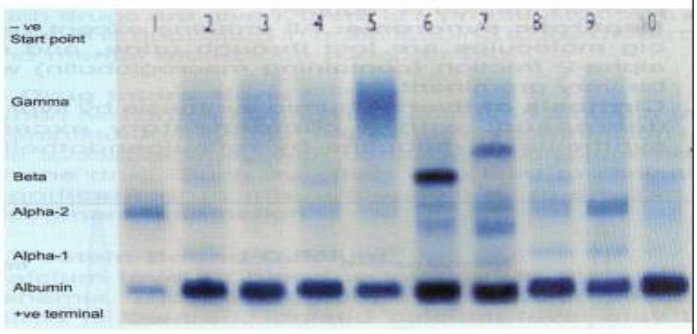
2*Proteins are soluble compounds in the plasma, but here we have a competition between them and the salts to

interact with water molecules - as we know the salts have a high ionization ability- so the proteins will lose this competition. In other words, proteins will precipitate because they don't find a water molecule to interact with.

3* Precipitation depends on the *solubility degree* ofthis protein:

The high soluble one, the last one precipitate.

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

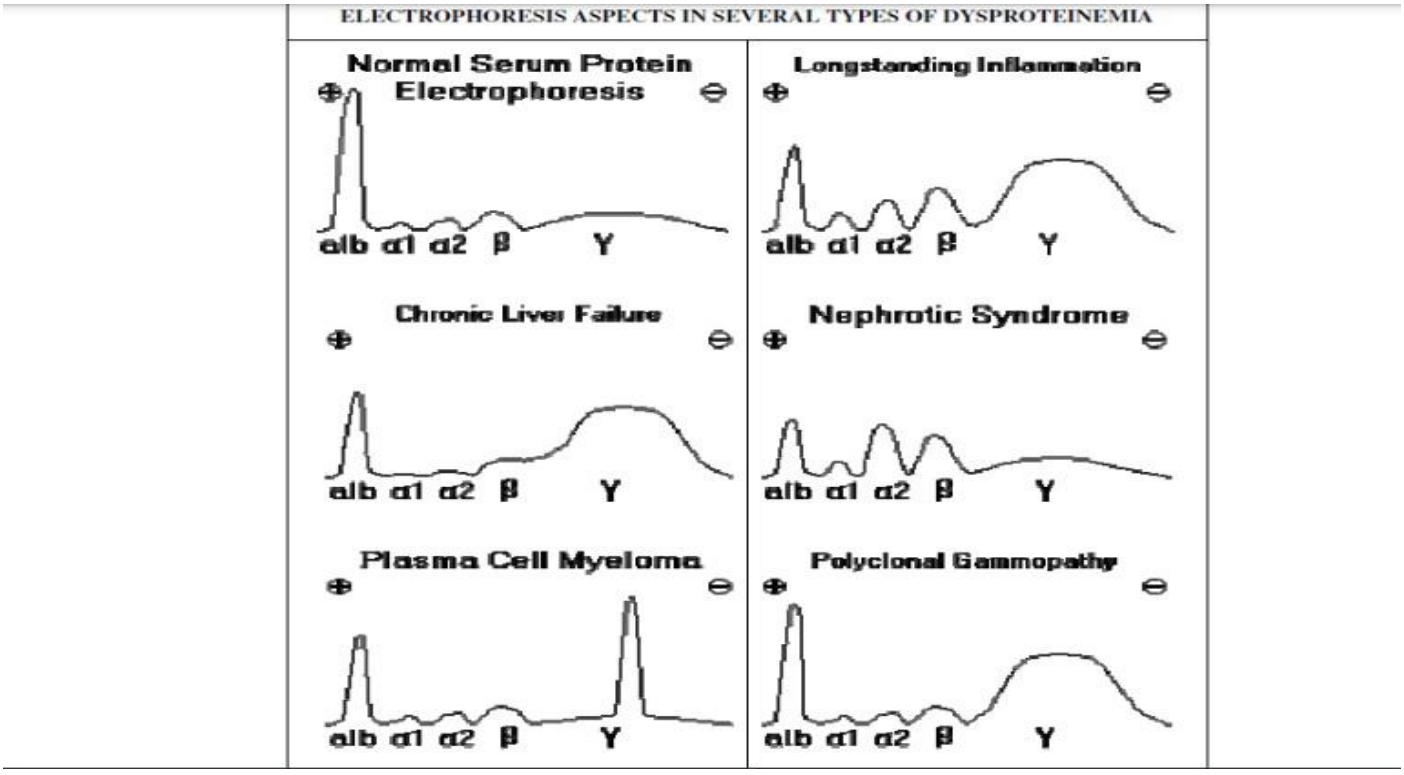
We use a plate of a gel (Agarose as an ex) and putting the sample in it,apply a current pushing the proteins from the (-ve) electrode to the(+ve) electrode depending on the MW – if we cancel the charge and the shape factors – (as known from the Molecular part).

But, we can't use the plasma as it because it contains blotting factor – mainly the fibrinogen- so it will close the pores and the proteins won't move. So logically using (plasma – fibrinogen) = serum which called -defibrinated plasma-.

By electrophoresis, we get the following chart we can take these details:

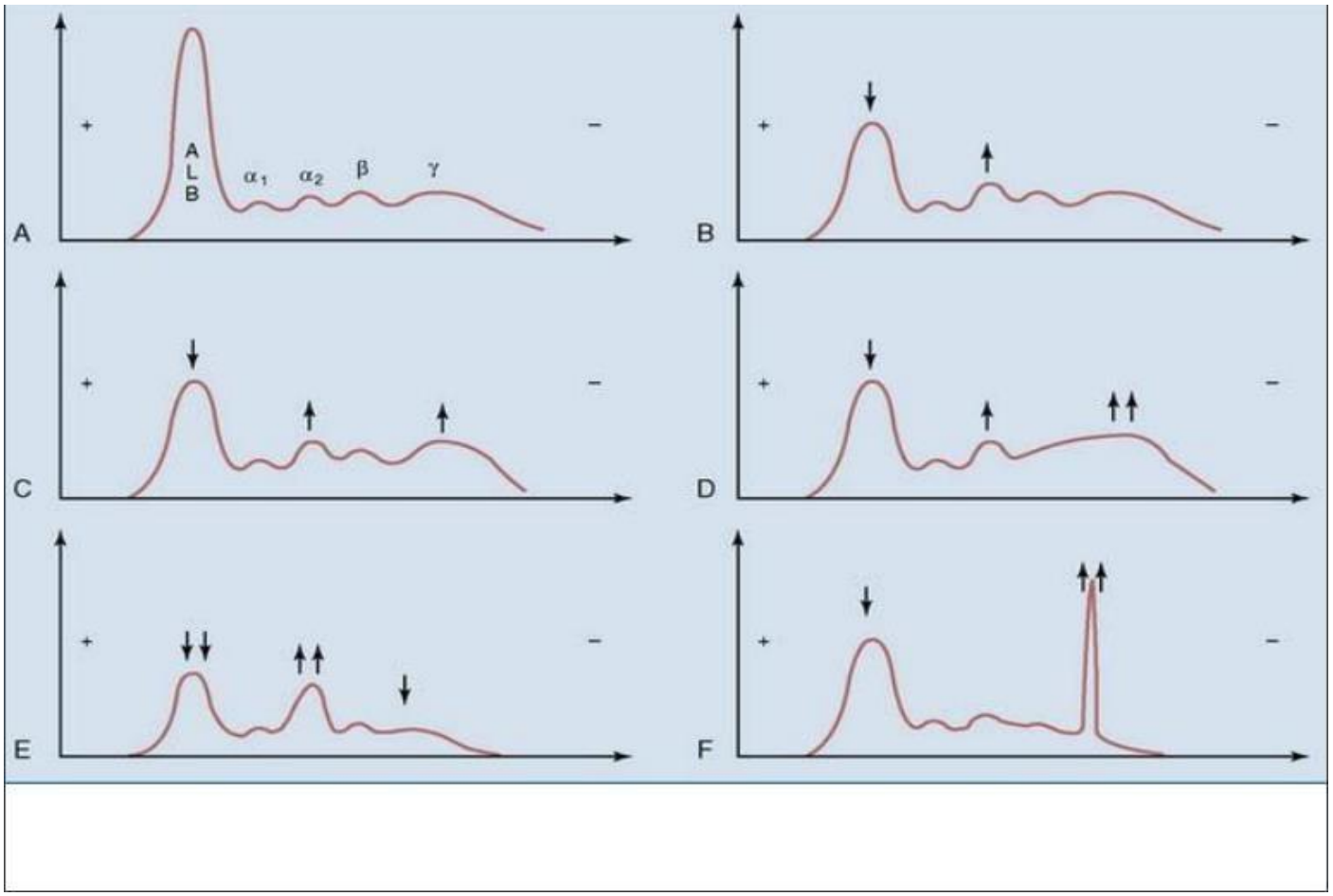
1* The albumin is the more negative protein because it is the nearest one for the (+ve) electrode.

2*The huge albumin band reflects the more concentration of it in the sample (the more concentrated protein in the plasma).



3* Any change in one of the protein concentration(as chronic liver), is followed by increasing in others.

4* The γ proteins almost have the same concentration.



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

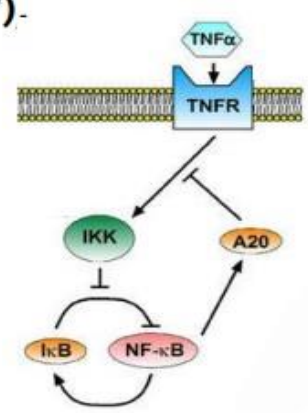
- A nutritive role
- Maintenance of blood pH (amphoteric property)
- Contributes to blood viscosity
- Maintenance of blood osmotic pressure

Specific functions

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

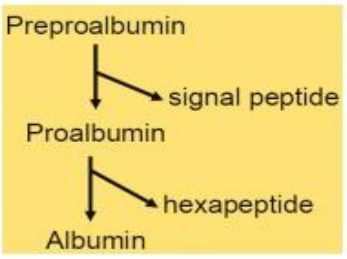
Acute-phase proteins

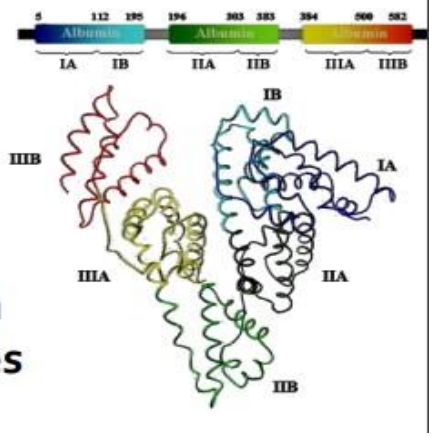
- Levels increase (0.5-1000 folds), acute inflammation, tissue damage, chronic inflammation & cancer. C-reactive protein (CRP), α1 -antitrypsin, haptoglobin, & fibrinogen
- Interleukin-1 (IL-1), main stimulator (gene transcription)
- Nuclear factor kappa-B (NFkB): Exist in an inactive form in cytosol, 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

*The Major Protein in Human Plasma

*Its small molecule =69 kDa, half-life (20 days)

*The main contributor to the osmotic pressure due to the proteins = oncotic pressure (75-80%)

*It's synthesized by the Liver: 12 g/day (25% of totalprotein synthesis) (liver function test)

Synthesized as a preproprotein:

-One polypeptide chain, 585 amino acids, 17 disulfide bonds.

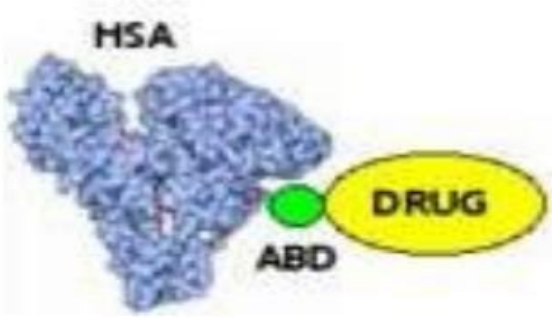
*Proteases (enzymes work in degradation of the proteinsas trypsin) subdivide albumin into 3 domains.

Ellipsoidal shape (viscosity) vs. fibrinogen (used in the clotting process, the small MW of albumin and unglycosylated (no sugar bounded) make it less viscous so the efficiency of it in transporting and binding will increase).

It gets two steps of 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)



Binds various ligands: noncovalent interactions.

-Free fatty acids (FFA)

hormonal signal in the blood from *adipose tissue*.

-Certain steroid hormones

-Bilirubin: intermediate of degradation of heme group.

*The RBC get regeneration every 120 days, so the components will be broken down (except the ferrous). One of these products is bilirubin (insoluble molecule),

we should convert it to soluble form to excrete outside of the body.

*It should go to the hepatocyte. transporting facilitated by the albumin.

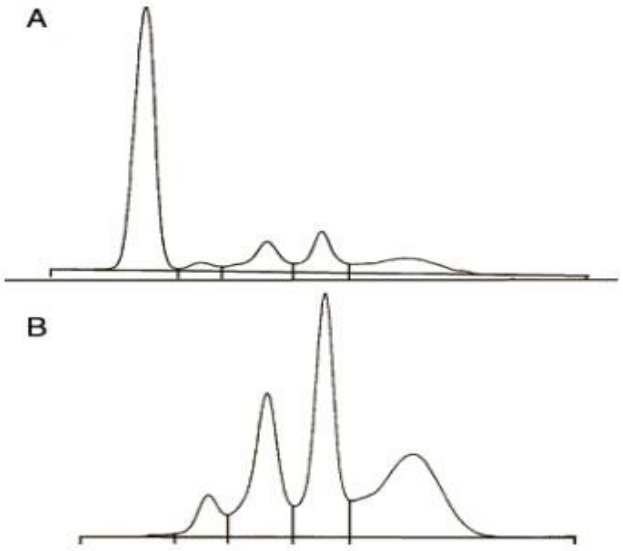
-Plasma tryptophan (very hydrophobic with large R group)

-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!!!



There are human cases of an albuminemia(rare)

*Autosomal recessive inheritance: both copy of the genes should be abnormal to this condition.

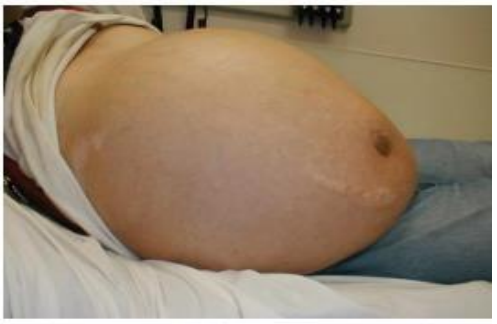
*One of the causes: a mutation that affects splicing, cause it is genetic, it leads to the absence of the expression of the albumin = analbuminemia

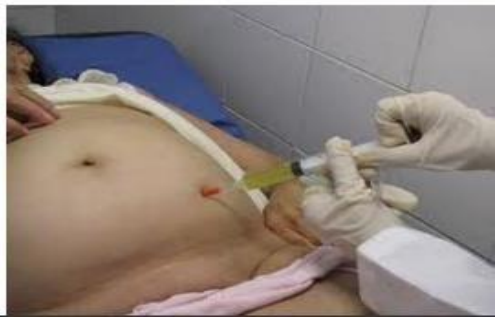
-Patients show moderate edema (generalized edema)

(when the concentration of any proteins drops, the concentration of the others will increase to compensate, so we have moderate edema because of the presence of other proteins (oncotic pressure still exists).

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 of proteins
- Hyperalbuminemia: dehydration (relative increase)





Hypoalbiminemia

Not complete absence of it, but we have low concentration.

-Edema seen in conditions where albumin level in blood is less than 2 g/dl

*Malnutrition (generalized edema) as famine.

*Nephrotic syndrome (more excretion of the kidney of the albumin) not a problem with the production.

*Cirrhosis (mainly ascites) a problem in the production

Ascites: edema in the stomach area.

*Gastrointestinal loss of proteins

**Severe burns (plasma loss in the absence of skin barrier) من عندي هاي

Hyperalbuminemia:

Cause: dehydration (relative increase)

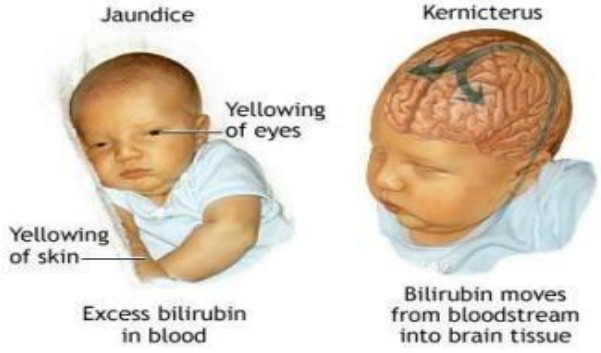
Actually, the concentration of the protein does not reduce. The volume of the water (as diarrhea) decreases so the concentration of the albumin increase.

Cases: diarrhea.

No clinical conditions are known that cause the liver to produce large amounts of albumin.

Other clinical disorders

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



Drug-drug interaction:

*Bilirubin toxicity (aspirin is a competitive ligand of albumin): kernicterus and mental retardation

*Phenytoin-dicoumarol interaction

Bilirubin Non-polar, intermediate of breaking down the heme group, happens in the macrophage especially in the spleen .

During the pathway, each molecule has its own color, we use it to stain(or colored) the compounds : such as the yellow in urine , the brown in fees.

Bilirubin increases in the fetus -special in pre-born-. enzyme that converts bilirubin to soluble form is in minimal rate, so it is not enough to this function. That's why new-born seems yellow in the first week and with time they get thenormal color.

But bilirubin must not accumulate in the blood, because it overcomes the BBB and enters the brain tissue causing mental retardation. We call this case as kernicterus.

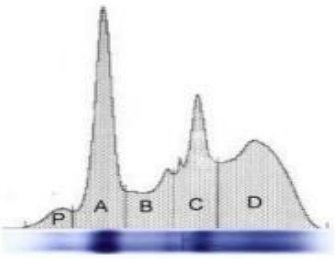
*Aspirin is a competitive ligand of albumin.

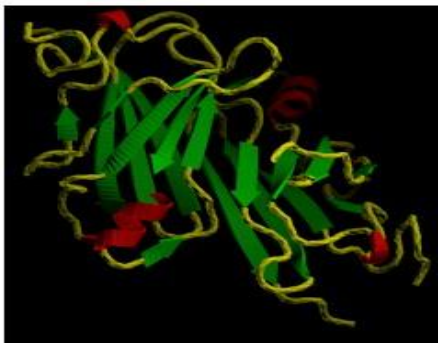
-The site where bilirubin binds to the albumin is the same as the aspirin, so when we give the baby aspirin, it will compete with bilirubin in binding. That is results in accumulation of bilirubin in the blood, which causes as above. So don't give them that.

*Another medication with the same story (Phenytoin (anti-dicoumarol(interaction)). Although They bind to differentbinding sites on albumin, they interact with each other. We avoid the interaction between drugs by reducing the dose one of them *or* not using it at all.

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





*Migrates faster than albumin in electrophoresis, MW = 62 kDa (smaller than albumin)

*It is a small glycoprotein (rich in tryptophan, 0.5% carbohydrates) not like albumin.

*Blood level is low (0.25 g/L) remember the concentration of albumin was 6 g/dL

*It has short half-life (≈2 days):

sensitive indicator of disease or poor protein nutrition.

*Main function:

-T4 (Thyroxine) and T3 carrier. where T3 is the active form with 3 iodine groups.

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 (52 kDa)
- Neutralizes trypsin & trypsin-like enzymes (elastase)
- 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 (ZZ, SZ). MS, MZ usually not affected
- Increased level of α1- 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

α1-antitrypsin.

*α1-Antiproteinase (works against the proteinases)

-small protein with MW = 52 kDa.

*Neutralizes (cancel the effect) of the trypsin.

-Trypsin and elastase are considered as proteinases (enzymes that break down the proteins).

Elastase found in the lung, works in destroy elastic fibers for a purpose.

*90% of α1-globulin band (the most concentrated).

*Many polymorphic forms (at least 75)

*Alleles PiM, PiS, PiZ, PiF(MM is the most common)

Deficiency (genetic):

Emphysema (a lung condition where the air sacs –alveoli- are damaged which causes the shortness of the breath).

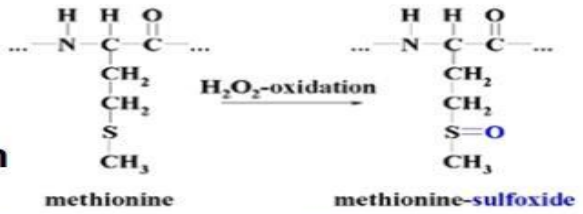
When these two alleles combine (ZZ, SZ), we get this deficiency of the antitrypsin.

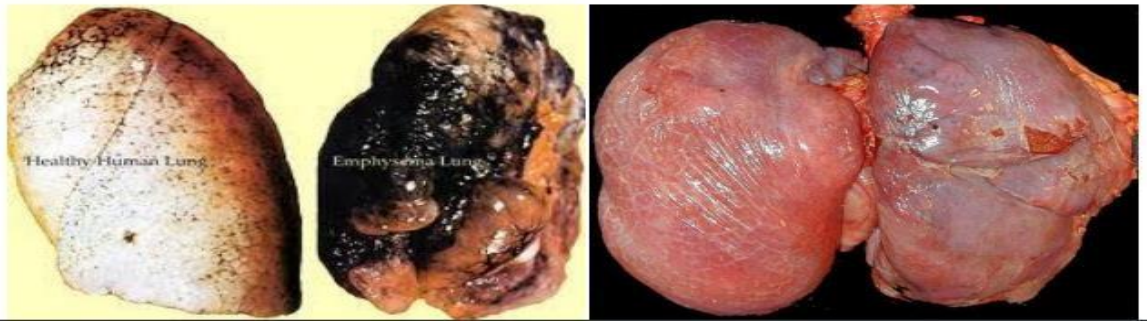
MS, MZ alleles usually not affected.

*Increased level of α1-antitrypsin (acute phase response) considered as positive acute phase protein). *Since* it is existed, it will bind to elastase preventing it from damaging the lung tissues.

Smoking & α1- antitrypsin deficiency

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





A relationship between smoking and deficiency in a –antitrypsin.

***Chronic inflammation* (neutrophil elastase)**

-Smokers get toxins in a continuous way so they get *Chronic inflammation*. The neutrophil elastase (molecules containing elastase) gets over activated so it will cause damage to the lung tissue –as deficiency in a-antitrypsin-.

***Oxidation of Met358**

Met is a building amino acid in the lung's cells. Neutrophil elastase affects the Met by oxidizing it to be ineffective in the building of the cell.

***Devastating(fatal) in patients with PiZZ here we have doubledeffect- genetic and acquired disease-.**

***Elaboration:**

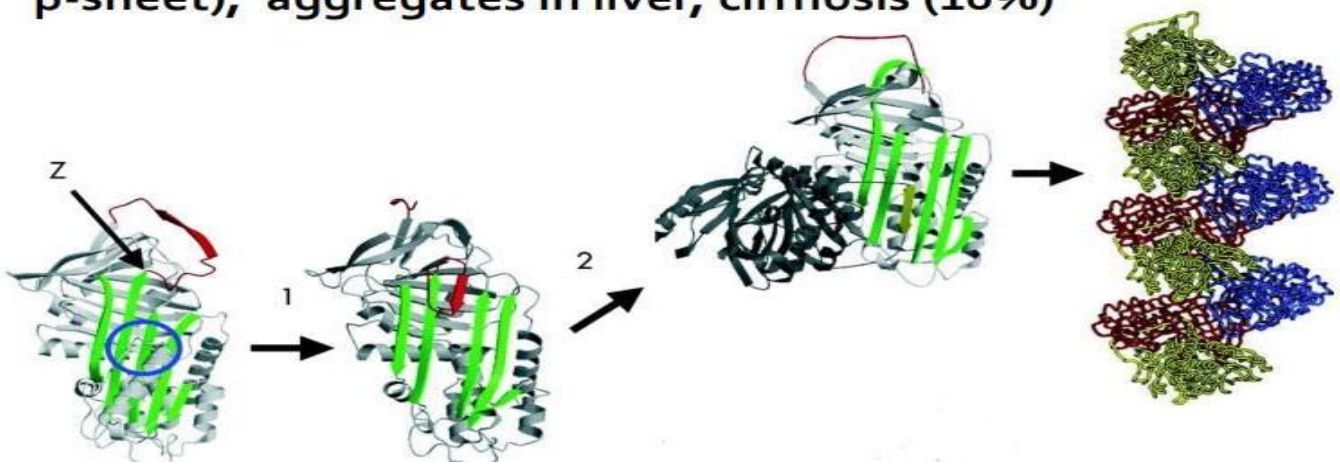
Genetic deficiency of α1 -Antitrypsin.

-Synthesis of the defective α1 -Antitrypsin occurs in the liver but it cannot secrete the protein

-α1 -Antitrypsin accumulates in hepatocytes and will be deficient in plasma.

Liver disease & α1- antitrypsin deficiency

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



Normally, the alpha-1-antitrypsin structure is 3D

ZZ phenotype (change the sequence of amino acids that's forming loops. These loops have very high affinity of Beta sheet (binding to each other) that's lead to aggregation of alpha 1 anti-trypsin (synthesis in liver) → cirrhosis

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 α1-fetoprotein increases in:
  - ✓ Fetus and pregnant women Normally
  - ✓ Hepatoma & acute hepatitis

***Synthesis in fetal (related to fetus) yolk sac.**

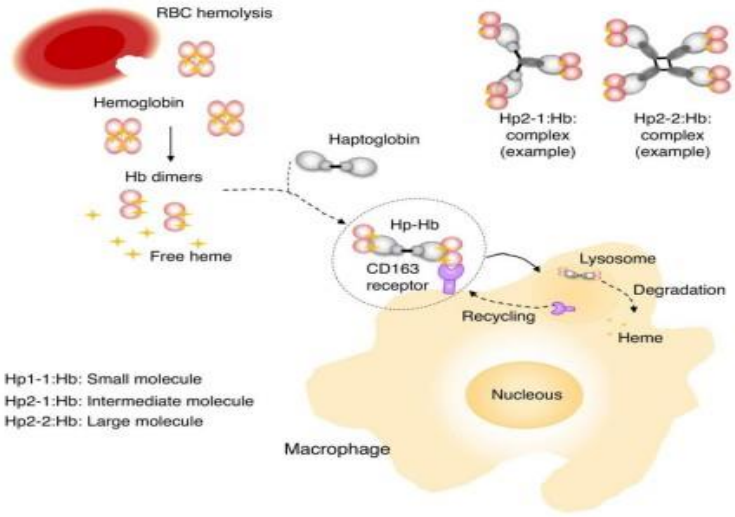
*** Very low level in adult, except:**

-Normally → pregnant woman

-pathophysiology→ cancer disease.

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



-Hemoglobin is normally found within red blood cells ,but gets released when they rupture – this process is called hemolysis. Hemolysis occurs in some diseases, including infections, malaria, and some types of anemia.

* Haptoglobin is mainly produced by liver cells

*Free hemoglobin in the blood causes oxidative damage to cells and tissues, Haptoglobin binds free hemoglobin in the bloodstream. A stable haptoglobin-hemoglobin complex is formed and cleared by white blood cells.

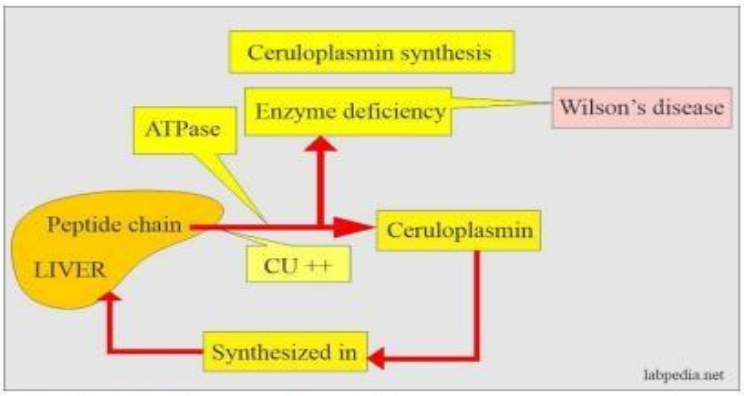
This prevents hemoglobin-induced inflammation and oxidative tissue damage.

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



* present in blood.

* Not responsible for the copper transfer process (it is transferred by albumin)

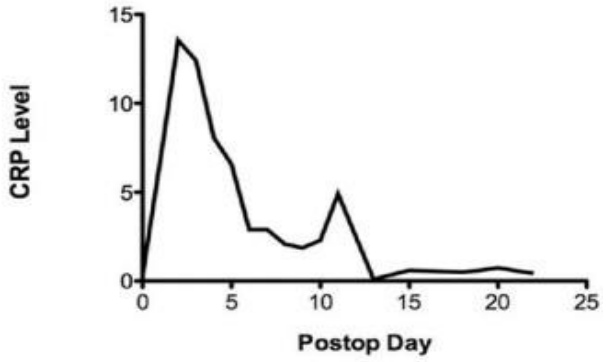
*An enzyme defect leads to a defect in the manufacture of the ceruloplasmin protein, and this affects the iron level(because the body proteins accept it, it must gain an electron from Cu^{+2}) in this case it acts as an oxidizing agent.

Enzyme deficiency→ A defect in the production of the protein ceruloplasmin→ Low concentration Cu^{+2} → Low fe^{+3} → Problems linking iron with oxygen

This leads to the accumulation of Cu.

C- reactive protein (CRP)

- A homopentameric acute-phase inflammatory protein
- 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)

*Normal range ≤ 5

-If it is higher than normal, this indicates a problem such as : trauma , cancer …