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Med Learn

Metabolism

Plasma proteins

ARKAN STUDY HOUSE

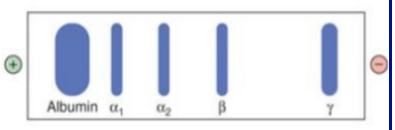
❖ Plasma proteins

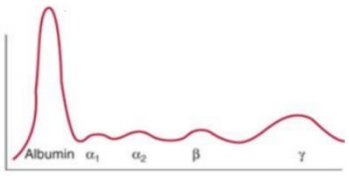
- **Plasma:** It is the liquid medium of the blood where the cells are suspended
- The composition of the plasma:
  1) Water (92%)
  2) Solids (8%)
- **Organic solids**
  - Plasma proteins: **albumin**, globulins, fibrinogen
  - Non-proteins nitrogenous compounds, such as urea, uric acid, creatinine, creatine, ammonia (NH_3) and free amino acids
  - Lipids such as cholesterol, triglycerides (TG), phospholipids and fatty acids
  - Carbohydrates such as glucose, fructose and pentoses but not glycogen
  - Other substances as: Ketone bodies, bile pigments, vitamins, enzymes & hormones
- **Inorganic (ions):** Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO^{3-} , HPO_4^{2-}, SO_4^{2-}

Plasma: Contains fibrinogen
- When plasma is taken it is placed in a tube containing anticoagulant, so the sample remains fluidic

Serum: Without fibrinogen (defibrinated)
- The sample is placed in a tube without anticoagulant, so clotting occurs (fibrinogen → fibrin)
- The sample forms 2 layers: a solid layer (clots) and a supernatant layer (serum)

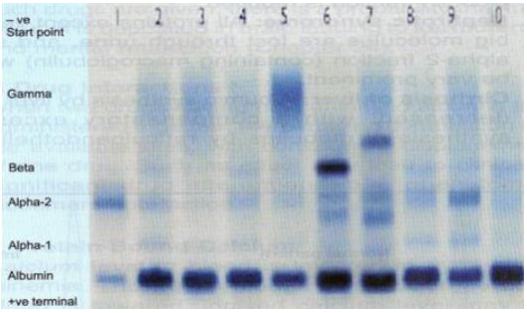
- The amount of plasma proteins is very important in the diagnosis of many disorders
  - It is measured by **biuret method** which uses an alkaline-copper sulfate that binds to the peptide bonds of the proteins producing a violet complex
  - The density of the violet color determines the amount of the protein
  - The normal range of plasma proteins 6-8 g/dcL
  - An increase in the amount of plasma proteins (hyperproteinemia), decrease (hypoproteinemia)
- Plasma proteins can be separated and purified by:
  - **Salting out:** using large concentrations of ammonium sulfate
    - ✓ It purifies fibrinogen, albumin and globulins from each other
  - **Electrophoresis:** it is the most common (the best) method and uses the **serum** instead of plasma
    - ✓ Forms 5 bands (Albumin, globulin α1, α2, β, and γ)
    - ✓ In electrophoresis, the proteins are separated according to their **charges** (negatively charged proteins migrate toward the positive pole, positively charged proteins remain in their position)
    - ✓ Proteins in the same band have the same charge
    - ✓ The density (thickness) of the band indicates the amount (concentration) of the protein
    - ✓ When results are shown in a curve, area under the curve represents the amount of proteins

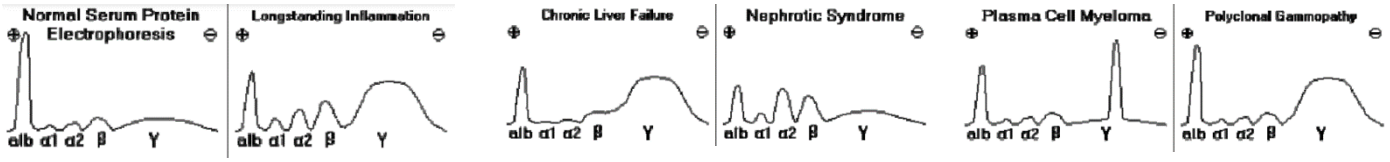




- Albumin is smaller than globulin and slightly negatively charged
- Globulins (3 bands):
  - **α band:**
    - ✓ α1 region consists mostly of α1-antitrypsin
    - ✓ α2 region is mostly haptoglobin, α2-macroglobulin and ceruloplasmin
  - **β band:** transferrin, LDL, complement system proteins
  - **γ band:** immuno-globulins (anti-bodies)

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





❖ Synthesis of plasma proteins

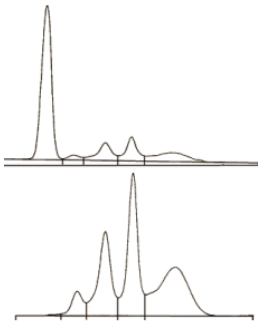
- **Albumin** and **globulins** are synthesized in the **liver**
- **γ-globulins** are synthesized in the **plasma cells**, lymph nodes, bone marrow and spleen
- Most plasma proteins are synthesized as **preproproteins**
  - Preproproteins are inactive proteins with a signal peptide on their N-terminus, which must be cleaved in many mechanisms to become actively functional
  - They undergo various posttranslational modifications (proteolysis, glycosylation, phosphorylation)
- Most plasma proteins are **Glycoproteins** (N- or O linked)
  - Albumin is the major exception (not a glycoprotein)
- Functions of the plasma proteins:
  - General functions
    - ✓ A nutritive role
    - ✓ Maintenance of blood pH (amphoteric property), so they can act as buffers due to the presence of carboxyl and amino groups in addition to **histidine** amino acid
    - ✓ Contributes to blood viscosity
    - ✓ Maintenance of blood osmotic pressure
  - Specific functions
    - ✓ Enzymes (such as **rennin**, **coagulation factors**, **lipases**)
    - ✓ Humoral immunity (**immunoglobulins**)
    - ✓ Blood coagulation factors
    - ✓ Hormonal (**Erythropoietin**)
    - ✓ Transport proteins (**Transferrin**, **Thyroxin binding globulin**, **Apolipoprotein**)

♦ Albumin

- The **Major** Protein in Human Plasma, 69 kDa, half-life (**20 days**)
  - Synthesized in the liver in a rate of 12 g/day
  - Synthesized as a preproprotein, which undergo 2 cleavage processes (cleave signal peptide then hexapeptide) to become active
  - 25% of total protein synthesis in the liver, so its concertation is utilized in the liver function test
  - It is a monomer (1 polypeptide) with 585 amino acids and 17 disulfide bonds
  - Anionic at pH 7.4 with 20 negative charges
- The main contributor to the osmotic pressure (75-80%)
  - It plays a predominant role in maintaining **blood volume and body fluid** distribution
- Binds various ligands:
  - Free fatty acids (FFA)
  - Certain steroid hormones
  - Bilirubin (a metabolite of heme)
  - Plasma tryptophan
  - Metals: Calcium, copper and heavy metals
  - Drugs: sulfonamides, penicillin G, dicumarol, aspirin (**drug-drug interaction**)

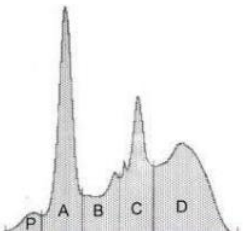
- **Clinical note (1): Congestive heart failure**
  - The arteriolar hydrostatic is higher than the venous hydrostatic pressure, causes the filtration of fluids and plasma from the capillary
  - The high concentration of albumin causes a high osmotic (colloid) pressure which leads to the reabsorption of fluids into the blood circulation
  - **Congestive heart failure** is a cycle of hypertension, weakening of cardiac muscle, kidney damage and edema
    - ✓ Hypertension causes a damage in the kidney, which leads to loss of albumin in urine
    - ✓ Less albumin and less osmotic force at capillaries result in edema which increases hypertension
    - ✓ Hypertension increases weakening cardiac muscle which reciprocally increases hypertension

- **Clinical note (2): Analbuminemia, Hypoalbiminemia, Hyperalbuminemia**



  - **Analbuminemia:** It is an autosomal recessive disease, Albumin genetically is **not synthesized**
    - ✓ It can be caused by a mutation that affects the splicing of mRNA
    - ✓ The patient shows a moderate edema
  - **Hypoalbiminemia:** edema seen in conditions where **albumin level in blood is less than 2 g/dl**, and can be caused by:
    - ✓ Malnutrition (**generalised** edema)
    - ✓ Nephrotic syndrome
    - ✓ Cirrhosis (mainly **ascites**)
    - ✓ Gastrointestinal loss of proteins
  - **Hyperalbuminemia:** relative increase in the albumin concentration, cause the fluids to get out of the cells toward the blood, causing **dehydration** of the cells

♦ Prealbumin (transthyretin)



- Very minor plasma protein with a low blood level (0.25 g/L), 62 kDa
  - Migrates ahead of albumin (highly negative)
  - It is a small glycoprotein (rich in tryptophan, 0.5% carbohydrates)
  - It has short half-life (≈ 2 days)
    - ✓ Sensitive **indicator** of disease or poor protein nutrition
- Its main function: **T4 (Thyroxine) and T3 carrier**

♦ α_1-Globulins

- They include:
  - **α1-antitrypsin (α1-antiprotease)**, 52 kDa
    - ✓ Neutralizes and controls the activity of trypsin & trypsin-like enzymes (elastase in the lungs)
    - ✓ 90% of α1- globulin band (the most abundant α1 protein)
    - ✓ Many polymorphic forms (at least 75)
    - ✓ Alleles Pi^M, Pi^S, Pi^Z, Pi^F (**MM is the most common**)

Emphysema: a lung disease affects mostly ZZ and SZ, but MS and MZ are less affected by the disease

  - **α1-fetoprotein**
    - ✓ Synthesized primarily by the **fetal yolk sac** and then by **liver** parenchymal cells in a very low levels in adult
    - ✓ Functions of α1-fetoprotein: Protect the fetus from immunolytic attacks, modulates the growth of the fetus and transport compounds such as steroids

- ✓ **Low level** of this protein increases the risk of **Down's syndrome**
- ✓ Its level increases in **Fetus** and **pregnant** women Normally **Hepatoma** & **acute hepatitis**

➢ **Orosomucoid (alpha-1-acid glycoprotein)**

- ✓ Concentration in plasma (0.6 - 1.4 G/dcL)
- ✓ Carbohydrate-rich (carbohydrate content 41%)
- ✓ Acts as a transporter of progesterone and transports carbohydrates to the site of tissue injury
- ✓ Marker of acute inflammation
- ✓ Concentration **increases** in inflammatory diseases, cirrhosis of liver and in malignant conditions
- ✓ Concentration **decreases** in liver diseases, malnutrition and in nephrotic syndrome

• **Clinical note (3): α1-antitrypsin deficiency**

➢ **Smoking** causes defects in the α1-antitrypsin by the oxidation of Met^{358} forming Met-sulfoxide

- ✓ It causes chronic lung inflammation due to the activity of neutrophil elastase
- ✓ Devastating in patients with Pi^{ZZ}

➢ Individuals with ZZ phenotype have a higher risk to develop liver diseases

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

♦ α_2-Globulins

• They include:

➢ **Haptoglobin (HP)**, 90kDa

- ✓ A tetramer (2α, 2β)
- ✓ 3 phenotypes:
  1) Hp 1-1 → α1, α1 + 2β
  2) Hp 2-1 → α1, α2 + 2β
  3) Hp 2-2 → α2, α2 + 2β
- ✓ **Binds the free hemoglobin** (65 kDa) and prevents loss of hemoglobin & its iron into urine
- ✓ Hb-Hp complex has shorter half-life (90 min) than that of Hp (5 days)
- ✓ Hp has a **decreased** level in hemolytic anemia

➢ **Ceruloplasmin,** 160 kDa

- ✓ A copper containing **glycoprotein** with 6 atoms of **copper**
- ✓ **Metallothioneins:** Proteins that regulate tissue level of metals
- ✓ Ceruloplasmin regulates copper level and **contains (stores)** 90% of serum Cu
- ✓ Copper is **transported** in the blood mainly by **albumin**
- ✓ Ceruloplasmin decrease in a liver disease called Wilson's

Cu-containing enzymes:

- ➢ Amine oxidase
- ➢ Cu-dependent superoxide dismutase
- ➢ Cytochrome oxidase
- ➢ Tyrosinase

Ferroxidase: oxidizes ferrous to ferric

➢ **α_2 Macroglobulin**

- ✓ Comprises 8–10% of the total plasma protein in humans
- ✓ Synthesized by hepatocytes and macrophages
- ✓ Inactivates all the proteases and thus is an important in vivo anticoagulant
- ✓ Carrier of many growth factors
- ✓ Normal serum level 130-300 mg/dl
- ✓ Concentration is markedly **increased** in nephrotic syndrome, since other proteins are lost through urine in this condition

♦ β-Globulins

- They include:
  - **Haemopexin**
    - ✓ Normal level in adults (0.5 - 1.0 g/L)
    - ✓ Low level at birth, reaches adult value within first year of life
    - ✓ Synthesized in liver
    - ✓ Function is to bind heme formed from breakdown of Hb and other hemoproteins
    - ✓ **Low level:** found in hemolytic disorders, at birth and drug induced
    - ✓ **High level:** pregnancy, diabetes mellitus, malignancies and Duchenne muscular dystrophy
  - **C-reactive protein (CRP)**
    - ✓ Able to bind to a polysaccharide (fraction C) in the cell wall of pneumococci
    - ✓ Help in the defense against bacteria and foreign substances
    - ✓ Undetectable in healthy individuals, detectable in many inflammatory diseases (Acute rheumatic fever, bacterial infection, gout) & Tissue damage
    - ✓ Its level reaches a peak after 48 hours of incident (monitoring marker)
  - **Complement C1q**
    - ✓ First complement factor to bind antibody, this binding triggers the classical complement pathway
    - ✓ Thermolabile (destroyed by heating)
    - ✓ Normal level – 0.15 gm/L
    - ✓ Decreased level is used as an indicator of circulating Ag– Ab complex
    - ✓ **High levels** are found in **chronic infections**

♦ γ-Globulins

- Immunoglobulins play a key role in the defense mechanisms of the body
- There are five types of immunoglobulins:
  - **IgG:** Main antibody in the secondary response
    - ✓ Opsonizes bacteria, Fixes complement, neutralizes bacterial toxins and viruses, crosses placenta
  - **IgA:** prevents attachment of bacteria and viruses to mucous membranes, but doesn't fix complement
  - **IgM:** Produced in the primary response to an antigen, fixes complement
    - ✓ Represents antigen receptor on the surface of B cells
  - **IgD:** Found on the surface of many B cells as well as in serum, but its function is uncertain
  - **IgE:** Mediates immediate hypersensitivity and defends against worm infections

♦ Fibrinogen (clotting factor 1)

- Constitutes 4-6% of total protein
- Highly elongated with axial ratio of 20:1
- Imparts maximum viscosity to blood
- Synthesized in liver
- Made up of 6 polypeptide chains
- Chains are linked together by S-S linkages (disulfide)
- Amino terminal end is **highly negative** due to the presence of **glutamic acid**
  Negative charge contributes to its solubility in plasma and prevents aggregation due to electrostatic repulsions between the fibrinogen molecules

- Transport proteins:
  - Albumin: Fatty acids (NEFAs), bilirubin, hormones, calcium, heavy metals, drugs
  - Prealbumin (Transthyretin): Steroid hormones thyroxin, Retinol
  - Retinol binding protein: Retinol (Vitamin A)
  - Thyroxin binding protein (TBG): Thyroxin
  - Transcortin (Cortisol binding protein): Cortisol and corticosteroids
  - Haptoglobin: Hemoglobin
  - Hemopexin: Free heme
  - Transferrin: Iron
  - HDL (High density lipoprotein): Cholesterol (Tissues to liver)
  - LDL (Low density lipoprotein): Cholesterol (Liver to tissues)

Bilirubin: is a metabolite of **heme** (protoporphyrin)
- Transported by albumin
- **Yellow** color
- Used in the diagnosis of liver diseases

- **Acute Phase Proteins**
  - In cases of inflammation, tissue damage and neoplasm their concentration **increases** (0.5-1000 folds)
  - Examples: **C-reactive protein (CRP)**, Ceruloplasmin, α1-antitrypsin, haptoglobin, fibrinogen, α2-macroglobulins and α1-acid glycoprotein
- **Negative acute phase proteins**
  - The levels of certain proteins **decrease** in blood in response to certain inflammatory processes
  - Examples: prealbumin, albumin, transferrin, Transthyretin, Retinol binding protein

❖ Clinical significance of plasma proteins

- **Hyperproteinemia** (Levels higher than 8.0gm/dl)
  - **Hemoconcentration:** caused by dehydration, where the concentration of albumin and globulin increases but the ratio of albumin to globulin remains the same
    - ✓ It is caused by: Excessive vomiting, Diarrhea, Diabetes Insipidus, Diuresis, Intestinal obstruction
- **Hypoproteinemia** (Decrease in total protein concentration)
  - **Hemodilution:** Both Albumin and globulins are decreased, A:G ratio remains same, as in water intoxication
  - **Hypoalbuminemia:** low level of Albumin in plasma
    - ✓ It is caused by: Nephrotic syndrome, Protein losing enteropathy, Severe liver diseases, Mal nutrition or malabsorption, Extensive skin burns, Pregnancy, Malignancy
- **Hypogammaglobulinemia,** caused by:
  - Losses from body through urine, GIT or skin, decreased synthesis, primary genetic deficiency
  - **Secondary** causes, drug induced (Corticosteroid therapy), uremia, hematological disorders
  - AIDS (Acquired Immune deficiency syndrome)
- **Hypergammaglobulinemia**
  - Polyclonal, caused by:
    - ✓ Chronic infections, Chronic liver diseases, Sarcoidosis and Auto immune diseases
  - Monoclonal, caused by:
    - ✓ Multiple myeloma, Macroglobulinaemia, Lymphosarcoma, Leukemia and Hodgkin's disease

Past Paper

1. **Select the one of the following statements that is NOT CORRECT:**
   A. Albumin is synthesized as a preproprotein.
   B. Albumin is stabilized by multiple intrachain disulfide bonds.
   C. Albumin is a glycoprotein.
   D. Albumin facilitates the movement of fatty acids through the circulation.
   E. Albumin is the major determinant of plasma osmotic pressure.

2. **Select the one of the following statements that is NOT CORRECT:**
   A. Wilson disease caused by increased the concentration of the Ceruplasmin in blood
   B. Wilson disease is characterized by copper toxicosis (abnormally high levels of copper).
   C. Wilson's disease is an autosomal recessive genetic disease.
   D. Wilson caused bronzy skin and eyes tissue

3. **The functions of plasma albumin are:**
   A. Osmosis
   B. Transport
   C. Immunity
   D. both (A) and (B)

4. **In one molecule of albumin the number of amino acids is:**
   A. 510
   B. 585
   C. 610
   D. 650

5. **Ceruloplasmin is:**
   A. α1-globulin
   B. β-globulin
   C. α2-globulin
   D. None of these

6. **In the total proteins, the percentage of albumin is about:**
   A. 20–40
   B. 50–60
   C. 30–45
   D. 80–90

7. **Molecular weight of human albumin is about:**
   A. 156,000
   B. 69,000
   C. 90,000
   D. 54,000

8. Albumin is involved in the transport of all of the following except:

A. Free fatty acids
B. Aspirin
C. Steroids
D. Some cations
E. Hemoglobin

9. A deficiency in which of the following proteins causes Wilson disease:

A. Ceruloplasmin
B. Albumin
C. C reactive protein
D. Haptoglobin
E. Alpha 1 antitrypsin

10. Choose the correctly matched pair of words:

A. Liver disease – Increased albumin concentration
B. Bacterial infection – Decreased C reactive protein concentration
C. Increased alpha 1 antitrypsin concentration –trypsin inactivation
D. Smoking – oxidation of methionine in elastase
E. C+D

11. Choose the mismatched pair among the following:

A. Hemolytic anemia – Elevated Haptoglobin levels
B. Acute inflammation – Elevated C-reactive protein levels
C. Pi^{ZZ} genotype – Decreased activity of Alpha 1 antitrypsin
D. Down syndrome – Low alpha 1 fetoprotein levels
E. None of the above

12. True about Prealbumin:

A. Migrates at a lower speed than albumin in gel electrophoresis
B. Converted to albumin after cleavage of hexapeptide
C. Is a sensitive marker of protein malnutrition due to its long half-life
D. A+B
E. None of the above

13. A 50 g sample of plasma was obtained. How many grams of plasma proteins (approximately) would there be in this sample:

A. 10 grams
B. 3.5 grams
C. 45 grams
D. 35 grams
E. 1 gram

14. Which of the following statements regarding blood composition is FALSE:

A. Fibrinogen is present in plasma while absent in serum
B. The blood cells that makes up the most of hematocrit are: Red Blood Cells
C. The most abundant plasma protein is synthesized in the liver
D. None of the above is false

15. Which of the following represents a TRUE statement:

A. Albumin is glycosylated
B. The main copper-binding plasma protein is albumin
C. Transferrin oxidizes Fe2+ to Fe3+
D. Elastase activity is elevated in smokers
E. Alpha 1 antitrypsin is the main contributor to blood oncotic pressure

16. Which plasma protein binds iron?

A. Fibrinogen
B. Albumin
C. Transferrin
D. Gamma-globulins
E. Haptoglobin

17. What is the most abundant plasma protein in normal individuals?

A. alpha1-antitrypsin
B. haptoglobin
C. albumin
D. gamma globulin
E. fibronogen

18. Which major class of plasma proteins is not synthesized in the liver?

A. alpha1-antitrypsin
B. haptoglobin
C. albumin
D. gamma globulin
E. fibronogen

Q1: C	**Q2: A**	**Q3: D**	**Q4: B**	**Q5: C**
Q6: B	**Q7: B**	**Q8: E**	**Q9: A**	**Q10: E**
Q11: A	**Q12: C**	**Q13: B**	**Q14: D**	**Q15: D**
Q16: C	**Q17: C**	**Q18: D**		