METABOLISM

Sheet n:

Writter:

Correcter:

Plasma proteins

Plasma proteins are not directly related to metabolism, but they're related to a topic we already covered in biochemistry, which is the protein structure and function.

This topic here has a very important clinical significance for the diagnosis of diseases. Any protein in the plasma will help you in the future to diagnose diseases and manage them.

What is the difference between plasma and serum ?

Plasma originates from blood. If you take a blood sample from one patient and put it in a tube that has anticoagulant, you will prevent coagulation without a blot. And if you take that anticoagulated blood and centrifuge it (put it in a machine and revolve that blood sample in a test tube) for about 3000 rounds/minutes for 10 minutes, all the solid materials will be precipitated and the fluid (supernatant) will be separated from the solid material. That fluid component after centrifugation is called plasma.

On the other hand, if you take the blood sample from a patient and put it in a tube that doesn't have anticoagulant, the blood will be coagulated after a few minutes. And if you take that coagulated blood and centrifuge it, two layers will be formed: a solid one AND a supernatant. That supernatant is serum.

In terms of composition, plasma and serum are 99% the same, except for one thing; plasma contains a protein called **fibrinogen** while serum doesn't contain that protein. Why? Because in serum, fibrinogen is converted to **fibrin** due to the clotting.

So…

PLASMA

Is the liquid medium where cells are suspended.

Composition:

- **Water (92%).** Major
- **Solids, or cellular components in terms of platelets and cells: RBCs & WBCs (8%).** Minor
  - **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, pentose.

BUT NOT glycogen!! Why? Because it is stored in the liver or muscle and when it's required, it'll be phosphorylated or degraded into glucose. So you'll see glucose from a glycogen, not glycogen.

- Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones.

Examples on enzymes: carbonic anhydrase, trypsin, a1-antitrypsin, prothrombin, fibrinogen... these are proteolytic enzymes that convert pro-proteins into proteins.
معلومات خارجية من النت، فقط للتوضيح ***Proteolytic enzymes** (**proteases**) are enzymes that break down proteins.

***A pro-protein** (or pro-peptide) is an inactive protein/peptide.

- **Inorganic:**

Na+ , K+ , Ca2+ , Mg2+ , Cl- , HCO3- , HPO42- , SO42-

All of these components are very important to know about. And in this course, you will be exposed to the metabolism of all of these components because each component of these (including the plasma proteins) has a specific physiological concentration in the blood. **Examples**: سهلة، مجرد أمثلة واقعية عالفقرة السابقة

1- **Albumin** has a physiological normal concentration of (3.5-5) g/dcL, and it's synthesized in the liver. If the concentration in blood goes less that, it means that some disorder in an organ (the liver) has occurred in your body, which means that your liver is not working well, not doing its function, not synthesizing albumin in the proper concentration. For many reasons (chronic infection, cancer…etc).

2- **Urea** is synthesized and excreted in the liver, kidney respectively. if there's a problem in the liver or the kidney's function, urea concentration will be deviated from the normal. So determining the urea concentration in blood will tell you that there's something wrong in the liver or the kidney.

3- High concentration of **uric acid** is a marker for "Gout" disease and other metabolic diseases.

4- Also **cholesterol** and **triglyceride**, all of these if deviated from the normal concentration, it will indicate something wrong in the function of vital/important organs like liver, kidney or muscle.

5- **Glucose** concentration is usually about 80-100mg/dcL. If it gets to 300mg/dcL that will indicate hyperglycemia (diabetes). : الخ الصحة

So, each of these metabolites concentration is important to know when we want to compare with healthy persons or the normal physiological concentrations. Deviations from the normal will be markers for diseases.

Okay now, let's talk more about our main topic: ***plasma proteins***

Albumin, globulin, a-globulin are not the only proteins present in the plasma, there are actually hundreds of proteins. But they are categorized into:

-albumin -globe -globulins

And the concentration of each of these is also very important. For example, some people don't have enough **fibrinogen**, which causes bleeding.

So, knowing the concentration of fibrinogen of a blood sample of a patient always suffering from bleeding will help you in diagnosis. And then you could provide that patient with fibrinogen. Problem: solved! ✓✓

Now, the question is: how can one determine the concentration of these in the blood? Very simple…

1. Take a blood sample, centrifuge it, take a specific volume of the serum or plasma.
2. determine the concentration by using **"biuret method"**.

what is important in biuret method is the reaction of alkaline-copper sulfate with peptide bonds (the covalent bonds found within the protein), making a complex as a violet color

3. Then you measure the optical density of that violet complex color and then relate it to a standard complex. You could determine how many grams of total proteins you have per dcL.

This value that you have just determined is very important, because if it's below the normal range, then the patient has **hypoproteinemia**. And if above, the patient has **hyperproteinemia**.

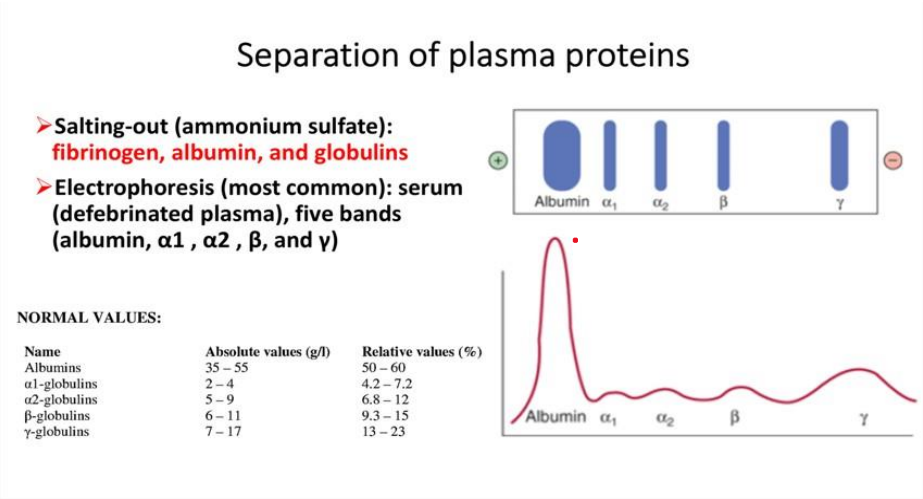
Each of these conditions will indicate a disorder in kidney, liver or others. So, to know the concentration of total proteins AND to ask about the concentration of albumins and globulins is a routine medical test for the diagnosis of some diseases, by comparing to the normal concentrations. Any deviations will indicate diseases or disorders.

life works out in the strangest, funniest ways… don't worry, you'll get there :))

So, how can we know if the elevated concentration belongs to which exact protein (albumin, fibrinogen or globulin) ?

The tedious task that may be the solution is protein purification, being time-consuming! This is principally right, but a simpler method could be done.

Electrophoresis

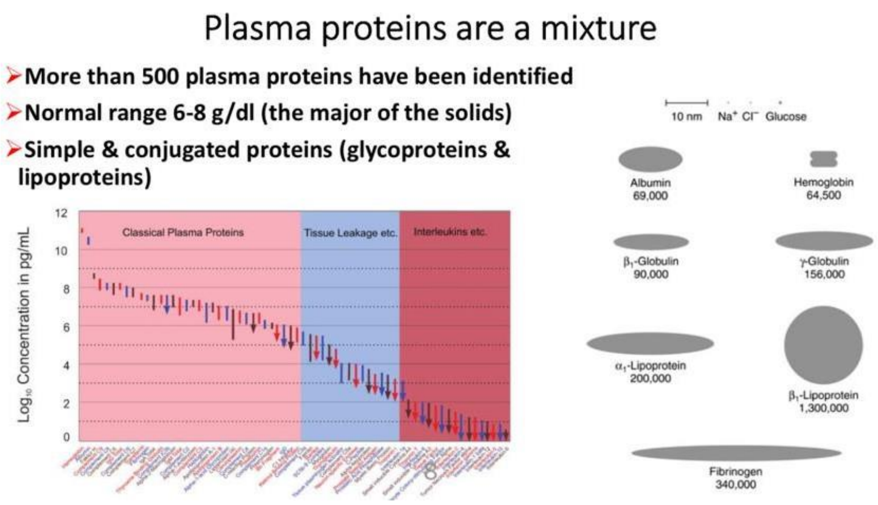


The diagram shows electrophoresis, beneath is an electrogram.
Each band represents a protein or category of proteins.

What's involved here is gel or cellulose paper and you take few microliters of plasma or serum, and apply it at a point on the gel or paper, then subject the sample to an electrical current, passing it in one direction for half an hour, and because they have different charges and isoelectric points (as a result of structural differences), is that it is going to be separated based on **charge**. The one with a highly negative charge will migrate faster, as albumin, while others with positive charges will stay near the starting point. So, they are separated via electrophoresis according to charge.
After electrophoresis is done, bands are not visualized unless the proteins are stained. The higher the concentration the higher the density of the band. After that, the strip is scanned, ending up with peaks. Each band has its peak below it, as shown in the figure above.

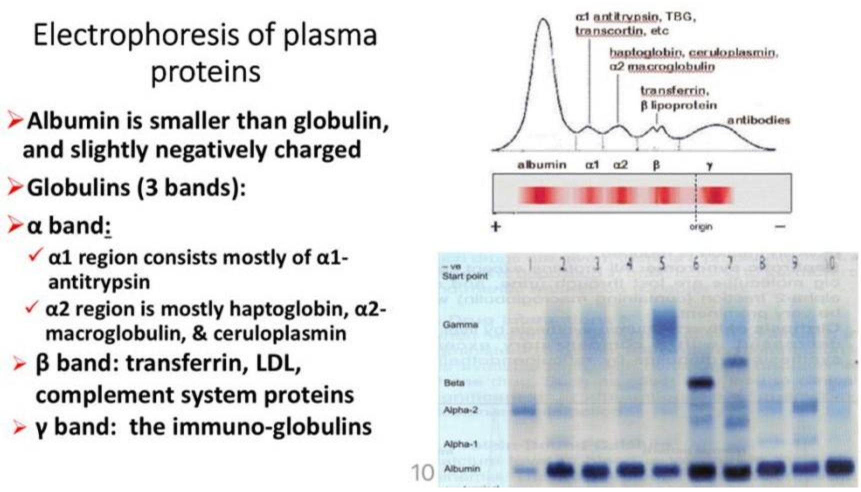
According to this gram, plasma proteins are categorized into two categories: albumin and globulins.
Albumin peak represents a pure protein, that means that under the albumin peak, you will only see albumin. While on each of the other peaks, there are a lot of proteins, categorized under each peak. What is common between them?
Suppose we have fifty proteins under the gamma peak, what is in common? Charge. Same charge, that's why they are not separated from each other. But when comparing to other peaks, they differ in the charge.
You could determine the amount of each protein of these by entering this data in a computer that will calculate the area under each peak.(area represents concentration of protein). So these areas are concentrations of these proteins under physiological conditions, here indicated as the absolute values beside the graph.
Remember that previously we have said that proteins account for 6-8 grams/dL, and here is shown for albumins as 35-55 grams/L, meaning: 3.5-5.5 grams/dL for albumins. dL=100mL. 10dL=1 Liter

So these are the absolute values for proteins in the plasma at normal conditions.


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)
Classical Plasma Proteins
Tissue Leakage etc.
Interleukins etc.
Log10 Concentration in pg/mL
10 nm
Na+ Cl- Glucose
Albumin 69,000
Hemoglobin 64,500
β1-Globulin 90,000
γ-Globulin 156,000
α1-Lipoprotein 200,000
β1-Lipoprotein 1,300,000
Fibrinogen 340,000

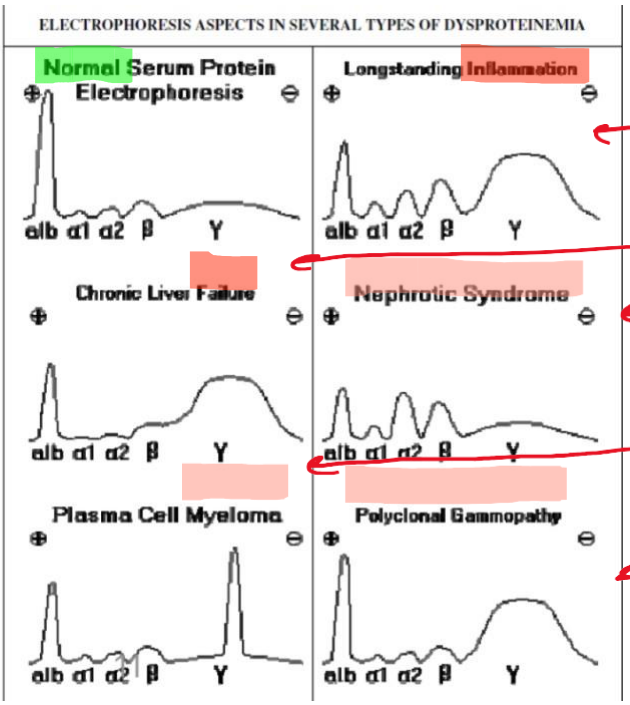
هذا السلايد غير موجود ضمن السلايدات التي نزلها الدكتور على تيمز للاستزادة فقط.

If the absolute number of the relative percentage of albumin deviates from what is shown, a problem in the production of urea occurs, or in the production of albumin which is synthesized in the liver, and when the liver is functioning wrongly, this amount will not be synthesized!
The same thing happens when there's a kidney problem, albumin becomes secreted in the urine, thus reducing albumin concentration, such as in blood, indicating the presence of a kidney disease.


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
α1 antitrypsin, TBG, transcortin, etc
haptoglobin, ceruloplasmin, α2 macroglobulin
transferrin, β lipoprotein
antibodies
albumin α1 α2 β γ
origin
Start point
Gamma
Beta
Alpha-2
Alpha-1
Albumin
10

in a previous slide, alpha 1 peak contained alpha 1 antitrypsin which is a protease inhibitor, <u>you shall remember this one</u>.
-under the alpha 2 globulin region, these are samples of proteins found under this category, haptoglobin and ceruloplasmin are important.
- the beta band of the globulin contains transferrin, LDL, and complement system
- the gamma globulin, <u>which appears the first on electrophoresis</u>.

Clinical Cases



These are not for memorization, just to show you patterns of electrophoresis when they differ between normal and abnormal conditions. So, different cases lead to different plasma protein electrograms when compared to the normal one, in terms of the amount of protein and the presence or absence of a different category of plasma protein. For example, less albumin is seen in a chronic liver failure, alpha 1 and 2 are very low, gamma is high. Low albumin and high gamma indicate a specific disease, meaning the production of monoclonal antibodies of a specific type.

In the nephrotic syndrome, albumin is very low, alpha and beta globulin are high, and diffused gamma is observed.

V2: PAGE 4

alkaline-copper sulfate

بدل

alkaline-cover sulfate