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Bio chemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Hello friends, this is the first lecture in Metabolism, hope you have a nice semester full of A's.

Metabolism is Biochemistry 2.

The first lecture is about PLASMA PROTEINS, in which we started discussing in BIOCHEMISTRY 1 course in summer semester when we learned about Immunoglobulins.

This lecture is found in:
Harpers Biochemistry 31st edition; Chapter 52.
Plasma Proteins and Immunoglobulines.

Plasma Proteins
General properties and selected examples.

What is **Plasma**?

Plasma is everything found in blood except for cells, and plasma is obtained from **Centrifugation**.

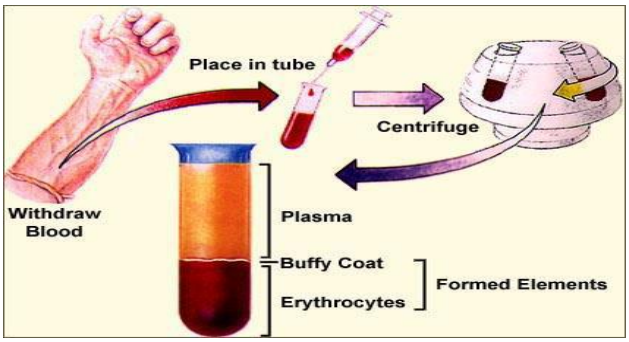
Centrifuged Blood means spinning blood in order to get separation of its components according to density (Liquid part and Cellular Part).

When we withdraw a blood sample from a patient and we centrifuge this blood sample, we obtain two phases:

1) Dense with red precipitation (Erythrocytes/ Red Blood Cells)
2) Plasma (Liquid part)

Between these two phases we can find the **Buffy Coat** which is a white coat made of White Blood Cells as well as Platelets.

The Buffy coat plus to the Erythrocytes is the Cellular part, but the Plasma is the Liquid Part of the blood sample.



Plasma Vs. Serum

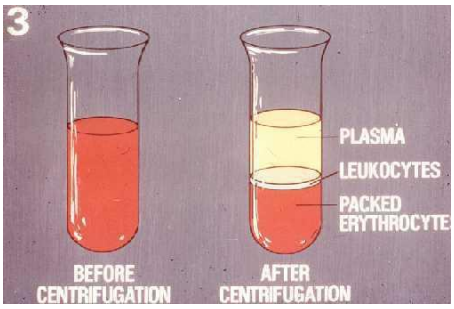
The Serum is the same as Plasma but they only differ in the availability of Clotting Factors (عوامل التخثر).

* **Plasma** is Blood with Anticoagulant & Clotting Factors (Fibrinogen).

* **Serum** is clotted blood. (No Anticoagulant nor Clotting Factors are present - which means no Fibrinogen is present but Fibrin is present).

- The Anticoagulant prevents Clotting Factors from clotting blood.

Note: Prevention of clotting factors from clotting is presented by prevention of Fibrinogen of clotting to form Fibrin (Fibrin is present in Serum only).



So to sum up, after Centrifugation and separation of blood contents according to density, we can find out that Plasma has the lowest density compared to the cells part.

Plasma = liquid part of blood + Anticoagulant+ Clotting factors. -> No clotting occur.

Serum = liquid part of blood . (Clotted Blood). -> No Anticoagulant, and Clotting factors (Fibrinogen) had been turned to their active form (Fibrin) .

Separation of Components in terms of percentages (After Centrifugation):

Plasma constitutes around **55%** of total blood sample.

RBC (Erythrocytes) constitutes around **45%** of total blood sample, which is precipitated and this phase is called: "Packed Cells" or "Hematocrit" and it is more dense than plasma.

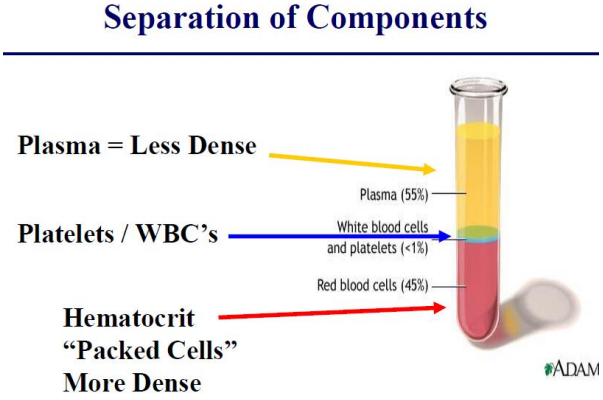
Which means that the Buffy Coat constitutes a very **small** percentage of blood sample, which is **less than 1%** and it is the **minor** component in relative to the other

components in the sample. (Even if in the picture below it is written that it is 1%, it is less than that).

NOTES:

1) As I mentioned earlier, plasma is the least dense component, and that's why it is found on the top of the test tube.

2) **Hematocrit (HCT)** that I mentioned earlier is one of the variables that is measured in Protein Blood Tests such as Protein blood count or CBC, also it helps in finding out about some diseases or conditions processes that we're going to learn about later on.



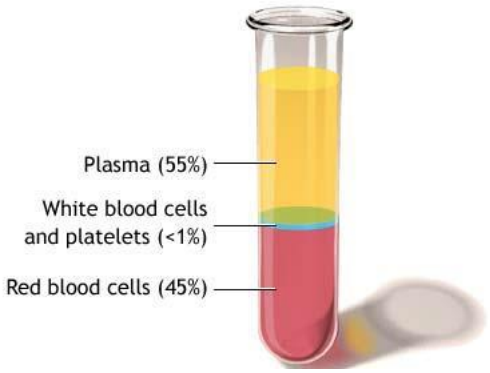
3) Most of the Blood Functions are carried by the Plasma.

1. **Respiration**—transport of oxygen from the lungs to the tissues and of CO_2 from the tissues to the lungs
2. **Nutrition**—transport of absorbed food materials
3. **Excretion**—transport of metabolic waste to the kidneys, lungs, skin, and intestines for removal
4. Maintenance of the normal **acid–base balance** in the body
5. Regulation of **water balance** through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid

6. Regulation of **body temperature** by the distribution of body heat
7. **Defense** against infection by the white blood cells and circulating antibodies
8. Transport of **hormones** and regulation of metabolism
9. Transport of **metabolites**
10. **Coagulation**

What are the components of Plasma?

1. **Liquid part** which constitutes of mainly **water**. (90% of plasma's composition is made of water molecules)
2. **Plasma proteins** 6-8%.
3. **Electrolytes** (Na+ & Cl-) 1%, which are the **minor** component.



Other components:

1. Nutrients (e.g. glucose and amino acids)

2. Hormones (e.g. Cortisol, thyroxine)
3. Waste products (e.g. Urea)
4. Blood Gases (e.g. CO2, O2)

So plasma proteins that constitute around 6-8% have so many types of proteins that can be distributed in some groups that constitute a mixture of hundreds of proteins in plasma, such as:

1. Albumin
2. Globulins
3. Fibrinogen

Main functions of plasma proteins in general:

1. **Buffering** – to maintain PH (Proteins that contain Histidine act as a buffer), it is not the main buffering system in the body but it is important.
2. **Maintaining colloid osmotic pressure**, as solutes- they can balance and maintain the osmotic pressure (albumin).
3. **Transport of molecules through blood** (such as water-insoluble hormones, e.g. steroid hormones).

So many molecules such as hydrophobic molecules need to be carried by plasma proteins because they cannot be transported through blood stream alone (because blood stream is hydrophilic).

Examples: a. Bilirubin molecule which is a product of metabolism.

b. Fatty acids that send a message or stimulation for fatty acid metabolism.

c. Steroid hormone.

d. Lipoproteins such as LDL/HDL (proteins that transport lipid molecules that are water insoluble from one side to another).

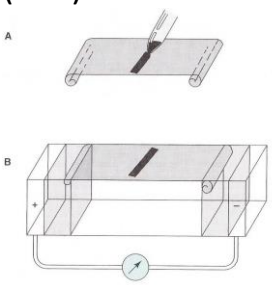
4. **Immune responses that act as Antibodies** (Immunoglobulins A/G/D/E/M).
5. **Clotting factors** (e.g. Fibrinogen).
6. **Antiproteases:** which are proteins that protect other proteins from hydrolysis of the peptide bond.

How can we separate these plasma proteins I mentioned earlier?

We can do that by biochemical techniques that we studied in Biochemistry 1.

For example:

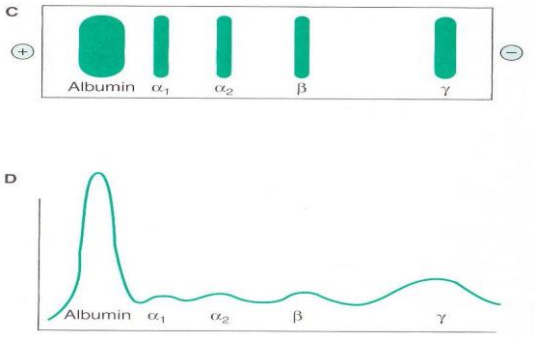
1. Salting out (**Solubility**).
2. **Electrophoresis**: separation according to size, in which proteins move from the Cathode (minus) to Anode (Plus) because of having total negative charge (SDS).



3. **Isoelectric focusing**: separation of proteins according to their isoelectric point (the point in which the total net charge of a protein equals 0).
4. **Use of antibodies**: this is the most specific, especially when using monoclonal antibodies.

Abundance:

As we can see in the picture below, Albumin is the most abundant plasma protein in comparison to the different types of globulins.



Note: Gamma-globulins are immunoglobulins, and relatively to the other types of globulins they're the most abundant.
Alfa1-globulins are the least abundant proteins.

Fractions of Plasma Proteins:

Fraction	*amount(%)*	*c (g/l)*
Albumins: albumin pre-albumin (transthyretin)	52 – 58	34 – 50
α_1-globulins: thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein	2.4 – 4.4	2-4
α_2-globulins: haptoglobin, macroglobulin, ceruloplasmin	6– 10	5 – 9
β-globulins: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system	8.5 – 14.5	6 – 11
γ-γ-globulins: IgG, IgM, IgA, IgD, IgE	10 – 21	8 – 15

Notes on the table above: Dr. Diala mentioned that we should not memorize the exact percentage, but we have to know the order of the abundance of the proteins.

Examples on Plasma Proteins and their function:

Function	Plasma Proteins
Antiproteases	Antichymotrypsin α_1-Antitrypsin (α_1-antiproteinase) α_2-Macroglobulin Antithrombin
Blood clotting	Various coagulation factors, fibrinogen
Enzymes	Function in blood, for example, coagulation factors, cholinesterase Leakage from cells or tissues, eg, aminotransferases
Hormones	Erythropoietin[a]
Immune defense	Immunoglobulins, complement proteins, and β_2-macroglobulin
Involvement in inflammatory responses	Acute phase response proteins (eg, C-reactive protein, α_1-acid glycoprotein [orosomucoid])
Oncofetal	α_1-Fetoprotein (AFP)
Transport or binding proteins	Albumin (various ligands, including bilirubin, free fatty acids, ions [Ca^{2+}], metals [eg, Cu^{2+}, Zn^{2+}], metheme, steroids, other hormones, and a variety of drugs) Corticosteroid-binding globulin (transcortin) (binds cortisol) Haptoglobin (binds extracorpuscular hemoglobin) Lipoproteins (chylomicrons, VLDL, LDL, HDL) Hemopexin (binds heme) Retinol-binding protein (binds retinol) Sex-hormone-binding globulin (binds testosterone, estradiol) Thyroid-binding globulin (binds T_4, T_3) Transferrin (transport iron) Transthyretin (formerly prealbumin; binds T_4 and forms a complex, with retinol-binding protein)

Notes on the table above:

1) Dr. Diala said that this table is important and sooner or later we are going to memorize it.
   But, Dr. Faisal said that this table is not for memorizing and you only have to scan it.
2) These are the functions of the plasma proteins and their examples.
3) **Antithrombin**: anti-clotting protein.
4) Leaking enzymes from tissues such as **AST/ALT** that are leaked either by damage or normally. (Amount of these enzymes is found normally but it is increased when there's damage or serious problem in that organism).
   Erythropoietin is a hormone that activates **Erythropoiesis** (the process which produces red blood cells (erythrocytes), which is the development from Erythropoietic stem cell to mature red blood cell), so it acts as a stimulus for stem cells.
5) **Oncofetal**: proteins which are present only during fetus development.

****Onco means cancer.

Example-**alpha1-fetoprotien (AFP):** made in the liver, and it is used as a tumor marker test in adults.

6) **Transport or binding proteins**: a large group of Plasma Proteins in which albumin is the most important one of them.

a. Retinol-binding protein binds to retinol. And Retinol is also known as vitamin A or Retinoic acid, which is a lipid soluble molecule who needs a carrier (specific binding Protein) through blood stream.

b. Sex-hormone-binding globulin: Sex hormones are made of cholesterol molecules that are considered hydrophobic.

c. Thyroid hormone is also hydrophobic so it needs a carrier

d. Transferrin transport iron ions, but Ferritin stores iron ions in the liver.

These proteins that we discussed earlier are encoded by genes that contain nucleotides by a certain sequence of nucleotides, but a change might happen in the sequence which can either cause a **mutation** or **POLYMORPHISM**.

تعدد اشكال :Polymorphism : Different shapes of proteins.

It is the change in a sequence of nucleotides that occur in more than 1% of the whole population.

It is considered as a normal variation that is present between different populations.

Normally more than 1 polymorphism can be founded in 1 gene.

Polymorphism is the main reason for people having different hair/ eye color.

Polymorphisms that happen between different races (Asians/Africans…) might:

1) Have no effect (Negligible effect).

Example: G might turn into T but at the end Albumin protein is translated with the same primary sequence, so it is considered as a silent mutation.

Example: An enzyme in Folic Acid Metabolism: *LTHFR* is found to be in 24% of Jordanian culture.

2) Cause a disease.

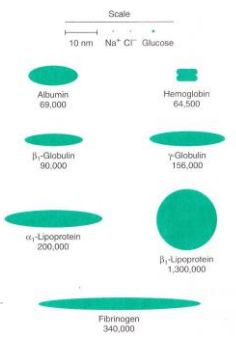
Example: we might see that a disease is common in a specific race more than the other.

Mutation:

Is the change of a sequence of nucleotides in less than 1% of the whole population.

How can we distinguish between proteins that have polymorphism?

We can do that by checking of the proteins to see if they have different amino acid sequence by *separation by electrophoresis* (change in size), or by *sequencing* (which is more accurate way because a change in 1 amino acid in terms of size might have a really small effect).



Polymorphism of Plasma Proteins

- The presence of two or more genetic variants of the same protein.
- The variants are encoded by a single locus.
- The variant appears in at least 1% of the population.
- The variants differ slightly in their amino acid sequence.
- Migrate differently under electrophoresis.

Half-life

Some of Plasma Membrane Proteins change because of the change of some conditions: **Acute phase proteins.**

The percentage of this change might be large enough to be multiplied by 1000, or small enough to be 1.5 (x50% of the normal protein).

Proteins get the most effect of acute conditions in cancer or chronic inflammatory processes.

In cancer, cancerous cells might produce a large amount of a specific type of protein.

In chronic inflammations, even if it is chronic and happens for a long time, this chronic condition does not stay stable for the whole time:

The condition of a patient might get worse: proteins that are related to this medical condition are multiplied.

Example: alfa1-antitripsin.

So after studying this, scientists discovered that each protein exists in plasma or in cells have **half-life**.

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NOTES:

1) Proteins do not stay in the same concentration or activity, they get degraded.

2) Proteins have wide variation in their half-life. *Example:*

- Collagen protein (not plasma protein) stays in body for 3 years (structural protein)

- Kinases proteins need to recycle and be degraded so they have shorter half-life.

3) If clotting factors are expressed highly, they need to be degraded later on.

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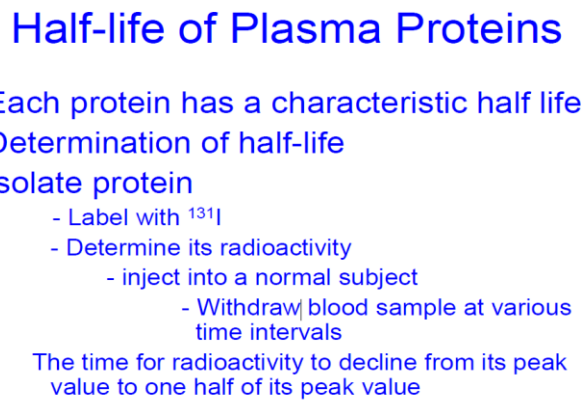
So we conclude from the examples we discussed earlier that proteins half-life are mostly related to their function.

We can determine the half-life of a protein by isolating the protein and labeling it with radioactive Iodine. (131I)

Radioactivity has a certain level that we can measure, and its level goes down after degrading a certain protein, so we can measure the change of the radioactivity level, and calculate the change of the amount of a certain protein, and that's how we can figure out the half-life of a certain protein.



THE END.

If anyone finds anything not correct please tell us, and if you've got any questions do not hesitate to ask us! Thank you all.