COMPONENTS OF BLOOD TISSUE & FUNCTIONS OF PLASMA PROTEINS

Hematopoietic System and Disorders (MED202)

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Lecture outline

- ✓ Reminding of body fluids and body fluid compartments
- ✓ Component of the blood tissue
- ✓ Components of plasma
- ✓ Major types of plasma proteins
- ✓ Electrophoretic distribution of plasma proteins
  - Albumin
  - Globulins
  - Fibrinogen
- ✓ General properties of plasma proteins and their functions

Water (H_2O)

- The chemical reactions of life take place in aqueous solutions.
- Human beings are mostly water, ranging from about 75% of body mass in infants to about 50–60% in adult men and women.
- «**Polar**» molecule
  - H atoms have partial positive; O atom has partial negative charge
- Beucause of its polar nature water molecules can
  - form hydrogen bonds with each other and with other polar molecules
  - interact with positively or negativeley charged ions.

Water (H_2O)

- In a 70-kg adult man, the total body water is about

  60% **~ 42 liters**

- This percentage depends on age, gender, and degree of obesity.

Decreased body water (~40%)	Increased body water (~80%)
Elderly people	Premature and new born babies
Women	People with edema
Obese people	

The total body fluid is distributed mainly between two compartments

1- Intracellular fluid (inside the cell)
40 % body weight
=28 liters

2- Extracellular fluid (outside the cell)
20 % body weight
= 14 liters

a) **Interstitial fluid** (Intercellular)
15 % body weight = 10.5 liters

b) **Intravascular fluid** (Blood Plasma)
5 % body weight = 3.5 liters

c) **Transcellular fluid** (synovial, peritoneal, pericardial, CSF, intraocular spaces)

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BLOOD VOLUME

- Blood contains both
  - extracellular fluid (the fluid in **plasma**)
  - intracellular fluid (the fluid in the blood cells)

- Blood is considered to be a separate fluid compartment because it is contained in a chamber of its own: the circulatory system

- Especially important in the control of cardiovascular dynamics.

Total body weight x **0.07** = (**~5 Liters**)

Blood functions

- ✓ Transportation of nutrients, gases, wastes, hormones
- ✓ Regulation of pH
- ✓ Restriction of fluid loss during injury
- ✓ Defense against pathogens and toxins
- ✓ Regulation of body temperature

Components of the Blood

- 60% plasma

- %40 red blood cells (i.e., erythrocytes)

These percentages can vary considerably in different people, depending on gender, weight, and other factors.

Hematocrit: Packed Red Blood Cell Volume

- ✓ Fraction of the blood composed of red blood cells, as determined by centrifuging blood in a **hematocrit tube** until the cells become tightly packed in the bottom of the tube.
- ✓ ~3 to 4 % of the plasma remains entrapped among the cells
- ✓ **True hematocrit** is only about 96% of the measured hematocrit.

Hematocrit: Packed Red Blood Cell Volume

Men: 0.40

Women: 0.36

Severe anemia: 0.10

Polycytemia: 0.65

Constituents of Extracellular and Intracellular Fluids

Extracellular fluid (plasma + interstitial fluid) contains

Large amounts of

- Na^+
- Cl^-
- HCO_3^-

Small amounts of K^+

- Ca^{2+}
- Mg^{2+}
- PO_4^{3-}
- Organic anions

Ionic composition of plasma and interstitial fluid is similar

- ✓ Because the plasma and interstitial fluid are separated only by highly permeable capillary membranes, their ionic composition is similar.
- ✓ The most important difference between these two compartments is the **higher concentration of protein in the plasma**
- ✓ Because the capillaries have a low permeability to the plasma proteins, only small amounts of proteins are leaked into the interstitial spaces in most tissues

Ionic composition of plasma and interstitial fluid is similar

	Plasma (mOsm/L H_2O)	Interstitial (mOsm/L H_2O)	Intracellular (mOsm/L H_2O)
Na^+	142	139	14
K^+	4.2	4.0	140
Ca^{++}	1.3	1.2	0
Mg^{++}	0.8	0.7	20
Cl^-	106	108	4
HCO_3^-	24	28.3	10
HPO_4^-, $H_2PO_4^-$	2	2	11
SO_4^-	0.5	0.5	1
Phosphocreatine			45
Carnosine			14
Amino acids	2	2	8
Creatine	0.2	0.2	9
Lactate	1.2	1.2	1.5
Adenosine triphosphate			5
Hexose monophosphate			3.7
Glucose	5.6	5.6	
Protein	1.2	0.2	4
Urea	4	4	4
Others	4.8	3.9	10
Total mOsm/L	299.8	300.8	301.2
Corrected osmolar activity (mOsm/L)	282.0	281.0	281.0
Total osmotic pressure at 37°C (mm Hg)	5441	5423	5423

Blood Plasma

If a test tube of blood is left to stand for half an hour OR is centrifuged, the blood separates into three layers

- Denser components sink to the bottom of the tube: Cellular fraction (1. Red blood cells, 2. White blood cells and platelets)
- Fluid remains at the top: 3. **Plasma**

Blood Plasma

- 55% of the whole blood
- 5% of total body weight
- In a 70 kg man: 3,5 L
- Constituents:
  - Mainly water
  - Ions, organic and anorganic molecules

Plasma vs. Serum

- If the blood is waited in normal conditions, it is coagulated.
- If anticagulants are added to the blood, it remains fluid.
  - EDTA
  - Heparine
  - Sodium citrate

Composition of the Plasma

- 91.5 % water
  - Provides a **solvent** environment to facilitate the transport of molecules
- 8.5 % **solutes**

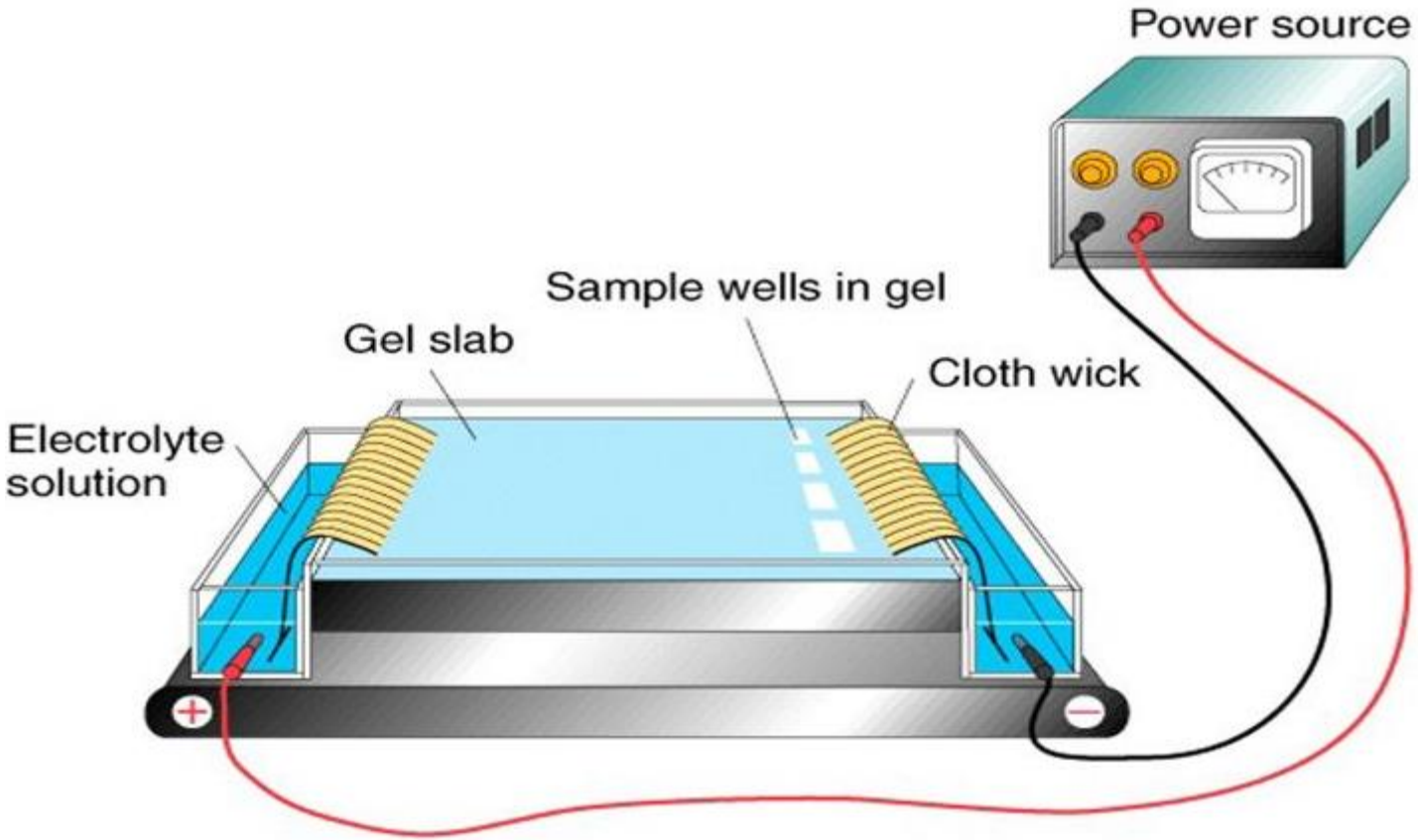
Components of the Plasma: Solutes

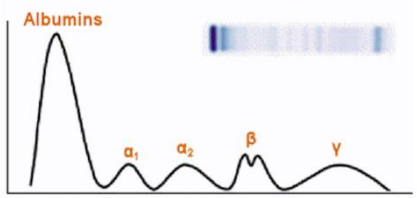
- **Proteins**
- Electrolites (Ions): Na^{+}, K^{+}, Cl^{-}, Ca^{++}, Mg^{++}
- Food substances: Amino acids, Glucose, Minerals, Vitamins
- Waste products: Waste nitogen→ Amonnia →Urea, Metabolites
- Hormons: Glukagon, Insülin, TSH,…
- Gases: O_2 (%1), CO_2
- Lipoproteins: Lipids do not directly dissolved in the plasma. They are carried by carrier proteins.

Methods of Plasma Protein Separation

- Precipitation by salting out
- Electrophoresis separation of protein fractions
- Immunoelectrophoresis technique
- Affinity chromatography
- Ultra-centrifugation technique

Seperation of Plasma Proteins: Electrophoresis

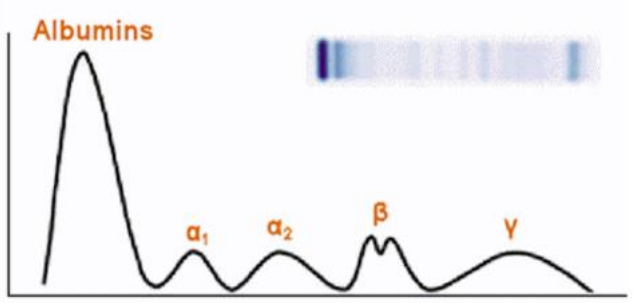




Major Plasma Proteins

7% of the plasma

- **Albumin** 60%
- **Globulins** 36%
  - **α1-Globulins** 5.3%:
    α1-Antitripsin, Transcortin, etc.
  - **α2-Globulins** 8,6%:
    Haptoglobulin, seruloplasmin,
    α2- makroglobulin, etc.
  - **β-Globulins** 13,4%:
    β1-transferin, β-lipoprotein, etc.
  - **ɣ-Globulins** 11.0%:
    Antibodies etc.
- **Fibrinogen** 4%



PROTEIN FRACTIONS OF HUMAN PLASMA	MOLECULAR WEIGHT (kDa)	FUNCTION
Transthyretin	62	Binds T_3 and T_4 Binds vitamin A
Albumin	69	Oncotic pressure Binds steroids, T_3, bilirubin, bile salts, fatty acids
α_1-antitrypsin (α_1AT)	54	Protease inhibitor Deficiency causes emphysema
α_2-macroglobulin	725	Broad-spectrum protease inhibitor Synthesized by liver
Haptoglobin	100	Binds hemoglobin
β-lipoprotein = low-density lipoprotein (LDL)	380	Binds lipid
Transferrin	80	Binds iron
Complement C3	185	Third component of complement system
Fibrinogen	340	Clotting protein Precursor of fibrin
Immunoglobulin A (IgA)	160	Mucosal immunity Synthesized by plasma cells in exocrine glands
Immunoglobulin D (IgD)	170	Synthesized by B lymphocytes
Immunoglobulin E (IgE)	190	Synthesized by B lymphocytes Binds to mast cells or basophils
Immunoglobulin G (IgG)	150	Humoral immunity Synthesized by plasma cells
Immunoglobulin M (IgM)	970	Humoral immunity Synthesized by B lymphocytes

Functions of the plasma proteins

- ✓ Maintenance of osmotic pressure in the blood: **Oncotic pressure**
- ✓ Transport mechanism: Hormones, enzymes, lipids, metal ions in the blood
- ✓ Immune defence
- ✓ Blood coagulation
- ✓ Blood viscosity
- ✓ Buffering
- ✓ Source of amino acids for the tissues

Functions: Maintenance of oncotic pressure

- Since most capillary walls are relatively impermeable to plasma proteins, the plasma is rich in protein.
- Protein concentration
  - Plasma : 7.3 g/dL
  - Interstitial fluid: 2-3 mg/dL
- Plasma proteins exert an osmotic force of about 25-28 mmHg to pull the interstitial fluid from tissues into the blood.
- **Colloid osmotic pressure** or **Oncotic pressure**

- Additional cations in the region of proteins increase the number of osmoactive molecules.

- Donnan effect causes the plasma colloid osmotic pressure to be about 50% greater than the pressure that can be generated only by the action of proteins.

The colloid osmotic pressure of normal human plasma averages about 28 mm Hg

19 mm Hg of this pressure is caused by molecular effects of the dissolved **plasma proteins**

9 mm Hg is caused by the ***Donnan effect***

i.e. extra osmotic pressure caused by Na^+, K^+, and the other cations held in the plasma by the proteins

Oncotic pressure vs. Hydrostatic pressure

- **Hydrostatic pressure** refers to force that is exerted by the fluid inside the blood capillaries against the capillary wall.
- **Oncotic pressure**
  - Refers to the force that is exerted by plasma proteins in the blood vessels.
  - Oncotic pressure is the opposing force to hydrostatic pressure
  - Prevents plasma loss from the capillaries

Relative mass concentrations (g/dl) of the different types of proteins in normal plasma and their respective contributions to the total plasma colloid osmotic pressure

		g/dl	Πp (mm Hg)
Albumin	80%	4.5	21.8
Globulins	20%	2.5	6.0
Fibrinogen		0.3	0.2
Total		7.3	28.0

Functions: Buffering

- Plasma proteins are responsible for 15% of the buffering capacity of the proteins in the blood (including hemoglobin), due to the weak ionization of their substituent COOH ve NH_2 groups
- Normal plasma pH: 7.40
- The proteins are mostly in the anionic form

Functions: as a source of amino acids for the tissues

- When the tissues become depleted of proteins, the plasma proteins can act as a source of rapid replacement.
- Whole plasma proteins can be taken by tissue macrophages through the process of pinocytosis.
- Once in these cells, they are split into amino acids that are transported back into the blood and used throughout the body to build cellular proteins wherever they are needed.

The plasma proteins function as a **labile protein storage medium** and represent a readily available source of amino acids whenever a particular tissue requires them.

Albumin

- Most abundant protein in te plasma
- 618 aa, MW: 69.000 Da
- The fastest plasma fraction during protein electrophoresis
- Synthesized in the **liver**
- Normal concentration:

  3.5-5.5 g/dL
- Dissolved in water
- Rich in some of the essential amino acids: Lys, Leu, Val, Phe, Tre, Arg, His
- Asp and Glu are also abundant

Albumin: *Functions*

- One of the most important functions of the albumin is the maintenance of oncotic pressure and the regulation of fluid distribution between blood and tissues: Preventing loss of plasma from the capillaries
  - Responsible for the 80% of the oncotic pressure
  - Decreased albumin levels causes generalized edema.
- In addition to being a source of nutrient, it is also responsible for the transport of fatty acids, trace elements and certain drugs.

Globulins

- Can be divided into four different fractions by their electrophoretic mobility:
  - α1-, α2-, β- and ɣ-globulins
- α1-, α2- and β-globulins are synthesized in the liver
- ɣ-globulins are synthesized in the lymphatic tissues.
- Concentration of total serum globulins:

  2.3-5.5 g/dL

α1-Globulins

- Complex proteins containing carbohydrate and lipid moieties.
- **Orosomucoid (α1-glycoprotein)**
  - MW: 44.000 Da
  - rich in carbohydrates, soluble in water, heat resistant
  - Responsible for transport of hexozamines to the tissues
  - Acute-phase protein and indicator of inflammation
  - 0,6-1,2 mg/mL
- **Alpha-1-antitrypsin**
  - Produced by the lungs and liver
  - Serine protease inhibitor
  - Inhibits proteolytic enzymes produced by neutrophils during inflammation

α2-Globulins

- This fraction contains complex proteins
- Plasminogen, Protrombin, Haptoglobulin, Seruloplasmin and α2-macroglobulin.
- Normal serum level: **67 mg/dL.**
- **Plasminogen** ve **protrombin** are the inactive forms of plasmin and trombin, respectively. They play an important role in blood coagulation.
- **Seruloplasmin** has a glycoprotein structure. It is an important component of Cupper metabolism. 95% of the plasma Cu^{2+} is found bound to seruloplasmin.
- **Haptoglobulins** have glycoprotein structure and are synthesized in the liver. By binding to free Hb formed by the breakdown of erythrocytes they prevents the loss of Hb and related-iron
- **α2-macroglobulin** is the inhibitor of serum endoproteases.

β-Globulins

- **Hemopexin**: Binds to porphyrins, particularly heme for heme recycling. Protects cells against oxidative damage by heme.
- **Transferrin:**
  - Transport of free iron in the plasma.
  - Almost 33% of plasma transferrin are saturated by iron.

ɣ-Globulins:

- Also known as «immunoglobulins»
- Act as antibodies
- Classified according to their electrophoretic properties: IgG, IgA, IgE, and IgM

IgG

- 75-80% of the gamma-globulins
- 4 different isoforms: G_1, G_2, G_3 and G_4
- MW: 51.000-60.000 Da
- Generates secondary response in the immune system.
- Acts against bacteria and viruses by opsonizing.
- Neutralizes tox,ns
- Act,cate complement by classical pathway.
- It passes through the placenta and provides immunity to the newborn.

IgA

- MW: 52.000-56.000 Da
- Constitutes 10-15% of total immunoglubulins
- The epithelial cells carrying the IgA Ab receptor form a common defensive barrier in the digestive, respiratory and urogenital tracts.
- Antigens in the mucus are captured and destroyed by IgA antibodies.

IgM

- MW: 970 kDa
- Accounts for 5-10% of total immunoglobulins
- The largest Ab
- It is the first group of antibodies formed as a primary response to antigenic stimulation
- Effective in complement fixation
- By opsonization, they fight the antigens that T-cells cannot cope

IgD

- MW: 185 kDa
- Constitutes 0.2% of total immunoglobulins
- The function of IgD is to signal the B cells to be activated.

IgE

- Involved in the stimulation of basophils and mast cells.
- Very important in hypersensitivity reactions and protection against infections.

Fibrinogen

- MW: 340.000 Da
- A fibrous protein
- Contains 6 polypeptide chains linked by disulfide bonds
- Plays an important role in blood clotting

Name	Principal Function	Binding Characteristics	Serum or Plasma Concentration
Albumin	Binding and carrier protein; osmotic regulator	Hormones, amino acids, steroids, vitamins, fatty acids	4500–5000 mg/dL
Orosomucoid	Uncertain; may have a role in inflammation		Trace; rises in inflammation
α_1-Antiprotease	Trypsin and general protease inhibitor	Proteases in serum and tissue secretions	1.3–1.4 mg/dL
α-Fetoprotein	Osmotic regulation; binding and carrier protein[a]	Hormones, amino acids	Found normally in fetal blood
α_2-Macroglobulin	Inhibitor of serum endoproteases	Proteases	150–420 mg/dL
Antithrombin-III	Protease inhibitor of intrinsic coagulation system	1:1 binding to proteases	17–30 mg/dL
Ceruloplasmin	Transport of copper	Six atoms copper/mol	15–60 mg/dL
C-reactive protein	Uncertain; has role in tissue inflammation	Complement C1q	< 1 mg/dL; rises in inflammation
Fibrinogen	Precursor to fibrin in hemostasis		200–450 mg/dL
Haptoglobin	Binding, transport of cell-free hemoglobin	Hemoglobin 1:1 binding	40–180 mg/dL
Hemopexin	Binds to porphyrins, particularly heme for heme recycling	1:1 with heme	50–100 mg/dL
Transferrin	Transport of iron	Two atoms iron/mol	3.0–6.5 mg/dL
Apolipoprotein B	Assembly of lipoprotein particles	Lipid carrier	
Angiotensinogen	Precursor to pressor peptide angiotensin II		
Proteins, coagulation factors II, VII, IX, X	Blood clotting		20 mg/dL
Antithrombin C, protein C	Inhibition of blood clotting		
Insulinlike growth factor I	Mediator of anabolic effects of growth hormone	IGF-I receptor	
Steroid hormone-binding globulin	Carrier protein for steroids in bloodstream	Steroid hormones	3.3 mg/dL
Thyroxine-binding globulin	Carrier protein for thyroid hormone in bloodstream	Thyroid hormones	1.5 mg/dL
Transthyretin (thyroid-binding prealbumin)	Carrier protein for thyroid hormone in bloodstream	Thyroid hormones	25 mg/dL

[a]The function of alpha-fetoprotein is uncertain, but because of its structural homology to albumin it is often assigned these functions.

Synthesis of Plasma Proteins

- Essentially all the albumin and fibrinogen of the plasma proteins, as well as 50 to 80 % of the globulins, are formed in the liver.
- The remaining globulins (i.e. gamma globulins) in lymphoid tissues.
- The rate of plasma protein formation by the liver can be extremely high—as much as 30 g/day.
- Certain disease conditions cause rapid loss of plasma proteins; for example, severe burns that denude large surface areas of the skin can cause the loss of several liters of plasma through the denuded areas each day
- Occasionally, a person with severe renal disease loses as much as 20 grams of plasma protein in the urine each day for months, and this plasma protein is continually replaced mainly by liver production of the required proteins

Increased plasma protein levels (Hyperproteinemia)

- Acute inflammatory conditions
- Acute tissue destruction
- Dehydration, diarrhea
- Multiple myeloma

Decreased plasma protein levels (Hypoproteinemia)

- Hemorrhage
- Liver cirrhosis
- Nephritis and nephrotic syndrome
- Malabsorption
- Extensive burns
- Conditions in which intestinal absorption is impaired

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