Lecture :1

Dr. Name :Nafez Abu Tarboosh

Done By :Mohammad Falahat

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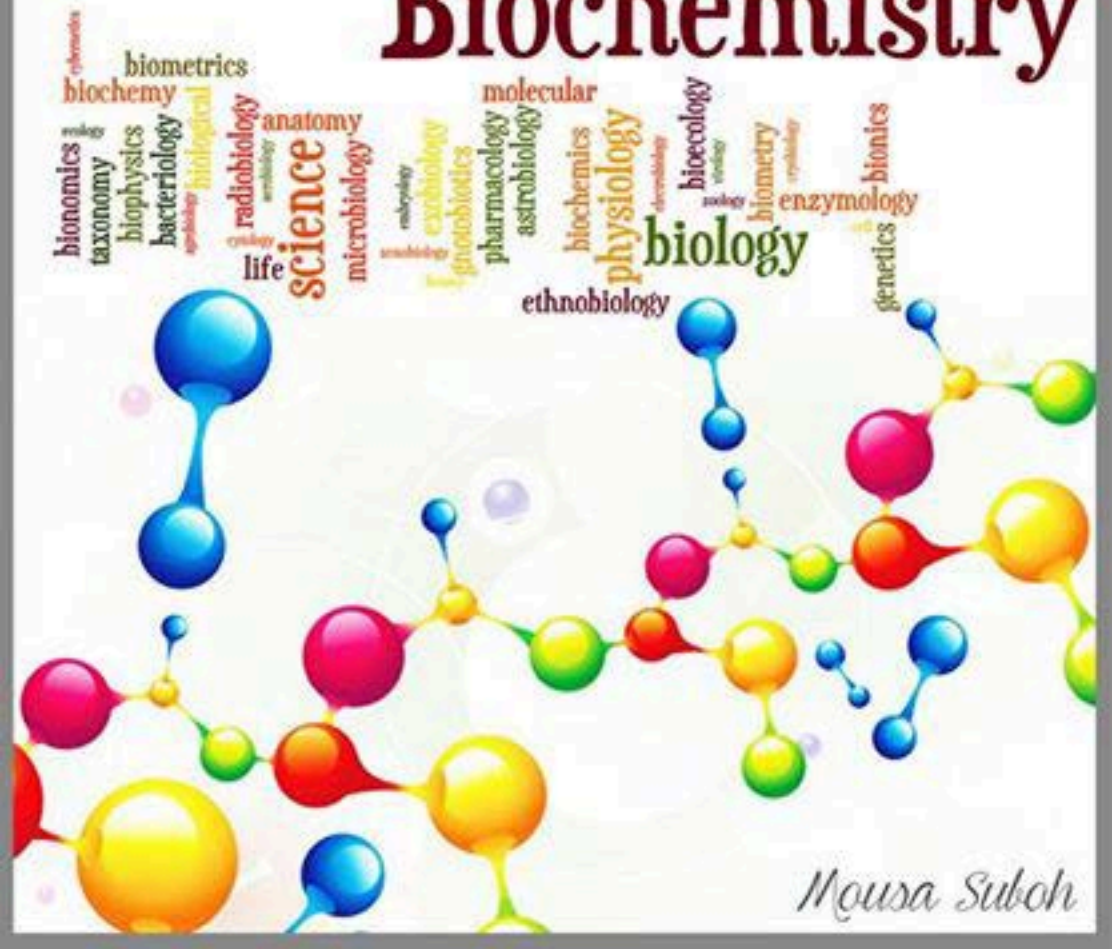
Medical Committee
The University of Jordan

Biochemistry



In the first semester in biochemistry 101 we talked about structural biochemistry which studies the structures of the chemical compounds in our bodies (Proteins, carbohydrates, lipids, and nucleic acids) . While in the second semester in biochemistry 102 we will study metabolism of these structures (what will happen to them within the body).

In the previous semester we talked about the *structure - function relationship* of some proteins. We talked about *fibrous proteins*, like collagen and elastin, and *globular proteins*, like hemoglobin and myoglobin briefly.

In the blood system we will study hemoglobin and myoglobin in details in 8 lectures, followed by the plasma proteins in 1.5 – 2 lectures, and immunoglobulins in 1.5 – 2 lectures, all as a *structure – function relationship*.

Plasma proteins

What is plasma?

It's the liquid part of the blood, and the liquid medium in which cells are suspended.

In other words, blood is composed of a liquid called plasma, and cells suspended in that liquid.

The composition of plasma: water (92%), solids (8%).

-respiratory gases can be found in the plasma.

The solids are classified into:

1. Organic:

Plasma proteins (Albumin; Globulins; Fibrinogens …), *nutrients* (glucose; free amino acids; triglycerides; cholesterol; free fatty acids …), *waste products* (ammonia; urea …).

2. Inorganic:

Ions (electrolytes, like : Na^+, K^+, Cl^-, SO_4^{-2}, HPO_4^{-2} …).

We will talk about how to get plasma, the different components of this plasma, the general and specific functions of plasma proteins and their classification. Also, we will talk about these proteins in particular(about their structures and the related functions): Albumin, Prealbumin , α1-antitrypsin, α1-fetoprotein, Haptoglobin (Hp), and Ceruloplasmin .

How can we get plasma (out of a blood sample)?

1. By **centrifugation** , which is the separation of materials according to their density, the higher the density, the earlier the aggregation (separation). Cells, membrane fragments, proteins, and other components can be separated in this method . According to the *density* and the *molecular weight* of the desired component, we set the centrifugation device to rotate at a certain speed to get that component separated (aggregated).

To take the cells out of a blood sample, we put the test tube containing the blood sample in a centrifuge, the cells will precipitate leaving the plasma.

2. If we put a blood sample in a test tube and leave it, by time the cells will precipitate leaving the plasma.

This method was used before as a daily test in labs and hospitals. You leave a blood sample in a test tube for an hour, and measure the speed of precipitation of the cells. Normally, all the cells usually precipitate in an hour. In males, the volume of the precipitated cells in an hour is 47% of the sample (not well packed like in centrifugation, but it's a used procedure) and the rest is plasma. In females, the volume of precipitated cells is about 42%, this is called **hematocrit** or **packed cell volume**.The speed of precipitation is called: **erythrocyte sedimentation rate (ESR)** (because most of the cells are RBCs (erythrocytes)), it can be higher than normal in many cases, like inflammation, cancer, and pregnancy; Which means that the required time to precipitate the cells is less than an hour. It was used as a detection method to detect cancer, pregnancy, and inflammatory processes because the tests and markers were not yet developed.

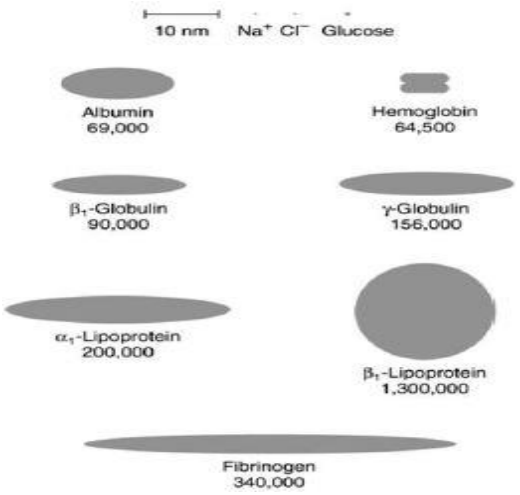
-Hematocrit : (also known as **packed cell volume (PCV)**) is the volume percentage (%) of red blood cells (erythrocytes) in blood.

Plasma proteins

The number of the discovered proteins in the plasma is increasing; due to the discovery of new better techniques in isolating proteins and identifying them. So far, more than **500** plasma proteins are identified, their normal total concentration is **(6 – 8 grams/deciliter)**. The main plasma protein is albumin. Hemoglobin(in high concentrations) is not a plasma protein because it(Hemoglobin) is found in RBCs. Free Hemoglobin can be found in the plasma but in low concentration .

Plasma proteins are usually *glycosylated* (attached to carbohydrates), the attachment on protein occurs either on the *nitrogen* atom of the side chain of **asparagine** residue *(N-linked).* Or on the *oxygen* atom of the side chain of either **serine**, **therionine**, **hydroxylysine**, or **tyrosine** residues *(O-linked).*

Plasma proteins differ from each other in their *size*, *shape*, molecular *weight*. As a comparison between albumin and fibrinogen. *Albumin* is ellipsoidal in shape, while *fibrinogen* is very elongated .*Albumin* has a small molecular weight (69 kilo Daltons), while *fibrinogen* has a large molecular weight (340 kilo Daltons). The shape is highly related to the function.



Most plasma proteins are glycosylated, most of them are negatively charged; due to the negatively charged carbohydrates and the negative charge on the protein itself. The carbohydrate portions(negatively charged ;so they will attract water)and surface area of the protein exposed to water, will affect the viscosity of the blood; the higher the surface area of the protein, the more viscous the solution. As a result, albumin (smaller surface area) has a lower viscosity than fibrinogen (larger surface area), which directly affects the viscosity of the blood.

- Most plasma proteins are Glycoproteins (N- or O-linked). **Albumin** is the major exception ; it does not contain sugar residues .

How to get plasma proteins out of plasma (separation of plasma proteins) ?

We introduced how to get the plasma out of a blood sample, now, we are going to know how to get out the proteins which are dissolved in this plasma, the following methods are used:

1. **Salting out**:

A process of separating proteins out of the solution through adding salts, addition of more salt will precipitate the protein out; because the salt will ionize and bind water at a higher affinity than proteins (protein – water bonds will be broken), hydrophobic features of proteins will start attaching them to each other and then precipitation occurs.

When salting out occurs, we get 3 main groups of proteins:

- Fibrinogen (which is responsible of the clotting of the blood, through changing fibrinogen into fibrin which forms the clot).

- Albumin (major protein) .

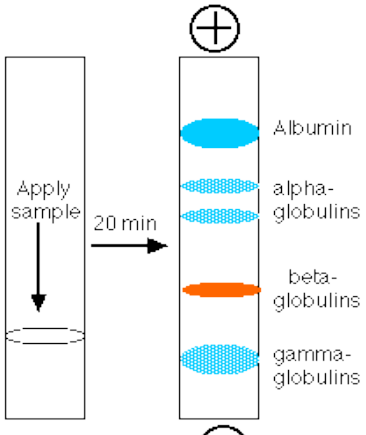
- Globulins (a collective group of proteins, many proteins are called globulins) .

2. **Electrophoresis**:

A technique of separating proteins, it has two procedures:

- Native gel electrophoresis: proteins will move according to their shapes, sizes, molecular weights in a gel material exposed to an electric field.

- Denaturing gel electrophoresis: proteins will move according to their molecular weights only, SDS is added to the proteins to denature them and give them negative charges (all the proteins are negatively charged, but some more strongly than others), then they are exposed to an electrical field, they will move towards the positive electrode(anode) due to their negative charges, the lower the molecular weight and the slightly higher the negative charge , the faster the movement of that protein, thus the fastest protein moving toward the positive electrode is albumin, which has smaller molecular weight and slightly higher



Separating serum proteins by electrophoresis

negative charge than the others.(Gamma globulins are the least negatively-charged serum proteins, the closest to the negative electrode) .

Electrophoresis gives less outcomes than salting out, it gives albumin and globulins only and does not give fibrinogen, because electrophoresis is operated on a serum sample, not a plasma sample. Serum is defibrinated plasma, plasma without clotting factors, as a result, fibrinogen does not appear with the products of electrophoresis (albumin and the collective group of globulins). Albumin is the fastest, then come several bands of globulins, which are named alphabetically according to their distance from albumin, α1, α2, β (with lower concentrated gel, after more time, β band will separate into β1 and β2 bands), and γ globulins .γ globulins are immunoglobulins (antibodies).

*(Band = a group of proteins inside the gel)

*Blood is composed of plasma(water 92%,proteins 7%,other solutes1%)and cells (RBC + WBC + platelets)
***serum**: is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma not including the fibrinogens

plasma = blood - (RBC + WBC + platelets)
serum = blood - (RBC + WBC + platelets + fibrinogens)

The most abundant protein in the plasma is albumin (about 60% of plasma proteins), (3.6 – 5 grams / deciliter) concentration (the product of the percentage of albumin times the total concentration of plasma proteins{60%*6-8 grams=3.6-5grams}). Albumin normal concentration is compared with the measured albumin concentration (in patients) to detect Hypoalbuminemia (low level of albumin), or Hyperalbuminemia (high level of albumin).

Inside **α1 band**, there are many proteins, *α1-antitrypsin* forms 90% of this band. Inside **α2 band**, the most abundant proteins are *haptoglobin* and *ceruloplasmin*. Inside **β band**, the most abundant proteins are *transferrin* (related to iron metabolism), *lipoproteins* (generally all of them), and *complement system proteins* (we'll study them in immunology). **γ band** is composed of *immunoglobulins* (antibodies).

γ globulins are synthesized by *plasma cells in the* bone marrow, spleen, and lymph nodes (*plasma cells are* products of immature B lymphocytes which divide to give memory cells and mature B lymphocytes which are the plasma cells), and these *lymphocytes* are products of bone marrow, spleen, and lymph nodes.

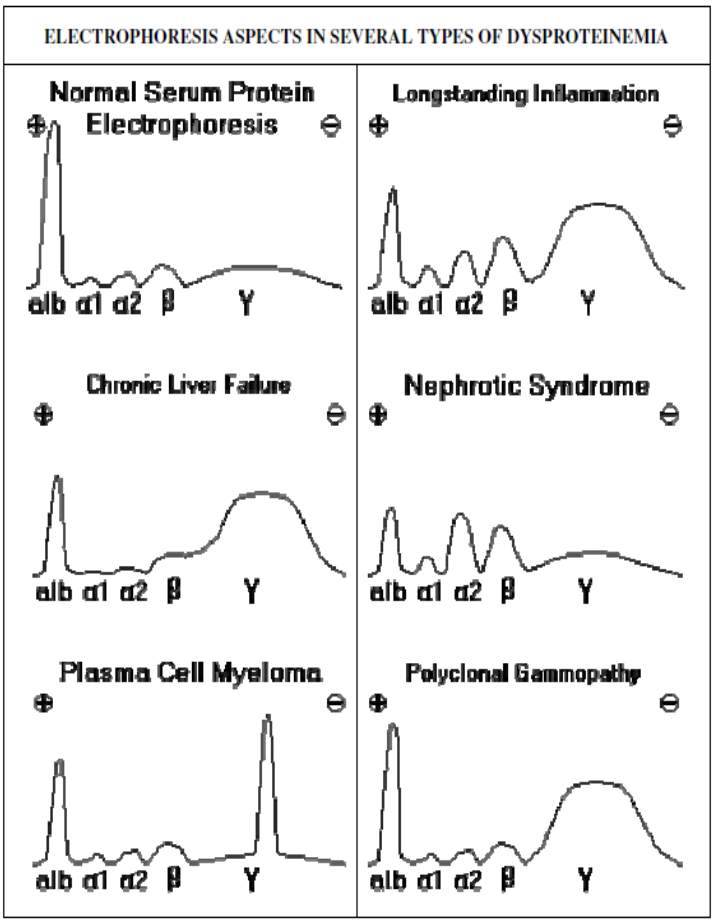
Q)Why most plasma proteins are synthesized as proproteins ?

Most plasma proteins (except γ globulins) are synthesized in the liver then they will be moved to the plasma, so they are first synthesized in an inactive form (proproteins). The second reason, they are synthesized within the liver cells and have to go outside those cells, so the synthesized protein should have a signal peptide which directs it's movement towards it's destination (to mitochondria, lysosomes, outside the cells …), the signal peptide is a certain group of amino acids at the beginning of the protein that direct it's movemnt, after the protein reaches it's destination, this part will be cleaved, thus; those proteins are synthesized as proproteins. The time of transit between the place of synthesis till they reach the plasma is different among different proteins, some proteins take about 30 minutes, and some take several hours.

Most plasma proteins are glycosylated, they go through several post translational modifications, like: proteolysis, glycosylation and phosphorylation. Proteolysis is the cleavage of the protein at more than one place to give the final form, like albumin (subjected to proteolysis to be active). Albumin is not a glycosylated protein (exception).

A device called densitometer measures the density of the proteins in each band in the electrophoresis gel, and expresses these densities as peaks like in the figure(densitometer representation), we can read these peaks, we can measure the exact amount of protein in the sample using these peaks, (this device converts the bands into readable, measurable, comparable data).

The highest peak is for albumin which constitutes 60% of plasma proteins, then come α1, α2, β, and γ, (γ usually has higher concentration than α1, α2, and β separately).



The first part of the figure represents a normal protein electrophoresis, and any disease or disorder in the body is reflected on the plasma proteins and as a result, on gel electrophoresis (densitometer representation).

If there is a problem in the *liver*, most plasma proteins will be affected (because most plasma proteins are synthesized in the liver), however, immunoglobulins (γ proteins) will not be affected by a liver disease because they are not synthesized in the liver. In this case the patient may be suffering from liver cirrhosis[Pronounced si-roh-sis], liver fibrosis, fatty liver, alcoholism affecting the liver (more than one reason related to the liver can lead to the third representation ,chronic liver failure).

Proteins usually do not appear in the urine, but if there is a *kidney* failure (like: nephrotic syndrome), the kidney loses it's ability to restrict proteins from getting out with the urine, it is not specific for a certain protein, all proteins will get out, so when we look at the presentation (the fourth one, nephrotic syndrome), we notice that all plasma proteins are getting out and decreasing in their densities.

If there is a cancer affecting the *plasma cells* (which produce immunoglobulins), the γ band will be higher than all other bands, (the fifth representation ,plasma cell myoloma).

This was the first time I write a sheet, I hope it was good enough.

Best wishes, your colleague:

Mohammad Nayel Falahat

Corrected by :Mohammed Nawaiseh