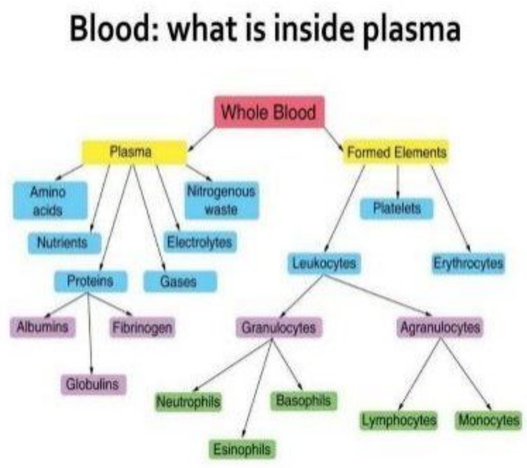
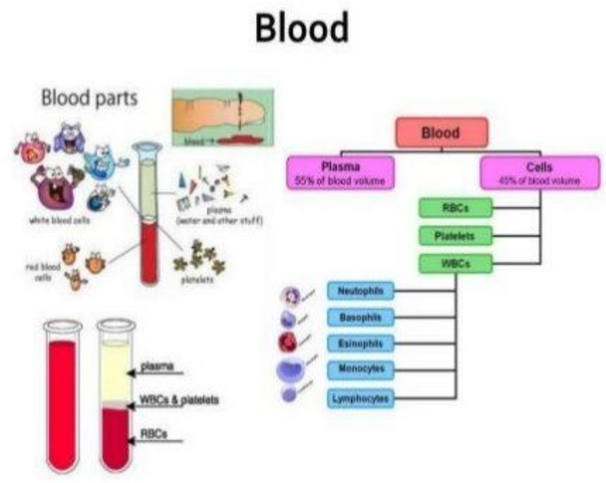
♡ Blood is made of 2 components: (everything)

the main way of communication between organs and the outside environment.

1. Plasma (liquid that cells are suspended (not soluble
2. Cellular component (RBCs (erythrocytes), WBCs (leukocytes) and Platelets)

the mixture of WBCs and platelets "Buffy Coat"





➢ Composition: ▪ Water (92%) ▪ Solids (8%)

➢ More than 500 plasma proteins have been identified

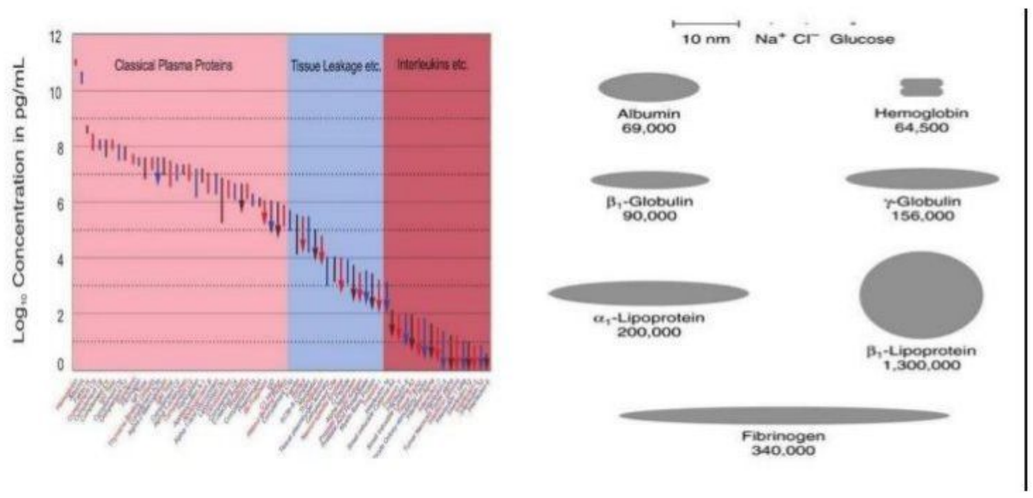
➢ Normal range 6-8 g/dl (the major of the solids)

➢ Simple & conjugated proteins (glycoproteins & lipoproteins)

Organic:

- 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
- **Inorganic:** $Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO3^-, HPO4^{2-}, SO4^{2-}$

♡ Proteins differ in MW and their shape



♡ Haematocrit OR Packed cell volume :

The haematocrit blood test determines ?

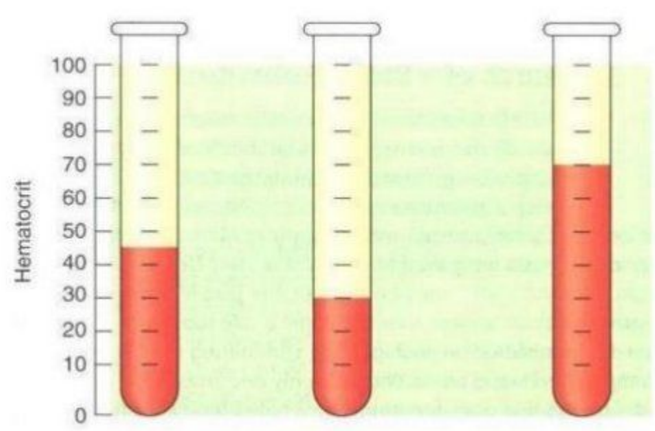
the percentage of blood cells in a given sample.

** RBCs the majority **

in males =47% ,

females it = 42%.

😉Any change (increase or decrease) = disease



♡ Separation of plasma proteins

(2 techniques):

1. Salting out :

☆(ammonium sulfate)☆

Plasma ✔

the precipitation of proteins depending on their solubility

The lower the solubility of a protein, the earlier it will precipitate (salts > proteins)

♤ fibrinogen, albumin, and globulins

2. Gel electrophoresis:

☆(most common)☆

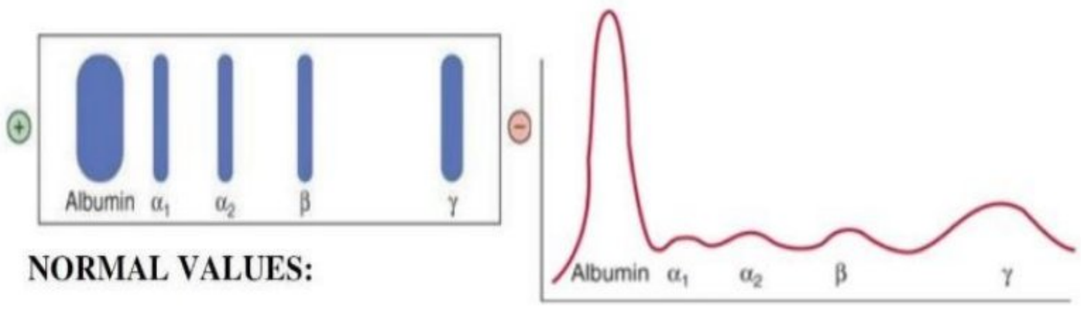
Plasma ✕

Serum ✔

(serum; Plasma without clotting factors (mainly fibrinogen)) (defebinated plasma)

Mechanisms :

The serum added to the wells and the plasma proteins (negative charge , MW) will result in 5 bands including : albumin, alpha1, alpha2,beta gamma



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

➢ Albumin is smaller than globulin, and slightly negatively charged (one band only)

Albumin makes (50% to 60%) of the 6-8 grams plasma proteins which means it is approximately (3.5-5.5 g/dl).

➢ Globulins (3 bands):

➢ α band: a1 MW = a2 MW

✓ 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

(5 types = Ig (MAGDE), B cell

Salting out ammonium sulfate	the precipitation of proteins depending on their solubility	fibrinogen, albumin, and globulins	Plasma ✔
Gel electrophoresis most common	The serum added to the wells and the plasma proteins (negative charge , MW)	albumin, alpha1, alpha2,beta gamma	Plasma ✕ Serum ✔

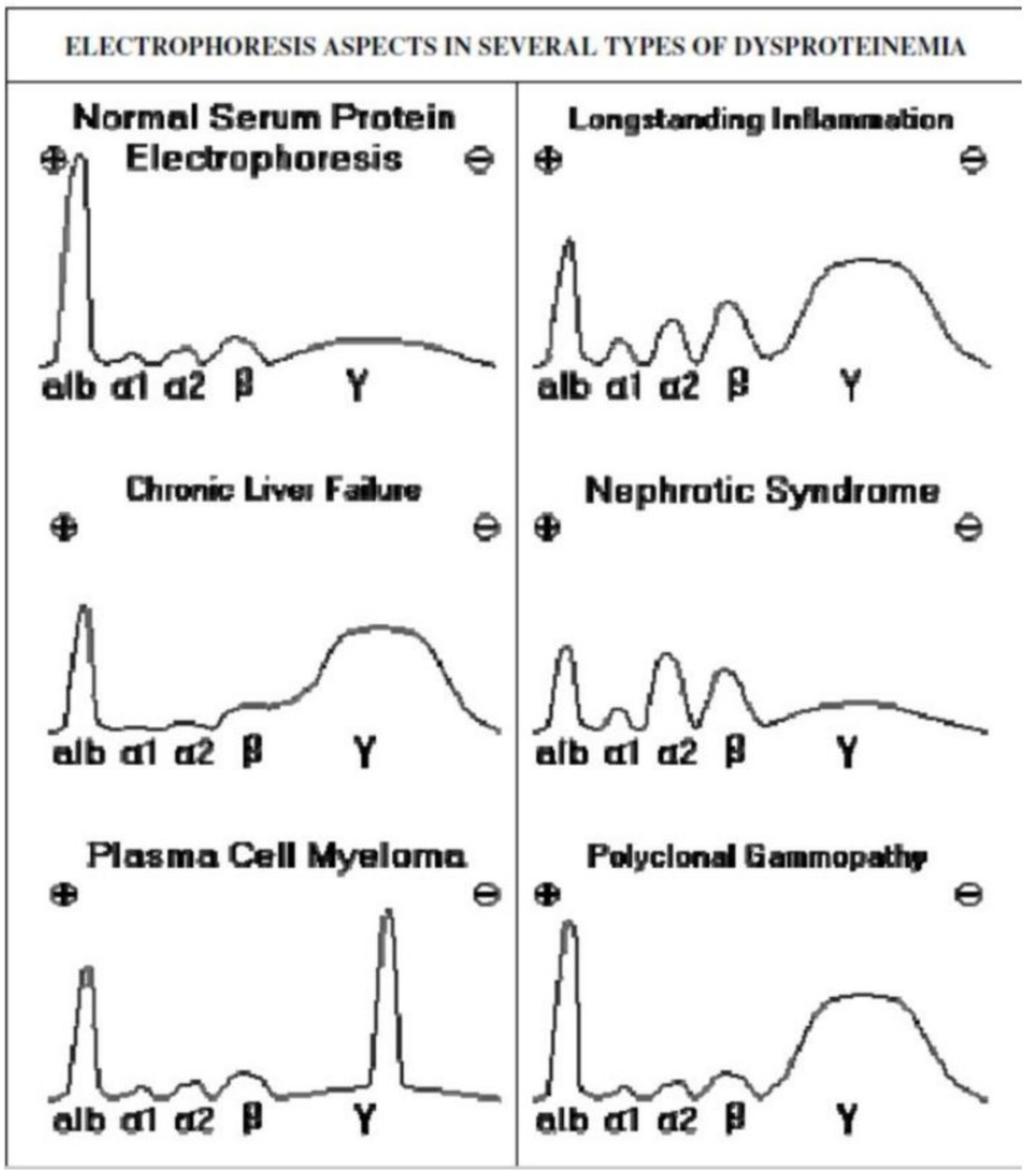
alpha band separated easily, and if we give more time, beta band will separated too.

** Densitometer measures the thickness of the band and convert it to a curve. 5)

** Concentrations ; albumin > Gamma > alphas and the beta :)

♡ DISEASE

Longstanding inflammation	globulins concentration increases
Renal failure (filtration failure or nephrotic syndrome)	some proteins were lost from the blood (in urine) not selective to any specific type of protein.
Liver failure (alcoholic liver, cirrhosis, fibroses hepatitis)	all proteins except gamma globulins their percentages will come down.
plasma cells with cancer (mature B lymphocytes)	gamma globulins will rise One type of gamma rise 5 types of gamma rise



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

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

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

✔ 30 mins up to several hours

** All plasma proteins are glycosylated except for albumin(carbohydrates are important to improve solubility, communication and attachment). 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

☆ **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

• Not all mutations result in diseases.

☆ POLYMORPHISM : When a mutation affects 1% or more of the population

** any mutation may result in a different sequence of amino acids, thus different shapes of a protein.

SINGLE-NUCLEOTIDE POLYMORPHISM (SNPs): change happens in one nucleotide

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

➢ 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

• 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

• Half-lives of plasma proteins are affected by diseases, mostly GI diseases because GI has a high blood supply

➢ Determined through isotope labeling studies (I131)

➢ 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)

" Protein-losing gastro enteropathy "

Functions of plasma proteins:

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 mmHg)

✓ Plasma proteins oncotic pressure is 25 mm Hg

✓ Edema can be a result of protein deficiency

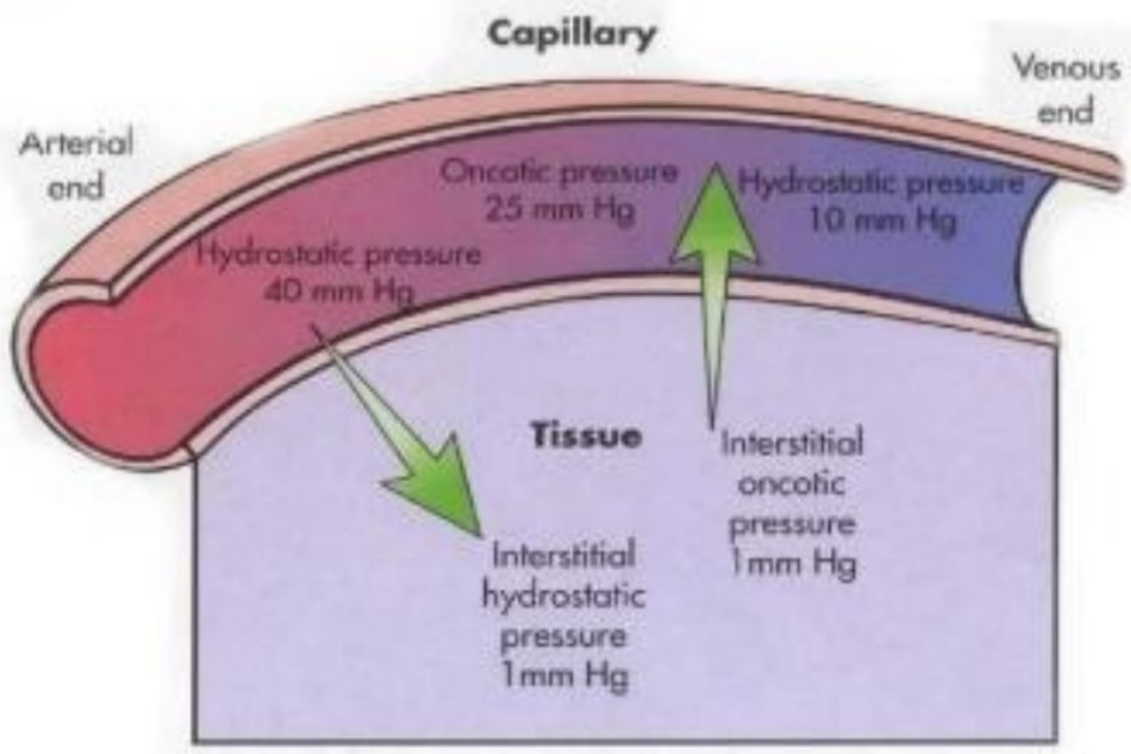
** Starling forces: two opposite forces controlling the exchange of nutrients between capillaries and tissues.

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 vessel

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



** In abnormal conditions (heart failure or kidney problems) it is not balanced

	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:

1. Inflammatory processes
2. Activate Interleukin-1 (IL-1) targets liver cells
3. translocation to a transcription factor* called Nuclear factor kappa B (NFkB) from the cytosol (inactive form) to the nucleus (active form).
4. In the nucleus (NFkB) binds to the DNA
5. start transcription (mRNA)
6. then translation to produce proteins (increasing their concentration)

For example

C-reactive protein (CRP)

α1 -antitrypsin

haptoglobin

fibrinogen

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

Prealbumin, albumin, transferrin

Plasma

Plasma proteins

➢ More than 500
6-8 g/dl of solids
➢ Simple & conjugated proteins

Synthesis

➢ Mostly liver (albumin, globulins), γ-globulins (plasma cells; lymph nodes, bone marrow, spleen)

➢ Most plasma proteins are synthesized as preproteins (signal peptide)

➢ Transit times (30 min to several hours)

➢ Most plasma proteins are Glycoproteins (N- or O-linked) . exception ; Albumin

half-lives

Proteins' half-lives are determined through a procedure known as isotopic labeling 🏷️ (I131)

• Half-lives of plasma proteins are affected by diseases, mostly GI diseases

Notes ✓💜

* One of blood contains

*liquid that cells are suspended (not soluble)

* the main way of communication between organs and the outside environment.

Separation

Subtopic

Salting out ammonium sulfate	the precipitation of proteins depending on their solubility	fibrinogen, albumin, and globulins	Plasma ✔
Gel electrophoresis most common	The serum added to the wells and the plasma proteins (negative charge , MW)	albumin, alpha1, alpha2,beta gamma	Plasma ✘ Serum ✔

Gel electrophoresis

polymorphism

☆ POLYMORPHISM : When a mutation affects 1% or more of the population

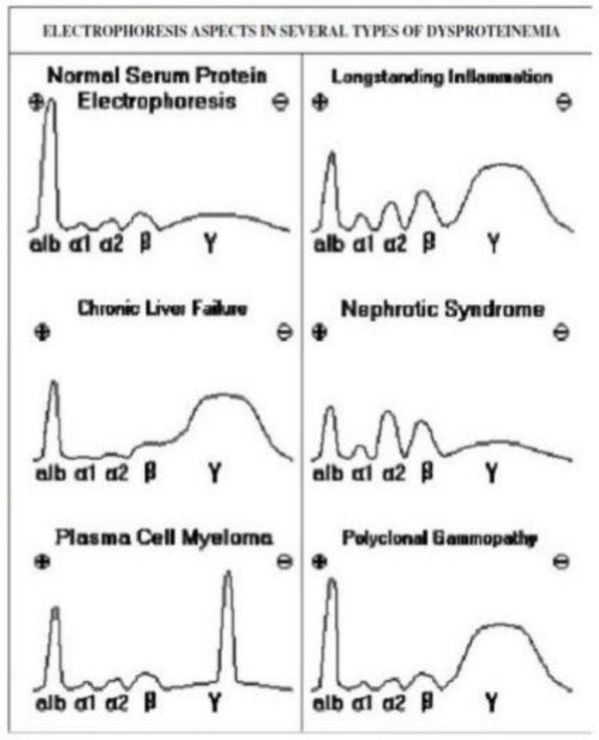
➢ A mendelian or monogenic trait

➢ 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

Longstanding inflammation	globulins concentration increases
Renal failure (filtration failure or nephrotic syndrome)	some proteins were lost from the blood (in urine) ✨not selective to any specific type of protein.
Liver failure (alcoholic liver, cirrhosis, fibroses hepatitis)	all proteins except gamma globulins their percentages will come down.
plasma cells with cancer (mature B lymphocytes)	gamma globulins will rise 💜One type of gamma rise 💜 5 types of gamma rise



Disease

Functions of plasma proteins

General functions

- A nutritive role
- Maintenance of blood pH (amphoteric property)
- Contributes to blood viscosity
- Maintenance of blood osmotic pressure

Specific functions

- Enzymes (e.g. rennin, coagulation factors, lipases)
- Humoral immunity (immunoglobulins)
- Blood coagulation factors
- Hormonal (Erythropoietin)
- Transport proteins (Transferrin, Thyroxin binding globulin, Apolipoprotein)

Functions

Albumin;

Function;

** The main contributor to the osmotic pressure (75-80%)

** major transporter for almost everything in blood by binding to ABD (albumin binding domain

Synthesis

Preproproteins

• Synthesized from liver 12g/day (25% of total liver protein products is albumin)

CLINICAL DISORDERS

Notes :

• The major protein in blood plasma

MW= 69 kDa

half-life = 20 days.

One polypeptide chain,

585 amino acids, 17 disulfide bonds.

Anionic at pH 7.4 with 20 negative charges

Ellipsoidal shape

** The normal albumin concentration is 3.5-5.5 g/dl

☆ Albumin is used in liver function test (increase or decrease in albumin means that there is a problem in liver)

	Analbuminemia	Hypoalbuminemi:	Hyperalbuminemia
What?	(no albumin)	Decease	Increase
Notes	very rare condition	less than 2 g/dl	
Cause	mutation that affects splicing # Autosomal recessive inheritance	• Malnutrition (generalised edema) • Nephrotic syndrome • Cirrhosis (mainly ascites) • Gastrointestinal loss	caused by dehydration, and some liver cancer cases.
Symptoms	moderate Edema (other proteins increase , not specific) life threating	EDEMA generalized (in the whole body) or localized (mostly in the abdomen (ASCITES)	
Treatment		This is treated by having a rough diet or by paracentesis	drink water (hydration).

☆ drug-drug interaction

Bilirubin toxicity	Phenytoin-dicoumarol interaction
** bilirubin: broken heme ** Babies: high bilirubin (7 – 10) days \ jaundice # Sun light as a treat # BBB : immature ASPIRIN + new baby = high accumulate bilirubin in brain "kernicterus " --→ mental retardation	# Phenytoin is an anti-epileptic drug . # Dicoumarol is an anti-coagulant. (High affinity to albumin) (Bind to the same spot)

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

Function: carrying T3 and T4

Notes and compare

** small glycoprotein (rich in tryptophan, 0.5% carbohydrates)

** MW=62 kDa

** (faster than albumin) in gel electrophoresis

** short half-life (only 2 days)

** Blood level is a lot lower than albumin (0.25 g/L).

** more sensitive indicator for liver function

Globulins

α1-globulins	α2- globulins	β- globulins	γ-globulins
▪α1-antitrypsin ▪α1-fetoprotein ▪α1- acid glycoprotein ▪Retinol binding protein	▪Ceruloplasmin ▪Haptoglobin ▪α2-macroglobulin	▪CRP ▪Transferrin ▪Hemopexin ▪β2-microglobulin	▪IGG ▪IGA ▪IGM ▪IGD ▪IGE

α1- fetoprotein

(Alpha 1 band)

Synthesis; fatal yolk sac → liver parenchymal cells.

** not produced in adults (very low level)

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.

α 1 – antitrypsin / a 1 - antiprotease

(52 kDa) , 90% of a1

function : neutralize (work against) trypsin & trypsin like enzymes (elastase).

antagonist (neutralizer) for trypsin { serine protease (hydrolase) }

1- Elastase (× Elastin) → skin, blood vessels and lungs

Elastase is produced by macrophages (WBC) during inflammation to break down the elastin of microorganism, but it will also affect the elastin in the alveoli walls (lungs)

** Antitrypsin breaks down elastase then the lung tissue is regenerated. So, Antitrypsin prevents excessive damage of tissues .

A person will face a problem when ;

1. A deficiency of Alpha-1 antitrypsin
2. Mutated alpha-1 antitrypsin

** Emphysema is characterized by having a barrel chest and difficulty in breathing

Active elastase + α_1-AT → Inactive elastase: α_1-AT complex → No proteolysis of lung → No tissue damage

Active elastase + ↓ or no α_1-AT → Active elastase → Proteolysis of lung → Tissue damage

2- Genetics and alpha1- antitrypsin

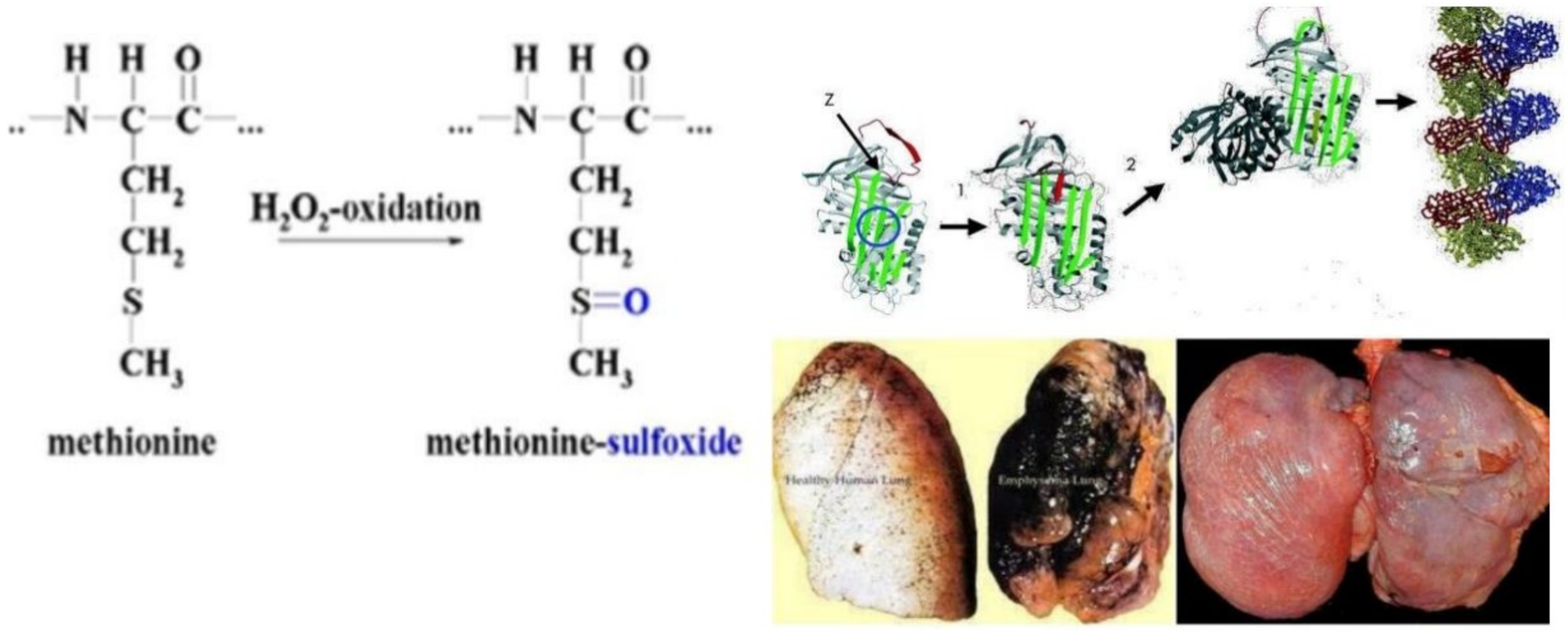
** plasma protein has at least 75 polymorphic forms.

alleles are: PiM PiS PiZ ,PiF , phenotype MM is the most efficient and common one

If one M allele is present, antitrypsin will be effective. So, having 2 copies other than M will cause a problem.

ZZ phenotype can lead to emphysema

♡ Smoking can oxidize the 358th amino acid methionine to methionine sulfoxide in antitrypsin. This residue is on the surface and supposed to bind to the elastase, so smoking will decrease the ability to bind drastically



♡ Liver : The ZZ phenotype antitrypsin has an extra loop and beta sheet. The beta sheet of an antitrypsin protein has high affinity towards the loop of another, so they will polymerize and form alpha-1 antitrypsin aggregates in the liver which can't leave and results in the killing of liver cells, and then leads to fibrosis then to cirrhosis of the liver.

" 10% of people with ZZ antitrypsin have cirrhosis "

Ceruloplasmin (a2 - band) • 6 atoms of copper

copper

A copper containing glycoprotein (MW=160 kDa)

• Copper is very important; many enzymes use it, such as:

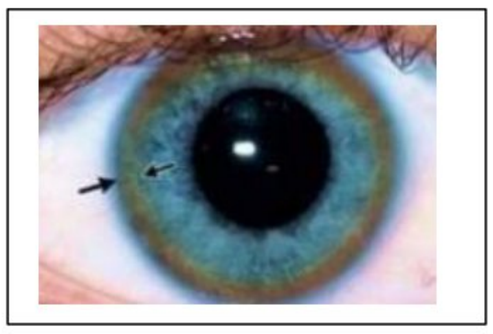
1. oxidative phosphorylation enzymes (complex IV) for ATP production
2. ferroxidase which oxidizes ferrous to ferric (transferrin) amine oxidase,
3. copper dependent superoxidase dismutase, cytochrome oxidase, tyrosinase.

FUNCTION:

1. 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)
2. The other 10% is bound to Albumin for transport (albumin has a lower affinity for Cu)

Pathological conditions:

1. Decreased levels in liver disease (it is produced in the liver) (Ex. Wilson's, autosomal recessive genetic disease)
2. 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



Haptoglobin (HP) a-2 band

acute phase reactant protein

• (MW=90kDa), a tetramer (2α, 2β)

• 3 phenotypes (polymorphs):

2 beta subunits are fixed

Hp 1-1→ α1, α1 + 2β

Hp 2-1→ α1, α2 + 2β

Hp 2-2 → α2, α2 + 2β

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.

Some hemoglobin molecules leave RBCs to plasma, the function of HP is to bind to free hemoglobin (65 kDa) to prevent them from getting filtrated in their 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

C Reactive Protein (CRP)

- Synthesis : first discovered, binds with the C fraction of the polysaccharide that is present in the cell wall of a type of bacteria called pneumococci.

- function : helps in the defense against bacteria and foreign object in the body.

- acute phase protein. It is undetectable in healthy individuals.

** Its level reaches a peak after 48 hours of the incident. Which is used as a monitoring marker **