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Sheet Slides

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Plasma proteins 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.

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.

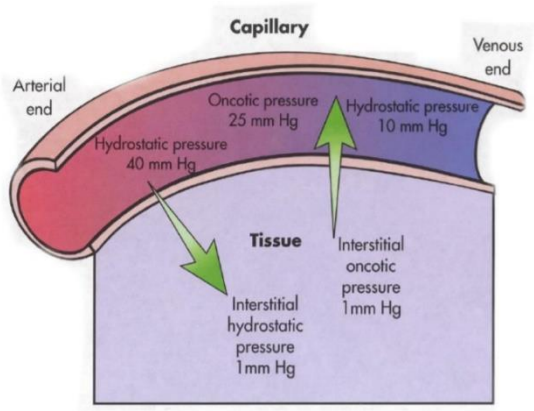
Blood pressure: it is the force applied by the volume of blood on the walls of the vessels (systolic and diastolic).

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 (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

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 kappa-B (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 acute-phase proteins)

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

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.

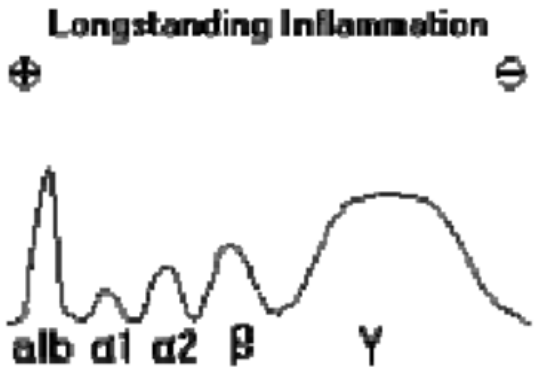
Ex: prealbumin, albumin, transferrin.

* Remember from sheet 1

In the case of inflammation

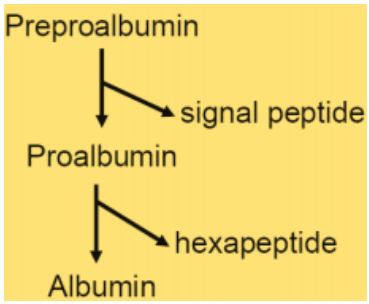
Some proteins increase (acute-phase)

and others decrease (negative)



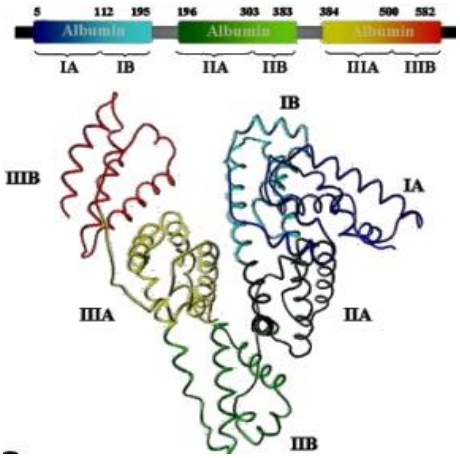
Now we will talk about specific functions of proteins

A) Albumin



- The dominant protein in blood plasma
- The main contributor to the osmotic pressure (75-80%)
- Synthesized as a preproprotein
- Synthesized from liver 12g/day (25% of total liver protein products is albumin)
- Albumin is used in liver function test (increase or decrease in albumin means that there is a problem in liver)
- MW= 69 kDa, half-life = 20 days.
- One polypeptide chain, 585 amino acids, 17 disulfide bonds.

- Proteases subdivide albumin into 3 domains (1A 1B, 2A 2B, 3A 3B)
- Anionic at pH 7.4 with 20 negative charges
- Ellipsoidal shape vs. fibrinogen (elongated)



- The fibrinogen shape exposes more negative charges to water thus more viscosity (that's why albumin is not elongated)
- Fibrinogen elongated shape increases blood viscosity in injuries to stop bleeding.
- **Always remember** that albumin is the major transporter for almost everything in blood by binding to ABD (albumin binding domain), **it transports:**
  Free fatty acids (FFA)
  Certain steroid hormones
  Bilirubin (from broken heme)
  Plasma tryptophan
  Metals: Calcium, copper and heavy metals
  Drugs: sulfonamides, penicillin G, dicoumarol, aspirin.

Since almost all drugs bind on the same protein (albumin), there is a chance of two drugs trying to bind on the same point on albumin and they will compete by their affinities and concentrations (drug-drug interaction)

One drug will bind and the other will be free in plasma (the free drug is the active one) it will go to the tissues and react there, so you can't know its concentration, and this might be fatal.

CLINICAL DISORDERS

1) Bilirubin toxicity

- **Normally,** New born babies have high concentration of bilirubin (enzymes are not mature enough=broken heme), bilirubin binds to albumin till the body deals with it.
- So, in the first 7-10 days babies will have physiological jaundice in skin, eyes, tissues

- Parents are advised to expose their babies to sunlight to mature the enzymes and break the bilirubin.
- The blood brain barrier (border that separates circulating blood from the brain) is not mature yet and brain can't deal with bilirubin, so there shouldn't be bilirubin in the brain.
- Aspirin binds on the same spot where bilirubin binds (competitive ligand), so giving aspirin to a new-born will lead to high concentration of bilirubin thus entering the brain and amassing there. This is called **kernicterus** and it causes mental retardation.

2) Phenytoin-dicoumarol interaction

Phenytoin is an anti-epileptic drug
Dicoumarol is an anti-coagulant

- When two drugs having high affinity to albumin are administered together, there may be competition for the available sites, with consequent displacement of one drug. such an effect may lead to clinically significant drug interactions
- So, they both bind on the same spot, you shouldn't take both of them at the same time

3) Hypoalbuminemia

- The normal albumin concentration is 3.5-5.5 g/dl
- When it is under 2g/dl, we have Hypoalbuminemia (aka hypoproteinemia) (because albumin is the main protein in plasma)
  This results in **EDEMA**

Edema could be generalized (in the whole body) or localized (mostly in the abdomen (**ASCITES**) depending on its cause

- Ascites (accumulation of fluids in the abdomen region) can be caused by liver cirrhosis (caused by drinking alcohol).
- when the cause is general (protein deficiency, starvation or famine) it will cause general EDEMA

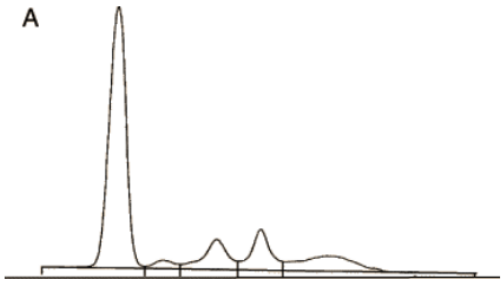
Remember the first general function, page 2

This is treated by having a rough diet or by paracentesis (removing fluid from the abdominal cavity)

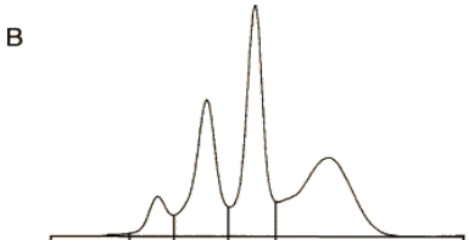
4) Hyperalbuminemia typically caused by dehydration, and some liver cancer cases. (this will affect a lot of proteins, not albumin only)

- What really happens is that water decreases (dehydration), so albumin has increased relative to water (pseudo increment), we call this a relative increase
- the simple solution is to drink water (hydration).

5) Analbuminemia (no albumin) a very rare condition.



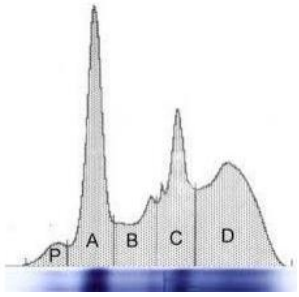
NORMAL



ANALBUMINEMIA

- This disorder absolutely causes edema, but it is not as severe as expected, because concentrations of other proteins have increased which compensates the decrease of albumin.
- Other proteins contribute to osmotic pressure too, so the loss in its value is not numerous.
- BUT it is life threating because these proteins can't do the **specific function** of albumin **(transporting food and waste)**.

B) Prealbumin or Transthyretin (transport, thyroid gland t3, t4)



- a small glycoprotein (rich in tryptophan, 0.5% carbohydrates) **unlike** albumin. It is different from albumin and called prealbumin because it migrates a head of albumin (faster than albumin) in gel electrophoresis.
- MW=62 kDa, short half-life (only 2 days)
- Blood level is a lot lower than albumin (0.25 g/L).
- Its main function is carrying T3 and T4
- It is more sensitive indicator for liver function (albumin takes a lot of time (20 day) unlike prealbumin (2 days)).
  However, its concentration is much lower than albumin.

C) Globulins

1) α1- fetoprotein (alpha 1 band)

- Synthesized primarily by the fetal yolk sac and then by liver parenchymal cells.
- normally it is not produced in adults, abundant in very low levels.
- Level of α1-fetoprotein increases in:
  Fetus and pregnant women Normally
  Hepatoma & acute hepatitis (cancer in liver)

Functions of α1-fetoprotein:

- Protecting fetus from immunotypic attacks
- Modulating the growth of fetus
- Transporting compounds e.g. steroids
- Low level in pregnancy: increased risk of Down's syndrome.

2) Haptoglobin (HP)

- It is an acute phase reactant protein
- α2 glycoprotein (MW=90kDa), a tetramer (2α, 2β)
- 3 phenotypes (polymorphs):
  Hp 1-1→ α1, α1 + 2β
  Hp 2-1→ α1, α2 + 2β *notice that the 2 beta subunits are fixed*
  Hp 2-2 → α2, α2 + 2β

Some hemoglobin molecules leave RBCs to plasma, the function of **HP** is to bind to free hemoglobin to **prevent** them from getting **filtrated in the kidneys** and leaving with urine, because even though our body can produce heme and globin, it can't produce **iron** (trace metal).

half-life of free **HP** is 5 days, when it is bound to hemoglobin the half-life of the complex becomes 90 mins (MW=150 kDa), so the complex is transported and broken in the liver quickly, and iron is extracted from the complex.

In cases of hemolytic anemia (damaged RBC→ more hemoglobin in plasma) the level of **HP** decreases as it binds to the hemoglobin and gets broken in the liver.

3) Ceruloplasmin

- A copper containing glycoprotein (MW=160 kDa)

- Copper is very important; many enzymes use it, such as:
  oxidative phosphorylation enzymes (complex IV) for ATP production
  ferroxidase which oxidizes ferrous to ferric (transferrin)
  amine oxidase, copper dependent superoxidase dismutase, cytochrome oxidase, tyrosinase.
- It contains 6 atoms of copper
- Regulates copper level in **blood** (A protein called metallothionein regulates the **tissue** level of Cu) as it contains 90% of serum Cu (it **stores** Cu)
- The other 10% is bound to Albumin for **transport** (albumin has a lower affinity for Cu)
- Decreased levels in liver disease (it is produced in the liver) (Ex. Wilson's, autosomal recessive genetic disease)
- Ceruloplasmin concentration is decreased, less affinity for binding to the copper, this results in increment of Cu in plasma thus it enters tissues, the person's skin and eyes will become bronzy.

THE END!