SHEET NO.

1

METABOLISM

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 017

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

DOCTOR : Dr.Nafeth

What is biochemistry?

في شوية ملاحظات اخر الشيت، اقرؤوها لو سمحتم..

It's the chemistry inside living cells.

Biochemistry consists of the structure and function of macromolecules (in the previous semester we talked about the structure. In this semester we will be discussing the related function of each molecule).

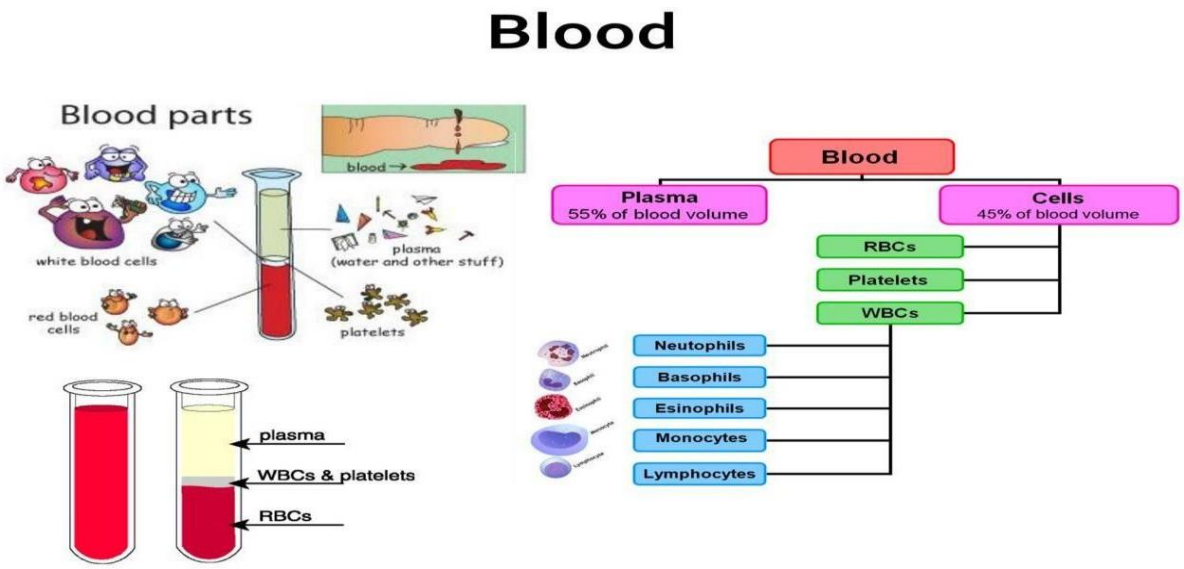
All macromolecules end up as acetyl coA when the body breaks them down for energy. This acetyl CoA enters Krebs cycle → oxidative phosphorylation→ to provide ATP.
***So, all macromolecules produce energy (but not the same amount) for example 1 molecule of Fat produces twice as much calories as 1 molecule of sugar (9 kcal/gm vs 4 kcal/gm) when burned.

1. What is plasma, and how can we extract it?
2. What are the different components of plasma?

Blood is made of 2 components:
1) Plasma (briefly speaking, it's the liquid that cells are suspended in).
2) Cellular component: RBCs (erythrocytes), WBCs (leukocytes) and Platelets

NOTE: we call the mixture of WBCs and platelets "Buffy Coat". (look at picture)

"cells are suspended in plasma"
What does that mean? Does the word **"suspended"** mean anything in biochemistry?
It means that the cells are not soluble, and they stay hanging in plasma.



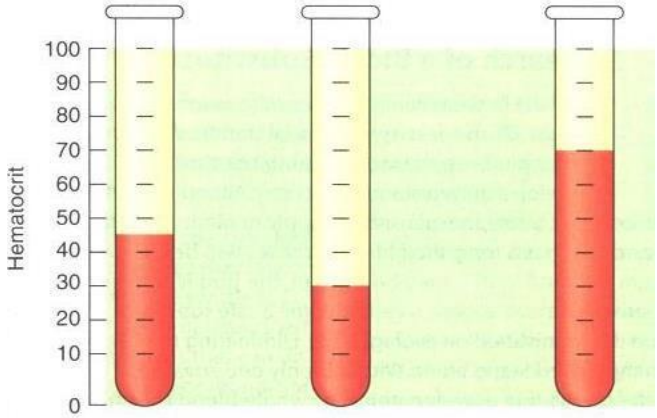
Hematocrit OR Packed cell volume:

The hematocrit blood test determines the percentage of blood cells in a given sample.

Note: on the internet you may find hematocrit as the percentage of RBCs only (its true that RBCs make the majority of cells but it is better to say that hematocrit is the percentage of all cells not only RBCs).

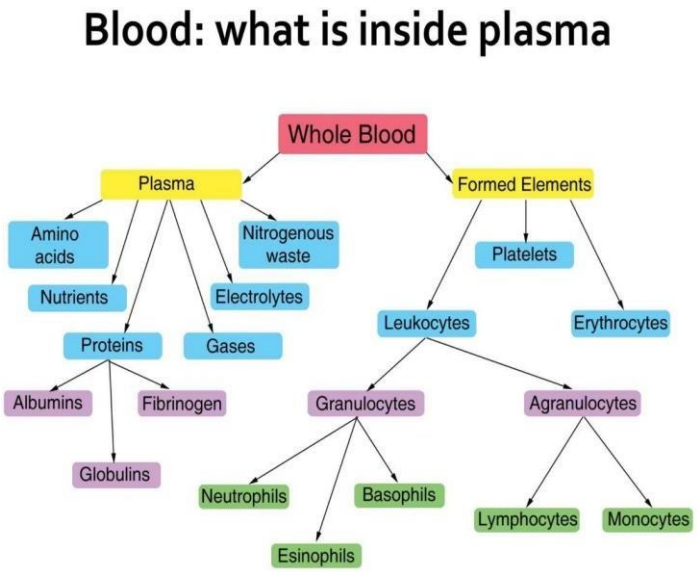
Hematocrit percentage in males =47% while in females it = 42%.

Any change (increase or decrease) on these percentages means that there is a pathological condition. For instance, in anemia we would find the percentages lower than the ones above.



Plasma:

As the Doctor said it is composed of everything Since it's the main way of communication between organs and the outside environment. Its responsible for messages from the blood to everywhere (anything that is found in cells or what cells need will be there. For example, metabolites/ wastes/ water/ nutrition / gases/ electrolytes).



Plasma (Slides)

- **Liquid medium where cells are suspended**
- **Composition: ▪ Water (92%) ▪ Solids (8%)**

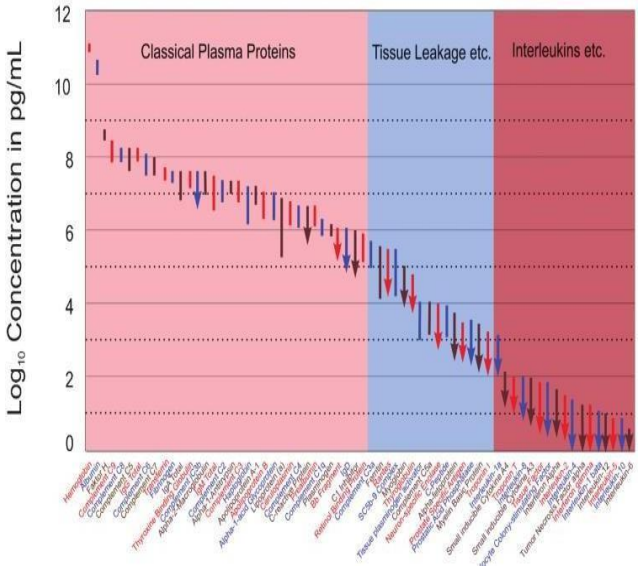
<u>Organic:</u>

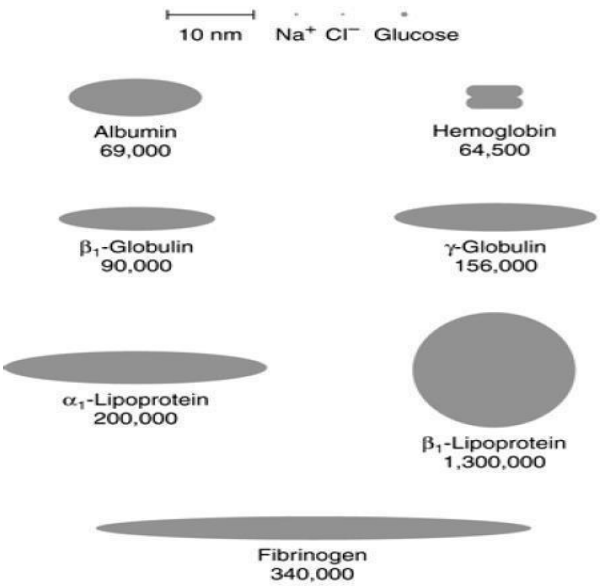
- **Plasma proteins: Albumin, Globulins & Fibrinogen**
- **Non-protein nitrogenous compounds: urea, free amino acids, uric acid, creatinine, creatine & NH_3**
- **Lipids: Cholesterol, TG, phospholipids, free fatty acids**
- **Carbohydrates: Glucose, fructose, pentose**
- **Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones**
- **<u>Inorganic:</u> Na^+,K^+,Ca^{2+},Mg^{2+},Cl^-,$HCO3^-$,$HPO4^{2-}$, $SO4^{2-}$**

There are more than 500 kind of plasma proteins (this number was figured out using the latest advanced techniques of discovering proteins).
NOTE: that most of the plasma proteins are modified (conjugated to either lipids or carbohydrates).
Proteins make up about 6-8 grams per deciliter (0.1 liter or 100 ml).
Main types are albumin, fibrinogen and globulins (alpha1, alpha2, beta and gamma proteins).
Proteins differ in MW and their shapes. For instance, Hemoglobin is 65K Daltons while albumin is 69k Daltons (the shape of the protein is so important and has a huge effect).

Plasma protein 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)**





Separation of plasma proteins (2 techniques):

1- Salting out: is the precipitation of proteins depending on their solubility, salts solubility is higher than proteins solubility in water (to be more soluble means to have more affinity) so when salts are added they become surrounded by what's called the hydration shells. In other words, the water interactions with salts are stronger than the interactions with proteins. Therefore, water gets away from proteins and the proteins start interacting with each other, which leads to aggregation and precipitation.

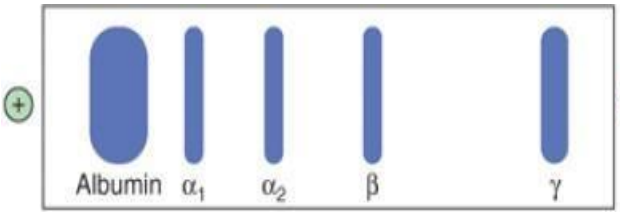
→ The lower the solubility of a protein, the earlier it will precipitate (and vice versa).

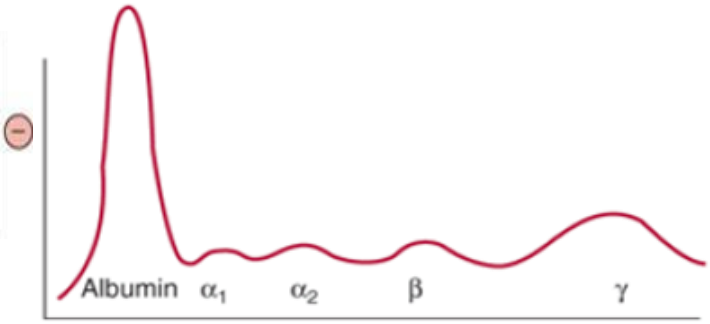
2- Gel electrophoresis: The serum (plasma without clotting factors (mainly fibrinogen)) is added to the wells and the plasma proteins (SDS added to make a uniform negative charge) will result in 5 bands including albumin, alpha1, alpha2, beta and gamma proteins. The fastest protein is albumin since it has the lowest MW and its the most negative (DR said that the main reason is MW since the SDS results in uniform negative charge).

→ We didn't use not plasma because when plasma is added to the well its exposed to air and that leads it to coagulate.

Slides:

- **Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins**
- **Electrophoresis (most common): serum (defebinated plasma), five bands (albumin, α1 , α2 , β, and γ)**





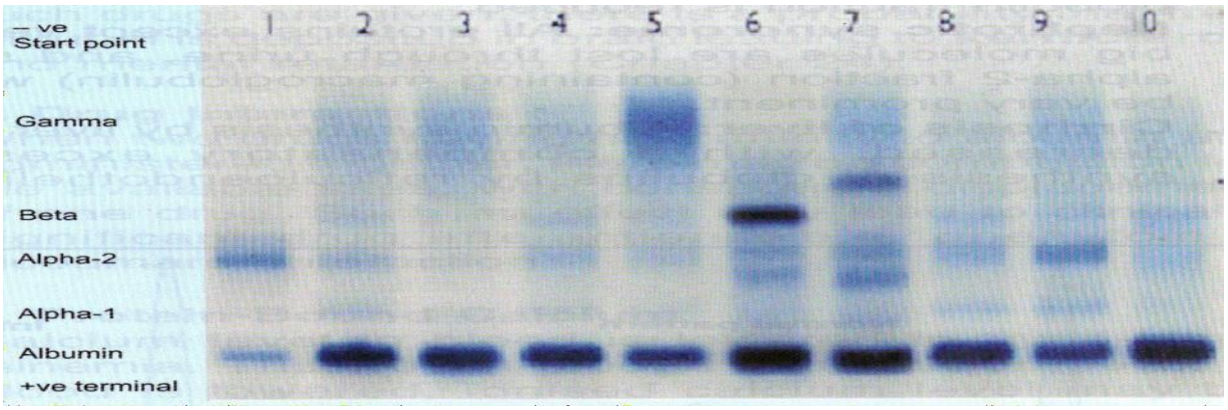
NORMAL VALUES:

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

Electrophoresis of plasma protein

- **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**

alpha band separated easily, and if we give more time, beta band will separated too.



1) Each band represents a group of proteins (only albumin band contains 1 protein).
2) There is beta 1 and beta 2 but it needs longer time to separate, gamma proteins don't separate at all. (alpha 1 and 2 have approximately the same MW).
3) Gamma globulins are immunoglobulins (antibodies) and there are 5 types of antibodies (IGG,IGM,IGA,IGD,IGE). Gamma globulins are the only ones made by mature B lymphocytes (mature B lymphocytes are called plasma cells), the rest of plasma proteins are made in the liver.
4) Densitometer measures the thickness of the band and convert it to a curve. 5) Concentrations (albumin>gamma>alphas and the beta).
6) Albumin makes (50% to 60%) of the 6-8 grams plasma proteins which means it is approximately (3.5-5.5 g/dl).
7) 90% of the Alpha 1 band is the alpha1-antitrypsin protein.

The DR said that we should be able to predict what happens to the graph 1 when particular changes happen. (liver or kidney failure, cancer or inflammation).

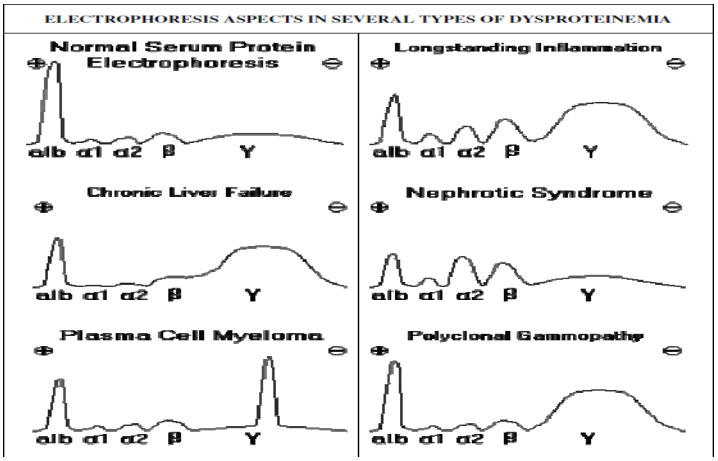
EXAMPLES:

In the case of inflammation globulins concentration increases in the body. (1st graph to the right)

Renal failure (filtration failure or nephrotic syndrome) in urine analysis will show that some proteins were lost from the blood (note that in this case the lost is not selective to any specific type of protein.

Liver failure (alcoholic liver, cirrhosis, fibroses hepatitis) since the liver is responsible for the production of all proteins except gamma globulins their percentages will come down.

plasma cells with Cancer (mature B lymphocytes) gamma globulins will rise (we have 2 cases, the 1st one happens when only one type of proteins increases, we will have a sharp increase in the graph, 2nd is that all 5 gamma were effected, we will notice a broad increase in the graph). The two cases are represented by the last two graphs in the diagram below.



Most of the plasma proteins are made inactive (preproproteins) and they are modified later, this is done for 2 reasons:

1st,no need for the protein to function unless its needed (it will be activated later as in fibrinogen).

2nd,if the site of synthesis differs from the site of function.

It takes from 30 mins up to several hours from the beginning of synthesis until it becomes mature and functional.

All plasma proteins are glycosylated except for albumin (carbohydrates are important to improve solubility, communication and attachment). But in the case of albumin if it was glycosylated its solubility increases and because albumin is the most abundant protein it will have a huge impact on the blood so it becomes denser (viscosity increases) and harder to move.

Synthesis of plasma proteins

- **Mostly liver (albumin, globulins), γ-globulins (plasma cells; lymph nodes, bone marrow, spleen)**
- **Most plasma proteins are synthesized as preproteins (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 because the concentration of albumin is the highest, and attachment of the carbohydrates increase the solubility and that lead to increases viscosity.**

Plasma protein and polymorphism

Before we start:
• A mutation is a permanent alteration in at least one nucleotide in the DNA, it might result in a change of one or more amino acids, at also may be harmless (silent mutation).
• Mutations can be common and spread in some societies more than others, so we see variations in the distribution of genetic diseases.
• Not all mutations result in diseases.

When a mutation affects 1% or more of the population -we choose a certain population (Jordanians, Asians, in middle east, universal)-, we call it **POLYMORPHISM** (different shapes of proteins), because genes are responsible for making proteins, so any mutation may result in a different sequence of amino acids, thus different shapes of a protein.

Usually the change happens in one nucleotide, we call this a **SINGLE-NUCLEOTIDE POLYMORPHISM (SNPs)**.

Examples on polymorphism:
1) ABO blood type (obviously not a disease). Most common type is O, other blood types are polymorphisms (represented in more than 1% of population)

2) Eye color differs from place to another (also not a disease). In Arab world black and brown are the common eye colors, whereas green and blue are uncommon traits, unlike the western countries where we notice the opposite.

Almost all plasma proteins have polymorphisms (not all people have the same sequence of amino acids for plasma proteins)

• Plasma proteins vary in half-lives (albumin 20 days, haptoglobin only 5 days)

Proteins' half-lives are determined through a procedure known **as isotopic labeling**, in which we label a protein and then calculate its concentration in the plasma after a certain time until we reach half of the labeled concentration.

• Half-lives of plasma proteins are affected by diseases, mostly GI diseases because GI has a high blood supply → due to chronic inflammatory processes affecting GI (a group called Protein-losing gastro enteropathy) → more blood supply to GI → expansion of the vessels → proteins leave the vascular system to the GI system, and then exit the body with stool→ net loss of proteins
Ex. Albumin half-life may be reduced from 20 to 1 day due to diseases.

Slides:

- **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 protein half-lives

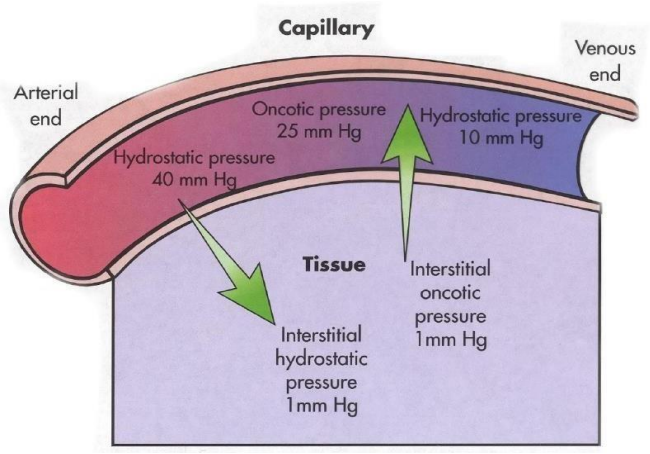
- **Determined through isotope labeling studies (I^{131})**
- **Albumin & haptoglobin (20 & 5 days)**
- **Diseases can affect half-lives (ex. Crohn's disease), albumin may be reduced (1 day)**
- **Protein-losing gastroenteropathy (gastro: stomach, entero: intestines, pathy: disease)**

Functions of plasma proteins:
Specific functions (vary from one protein to another)
1)Enzymes (e.g. rennin, coagulation factors, lipases)
2)Humoral immunity (immunoglobulins)
3)Blood coagulation factors
4)Hormonal (Erythropoietin)
5)Transport proteins (Transferrin, Thyroxin binding globulin, Apolipoprotein)

General functions (for all plasma proteins due to their common amino acid structures)
1) A nutritive role: when there is no food these proteins are broken down to provide energy.
2) Maintenance of blood pH (amphoteric property): all act as a buffer (H+ donor and acceptor) regardless to its nature because the existence of free carboxylic and amide groups at the terminus.
3) Contribution to blood viscosity: anything dissolve in water increases the viscosity.
4) Maintenance of blood osmotic pressure (oncotic pressure): it is the force applied by proteins themselves within blood on the plasma (water) to keep water inside the vessels (attract water), so it won't let water leak outside the vessels into the interstitial fluid.

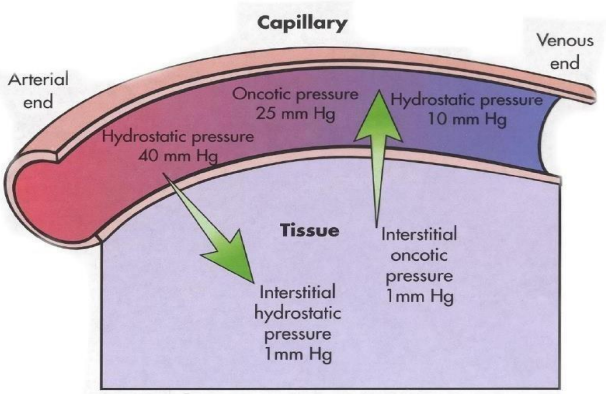
Starling forces

- ✓ **Arterioles, venules vs. tissue hydrostatic pressure (37 & 17 vs. 1 mm Hg)**
- ✓ **Plasma proteins oncotic pressure is 25 mm Hg**
- ✓ **Edema can be a result of protein deficiency**



Starling forces: two opposite forces controlling the exchange of nutrients between capillaries and tissues.
1) Oncotic pressure (directs water to the vessels).
2) Hydrostatic blood pressure: pressure applied by the fluid in the wall of vascular system. (to the interstitial fluid).
NORMALLY proteins control the process and don't allow water to get outside the vessels.

	Arteriole	Venule
Blood pressure (mm Hg)	40	10
Osmotic pressure (fixed)	25	25
Resultant	15 outside with nutrients	15 inside with wastes



- We notice there is a total balance, the amount of water that leaves the capillary from the arterial end re-enters from the venous end (15 outside and 15 inside).
- When there is a problem in plasma proteins (caused by heart failure or kidney problems), this will result in water accumulating in the tissues and therefore having a swelling (edema).
- In abnormal conditions it is not balanced, there is a total of 10 outside (20 outside and 10 inside) so water will accumulate in the tissues due to protein disorder.

	Arteriole	Venule
Blood pressure (mm Hg)	40	10
Osmotic pressure(fixed)	20	20
Resultant	20 outside with nutrients	10 inside with wastes

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**

A lot of plasma proteins are called **ACUTE-PHASE PROTEINS**, because under cases of acute inflammation, tissue damage, cancer or chronic inflammation, some proteins' concentrations increase dramatically (sometimes 1000-fold of their regular concentration)

THE MECHANISM:

Inflammatory processes activate a molecule called **Interleukin-1 (IL-1),** which targets liver cells and causes translocation to a **transcription factor*** called **Nuclear factor kappaB (NFkB)** from the cytosol (inactive form) to the nucleus (active form).

In the nucleus **(NFkB)** binds to the DNA to start transcription (mRNA) and then translation to produce proteins (increasing their concentration)

Ex: C-reactive protein (CRP), α1 -antitrypsin, haptoglobin, & fibrinogen (which are known as acutephase proteins)

Negative acute-phase proteins

Some proteins decrease in concentration (or do not get affected at all) in cases of acute inflammations, chronic inflammations or cancer. Ex: prealbumin, albumin, transferrin.

***A transcription factor** is a protein that binds to the DNA to form mRNA thus producing new proteins in the ribosomes by translation.

شوية ملاحظات صغيرة:

١- الشيت من ٠١٧ وحاولت قدر الامكان اجمع الاشياء الي موجودة بالسلايدز وشرحها لحد سلايد ١٦
٢- بفضل لو تسمعوا الريكورد وموجود ع ju med او تستعينوا باليوتيوب او الكتاب وبرضو موجود عالتيمز والموقع
٣- أي ملاحظة أو أي تعديل احكولي وما منقصر معكم جميعًا بإذن الله.
٤- استمتعوا وادرسوا بحب حتى لو في معيقات كثيرة كانت اليوم بالمحاضرة والشرح.
٥- https://doctor2017.jumedicine.com/wp-content/uploads/sites/7/2018/09/Biochem-1.pdf
https://doctor2017.jumedicine.com/wp-content/uploads/sites/7/2018/09/Biochem-2.pdf
هي رابط الشيتات الي اخدت منهم للي بحب يرجعلهم ودمتم في أمان الله.

نور شهوان