بسم الله الرحمن الرحيم

BioChemistry | FINAL 6

Plasma Proteins pt.1

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PLASMA PROTEINS

BLOOD

What are plasma proteins?

- Plasma: It's the non-cellular liquid part of the blood where the cells are suspended (the blood is suspential).
- Plasma proteins: are not cellular proteins like Hemoglobin (hemoglobin is cellular protein since it found in red blood cells), they are found in the plasma.

What do we mean by suspential?

- **Suspension** is a **heterogeneous mixture** in which solid particles are dispersed in a liquid, if you **left at rest,** the particles will **settle at the bottom,** but **shaking** the mixture will **temporarily make it appear homogeneous.**

- Blood is a **suspension**, but bumping gives it this **homogeneous appearance.**
- In a test tube, blood will gradually separate over time, however, we use a **centrifuge(الطرد المركزي)** because it makes the process faster. This process is called the **separation of blood into its component.**

BLOOD

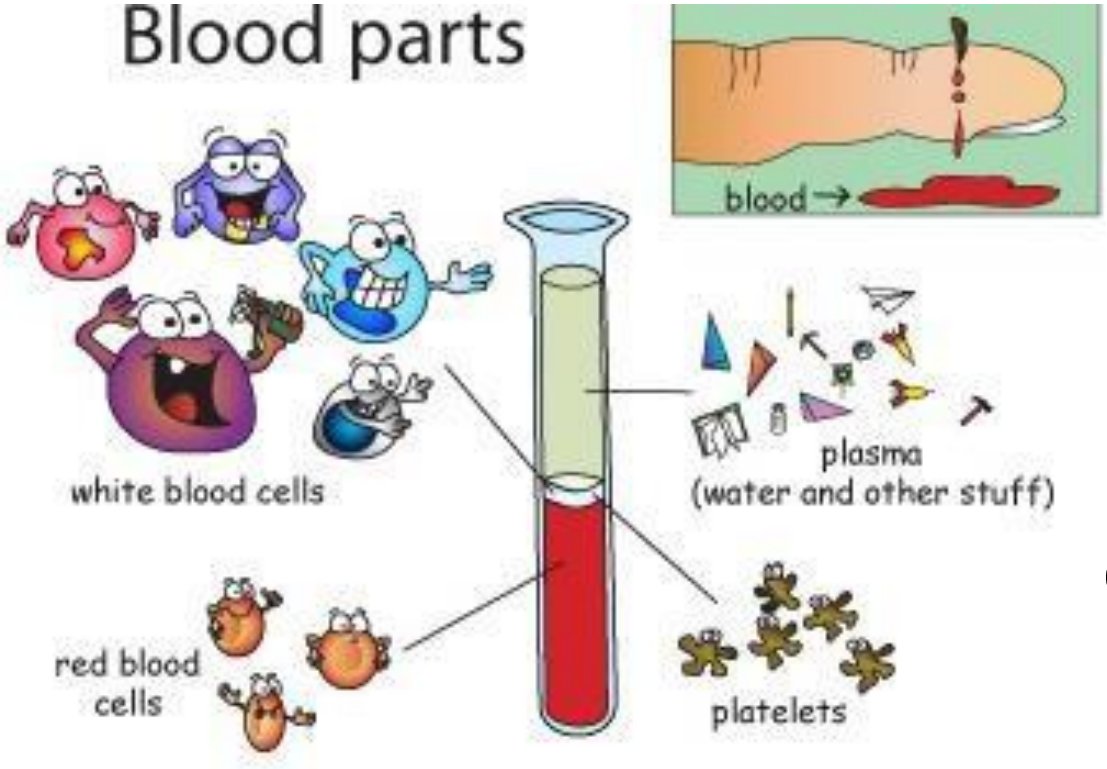
Why do we need blood in the body?

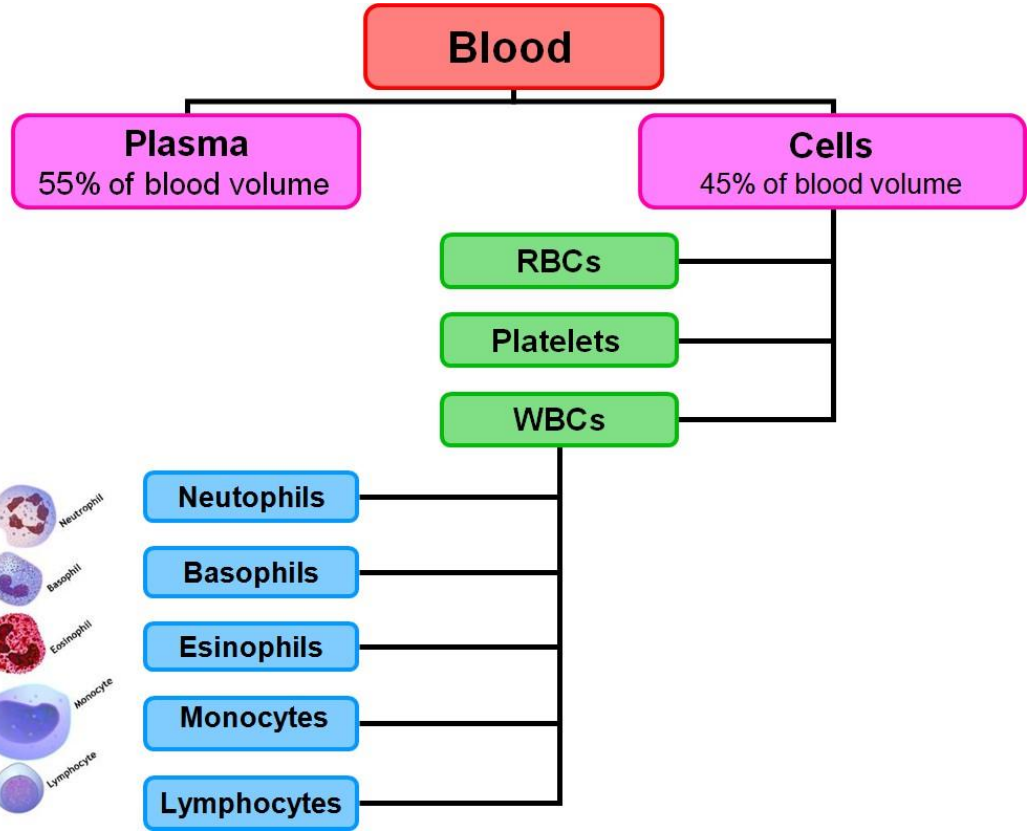
- Its the common medium that through its circulation connects the organs togather as well as connecting them with the molecules coming from external environment via (respiratory, GI and renal) systems.

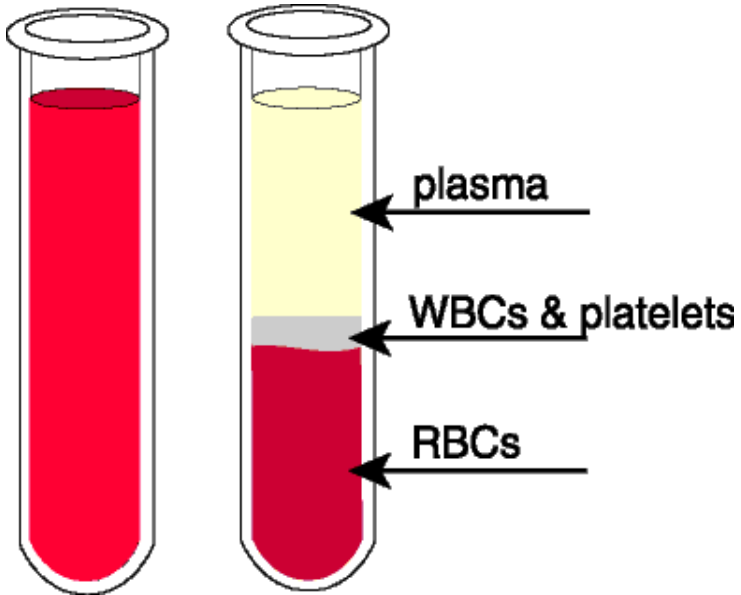
- **Every organ can have its own way to comunicate with environment** ,But having a common medium for all the organs and interface all the fluids has an important function , Blood able to do that by having red blood cells that contain hemoglobin for the transport of gases and plasma includes almost everything (what ever inside or from outside should be reflected on the blood)

- **Because blood reflects changes occurring in the body (internal or external), it acts like a biological "screen" that can reveal the body's physiological status through laboratory testing.**

BLOOD


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

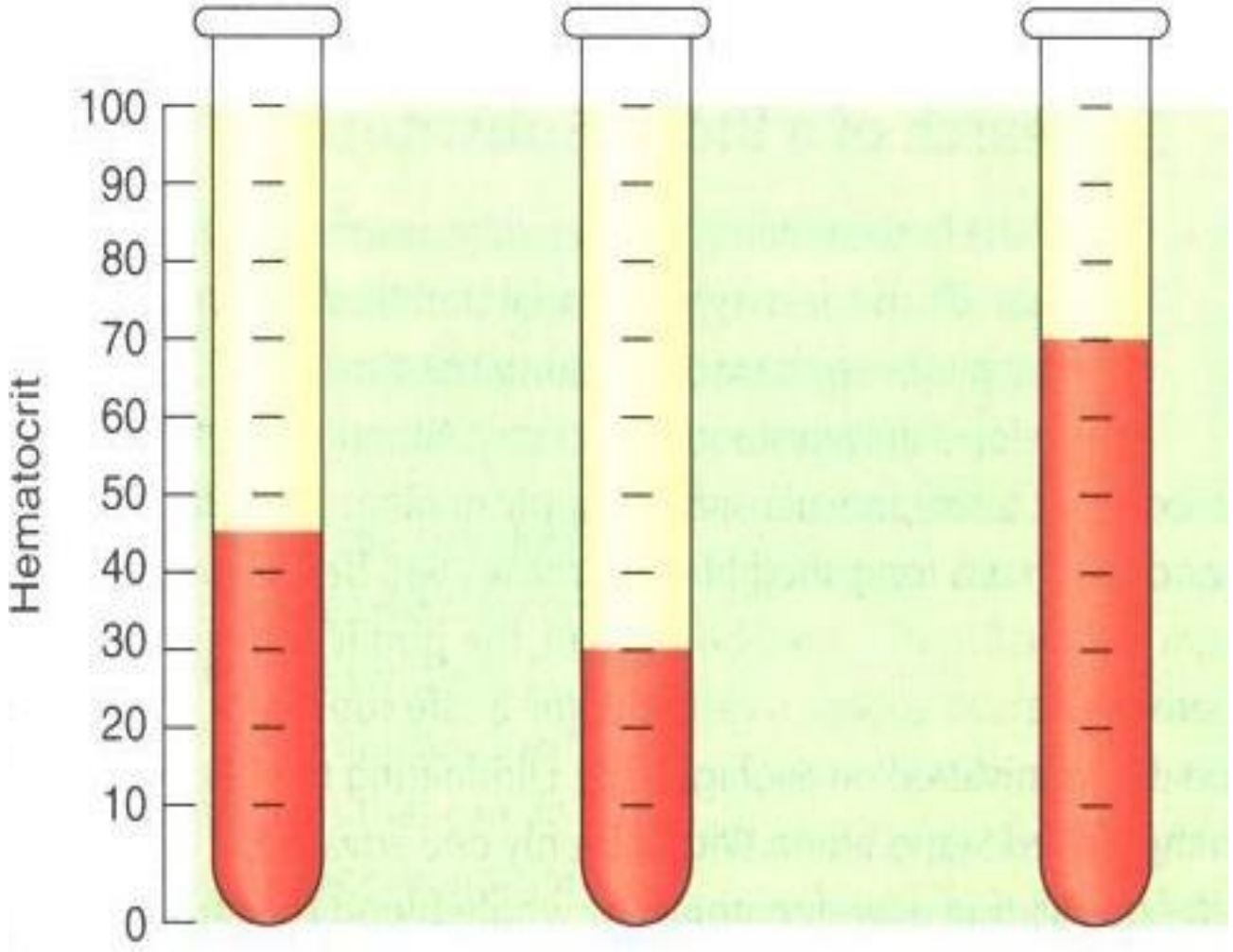

Blood
Plasma
55% of blood volume
Cells
45% of blood volume
RBCs
Platelets
WBCs
Neutophils
Basophils
Esinophils
Monocytes
Lymphocytes


plasma
WBCs & platelets
RBCs

BLOOD: PLASMA VS. HEMATOCRIT

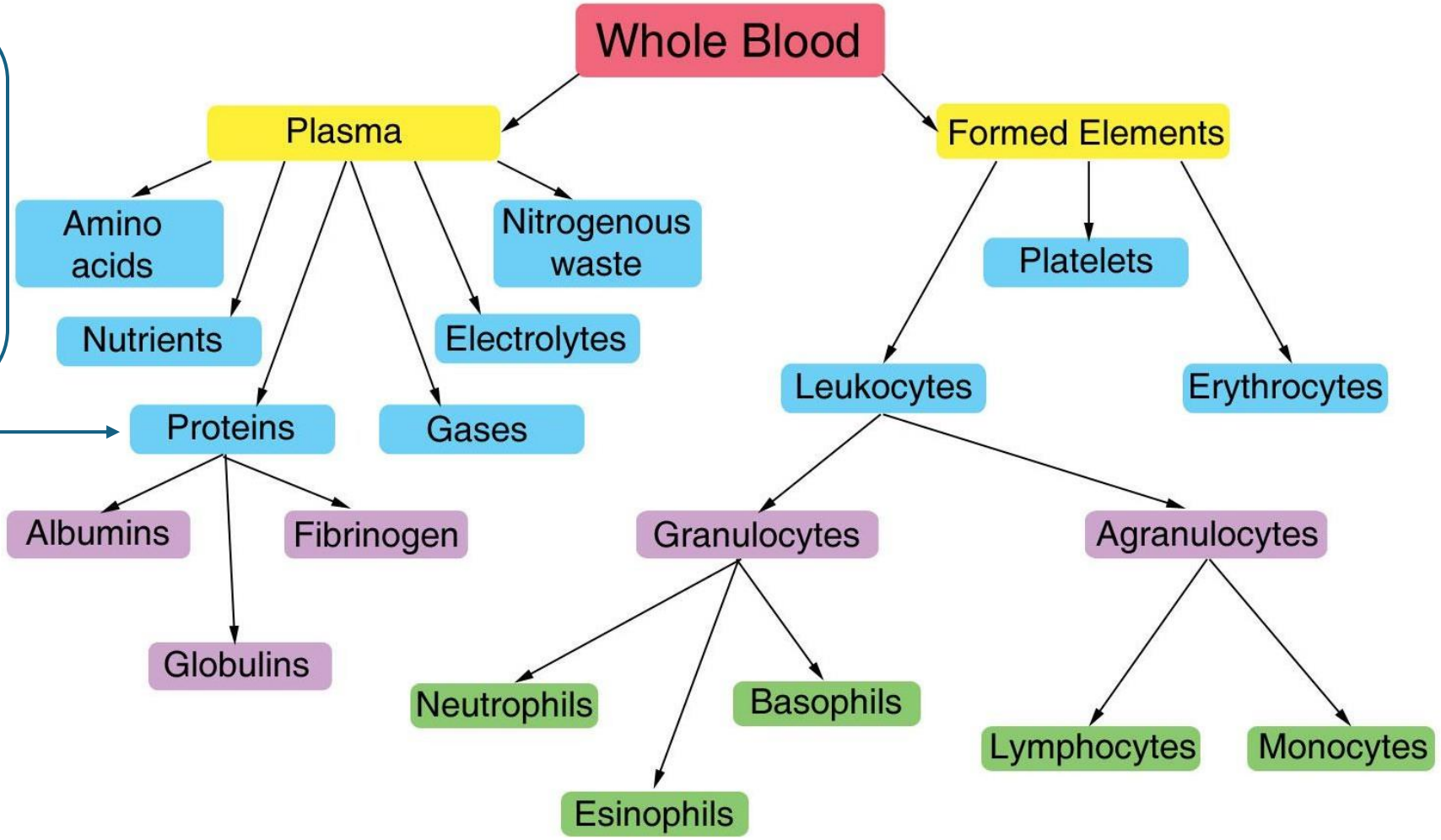
- Hematocrit or packed cell volume (Adult male: 47 %, Adult females:42%)

The percentage of cellular part of the blood differ between females and males and in certain diseases.



BLOOD: WHAT IS INSIDE PLASMA

As you can see here
Plasma contain lot of things but the proteins is our intrest now.



PLASMA

- **Liquid medium where cells are suspended**
- **Composition:** - **Water (92%)** - **Solids (8%)** Can be either organic or non organic
  - **Organic:** organic proteins are the proteins that we are going to discuss.
    - **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

Most plasma proteins used to be glycosilated in their nature and there is some exception

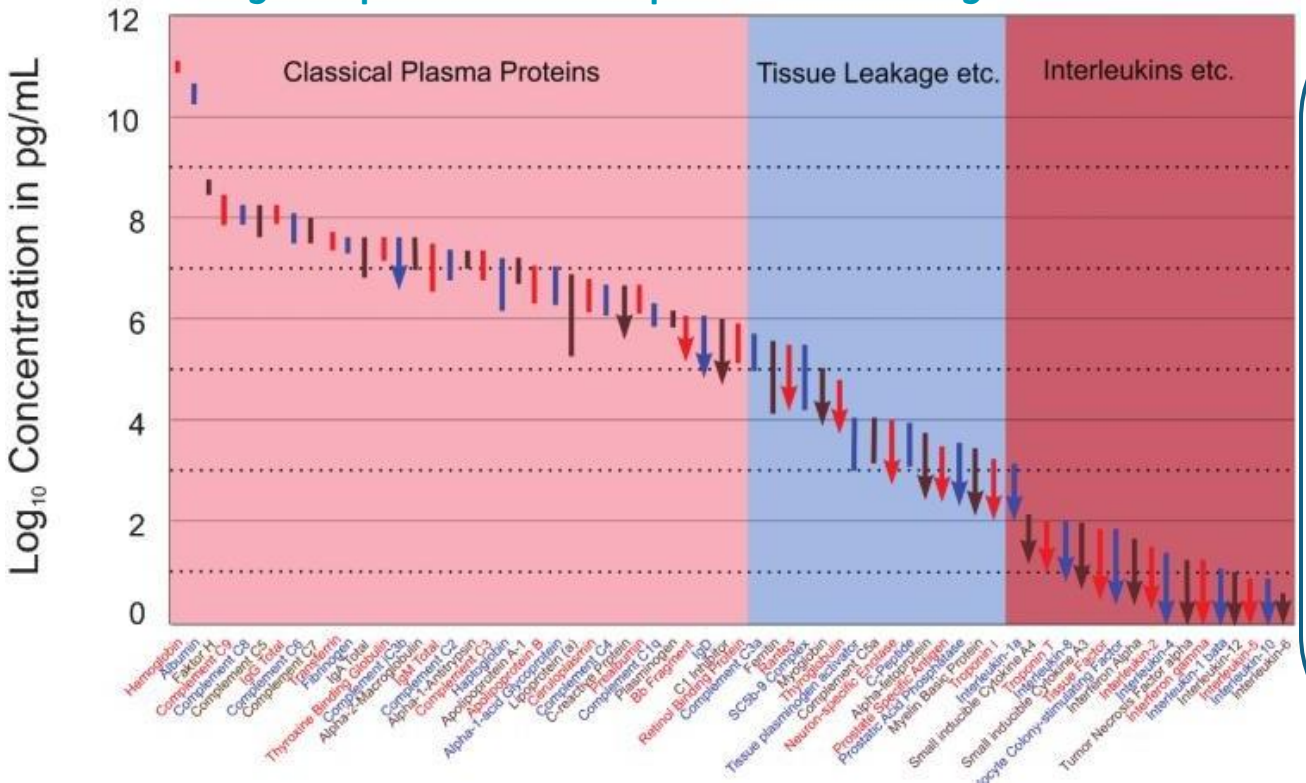
Plasma proteins are huge in number

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

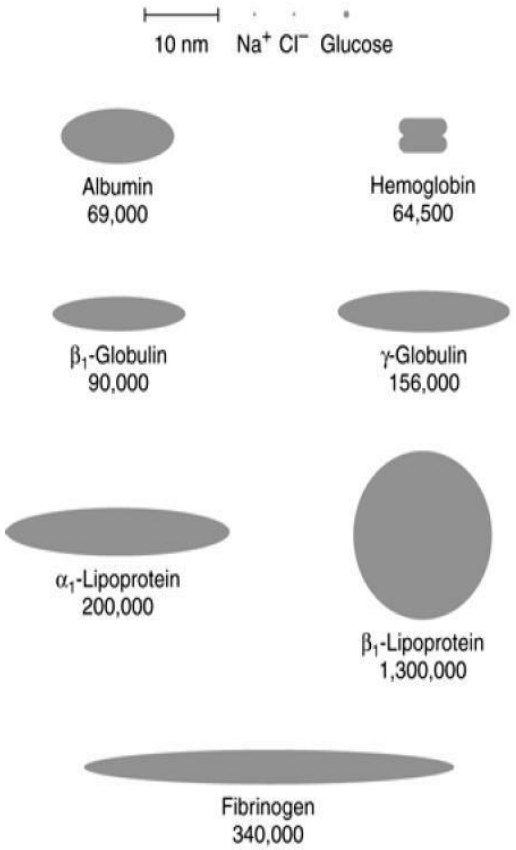
Deciliter = 100 mL

- Simple & conjugated proteins (glycoproteins & lipoproteins)

They adapt various shapes and the way the look



- Look at the hemoglobin it is **compact in shape** because it is **intracellular protein** But when you look on at albumin it is **episoldar** in shape not compact like hemoglobin
- **Other ones are more elongated and larger compared to albumin**
- Fibrinogen has the **highest molecular weight** and **comparing with albumin** it **much more elongated** which is have clinical applications.



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)

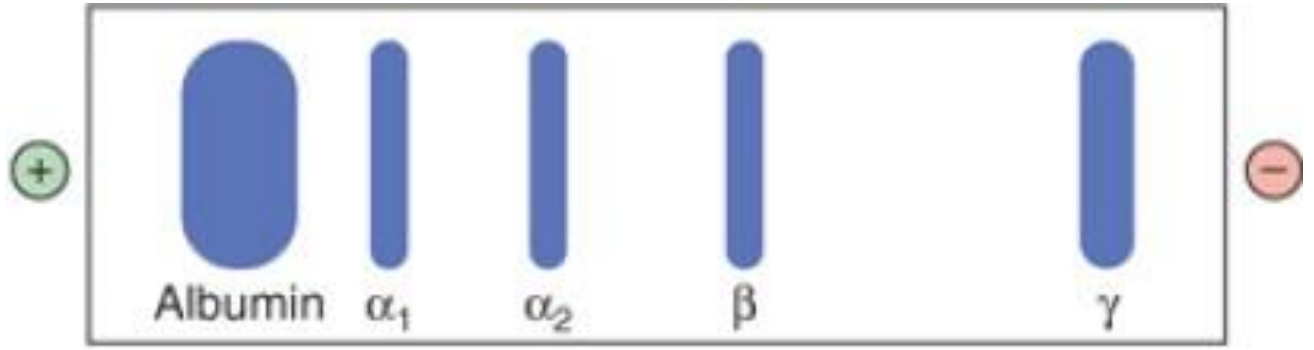


Figure 1

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

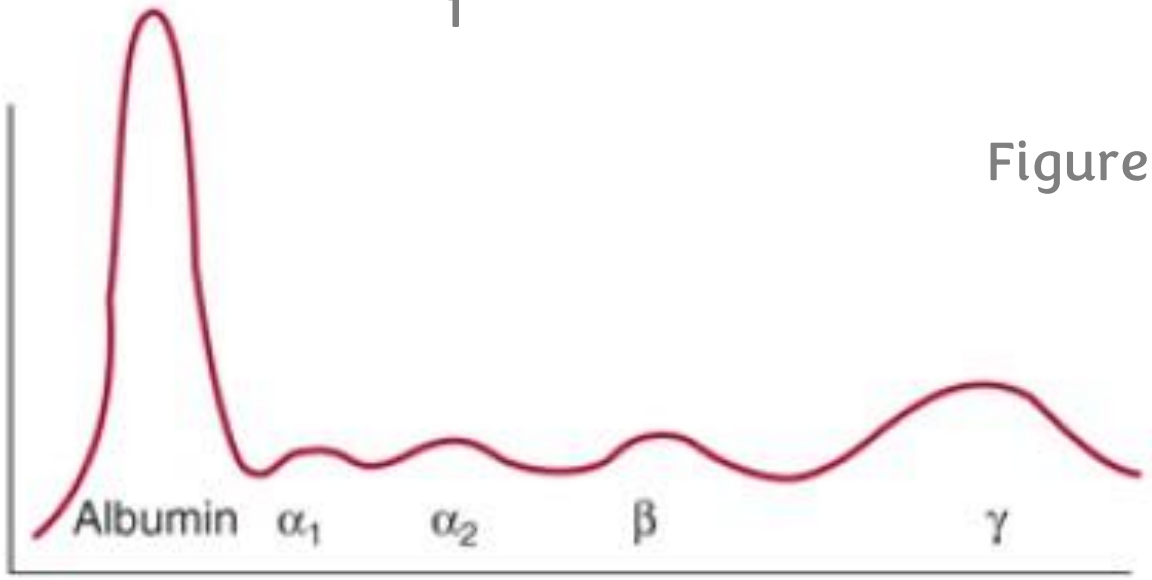


Figure 2

SEPARATION OF PLASMA PROTEINS

How to separate plasma proteins from a plasma sample?

- By a technique called **salting -out** it depends on **using salts.**

1) We add salts to the solution that contains plasma (plasma contains protons H+).
2) adding salts you are adding charges to the solution (- and +) ,this charges does not change the nature of the solution, but it **enhances the charge of the solution because of adding negative and positive in the same time and same quantities.**
3) **Enhancing the charge of the solution** makes the proteins **more soluble** because charges are better.
4) As continuing of adding of salts → the salts will **drag water from around the proteins** until you reach a state the protein won't be soluble any more (because the salts have the highest affinity to water more than proteins,and more than anything).

- **the higher the solubility of the protein the last will come out of the solution ,the lower the solubility of the protein the first will come out of the solution.**

By applying this technique you will have three major proteins precipitating at different times **(albumin ,fibrinogen and globulins)** and each of them will appear as one unit.

SEPARATION OF PLASMA PROTEINS

- **Thickening** and the **dinsity** of the bands reflects the **concentration** of that protein in the plasma compared with others.

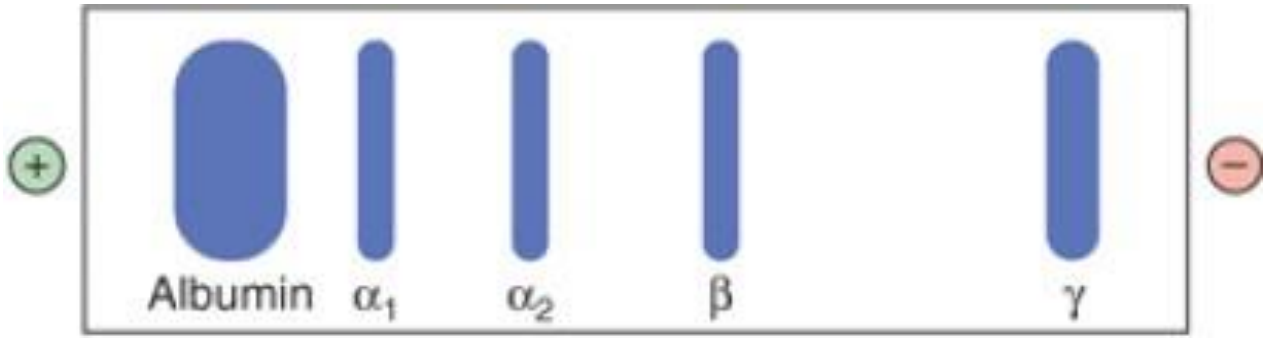
- The **dinsity** is measured by **dinsetometer** which measures **how much this band of gel can elamonate** (the light higher absorbency the higher concentration of the protein).

- This will give me different pecks ,is that right or logic?Yes take the biggest peck which is the albumin that is and you know that the albumin represent (60-70)% of plasma proteins which is (3.5-5.5)g/DL from the hole plasma proteins(6-8)g/DL and the rest is the other types of plasma proteins

SEPARATION OF PLASMA PROTEINS

- Fibrologien is responsible of the clotting of the blood

- Electrophoresis is another technique is used for separating plasma proteins from plasma **but it not useful in the presence of fibrologien** because fibrologian **causes clotting** ,and it is **very larg molecule** leading to the clogging of the gel.
- In order to use this technique we have to take out fibrologien from the plasma sample then it will be **defibrinated plasma** (plasma without fibrologien and any thing is related to clotting , it is called **serum**).
- We use serum in electrophoresis by applying serum sample in gel and when the process starts we will have different bands (**Thickening** in bands reflects the **concentration** of the protein and the **location** reflects the **speed**)



- ✓ **The fastest one in reaching the positive electrode is the smallest one in size** (which is albumin).

- Why the other-bands are named by alpha, beta and gama?

Because this bands represents many type of proteins that moved together so it can't be named with specific name of protein as the case of albumin.

If you give the sample more time beta and gamma also will separate into 1 and 2 etc...

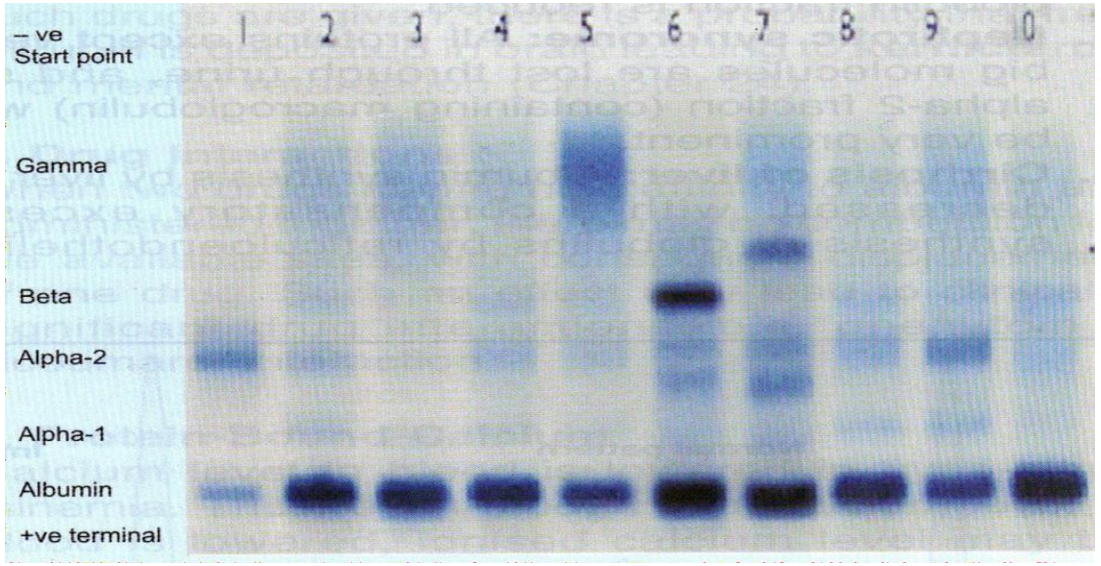
ELECTROPHORESIS OF PLASMA PROTEINS

- 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

They are also called antibodies

Globulins are a group of proteins not single protein

Gama globulins ,They are named with gama because they are the slowest proteins in electrophores technique



Alpha 1 region contain more than one type of proteins and the most abundant one antitrypsin with a percentage 91% of this region

SYNTHESIS OF PLASMA PROTEINS

Most plasma proteins

Immune proteins

- Mostly liver (albumin, globulins), γ-globulins (plasma 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

SYNTHESIS OF PLASMA PROTEINS

Where the plasma proteins are getting synthesis ?

- All **plasma proteins** are synthesized in the **liver** except for gama proteins (Immune proteins) are synthesized from the **plasma cells** which they are **mature B lymphocytes** which are found in **nodes ,bone marrow, spleen**.

- The proteins that synthesized in liver they are produced in its **inactive form** due to two reasons:-

- if you need them to function right away when you need them without waiting to synthesize them from the start so they are prepared them in an inactive form to activate them immediately once you need them.

- The site of synthesize is different from the site of action.
- In the case (synthesising of proteins in inactive form) of liver the proteins are synthesized as **preproproteins** , which :

- **Pro** means that the protein is **immature** by itself (functionally wise).
- **Pre** means it is a **babel that address the place of function** (location wise).

→"Pre pro" means there is **two steps** that the protein have to go through to be active.

SYNTHESIS OF PLASMA PROTEINS

- Most of proteins get post translational modifications before they start functioning.
- You can say (proinsulin) (preproinsulin) but not (preinsulin) cause it is another protein.

Why albumin are not glycosilated protein?

- Because it represent more than 50% of the protein so if it get glycosilated the blood becomes too heavy (viscous) and alter many physiological properties.

What if all plasma proteins are glycosilated except albumin?

- It won't cause a functional problem as many plasma proteins are required to be glycosilated, Because there are a high variety in this proteins and **the glycosilation of them contribute to functional diversity and give higher recognition** but the glycosilation of the albumin **won't give this variety of functions.**

POLYMORPHISMS AND HALF-LIVES

Polymorphisms

- 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

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 gastro-enteropathy

HALF-LIVES

- ## How to know the half life of proteins ?

- The half-life of proteins can be determined by **measuring their concentration at multiple time points** and monitoring the rate of decrease. By performing serial measurements over time, the half-life is identified **as the time required for the protein's concentration to drop to 50% of its initial value.**

- ## Why do we discus the half life of plasma proteins?

- Because it affected by certain diseases, **How diseases affect the half life of proteins?**

Diseases affects the concentrations of proteins inside the blood.

- ## Example :-

- The half life of albumin is 20 days so it supposed to stay at blood 20 day doing it function perfectly but **with certain diseases** the half life **will be shorter** so we will **lose the function of albumin which is general or specific function.**

HALF-LIVES

- **Example :-**

- **Kidney diseases** affect the half life of proteins by loosing amounts of proteins through excess filtration.

- **Gastrointestinal diseases** Like (Crohns disease) chronic inflammation diseases →the inflammation cause redness and vasodilation represented by swelling and because of this swelling the endothelium will have spaces in between resulting in excess lose of water as well as proteins that shouldn't get out the circulation to the lumen of intestine (albumins half life will decrease from 20 day to 1 day).

❖ These diseases are called collectively protein loosing Gastro intropathic diseases.

POLYMORPHISMS

- Polymorphic protein result from a **mutation** that lead to changing the efficiency of the function or totally stoping the function.
- If this mutation spread in communities and **exceeds 1%** between individuals →this mutation will be called a **polymorphism**.
- It could be **pathological (cause a disease)** or **not (like the eye color/ blood types).**
- The polymorphic plasma proteins (α1-antitrypsin, haptoglobin, transferrin, ceruloplasmin, and immunoglobulins) they have more than one copy.

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)

GENERAL AND SPECIFIC FUNCTIONS OF PLASMA PROTEINS

Each protein has specific function that is related to its structure as well as there are **general functions** that **all proteins share** because **they are protein :**

❖ General functions :-

1) They participate in nutrition which means any protein can be degraded to act as energy supply when it is needed in the body.

2) They mantain the PH which means all proteins can work as a buffer as it was discussed previously.

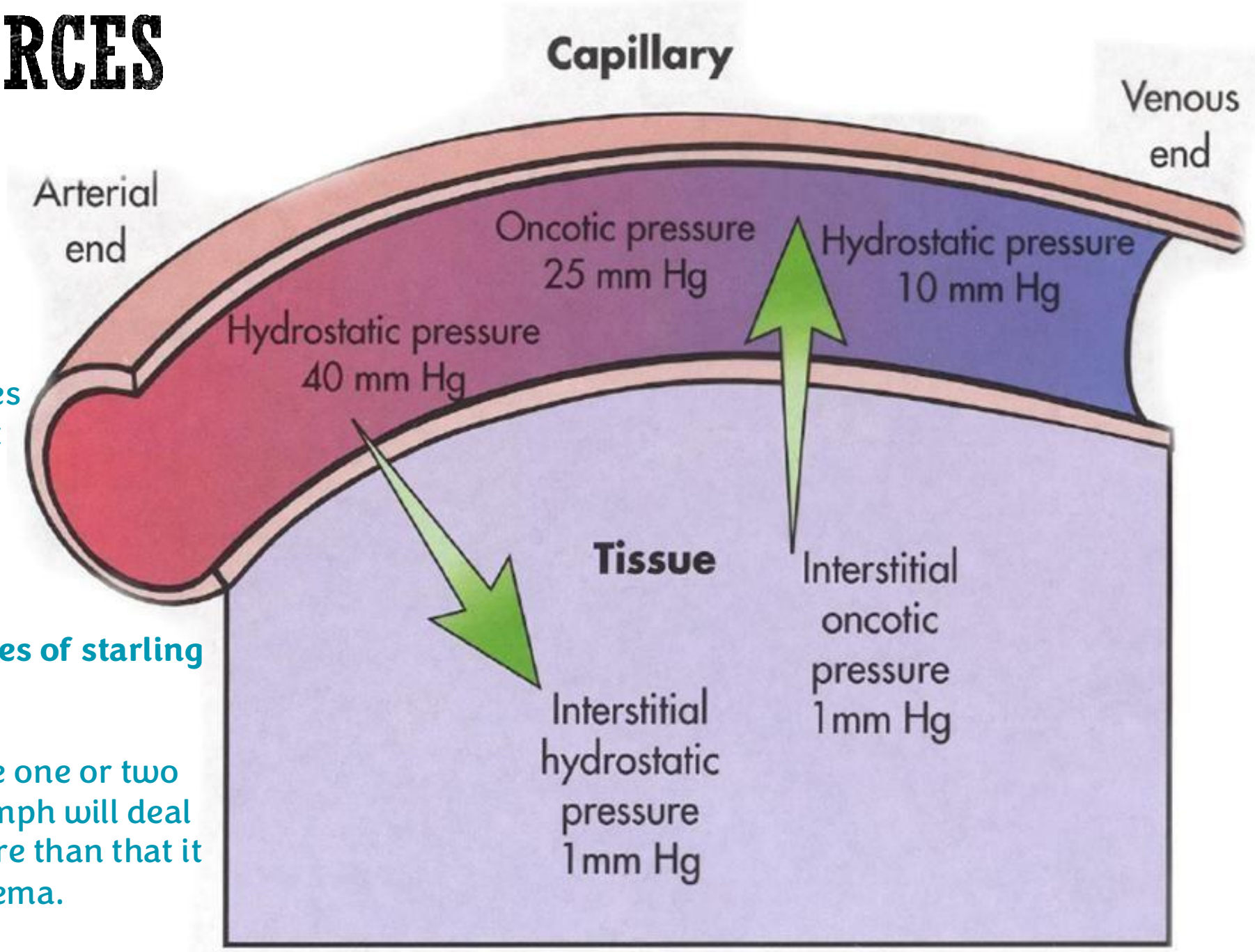
3) They can contribute to the blood viscosity because proteins are soluble so this increase the viscosity and they are glycosilated.

They are dissolved macromolecules that increase the internal resistance of plasma to flow and when they are glycosilated their size increase, further enhancing viscosity (additional explanation).

· They continbute to the blood oncotic pressure ,how? Because they need and drag water around them creating a force called oncotic pressure

STARLING FORCES

- Edema can be a result of protein deficiency



- Starling forces are the forces thats control the movement of fluids and neutrals and wastes in the arterial and veinous ends.

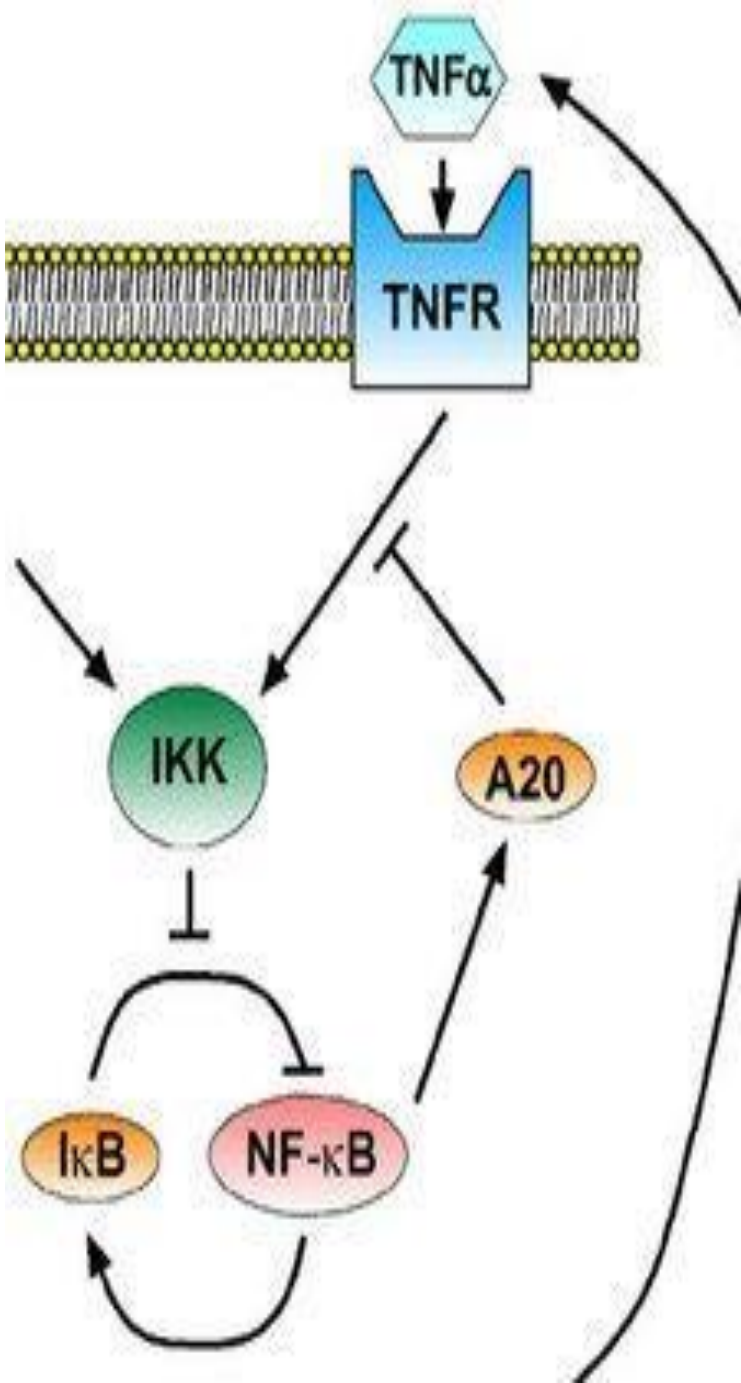
- **What will happen if the values of starling forces changes?**

Answer: If the difference where one or two mL it's not a big problem the lymph will deal with it ,but if the difference more than that it will lead to the formation of edema.

ACUTE-PHASE PROTEINS

We use this name for some of the plasma protein.

- Levels increase dramatically in concentration (up to 1000 folds), **acute inflammation**, tissue damage, **chronic inflammation & cancer.** C-reactive protein (CRP), α1 -antitrypsin, haptoglobin, & fibrinogen and this increase is an indicator that there is a problem and we must look for it.
- Interleukin-1(IL-1), main stimulator (gene transcription)
- Cytokines (material produced from the cell) stimulates Nuclear factor kappa-B (NFkB): Exist in an inactive form in cytosol, activated and translocated to nucleus (interleukin-1) where it initiate the process of transcription of acute phase proteins in large amounts.
- Negative acute phase proteins: prealbumin, albumin, transferrin proteins which don't increase in their concentration or decrease relatively.



ACUTE-PHASE PROTEINS

- Acute phase proteins they are **plasma proteins** whose concentration **changes significantly** in response to **acute inflammation, tissue physical damage, chronic inflammation & cancer.**

- The significant increase in the concentration of **acute-phase proteins** tells you that there are a problem but they can't tell you what is the problem.

- There are many acute phase proteins like **α1-antitrypsin, haptoglobin, fibrinogen and** (**CRP** which is the most universal one).

- (you will be punished as a physician if you discharge a patient with high concentrations of acute phase proteins). ☹

ACUTE-PHASE PROTEINS

❖ **How they increase in concentration?**

1) If there is an inflammatory process, factors (cytokines) will bind to its receptors on the cells.

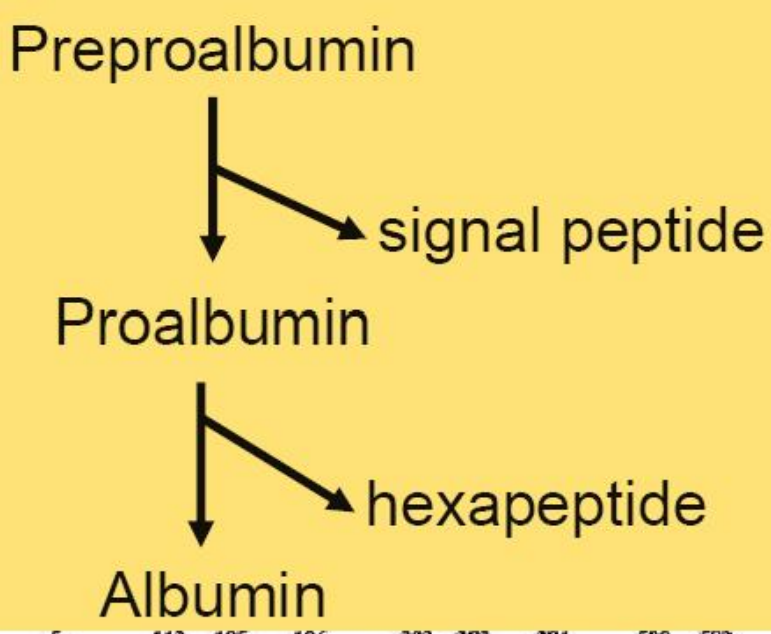
2) The receptors will convert an inactive (transcription factor found in the cytosol) which is called nuclear factor kappa-B to its active form.

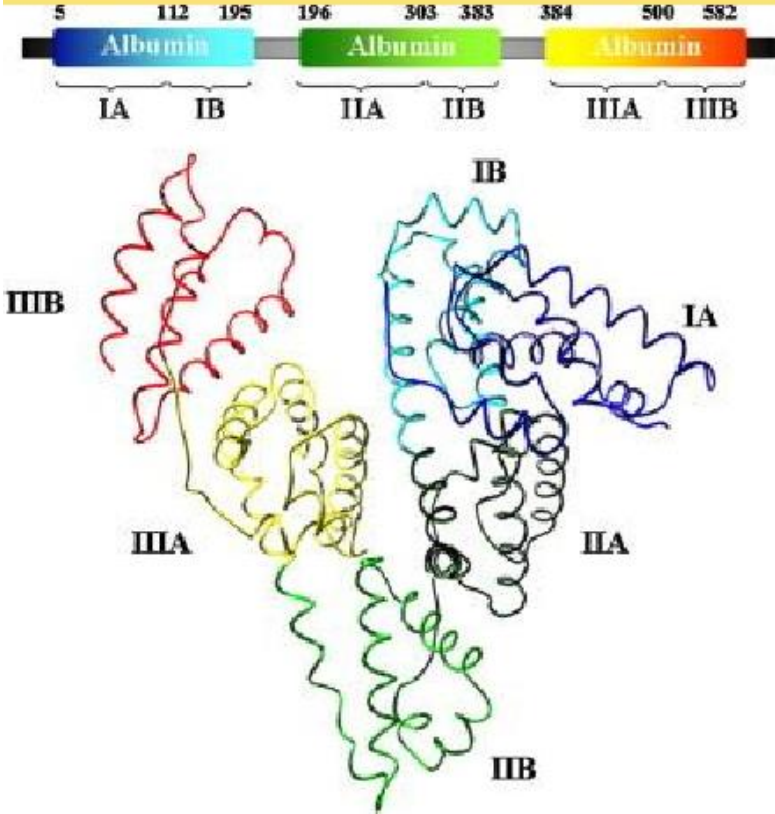
3) Once it activated ,it will translocate itself to the nucleus and **initiate the transcription of the DNA** to **produce more** copies of **acute phase proteins.**

➢ There are other other group of proteins which is called **negative acute phase proteins** and their concentrations **don't get to higher concentrations even relatively decrease.**

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

- Because the liver synthesize albumin in large quantities (relatively 25% of liver protein synthesis is albumin), and that **makes It an indicator of how the liver is functioning,** the obstrative of using it is that the half life of it is 20 days so it takes time to tell you that there is a problem in the liver Albumin takes a long time to signal liver problems because it has a **relatively long half-life (about 20 days)** and the liver maintains a large stored amount. Even if liver production starts to decline, albumin levels in the blood remain high for a while, so **the drop is not seen immediately.**
- Synthesized as a **preproprotein,** it gives a signal peptide (lose a single peptide) so as it get out of the liver cells it **becomes proalbumin** by removing the signal peptide , and then through processing steps such as materialisation or proteiazation by removing hexapeptide until it becomes in its fully functional albumin as premajor domains (1a,1b as domain one /2a,2b as domain 2 /3a,3b as domain 3)

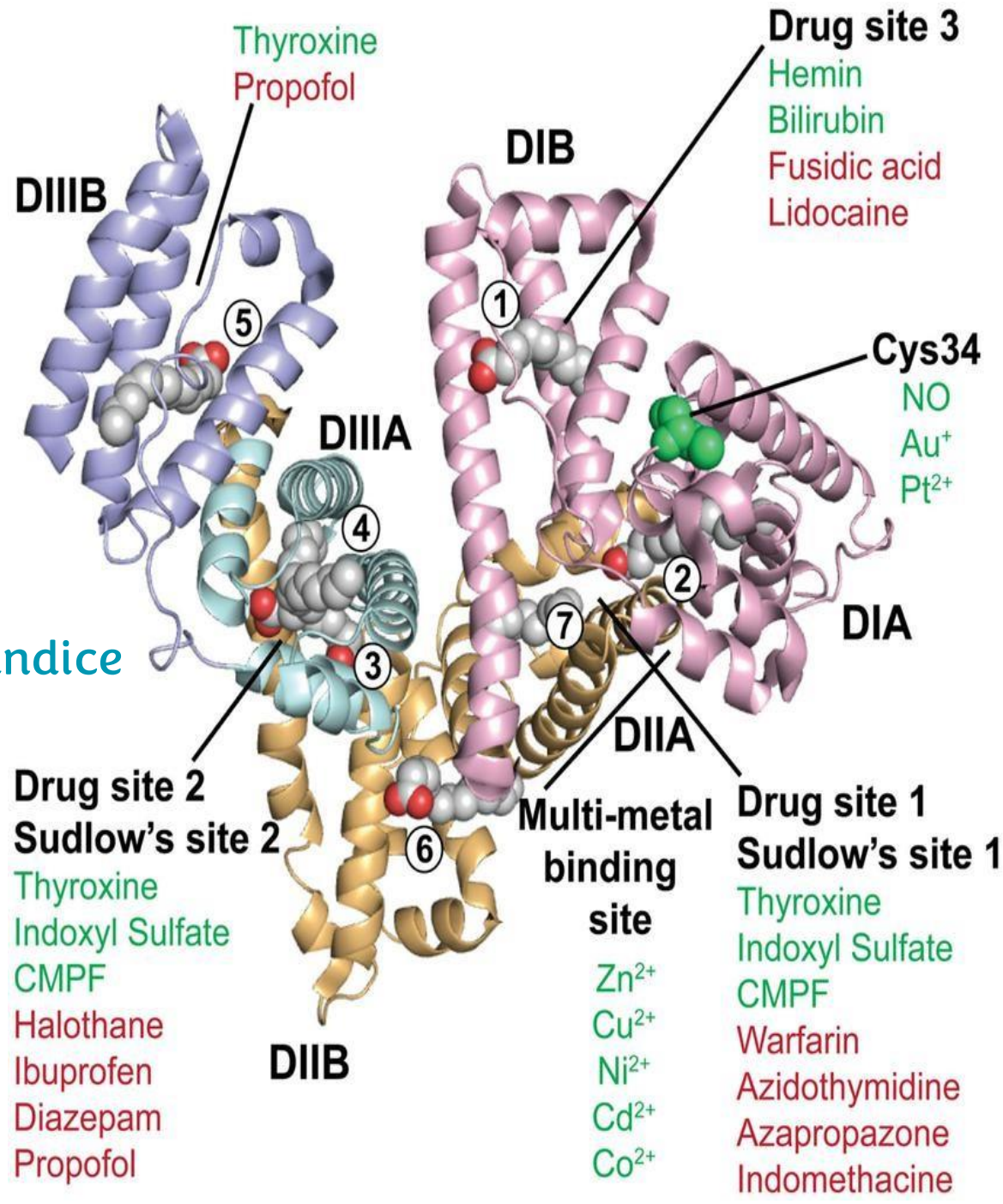
ALBUMIN

- It's structure must be conserved in a highly precise way because **it is a site of binding for a huge amount of materials** to transfer what you have from outside.
- When we transfer something it firstly bind to albumin in few seconds.
- For it's structure to be conserved **it contain 17 disulfide bond.**
- It is **ellipsoidal** not elongated, **because if it was elongated** this would increase the surface area which is exposed to water leading to dramatically increase in viscosity of the blood (interactions with water) because albumin constitute most of the plasma proteins.

ALBUMIN BINDING CAPACITY

A lot of materials can bind to albumin.

- binds various ligands:
  - Free fatty acids (FFA)
    Are carried in the blood bound to albumin
  - Certain steroid hormones
  - Bilirubin Bound to albumin is the reason of jaundice
  - Plasma tryptophan
  - Metals: Calcium, copper and heavy metals
  - Drugs: sulfonamides, penicillin G, dicumarol, aspirin (drug-drug interaction)



ALBUMIN BINDING CAPACITY

- Drugs are carried in the blood bound to albumin (drug binding domain).

- Most of the drug is transferred through albumin and these is why in each leaflet of drug the **drug-drug interaction** are written,**Why is this important ?!**

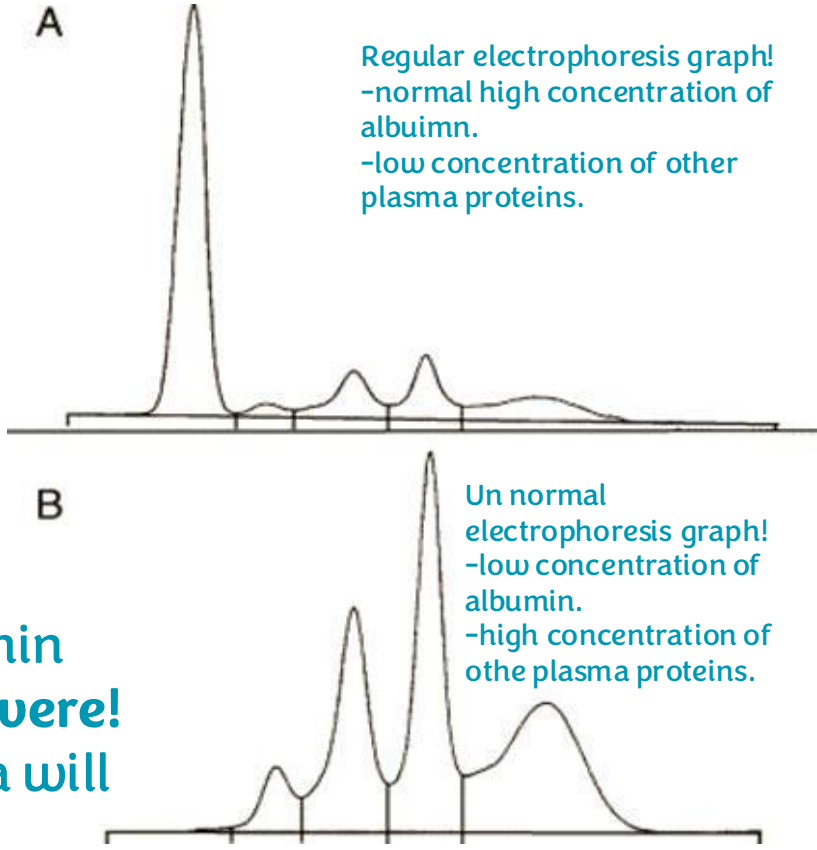
- **Answer:** Because both of these drugs are binding to the same place and **the one that is effective is the free** which is exposed to tissue, the one that **is bind is not effective** (if you increase the one that is free, the drug effect will increase ,and maybe we have side effect of that drug).

ANALBUMINEMIA

Complete lack of albumin ,will they live ? Yes ,but their life won't be easy because albumin is the major transporter inside the blood.

"a" before the word, means completely lack of it.

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



Oncotic pressure will decrease dramatically ,although it the albumin decrease to the half but it has a **moderate (medium effect) not severe!** Because the compensation mechanism, alpha 1,2 beta and gamma will increase dramatically in concentration so they replace general functions **but not the specific functions of albumin.**

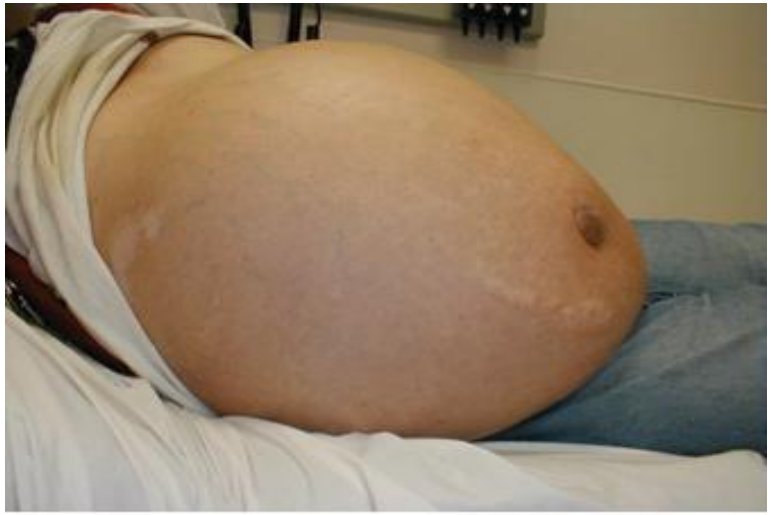
OTHER CLINICAL DISORDERS

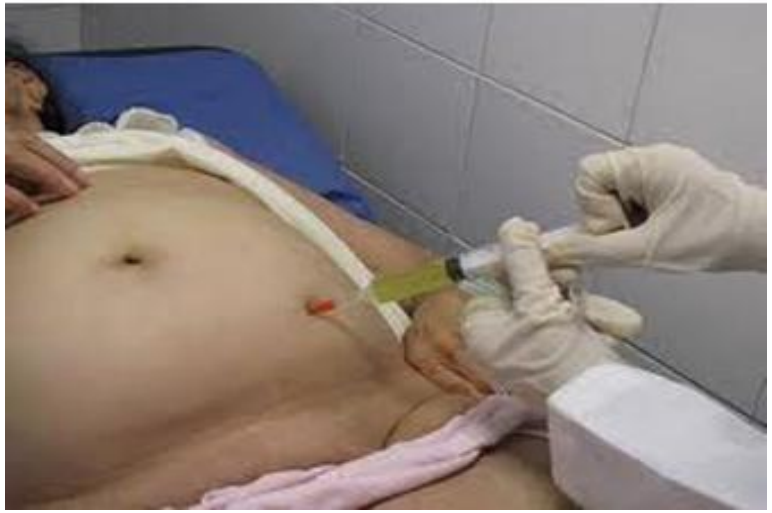
Hypo=low

- Hypoalbiminemia: edema seen in conditions where albumin level in blood is less than 2 g/dl Normal levels(3.5-5.5)g/dl
  - Malnutrition (generalised edema)
  - Nephrotic syndrome In kidney
  - Cirrhosis (mainly ascites) In liver
  - Gastrointestinal loss of proteins

Inflammatory process affecting the GIT

- Hyperalbuminemia: dehydration (relative increase) Increasing the concentration of albumin and it doesn't happen normally and this is happen relative mostly because of **dehydration so the albumin will in higher concentration** with respect to water unless you have cancer of the liver which cause its cells to increase in size (synthesis more of the protein)





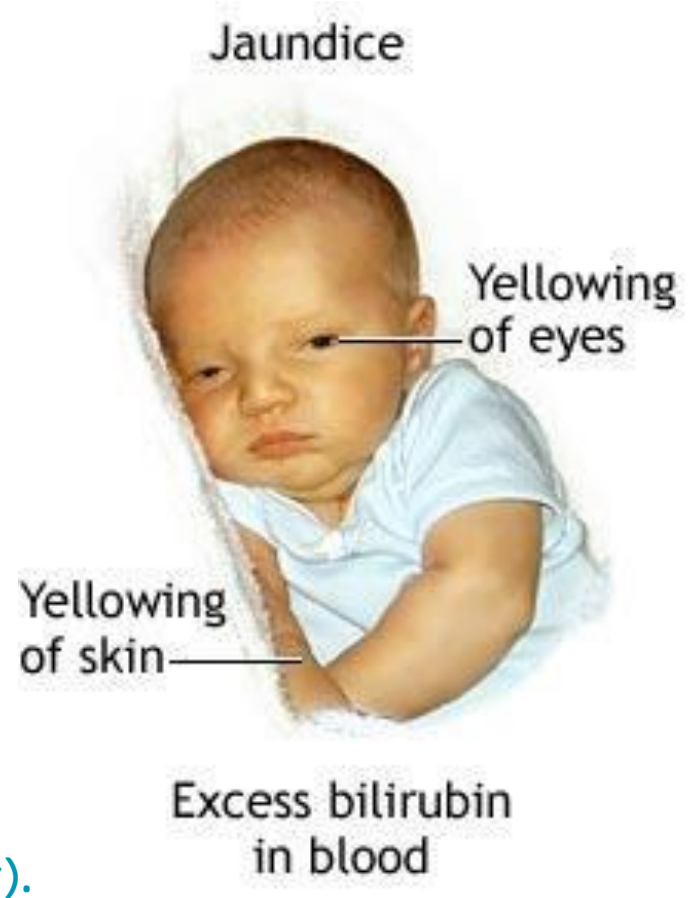
Ascites is the accumulation of fluid in the abdomen cavity

OTHER CLINICAL DISORDERS

- Drug-drug interaction:
- Bilirubin toxicity (aspirin is a competitive ligand of albumin): kernicterus and mental retardation, Reye's syndrome
- Phenytoin-dicoumarol interaction

Anti convulsant drug Anticoagulant (blood thinner).

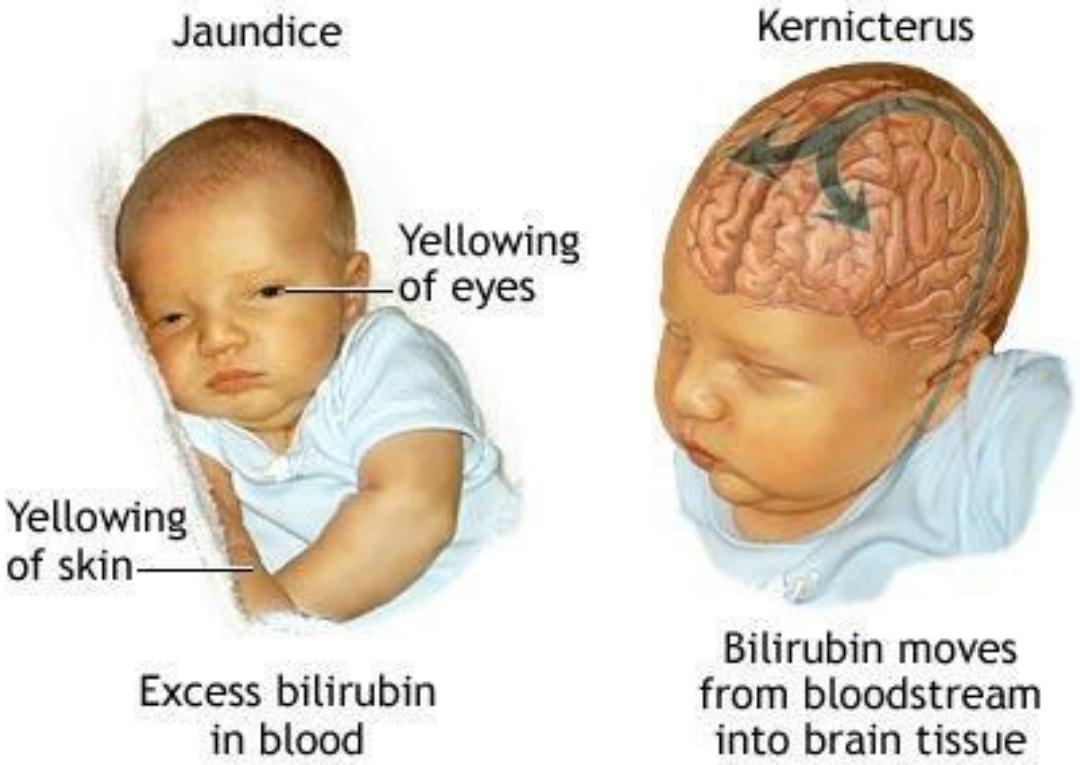
They both bind in the same place on albumin (if you are giving one of them you are increasing the free concentration of the other one and as a result anticoagulant and blood thinner leading to increasing in the bleeding of the blood.



Bilirubin toxicity

- In bilirubin toxicity bilirubin is getting bound to albumin ,**aspirin is connected to the same place of bilirubin on albumin** ,so if we take aspirin jaundice will increase noticeably because they are binding in the same place so they are competing in binding (more free bilirubin, more yellowing of eye and skin).

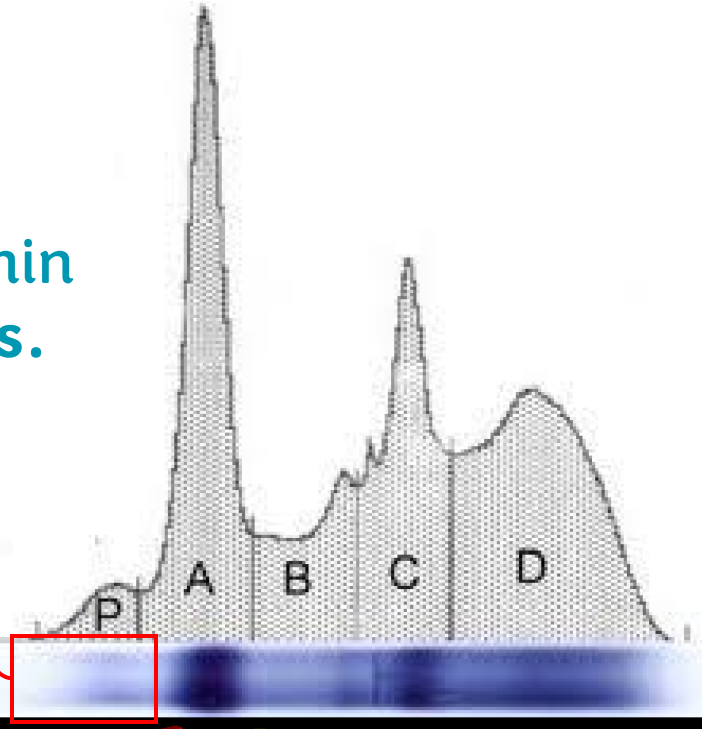
- Who determine which one will bind ?
  Answer: Affinity and Concentration.



- That's why aspirin is prohibited for new Norns (it gonna bind albumin) accordingly bilirubin will increase and the **blood brain barrier aren't formed yet** so bilirubin will get in brain cells which is called Kernicterus, which is having bilirubin inside brain cells and it can't deal with it so it will accumulate resulting in **mental retardation and Reye's syndrome.**

PREALBUMIN (TRANSTHYRETIN)

It is **completely different** than albumin we call it prealbumin because **it moves ahead of albumin in gel electrophoresis.**

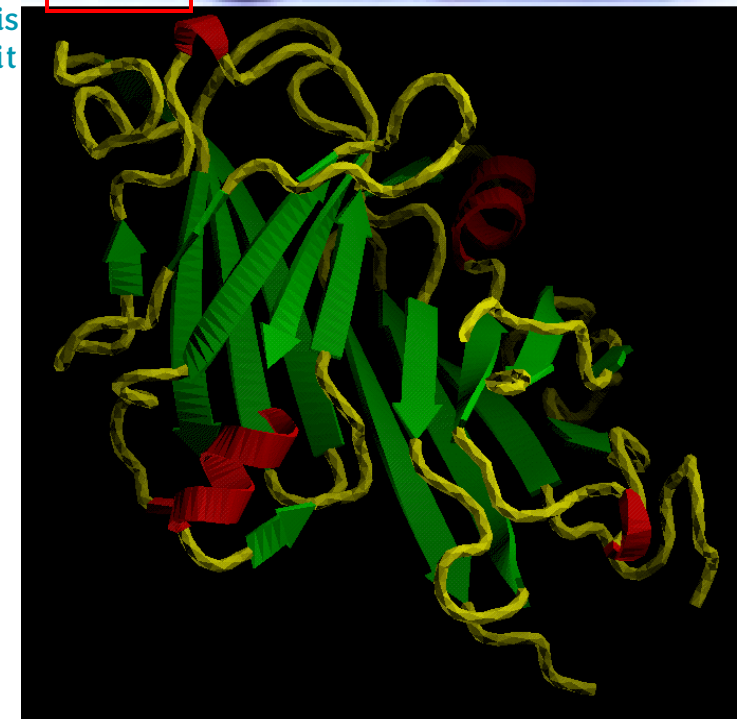


Band فاهي جدا
This is pre albumin.
Its concentration is very low because it is very فاهي

- 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:

Better protein core for liver functioning test because it has short half life but the problem is it's low concentration.

- T4 (Thyroxine) and T3 carrier
  It is the carrier of thyroid hormones.



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	Slide 30; Last Note	It is ellipsoidal not elongated **to** increase the surface area which is exposed to water leading to dramatically increase in viscosity(interactions with water) because it constitute most of the proteins which are there.	It is ellipsoidal not elongated, **because if it was elongated** this would increase the surface area which is exposed to water leading to dramatically increase in viscosity of the blood (interactions with water) because albumin constitute most of the plasma proteins.
V1 → V2			

رسالة من الفريق العلمي:

وَأَنْ لَيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَى

وَأَنَّ سَعْيَهُ سَوْفَ يُرَى