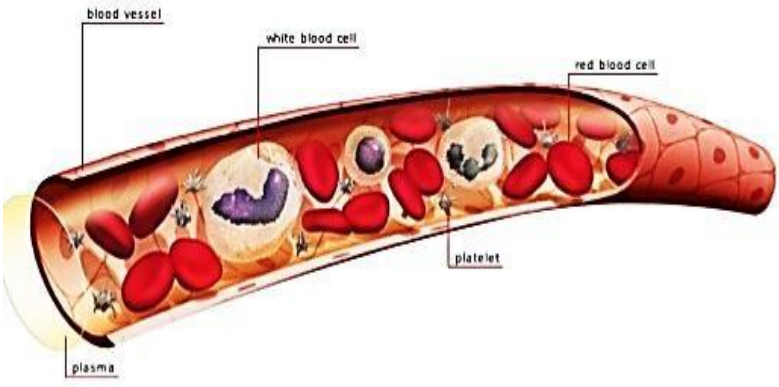
Plasma proteins



Regarding the course of metabolism, it's interested in discussing building up and breaking down of macromolecules that we have discussed last semester, so we will discuss degradation and building up of Carbohydrates, proteins, nucleic acids and lipids. Carbs, lipids, proteins, and nucleic acids, all of them at the end, the purpose of them is to be degraded and give you energy, so we will start with metabolism of energy at the beginning, before that there is a couple of lectures about plasma proteins, we will discuss the general properties for plasma proteins, some information about specific plasma proteins, next time we will start with the energy metabolism, starting with the plasma proteins, we are interested in discussing what proteins are present inside the plasma and when we say plasma we mean the liquid part of the blood.

What should we know?

What was mentioned in the slide:

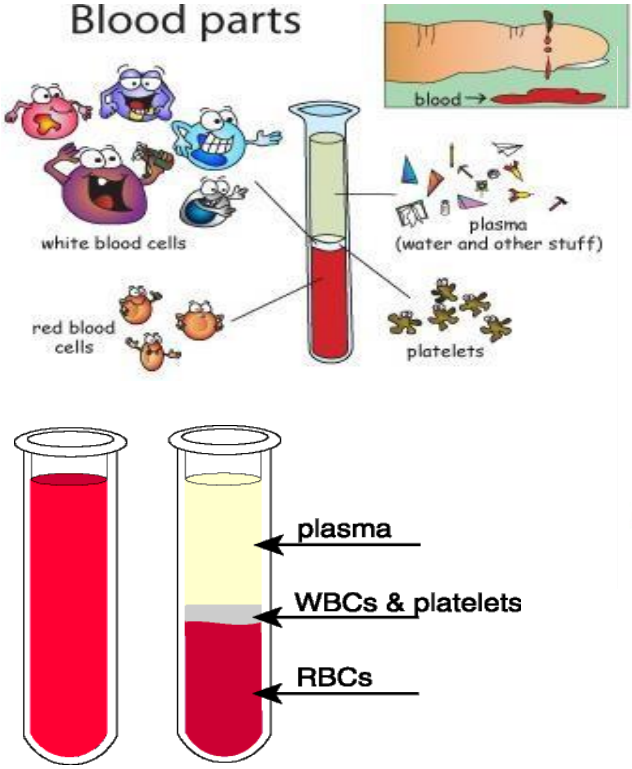
1. What is plasma, and how can we extract it?
2. What are the different components of plasma?
3. Plasma proteins (general functions, basis of classification, associated processes and molecules)
4. Plasma proteins: (structure, synthesis, function & diseases associated)

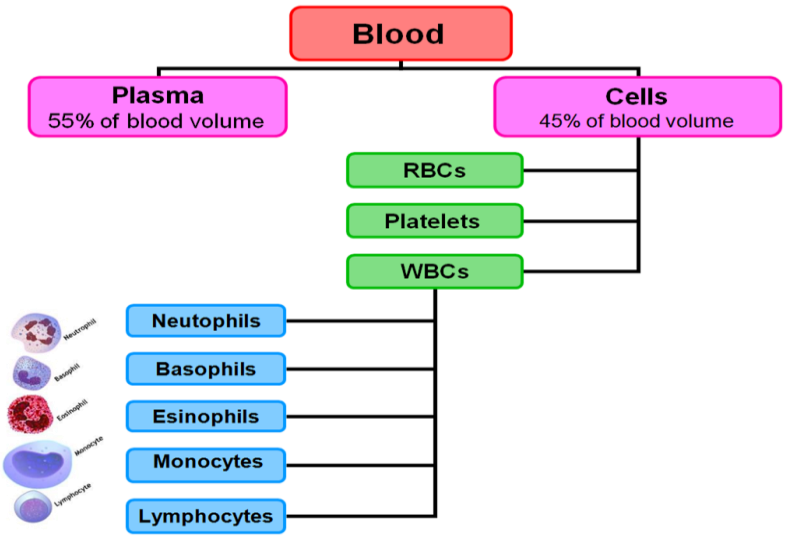
Albumin & pre-albumin	α1-antitrypsin	Haptoglobin (Hp)
α1-fetoprotein (AFP)	Ceruloplasmin	C-Reacrive Protein

What was mentioned in the lecture:

here is what we we're gonna cover through the lecture.

Blood





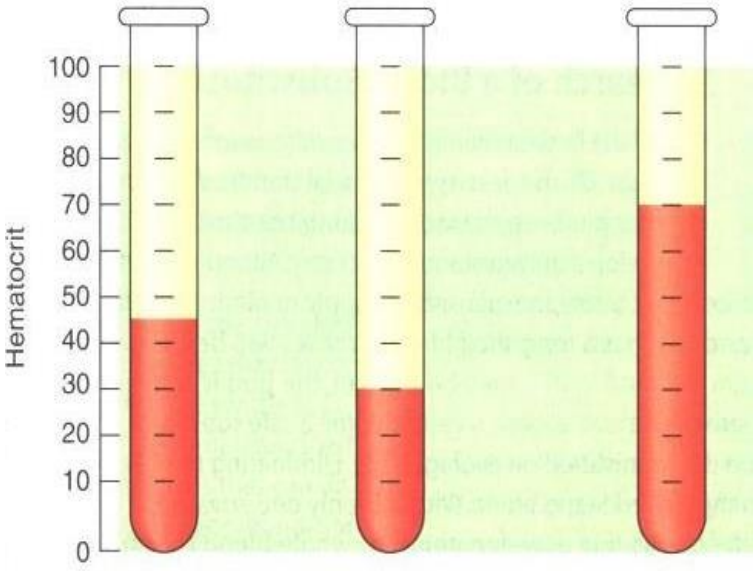
What was mentioned in the lecture:

the components of the blood are either plasma or cells, this distinction (in the figure) is based on volume (not on mass or any other thing), within the cells we have RBCs, platelets and white blood cells with its components, the plasma constitutes by volume approximately 55%.

Blood: plasma vs. hematocrit

What was mentioned in the slide:

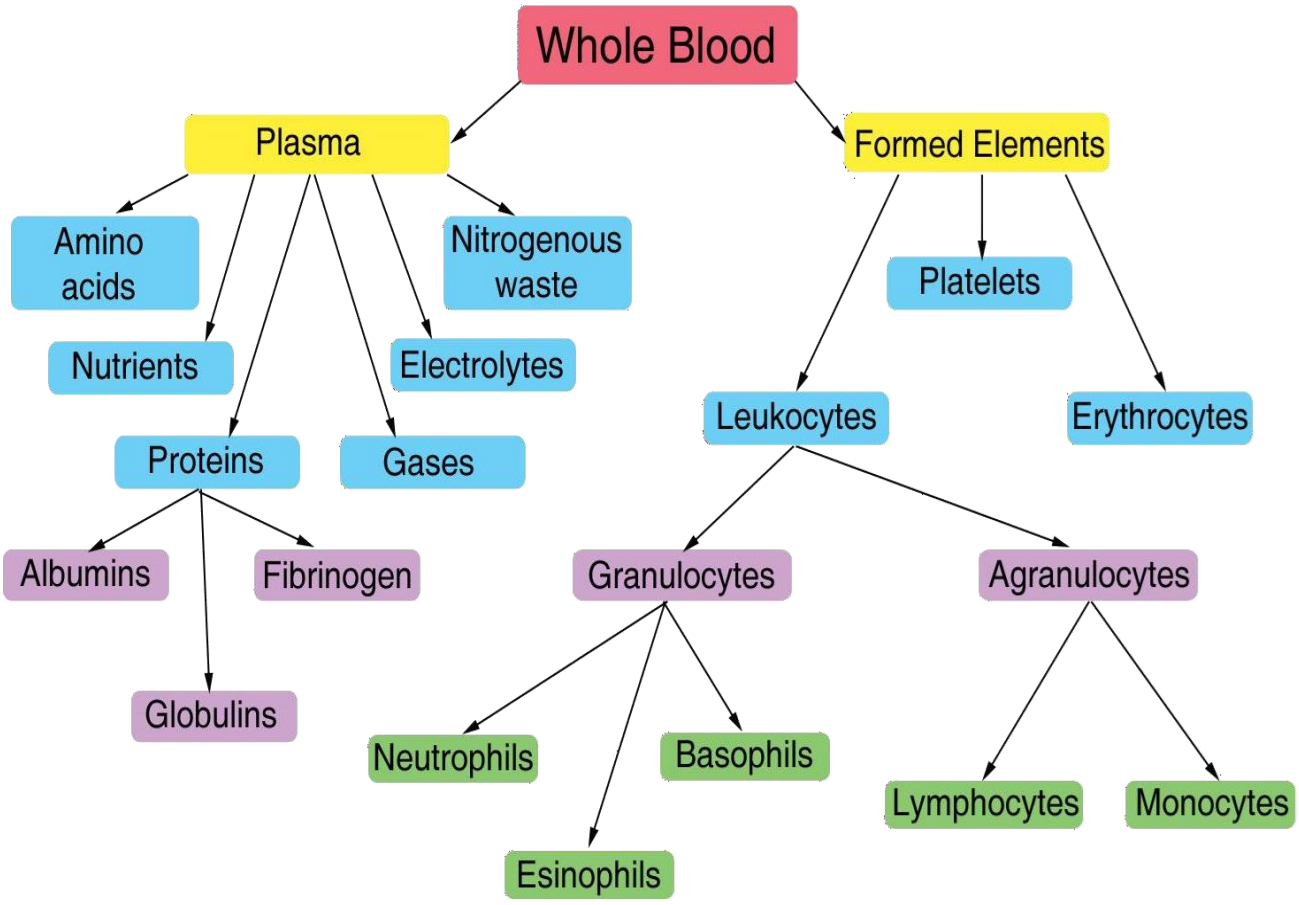
- **Hematocrit or packed cell volume (Adult male: 47 %, Adult females: 42 %)**



What was mentioned in the lecture:

and if we left 100 ml of the blood (deciliter of the blood) over an hour, the cellular components of the blood will precipitate leaving the plasma on the top (it is the supernatant), this procedure can be achieved if we leave the tube by itself or it can be achieved much faster if you will put this tube in a centrifuge to centrifuge the sample, now the cellular component of the blood in the adult male will constitute approximately 47% and in the females approximately 42±% .

Blood: what is inside plasma



What was mentioned in the lecture:

here are the whole components of the blood, what we are going to discuss is proteins which are present inside the plasma.

Plasma

What was mentioned in the slide:

- Liquid medium where cells are suspended
- Composition:
  - Water (92%)
  - Solids(8%)
    - 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 fattyacids
      - Carbohydrates: Glucose, fructose, pentose
      - Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones
    - Inorganic: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-}, SO_4^{2-}

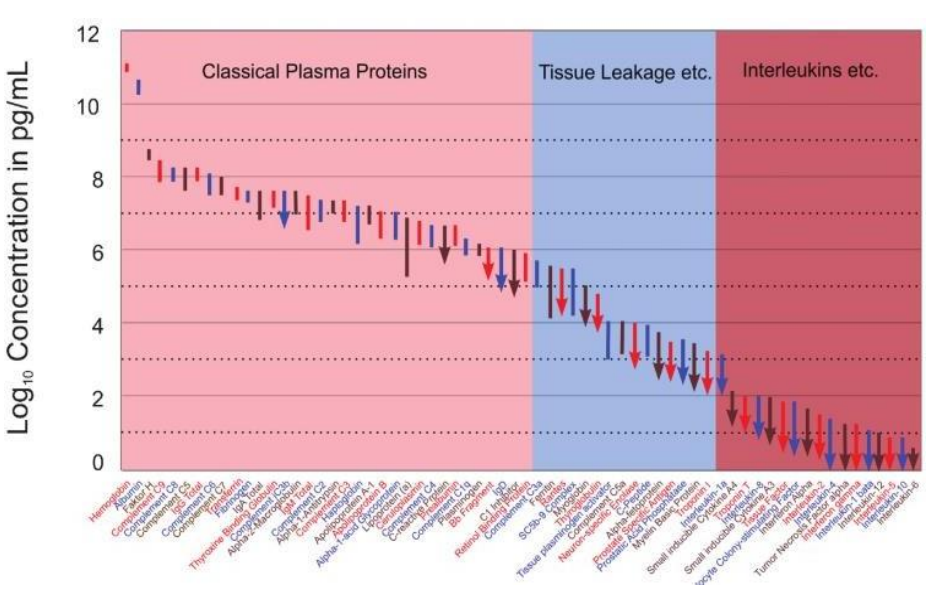
What was mentioned in the lecture:

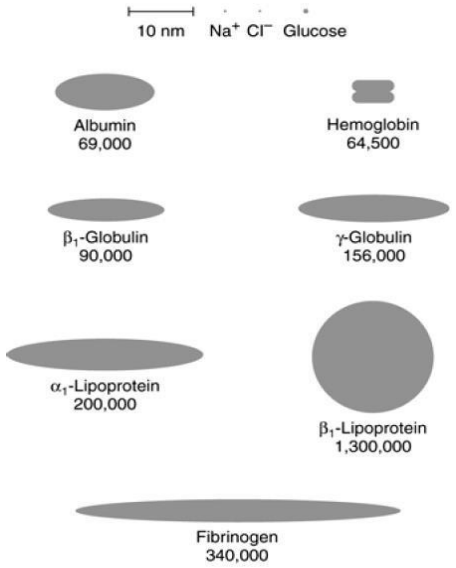
the plasma by constitution has 92% water, so it's a watery material most of it is water and the solids constitute about 8%, most of what we have in the solid are the plasma proteins including albumin, globulins, fibrinogen and the rest of these materials which you can read by yourself.

Plasma proteins are a mixture

what was mentioned in the slide:

- More than 500 plasma proteins have been identified
- Normal range 6-8 g/dl (the major of the solids)
- Simple & conjugated proteins (glycoproteins & lipoproteins)





What was mentioned in the lecture:

in the past, the proteins were low, they didn't discover too much of them, but with the advancement of the biochemical techniques more and more proteins have been discovered and up to date there is more than 500 plasma proteins that has been identified with their properties and their functions inside the plasma, the normal range for the plasma proteins inside the plasma is 6-8 grams per deciliter, which constitutes the major of the solids as we've discussed, the optimum concentration is between 7-7.5 grams per deciliter, but down to six up to eight is considered normal, most of these proteins are conjugated and a few are simple which are not conjugated to any other thing, most of them are conjugated to either carbohydrates (which is the most) and for lipids. This scheme (right figure) represents the dimensions and the shapes of the proteins just to illustrate how they are compared to hemoglobin, hemoglobin will not be discussed now because it's a corpuscular protein (it's inside cells) hemoglobin's molecular weight is approximately 65 kilodalton, albumin which is outside cells (it's inside the space inside the vascular system of the blood) has a molecular weight of 69 kilodalton, albumin's shape is much different, it's ellipsoidal in shape, we also have the γ globulins, the β globulins, the α globulins as you can see, and the fibrinogen which is the precursor of fibrin that makes the clot, and each shape has its own function, the shape participates to the function. As we've discussed last semester, always the structure is connected to the function and because the structure is connected to the function the structure gives the shape then the shape is connected to the function.

What was mentioned in the textbook:

BIOMEDICAL IMPORTANCE

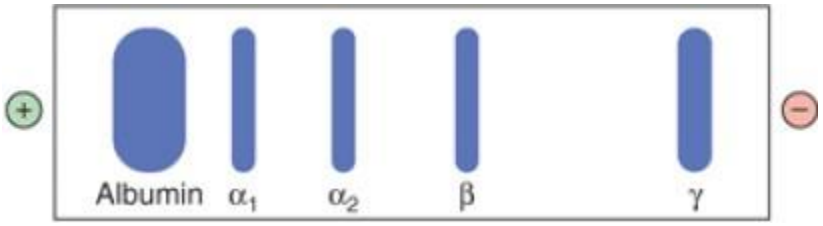
The proteins that circulate in blood plasma play important roles in human physiology. **Albumins** facilitate the transit of fatty acids, steroid hormones, and other ligands between tissues, while **transferrin** aids the uptake and distribution of iron. Circulating **fibrinogen** serves as a readily mobilized building block of the fibrin mesh (fibrin mesh is a mesh of long strands of tough insoluble protein that are adherent to platelets) that provides the foundation of the clots used to seal injured vessels. Formation of these clots is triggered by a cascade of latent proteases, or **zymogens**, called blood coagulation factors. Plasma also contains several proteins that function as inhibitors of proteolytic enzymes. **Antithrombin** helps confine the formation of clots to the vicinity of a wound, while α1-antiproteinase (antitrypsin) and α2-macroglobulin shield healthy tissues from the proteases that destroy invading pathogens and remove dead or defective cells. Circulating immunoglobulins called **antibodies** form the front line of the body's immune system. Perturbances in the production of plasma proteins can have serious health consequences. Deficiencies in key components of the blood clotting cascade can result in excessive bruising رضوض and bleeding (**hemophilia**). Persons lacking plasma ceruloplasmin, the body's primary carrier of copper, are subject to hepatolenticular degeneration (Wilson disease), while emphysema is associated with a genetic deficiency in the production of circulating α1-antiproteinase. More than one in every 30 residents of North America suffer from an **autoimmune disorder**, such as type 1

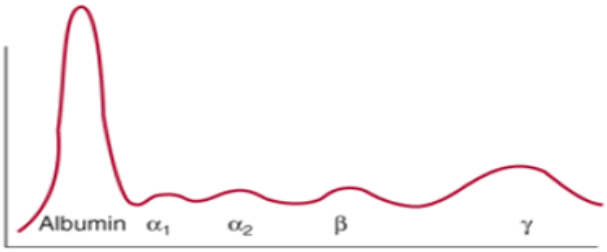
diabetes, asthma, and rheumatoid arthritis, resulting from the production of aberrant immunoglobulins. Insufficiencies in the production of protective antibodies, such as occur in many persons infected by the **human immunodeficiency virus** (HIV) or patients administered immunosuppressant drugs, render them immunocompromised, extremely susceptible to infection by microbial and viral pathogens. While the root causes of plasma protein–related diseases such as hemophilia are relatively straightforward, others—in particular many autoimmune disorders—arise due to the complex and cryptic interplay of genetic, dietary, nutritional, environmental, and medical factors.

The separation of plasma proteins

What was mentioned in the slide:

- **Salting-out (ammonium sulfate): fibrinogen, albumin, and globulins**
- **Electrophoresis (most common): serum (defebrinated 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

What was mentioned in the lecture:

how to separate these plasma proteins? how to get them outside of the blood? more than one technique has been applied, salting out (separation according to solubility) as we've discussed last semester can be used to separate plasma proteins from each other, and when applying salting out technique, fibrinogen will be one of the components that will get out as one unit, albumin will get out as one unit, and the globulins as a whole will get out as one unit, but when applying electrophoresis (separation by size) fibrinogen is out of the picture, so it's only albumin and globulins, why fibrinogen is out of the picture? Because electrophoresis is done on serum, it's not done on plasma and what do we mean by serum? what is the distinction between serum and plasma? it is a defibrinated plasma, which means the fibrin component is outside of the plasma then it will become serum so clotting will not occur inside the gel

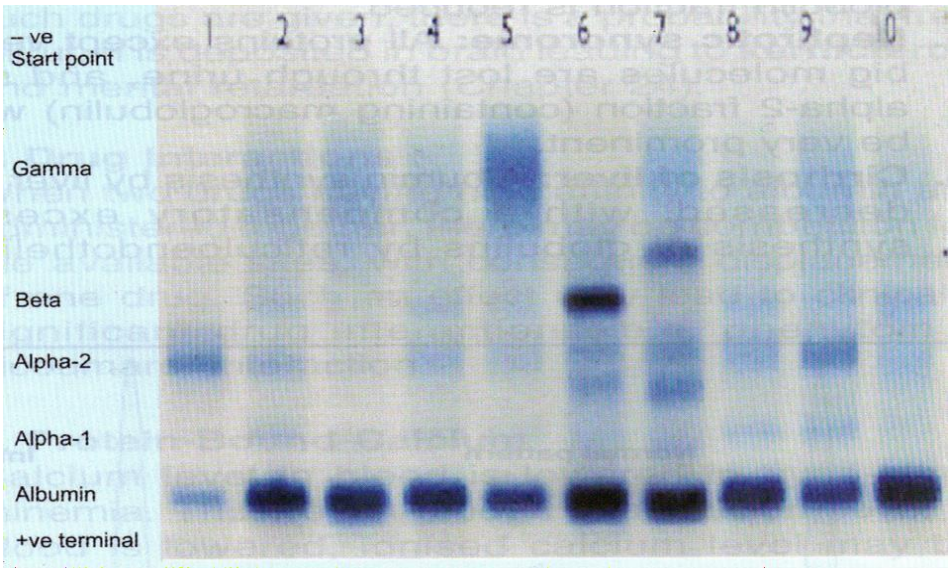
because the gel is very small, so when fibrinogen is out this is the picture that we will have inside the gel electrophoresis, albumin will be the closest to the positive electrode followed by different bands and the bands were named alphabetically randomly: α, β, γ, why it was named that way? albumin was named by itself because all of this band (see the figure) only contains albumin, whereas α1 contains more than one protein, α2 contains more than one protein, as the case of β and γ, so those are group of proteins, α bands separates easily in gel electrophoresis so it gives us
α1 and α2, if we give more time to the gel electrophoresis β also will separate into β 1 and β 2 bands but γ will stay the same, γ globulins are the immunoglobulins.
Notice: in medical laboratories, when we want to test plasma proteins, each protein has its own method to be tested (either salting out or gel electrophoresis), for gel electrophoresis, you need to test the serum , if we need to test serum, we put the plasma in a special tube that has a special substance that binds serum to remove fibrin.
Remember as we took in the last semester, the more bold the band on gel electrophoresis indicates higher concentration, so albumin is major component of plasma proteins, while the location of the band in the gel indicates molecular weight, (the less size the more it moves toward the positive electrode and that is also affected by the charge of the protein). while albumin as it is the closest to the positive electrode, it has in fact the lowest molecular weight compared to globulins and also is richly negatively charged (it has nearly 20 negative charges), it has nearly a molecular weight of 69 kilodaltons, then comes α1, α2, β and γ. We have a device called densitometer which reads the depth of color in gel on the forms of peaks (see the curve in the figure), when we see the peaks, we can tell that albumin has the highest concentration, so gel electrophoresis can tell the concentrations of these different proteins and the molecular weight to some extent (for example, you can tell that a protein in the A 1 band has a less molecular weight that a protein in α 2, however that is not true for all A2 proteins because the distance they move in the gel is affected by other factors that we're not going to discuss.)

The curve tell us that albumin constitutes 50-60% of the plasma proteins, which means that its concentration is 35-55 gram/liter

Electrophoresis of plasma proteins

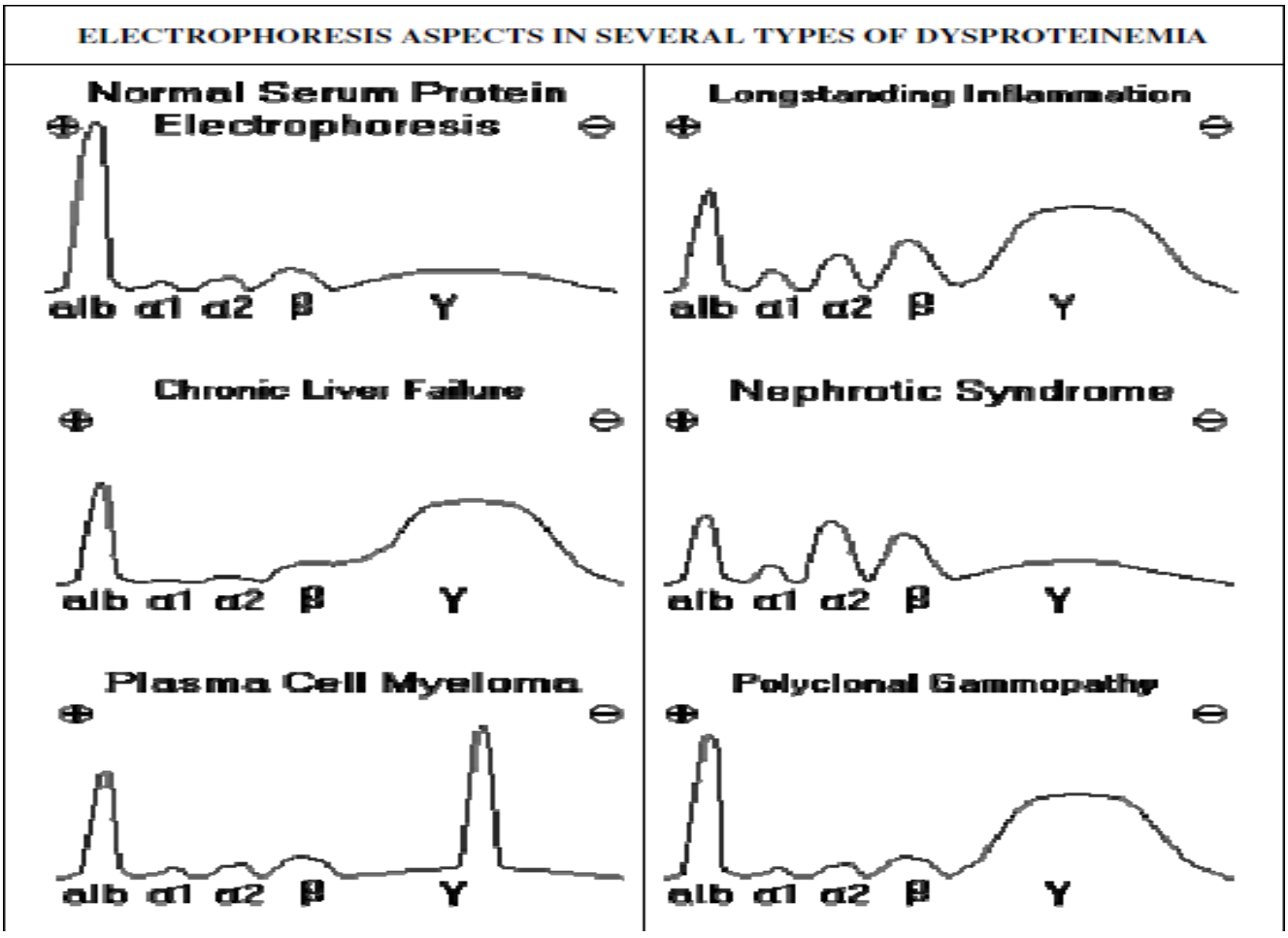
What was mentioned in the slide:

- **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.**



What was mentioned in the lecture:

Here we see the bands of gel electrophoresis, 90% of the α1 band is composed of a protein called α1-antitrypsin and the remaining 10% is composed of many proteins, α2 band is composed of many important proteins which include haptoglobin, α2-macroglobulin, & ceruloplasmin, β band contains transferrin, LDL, complement system proteins and we are not discussing these proteins here, γ band is composed of immunoglobulins (antibodies).



What was mentioned in the lecture:

This is a representation for albumin, α1,α2,β and γ ,we see that those are very very important to understand diseases, notice what happens to them (how their representation changes) in long standing inflammation, chronic liver failure, nephrotic syndrome (kidney failure), plasma cell myeloma which is canner affecting plasma cells which are antibody-producing , the representation of γ band in this case in a sharp peak, in this case the production of only one type of immunoglobulin is affected, while in polyclonal gammopathy, it affects more than one clone on antibodies resulting in a wide γ band, we'll discuss this in more details when we know the properties of plasma protein and why these changes happen.

Synthesis of plasma proteins

What was mentioned in the slide:

- **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**

What was mentioned in the lecture:

Most plasma proteins are synthesized in the liver like albumin and globulin, except for two: immunoglobulins (γ-globulins) are synthesized in plasma cells which are mature B-lymphocytes which exist in bone marrow (not blood) and Von Willebrand factor which is synthesized in the endothelial cells and precipitate in clotting, it cause platelets to adhere to each other.

Most plasma proteins are synthesized as preproteins, which means inactive proteins in the beginning then modified to be active, why? Because they're synthesized in liver cells but need to be secreted into the blood stream, therefore, the ribosomes synthesize then as preproteins with a signal peptide that drives then to endoplasmic reticulum, once inside the rough endoplasmic reticulum, the signal peptide is cleaved ,and they get modified by several methods may include glycosylation, proteolysis, and phosphorylation, etc. to become in the active form. then they are carried to Golgi apparatus by a vesicle for further modification, then they are carried by vesicles to the plasma membrane to be secreted, this process (synthesis and secretion) takes time that differs between plasma proteins, may range between 30 min – several hours . most plasma proteins are glycoproteins with carbs attached to them, but albumin is the major exception, it is not glycosylated why?? Because it has the highest concentration , and glycoproteins cause any substance to be more soluble, also more solubility of solutes makes water (solvent) more viscous, and carbohydrates in general are very soluble and have many negative charges, if albumin was glycosylated, that would make blood viscous and move hardly.

Plasma Proteins & Polymorphism

What was mentioned in the slide:

- 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

What was mentioned in the lecture:

let's talk about polymorphism, sometimes mutations happen in genes and is transmitted by mating between people though out generations, if a mutation is present in more than 1% of the population, then it is called polymorphism, polymorphism means presence of a protein in many forms in population, the other forms of this protein may carry the function well, or in some forms the function may be reduced causing a health problem, a common example on this is ABO blood groups, O is the most common, A and B is less common, and AB is much less common, polymorphisms might result in a disease or it might not result in a disease, this is important because when you study separate plasma proteins, there are diseases out of them because of certain polymorphisms, but there is no diseases in the polymorphism of blood types or eye color, polymorphism is population dependent (society dependent), it's not all the time fixed, for example, we can say that in the Arabian countries there are polymorphism in eye color where the black or brown color is the most common and green or blue color has the least percentage among the population, while the situation is opposite in the western countries, also it depends on the size of the population which can be Zarqa, Jordan, arab world, muslim world, or the whole world, and a trait can be a polymorphism in certain population nut not in another, examples on proteins that show polymorphism include α1-antitrypsin, haptoglobin, transferrin, ceruloplasmin, and immunoglobulins. How can we separate polymorphic proteins? (like if we have two forms of antitrypsin) because amino acids are changed by default, I can separate them by electrophoresis or isoelectric focusing depending on what is the type of amino acid that has been changed

Plasma Proteins Half-Lives

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What was mentioned in the slide:

- 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

What was mentioned in the lecture:

Plasma proteins also has half-lives, all of them, half-life means the amount of time required for the for a certain protein concentration to reach half of its original concentration, in albumin it is 20 days while in Haptoglobin it is five days, half-life is being used to detect for diseases, for example in Crohn's disease which is a disease affecting the intestines, the half-life of albumin can be reduced to one day, and it's not only in Crohn's disease, it's in a group of diseases called protein losing gastroenteropathy, (gastro from the stomach, entero from the intestines and pathy from disease), so any disease affecting the GIT (the gastrointestinal system) and causes protein loss, in any inflammatory process what happens is swelling? Why swelling happens? because the endothelial cells get away from each other allowing for fluid and proteins to come out from the plasma to the tissues, so swelling will occur, now because these diseases are affecting the GI tract, when the endothelial cells get away from each other the plasma and its proteins get inside the GI tract causing the loss of the protein from the blood , and because protein is being lost from the blood then the half-life will get decreased.

Functions of plasma proteins

What was mentioned in the slide:

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)

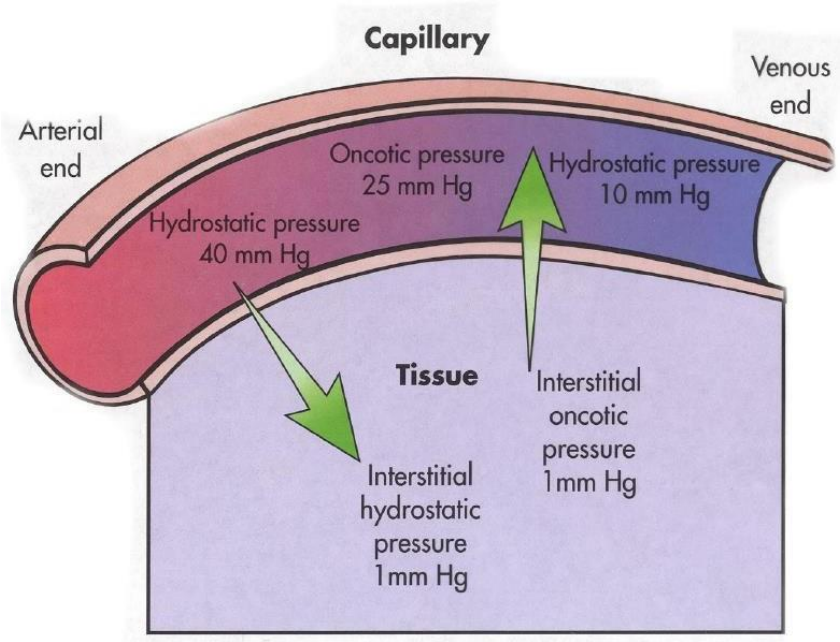
What was mentioned in the lecture:

Plasma proteins as a whole can do general functions where all plasma proteins as a unit perform these Functions, and there are specific functions where each protein has a specific function which is not applied to other proteins, some of them work as enzymes but not all of them, for example lipases work as enzymes, coagulation factors work as enzyme like factor VIII, factor III, factor II, renin works as an enzyme, some of them work in immunity as immunoglobulins. some of them work as hormones like erythropoietin , some of them participate in transport like transferrin which transports iron, thyroxine-binding globulin which transports thyroxine (a thyroid hormone), apolipoproteins which transports lipids. considering the general functions, all plasma proteins participate in these functions including nutrition that each plasma protein can be degraded to give you energy as any molecule inside the body, any protein can be broken to supply you with energy, maintenance of blood pH as all proteins inside the body, any protein which have at least one polypeptide chain has a free amino group and a free carboxylic group, both of these ends can donate a proton or accept a proton depending on the pH of the solution and accordingly they work as buffers, contribution to blood viscosity because proteins are soluble in water and we've said just before a while that any material which is getting dissolved in water increases the viscosity, so the more and more proteins you make the plasma more and more viscous, this is why albumin is not glycosylated, maintenance of blood osmotic pressure is discussed in the next slide.

Starling forces

What was mentioned in the slide:

- ✓ **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**



What was mentioned in the lecture:

we will cover in this slide the collective forces which are applied on the capillaries from the arterial side and from the venular side which are called starling forces, what do you mean by startling forces? They are the collective forces which are affecting the capillaries , what are the forces that we have? in the textbook you'll find in the chapter that I uploaded for certain numbers of the blood pressure and here you you'll find numbers here but all of them are close to each other, the important thing is the concept, now the what is the hydrostatic pressure (blood pressure)? It is the pressure applied by the water (because the blood is mostly water) or it is the force applied by the blood on the capillary walls or on the arteriolar walls (artery walls)or anywhere, so it is the pressure applied by the fluid on the walls of the vascular system, when it comes to the arteriolar side the pressure is going down (form 120/80 mmHg) to 40 mmHg and on the venular side it goes down and down to 10 mmHg, now plasma proteins collectively, because proteins are dissolved in water, they have to be dissolved in water, will keep attracting water around it to keep being soluble and this is a force that proteins apply on water molecules to keep attracting water molecules around it, so how much is the force applied by these proteins inside the plasma? it is close to 25 mmHg, the hydrostatic pressure on the arteriolar side is 40 mmHg, it is the force against the wall of the arteriole trying to get the fluid outside of the wall and the wall is preventing it. so we have 40 mmHg force getting the water out and we have 25 mmHg oncotic pressure which is the osmotic pressure applied by the proteins to keep water and the difference is 15 with outside direction, and this is how the exchange happens, fluid will get out of the vascular system at the capillary side by a force of 15 mmHg, getting the nutrients out, now on the venular side, the reverse happens, the hydrostatic pressure is 10 mmHg while the oncotic pressure will not change because it's applied by proteins and proteins are still inside the plasma, they are not getting outside they cannot get outside of the vascular system, so 10 mmHg trying to get the water out and 25 mmHg trying to get the water in, the difference is 15 to the inside, so the amount of fluid which gets out of the arterial side it gets back at the venular side with the wastes, and if it is not the same amount, if it is by a difference of one or two mmHg, the difference in an amount of fluid will get back through the lymphatic system, but if it is higher than that there will be accumulation of fluids inside tissues and this is what we call edema.

Acute-phase proteins

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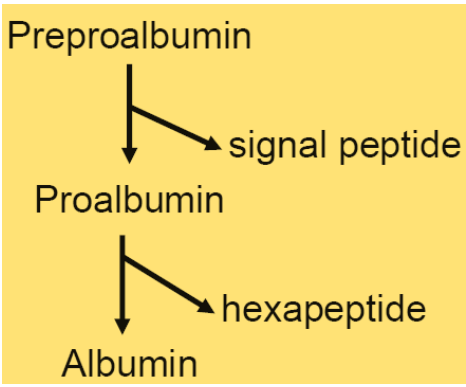
What was mentioned in the slide:

- **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**

What was mentioned in the lecture:

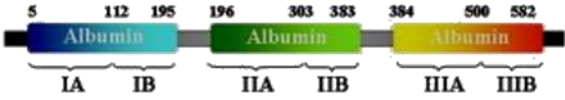
also one of the things that are characteristic for plasma proteins is that many of them are acute-phase proteins and as the name implies, those are proteins which increase in their concentration dramatically in cases of problems like acute inflammation, chronic inflammation, cancer, tissue damage, trauma, etc. some of these proteins increase in their concentration by 50 percent and some of them up to a thousand fold depending on the protein itself and depending on the problem itself if it's acute, chronic (chronic means has been there for a long time), etc. of these proteins is C-reactive protein (CRP), α1-antitrypsin, haptoglobin and fibrinogen, etc. now how these proteins getting increasing in their concentration? mechanistically what happens now in cases of acute inflammation, chronic inflammation, cancer, tissue damage, ….etc. whatever was the problem, the body will send messages to the different cells indicating that there is a problem, even if it is a chronic problem it will keep sending these messages, that there is a problem so try to correct, try to modify yourself, try to adapt, those molecules which are trying to do the connection in between cells and affect the same cells that has the problem, nearby cells, or even further cells away from the problem, we call them cytokines, of these cytokines are the interleukin-1 which is the main stimulator, interleukin-1 will come to the liver cells and cause activation for what we call nuclear factor kappa-B (NFKB), NFKB is a transcription factor (transcription factor is a protein that go and bind the DNA and makes the transcription faster), accordingly the proteins will increase in their concentration, so NFKB is a transcription factor which is inactive inside the cytosol because it is bound to its inhibitor, when interleukin one comes from outside and activates this cytosolic component, the inhibitor will dissociate and getting degraded, leaving the NFKB as an active one, NFKB will translocate from the cytosol to the nucleus, binds the DNA and starts its action as a transcription factor, it binds on promoters of certain genes, which genes?? the acute phase proteins as fibrinogen, haptoglobin α1 anti-trypsins, C-reactive protein and accordingly the transcription will be fast and accordingly the level of the protein will increase more and more and more. is that applied to all plasma proteins? No, there is a concept, what we call negative acute phase proteins, those proteins will not increase in their concentration in cases of acute inflammation, chronic inflammation, cancer, or tissue damage, etc. they might stay the same in their concentration or my they might get decreased in their concentration (because other proteins are being increasing in their concentration) of these are pre-albumin, albumin, and transferrin, pre-albumin is not the inactive form of albumin it's a separate protein.

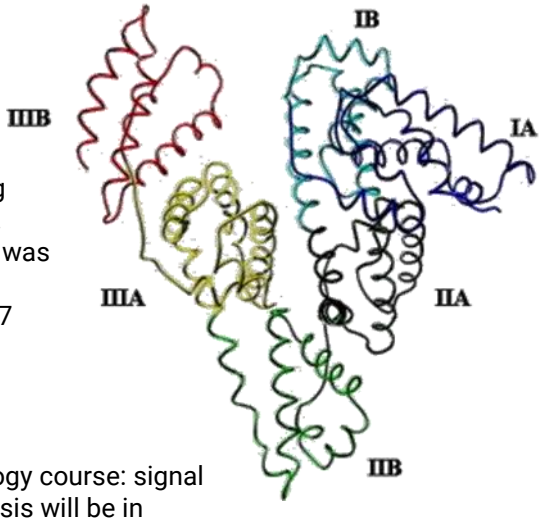
Albumin



What was mentioned in the slide:

- The Major Protein in Human Plasma, 69 kDa, half-life (20 days)
- The main contributor to the osmotic pressure (75-80%)
- Liver: 12 g/day (25% of total protein synthesis) (liver function test)
- Synthesized as a preproprotein
- One polypeptide chain, 585 amino acids, 17 disulfide bonds
- Proteases subdivide albumin into 3 domains
- Ellipsoidal shape (viscosity) vs. fibrinogen
- Anionic at pH 7.4 with 20 negative charges



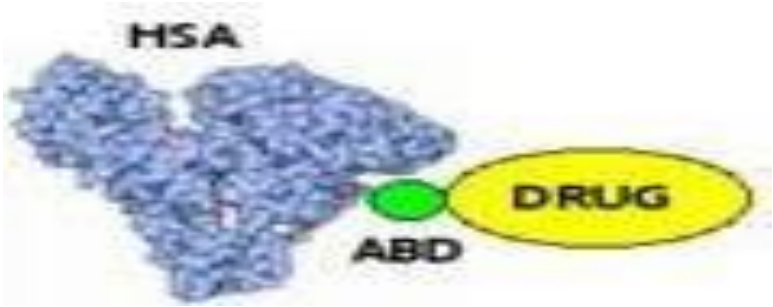


What was mentioned in the textbook:

The liver synthesizes approximately 12 g of albumin per day, representing about 25% of total hepatic protein synthesis and half its secreted protein. About 40% of the body's albumin circulates in the plasma (that's why if it was lost by for example kidney problems, we'll see edema), where it accounts for roughly three-fifths of total plasma protein by weight (3.4-4.7 g/dL). The remainder resides in the extracellular space. Because of its relatively low molecular mass (about 69 kDa) and high concentration, albumin is thought to contribute 75 to 80% of the **osmotic pressure** of human plasma. Like most other secreted proteins, albumin is initially synthesized as a **preproprotein**. Its **signal** peptide (remember from cytology course: signal peptide is a sequence of amino acids when exist in the protein, its synthesis will be in ribosomes bound to RER) is removed as it passes into the cisternae of the rough endoplasmic reticulum. A second **hexapeptide** (six amino acids) is cleaved from the new N-terminus farther along the secretory pathway.

Mature human albumin consists of a single polypeptide chain, 585 amino acids in length, that is organized into three functional domains. Its ellipsoidal conformation is stabilized by a total of 17 intrachain disulfide bonds.

Albumin binding capacity



What was mentioned in the slide:

- binds various ligands:
- ✓ Free fatty acids (FFA)
- ✓ Certain steroid hormones
- ✓ Bilirubin
- ✓ Plasma tryptophan
- ✓ Metals: Calcium, copper and heavy metals

Drugs: sulfonamides, penicillin G, dicumarol, aspirin (drug-drug interaction)

What was mentioned in the textbook:

A major role of albumin is to bind to and transport numerous **ligands**. These include free fatty acids (FFA), calcium, certain steroid hormones, bilirubin (a metabolite of heme), copper, and tryptophan. A variety of drugs, including sulfonamides, penicillin G, dicumarol, and aspirin, also bind to albumin; a finding with important pharmacologic implications. Preparations of human albumin have been widely used in the treatment of burns and of hemorrhagic shock (**Hemorrhagic shock** occurs when the body begins to shut down due to large amounts of blood loss, it is related with severe hypovolemia, and its main symptoms are tachycardia and hypotension)

Because a lot of drugs are transported by albumin, using 2 drugs of those that are transported by albumin at the same time can sometimes affect each other's fraction unbound, therefore, a drug can increase the excretion or the absorption of the other if both of them are transported by albumin.

Analbuminemia

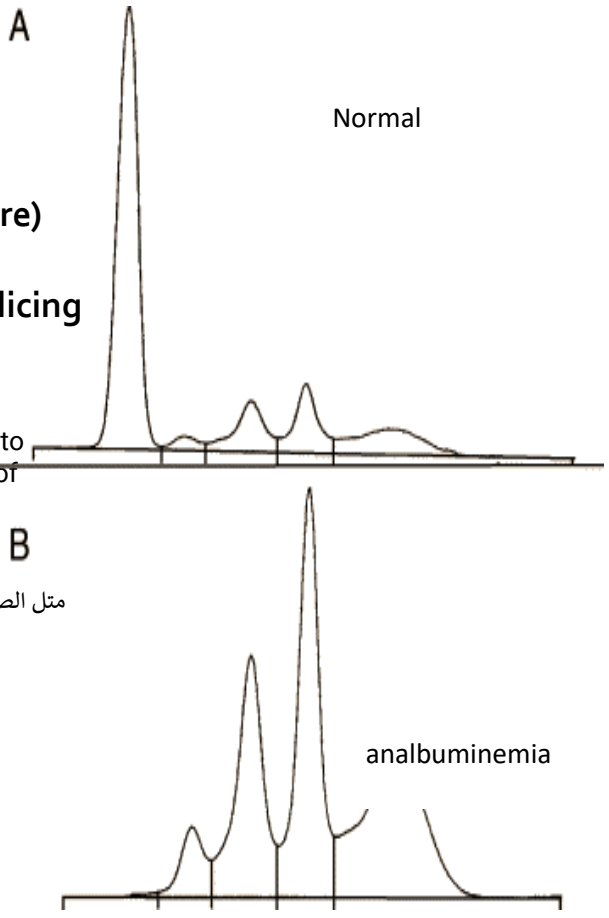
What was mentioned in the slide:

- There are human cases of analbuminemia (rare)
- Autosomal recessive inheritance
- One of the causes: a mutation that affects splicing
- Patients show moderate edema!!!

What was mentioned in the textbook:

Some humans suffer from genetic mutations that impair their ability to synthesize albumin. Individuals whose plasma is completely devoid of albumin are said to exhibit **analbuminemia**. Surprisingly, persons suffering from analbuminemia display only moderate edema.

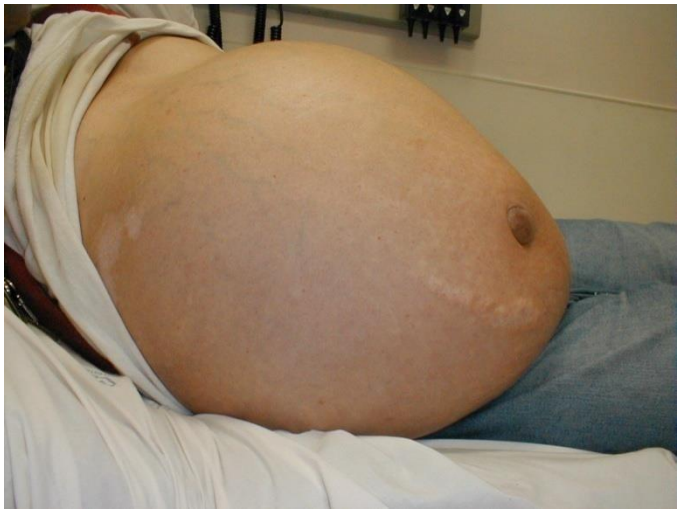
Autosomal recessive inheritance means that you must have two mutated genes to exhibit this disease متل الصفة المتنحية الي اخدناها بالتوجيهي

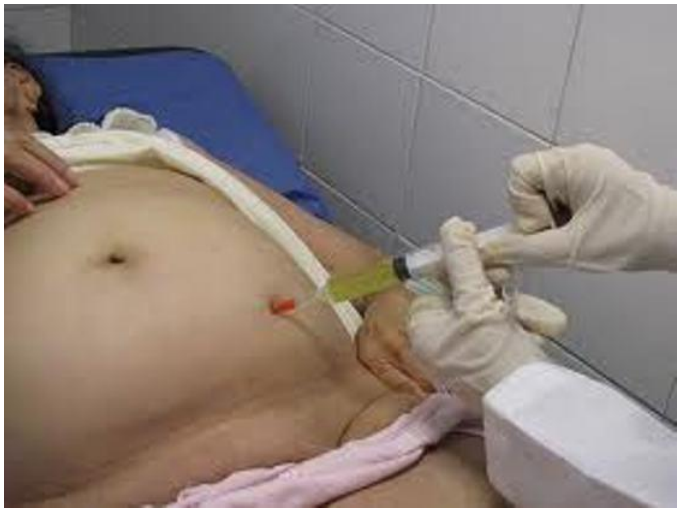


Other clinical disorders

What was mentioned in the slide:

- ➢ **Hypoalbuminemia: edema seen in conditions where albumin level in blood is less than 2 g/dl**
- ✓ **Malnutrition (generalized edema)**
- ✓ **Nephrotic syndrome**
- ✓ **Cirrhosis (mainly ascites)**
- ✓ **Gastrointestinal loss**
- ✓ **Hyperalbuminemia: dehydration (relative increase)**

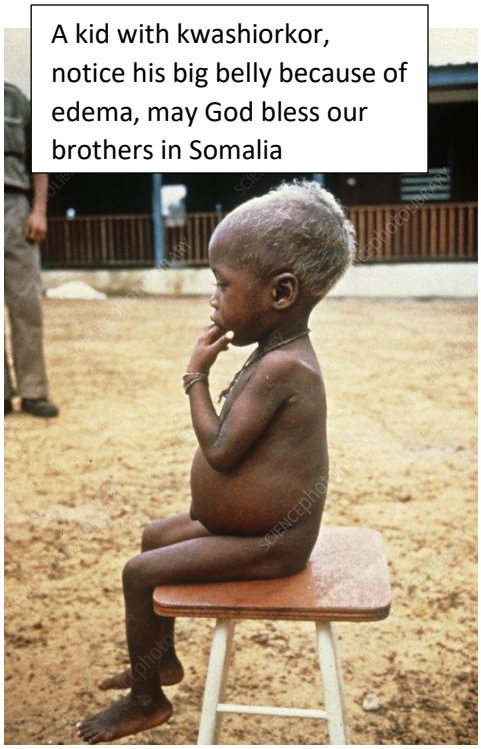


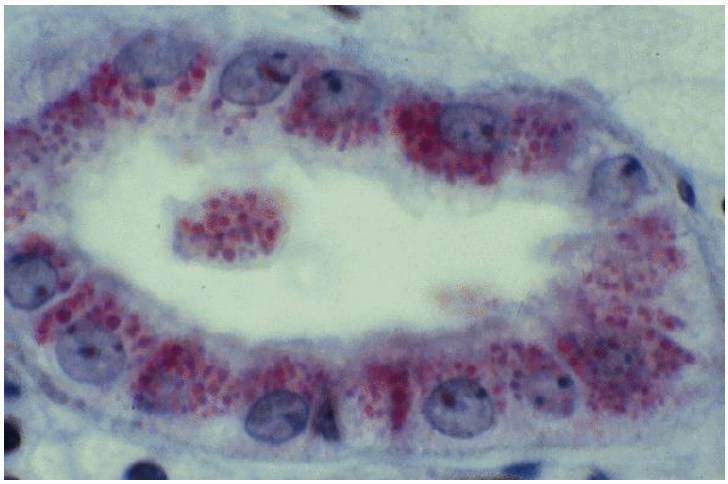


What was mentioned in the textbook:

Depressed
synthesis of albumin also occurs in a variety of diseases, particularly those of the liver. The plasma of patients with **liver disease** often shows a decrease in the ratio of albumin to globulins (decreased albumin-globulin ratio). The synthesis of albumin decreases relatively early in conditions of protein malnutrition, such as **kwashiorkor**.

A kid with kwashiorkor, notice his big belly because of edema, may God bless our brothers in Somalia





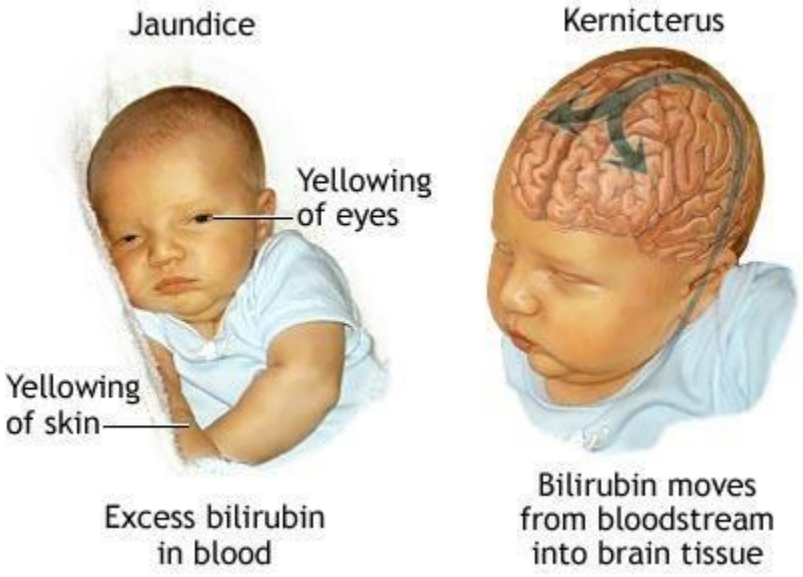
This is a section from a kidney with nephrotic syndrome, these pink granules are accumulations of albumin, this is how nephrotic syndrome can cause loss of albumin and hypoalbuminemia

cirrhosis is characterized by replacement of normal hepatic tissue with scar tissue which cannot synthesize albumin properly, the main symptom of cirrhosis is ascites which is accumulation of fluids in the peritoneal cavity because albumin is the main contributor to blood osmotic pressure, water will get out of blood vessels when albumin is deficient.
Protein losing enteropathy refers to any condition of the gastrointestinal tract (e.g. damage to the gut wall) that results in a net loss of protein from the body, faecal excretion of alpha 1-antitrypsin is a marker of protein losing enteropathy, it can cause loss of albumin.
Hyperalbuminemia is an increased concentration of albumin in the blood. Typically, this condition is due to dehydration.

Other clinical disorders

What was mentioned in the slide:

- **Drug-drug interaction:**
  - ✓ **Bilirubin toxicity (aspirin is a competitive ligand): kernicterus and mental retardation**
  - ✓ **Phenytoin-dicoumarol interaction**



What was mentioned in the textbook:

Kernicterus is a bilirubin-induced brain dysfunction.The term was coined in 1904 by Schmorl. Bilirubin is a naturally occurring substance in the body of humans and many other animals, but it is neurotoxic when its concentration in the blood is too high, a condition known as hyperbilirubinemia. Hyperbilirubinemia may cause bilirubin to accumulate in the grey matter of the central nervous system, potentially causing irreversible neurological damage. Depending on the level of exposure, the effects range from clinically unnoticeable to severe brain damage and even death. (Wikipedia)

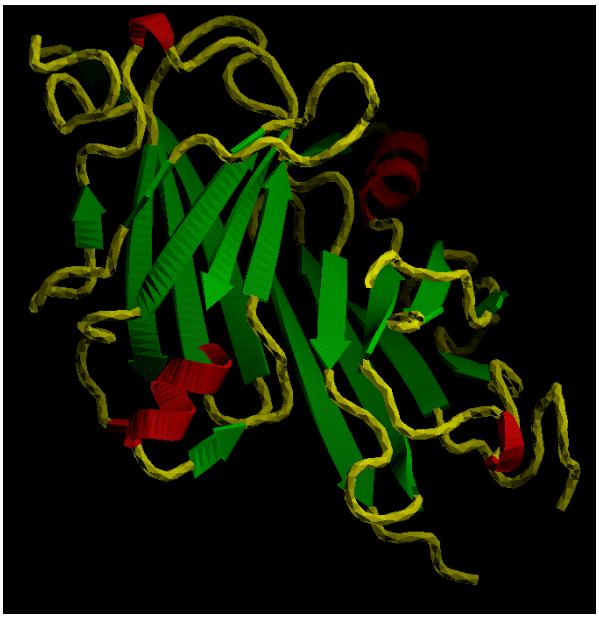
This can be caused by Administration of aspirin to neonates and infants. Aspirin displaces the bilirubin that was non-covalently attached to albumin in the blood stream, thus generating an increased level of free bilirubin which can cross the developing blood brain barrier. This can be life-threatening.

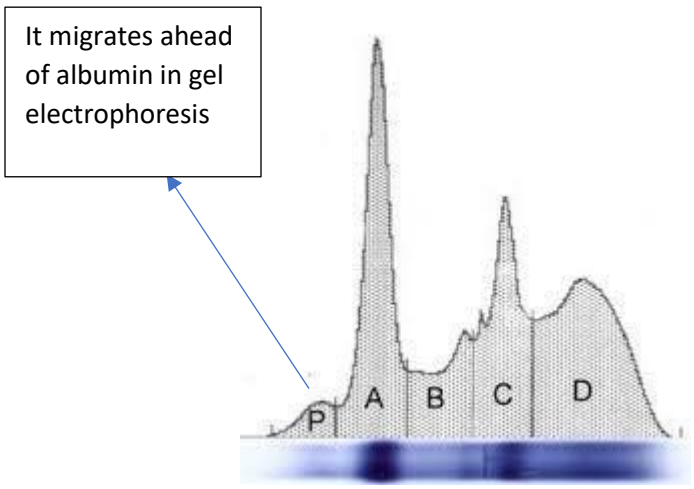
The metabolism of Dicoumarol (anticoagulant) can be decreased when combined with Phenytoin (antiepileptic)

Prealbumin (transthyretin)

What was mentioned in the slide:

- Migrates ahead of albumin, 62 kDa
- It is a small glycoprotein (rich in tryptophan, 0.5% carbohydrates)
- Blood level is low (0.25 g/L)
- It has short half-life (≈2 days): sensitive indicator of disease or poor protein nutrition
- Main function:
- ✓ T_4 (Thyroxine) and T_3 carrier
- ✓





What was mentioned in the textbook:

Familial amyloidosis results from accumulation of mutated forms of certain plasma proteins such as transthyretin. Over 80 mutationally altered forms of this protein have been identified.

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

Plasma proteins are divided into three groups: albumin (includes prealbumin), fibrinogen, and globulins which are those

α1- antitrypsin

What was mentioned in the slide:

- α1-Antiproteinase (52 kDa)
- Neutralizes trypsin & trypsin-like enzymes (elastase)
- 90% of α1- globulin band
- Many polymorphic forms (at least 75)
- Alleles Pi^{M}, Pi^{S}, Pi^{Z}, Pi^{F} (MM is the most common)
- Deficiency (genetic): emphysema (ZZ, SZ). MS, MZ usually not affected
- Increased level of α1- antitrypsin (acute phase response)

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

What was mentioned in the textbook:

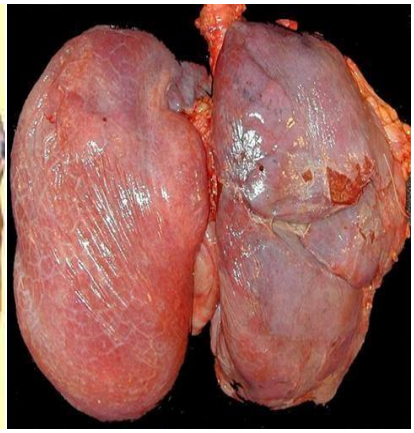
α1-Antiproteinase, a 394-residue glycoprotein that makes up >90% of the α1-albumin fraction, is the principal serine protease inhibitor (serpin) in human plasma. Formerly called α1-antitrypsin, α1-

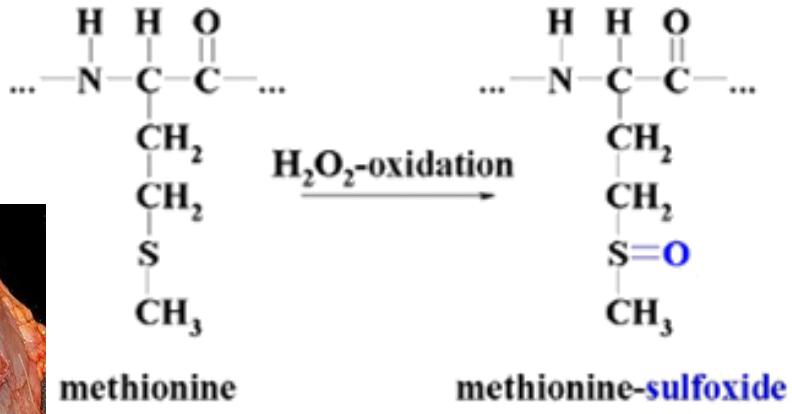
antiproteinase inhibits trypsin (a digestive enzyme), elastase (an enzyme that cleaves elastin which is the main component of elastic fibers in connective tissues and the lungs), and other serine proteases by forming an inactive covalent complex with them. α1-Antiproteinase is synthesized by hepatocytes and macrophages. At least 75 polymorphic forms of this serpin, or Pi, exist. The major genotype is MM, whose phenotypic product is PiM. A deficiency in α1-antiproteinase plays a role in some cases (~ 5%) of emphysema, particularly in subjects with the ZZ genotype (who synthesize PiZ) and in PiSZ heterozygotes, both of whom secrete lower levels of serpins than PiMM individuals. (note: there are four forms of the gene that encode this protein, they are Z, S, F,M, because we are diploid, we must have two forms of this gene, we can have any combination whether SZ, ZZ ,ZF, etc.)

Smoking & α1- antitrypsin deficiency

What was mentioned in the slide:

- Chronic inflammation
- Oxidation of Met^{358}
- devastating in patients with $_{Pi}ZZ$





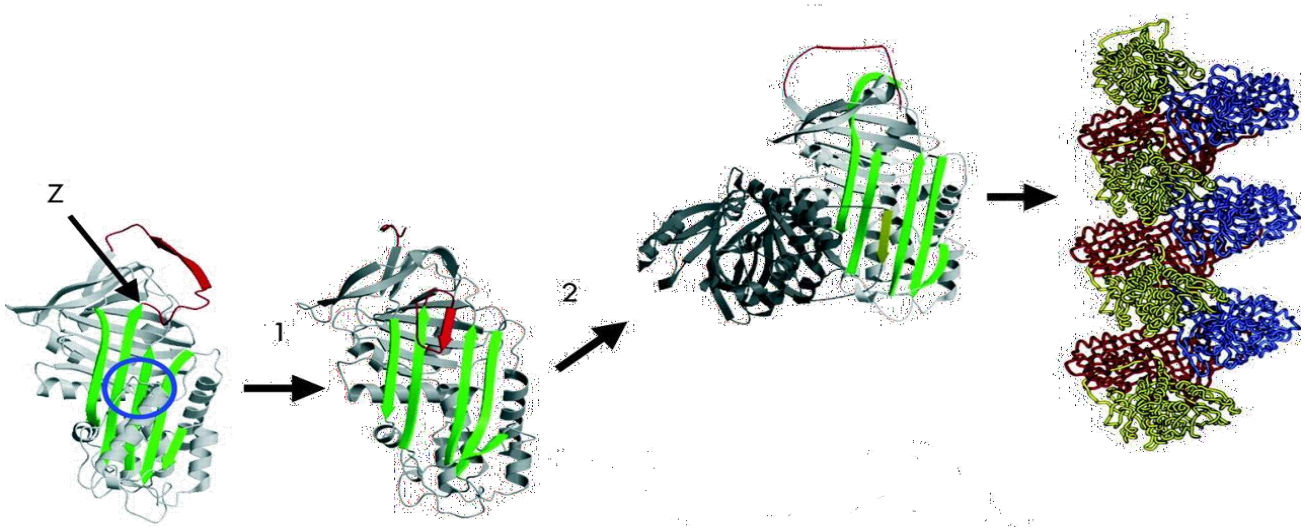
What was mentioned in the textbook:

In the lungs, components of the smoke produced by burning tobacco products and industrial activities can oxidize a key **methionine** residue, Met358, located in the protease-binding domain of α1-antiproteinase. Oxidation renders α1-antiproteinase unable to covalently bind and neutralize serine proteases. The subsequent damage produced by unchecked proteolytic activity in the lungs can contribute to the development of emphysema. Smoking can be particularly devastating for patients who already have low levels of α1-antiproteinase (eg, PiZZ phenotype). Intravenous administration of serpins (augmentation therapy) has been used as an adjunct in the treatment of patients with emphysema that exhibit α1-antiproteinase deficiency. Individuals deficient in α1-antiproteinase are also at greater risk of lung damage from pneumonia or other conditions that induce the accumulation of polymorphonuclear white blood cells in the lung. (neutrophils, because if you remember from immunology course, neutrophils contain elastase which is inhibited by antitrypsin, if antitrypsin is weak, elastase is not inhibited, therefore, inflammation will induce high damage)

Liver disease & α1- antitrypsin deficiency

What was mentioned in the slide

- Liver disease: ZZ phenotype polymerization (loop with β-sheet), aggregates in liver, cirrhosis (10%)



What was mentioned in the textbook:

Deficiency of α1-antiproteinase is also implicated in α1-antitrypsin deficiency liver disease, a form of cirrhosis that afflicts persons possessing the ZZ phenotype. In these individuals, substitution of Glu342 by lysine promotes the formation of polymeric aggregates of α1-antiproteinase in the cisternae of the endoplasmic reticulum in hepatic cells.

α1- fetoprotein

What was mentioned in the slide:

- Synthesized primarily by the fetal yolk sac and then by liver parenchymal cells
- Very low levels in adult
- Functions of α1-fetoprotein:
  - ✓ Protect the fetus from immunolytic attacks
  - ✓ Modulates the growth of the fetus
  - ✓ Transport compounds e.g. steroids
  - ✓ Low level: increased risk of Down's syndrome
- Level of α1-fetoprotein increases in:
  - ✓ Fetus and pregnant women <u>Normally</u>
  - ✓ Hepatoma & acute hepatitis

Haptoglobin (HP)

What was mentioned in the slide:

- It is an acute phase reactant protein
- α2 glycoprotein (90kDa)
- A tetramer (2α, 2β)
- 3 phenotypes:
- ✓ Hp 1-1→ α1, α1 +2β
- ✓ Hp 2-1→ α1, α2 + 2β
- ✓ Hp 2-2 → α2, α2 +2β
- Binds the free hemoglobin (65 kDa); prevents loss of hemoglobin & its iron into urine
- Hb-Hp complex has shorter half-life (90 min) than that of Hp (5 days)
- Decreased level in hemolytic anemia

What was mentioned in the textbook:

HAPTOGLOBIN PROTECTS THE KIDNEYS

Iron in Senescent Erythrocytes Is Recycled by Macrophages

Erythrocytes normally have a lifespan of approximately 120 days. Senescent or damaged erythrocytes are phagocytosed by macrophages of the reticuloendothelial system (RES) present in the spleen and liver. Around 200 billion erythrocytes are catabolized every day. Within the macrophage, heme derived from hemoglobin is converted by the enzyme heme oxygenase to biliverdin, releasing carbon monoxide and iron as by-products. Iron released from heme is exported from phagocytic vesicles in the macrophage by NRAMP 1 (natural resistance–associated macrophage protein 1), a transporter homologous to DMT1. Iron is subsequently secreted into the circulation by the transmembrane protein ferroportin. Thus, ferroportin plays a central role in both iron absorption by the intestine and iron secretion from macrophages.

In the blood, Fe^{2+} is oxidized to Fe^{3+} in a reaction catalyzed by the ferrioxidase ceruloplasmin, a copper-containing plasma enzyme synthesized by liver. Once oxidized, Fe^{3+} is then bound to transferrin in blood. The iron released from macrophages in this way (about 25 mg/d) is recycled, thereby reducing the need for intestinal iron absorption, which averages only 1 to 2 mg/d.

Haptoglobin Scavenges Hemoglobin That Has Escaped Recycling

During the course of red blood cell turnover, approximately 10% of an erythrocyte's hemoglobin escapes into the circulation. This free, extracorpuscular hemoglobin is sufficiently small at ≈65 kDa to pass through the glomerulus of the kidney into the tubules, where it tends to form damaging precipitates. Haptoglobin (Hp) is a plasma glycoprotein that binds extracorpuscular hemoglobin (Hb), forming a tight noncovalent complex (Hb-Hp). Human haptoglobin exists in three polymorphic forms, known as Hp 1-1, Hp 2-1, and Hp 2-2 that reflect the patterns of inheritance of two genes, designated Hp1 and Hp2. Homozygotes synthesize Hp 1-1 or Hp 2-2, respectively, while Hp 2-1 is synthesized by heterozygotes.

Normally, the level of haptoglobin in a deciliter of human plasma is sufficient to bind 40 to 180 mg of hemoglobin. Since the resulting Hb-Hp complex is too large (≥ 155 kDa) to pass through the glomerulus, the kidney is protected from the formation of harmful precipitates while the loss of the iron associated with extracorpuscular hemoglobin is reduced. Certain other plasma proteins bind heme, but not hemoglobin. They include a β1-globulin hemopexin, which binds free heme, and albumin,

which binds metheme (ferric heme) to form methemalbumin. Methemalbumin subsequently transfers this metheme to hemopexin.
Haptoglobin Can Serve as a Diagnostic Indicator In situations where hemoglobin is constantly being released from red blood cells, such as occurs in hemolytic anemias, the level of haptoglobin can fall dramatically. This decrease reflects the marked difference in the half-lives of free haptoglobin, approximately 5 days, and the Hb-Hp complex, approximately 90 minutes. The level of haptoglobin-related protein, a homologue of haptoglobin also present in plasma, is elevated in some patients with cancers, although the significance of this is not understood.

Ceruloplasmin

What was mentioned in the slide:

- A copper containing glycoprotein (160 kDa)
- It contains 6 atoms of copper
- Metallothioneins (regulate tissue level of Cu)

- Amine oxidase
- Copper-dependent superoxide dismutase
- Cytochrome oxidase
- Tyrosinase

- Regulates copper level: contains 90% of serum Cu
- A ferroxidase: oxidizes ferrous to ferric (transferrin)
- Albumin (10%) is more important in transport
- Decreased levels in liver disease (ex. Wilson's, autosomal recessive genetic disease)

What was mentioned in the textbook:

Oxidation by Ceruloplasmin Is a Key Feature of the Iron Cycle

Macrophages play a key role in the turnover of red blood cells. Following phagocytosis and digestion via lysosomal hydrolases, the iron is expelled largely in the ferrous, Fe^{2+}, state. In order to be recovered via the transferrin cycle, this iron must be oxidized to the ferric, Fe^{3+}, state by the ferroxidase ceruloplasmin, a 160-kDa α2-globulin synthesized by the liver. With six, catalytically essential, copper atoms, ceruloplasmin is the major copper-containing protein in plasma.

Deficiencies in Ceruloplasmin Perturb Iron Homeostasis

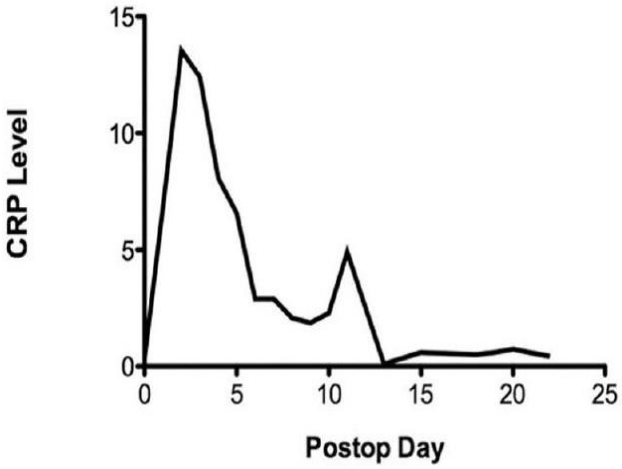
Ceruloplasmin deficiency can arise from genetic causes as well as a lack of copper, an essential micronutrient, in the diet. When adequate quantities of catalytically functional ceruloplasmin are lacking, the body's ability to recycle Fe^{2+} becomes impaired, leading to iron accumulation in tissues.
While persons suffering from hypoceruloplasmenia, a genetically heritable condition in which ceruloplasmin levels are roughly 50% of normal, generally display no clinical abnormalities, genetic mutations that abolish the ferroxidase activity of ceruloplasmin, aceruloplasminemia, can have severe physiologic consequences. If left untreated, the progressive accumulation of iron in pancreatic islet cells and basal ganglia eventually leads to the development of insulin-dependent diabetes and neurologic degeneration that may manifest as dementia, dysarthria, and dystonia.

In Wilson disease, a mutation in the gene for a copper-binding P-type ATPase (ATP7B protein) blocks the excretion of excess copper in the bile. As a consequence, copper accumulates in the liver, brain, kidney, and red blood cells. Paradoxically, rising levels of copper within the liver apparently interferes with the incorporation of this metal into newly synthesized ceruloplasmin polypeptides (apoceruloplasm) leading to a fall in plasma ceruloplasmin levels. If left untreated, patients suffering from this form of copper toxicosis may develop a hemolytic anemia or chronic liver disease (cirrhosis and hepatitis). Accumulation of copper in the basal ganglia and other centers can lead to neurologic symptoms. Wilson disease can be treated by limiting the dietary intake of copper while depleting any excess copper by the regular administration of penicillamine, which chelates copper and is subsequently excreted in the urine.

C- reactive protein (CRP)

What was mentioned in the slide:

- Able to bind to a polysaccharide (fraction C) in the cell wall of pneumococci (etymology)
- Help in the defense against bacteria and foreign substances
- Undetectable in healthy individuals, detectable in many inflammatory diseases (Acute rheumatic fever, bacterial infection, gout, etc.) & Tissue damage
- Its level reaches a peak after 48 hours of incident (monitoring marker)



What was mentioned in the textbook:

C-reactive protein stimulates the complement pathway