Blood Physiology

Lecture 1

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Blood Physiology - Syllabus

- Functions of the blood. Blood volume. Composition and physical-chemical properties of blood. The hematocrit. ESR.


Homeostasis

- Maintainance of nearly constant conditions in the internal environment
- Virtually all body components are homeostatic effectors
- Blood - links everything together!
Internal environment

Term introduced by Claude Bernard (milieu interieur) for the extracellular fluid
Fluid compartments of a prototypical adult human male weighing 70 kg.

**EXTRACELLULAR**

- **BLOOD PLASMA** 3 L
  - $[\text{Na}^+] = 142 \text{ mM}$
  - $[\text{K}^+] = 4.4 \text{ mM}$
  - $[\text{Cl}^-] = 102 \text{ mM}$
  - [Protein] = 1 mM
  - Osmolality = 290 mOsm

- **INTERSTITIAL FLUID** 13 L
  - $[\text{Na}^+] = 145 \text{ mM}$
  - $[\text{K}^+] = 4.5 \text{ mM}$
  - $[\text{Cl}^-] = 116 \text{ mM}$
  - [Protein] = 0 mM
  - Osmolality = 290 mOsm

- **Bulk interstitial fluid** 8 L
  - Bone 2 L
  - Dense connective tissue 3 L

- **TRANSCELLULAR FLUID** 1 L (Synovial fluid, CSP)
  - $[\text{Na}^+] = \text{Variable}$
  - $[\text{K}^+] = \text{Variable}$
  - $[\text{Cl}^-] = \text{Variable}$
  - [Protein] = Variable
  - Osmolality = Variable

**INTRACELLULAR**

- **INTERCELLULAR FLUID** 25 L
  - $[\text{Na}^+] = 15 \text{ mM}$
  - $[\text{K}^+] = 120 \text{ mM}$
  - $[\text{Cl}^-] = 20 \text{ mM}$
  - [Protein] = 4 mM
  - Osmolality = 290 mOsm

**TOTAL BODY WATER = 42 liters**
How do body compartments communicate?
How do body compartments communicate?

- **Plasma**: 3.0 L (not RBCs)
  - 4 – 5% of body weight

- **Extracellular fluid (ECF)**: 14.0 L
  - ECF = plasma + ISF
  - 20% of body weight

- **Intracellular fluid (ICF)**: 28.0 L
  - 40% of body weight

- **Output**: Kidneys, Lungs, Feces, Sweat, Skin

- **Intake**: Metabolism, Ingestion

- **Starling hypothesis**: Lymphatics

- **Osmotic gradient**: Cell membrane

The diagram illustrates the fluid compartments of the body, showing how fluids move between plasma, extracellular, and intracellular spaces.
Plasma - interstitial fluid exchange

Fluid, electrolytes, gases, small and large molecular weight substances can pass through the capillary endothelium by several different mechanisms:

- **diffusion** - important for gases, fluid and electrolytes (a)
- **bulk flow (convection)** - fluid and electrolytes - pores and intercellular clefts (d, e, f)
- **vesicular transport** - translocation of macromolecules across capillary endothelium (b)
- **active transport** - ions, glucose, AA
Blood

- component of the internal environment - Cl. Bernard, 1865
- component of the circulatory system
- liquid tissue

Blood is a dynamic and complex fluid that belongs histologically to connective tissue, consisting of:

1. **Plasma** - extracellular fluid: a pale-white watery solution of electrolytes & minerals, plasma proteins, carbohydrates, lipids, enzymes and metabolites

2. **Formed elements**: red blood cells (RBCs or erythrocytes), white blood cells (WBCs), and platelets (or thrombocytes).

Total blood volume is ~70 mL/kg body weight in the adult woman and ~80 mL/kg body weight in the adult man.

Medical terms related to blood often begin in hem/o- or hemat/o- from the Greek word “haima” for blood.
Blood facts

About 100 trillion cells in the entire body - 25 trillion of them are RBC!
All the blood in the circulation traverses the entire vascular circuit:
  - an average once a minute at rest
  - 6 times/min when a person is extremely active

Blood is considered a connective tissue for two reasons:

  (1) embryologically, it has *the same origin (mesodermal) as do the other connective tissue types*

  (2) *blood connects* the body systems together bringing the needed oxygen, nutrients, hormones and other signaling molecules, and removing the wastes.
Blood components

1 – plasma: plasmatic volume ~ 3 liters

Plasma is the non-cellular part of the blood and communicates continuously with the interstitial fluid through several processes.

2 – blood cells: volume ~2 liters

1) red blood cells (RBCs) or erythrocytes
   The fraction occupied by the red cells = the hematocrit (~ 45%).
2) platelets or thrombocytes
3) 5 kinds of white blood cells (WBCs) or leukocytes
   - 3 kinds of granulocytes:
     neutrophils, eosinophils, basophils
   - 2 kinds of leukocytes without granules in their cytoplasm:
     lymphocytes, monocytes
Blood Composition

- Plasma
- White blood cells, the "buffy coat"
- Red blood cells

Plasma

- Organic molecules such as:
  - Amino acids
  - Proteins
  - Globulins
  - Fibrinogen

- Trace elements and vitamins
- Nitrogenous waste
  - CO₂
  - O₂

Red blood cells

- White blood cells
- Platelets

Cellular elements

- Lymphocytes
- Monocytes
- Neutrophils
- Eosinophils
- Basophils
Blood functions

Maintenance of blood flow, tissue perfusion and capillary exchanges through the blood volume and pressure.

Transport (free in plasma, bound to plasma proteins, or within blood cells) through the body of:
- oxygen, carbon dioxide
- nutrients (glucose, lipids, amino acids, etc.), vitamins, cofactors
- ions, minerals, acids and bases, water.
- wastes (e.g., urea)
- hormones, other signaling molecules, antibodies
Blood functions

**Acid-base buffering power**
- RBC: CA $\rightarrow$ HCO$_3^-$; Hb (>deoxyHb);
- Buffer systems
  - Plasma proteins (polyanions at plasmatic pH)

**Defense** of the body against diseases, infections and other foreign materials. All the WBCs participate in these defenses together with complement system proteins

**Hemostasis** - arrest of bleeding, blood clotting, wound repair, vascular integrity

**Homeothermy** by distributing heat throughout the body
Blood functions: maintenance of AB balance by 2 mechanisms:

1. **Buffer systems** - composed of a weak acid and its salt with a powerful base, which have two origins: plasmatic and cellular (erythrocyte) - they fight against sudden shifts in AB balance

2. **Biological mechanisms** - in which lungs (regulate AB in minutes) and kidneys play a major role (regulate AB balance in days)
Buffer systems

- Take up H+ or release H+ as conditions change
- Buffer pairs – weak acid and its conjugate base
- Exchange a strong acid or base for a weak one
- Results in a much smaller pH change

Whenever a buffering reaction occurs, the concentration of one member of the pair increases while the other decreases.

<table>
<thead>
<tr>
<th>Table 9-1 Buffer System Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weak Acid</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Carbonic acid (H₂CO₃)</td>
</tr>
<tr>
<td>Hemoglobin (Hb)</td>
</tr>
<tr>
<td>Oxyhemoglobin (Hbo₂)</td>
</tr>
<tr>
<td>Plasma protein (HPr)</td>
</tr>
<tr>
<td>Acid organic phosphate (NaRHPO₄)</td>
</tr>
<tr>
<td>Acid inorganic phosphate (NaH₂PO₄)</td>
</tr>
</tbody>
</table>

Bicarbonate buffer

- The most important extracellular buffer

- Sodium Bicarbonate (NaHCO$_3$) and carbonic acid (H$_2$CO$_3$)

- Example of action:

  $\text{HCl} + \text{NaHCO}_3 \leftrightarrow \text{H}_2\text{CO}_3 + \text{NaCl}$

  $\text{NaOH} + \text{H}_2\text{CO}_3 \leftrightarrow \text{NaHCO}_3 + \text{H}_2\text{O}$
Blood volume

Blood volume: plasma volume (~55-60%) & cell volume (~40-45%)

Values: 5 liters; 65-80 ml/body weight in the adult; 7-8% body weight

Changes:
1. physiological: pregnancy, water loss/gain
2. pathological: hemorrhage…

Regulation mechanisms:
Renin-Angiotensin-Aldosterone axis, ADH, ANP, pressure natriuresis
Physiological blood volume variations

- gender: male > female - difference 1 l (androgens)
- effort - first 10-15 min- lowers (liquid extravasation in interstitial space - higher number of functional capillaries)
- posture
  - after 15 min of orthostatism- blood volume decreases with 15 % - high hidrostatic pressure in lower limbs- liquid extravasation in interstitial space
- pregnancy 20-100% higher volume - begins in 10th week → maximum in 30-34; after birth - normal in 2-8 wks (high aldosterone, ADH, vasodilation due to estrogens, low renal perfusion, higher conc of plasma proteins, higher no of erythrocytes - prolactin ++++ erythropoietin)
Blood volume regulation systems

- Renin angiotensin aldosterone
- ADH
- Atrial natriuretic peptide
- Thirst / salt appetite mechanisms
- Pressure natriuresis/ diuresis
Renin-angiotensin-aldosterone system

- Angiotensinogen → Angiotensin I → Angiotensin II
- Renin:
  - Decrease in renal perfusion (juxtaglomerular apparatus)
  - Kidney
- Tubular Na⁺ Cl⁻ reabsorption and K⁺ excretion, H₂O retention
- Aldosterone secretion:
  - Adrenal gland: cortex
  - Increase in blood pressure, arteriolar vasoconstriction
- ADH secretion:
  - Pituitary gland: posterior lobe
- Collecting duct: H₂O absorption
- Sympathetic activity
- Water and salt retention. Effective circulating volume increases. Perfusion of the juxtaglomerular apparatus increases.

Legend:
- Blue: Secretion from an organ
- Green: Stimulatory signal
- Red: Inhibitory signal
- Reaction
- Active transport
- Passive transport
Determination of blood volume

- Dilution method:
  - volume = mass of substance injected* / concentration
    - plasmatic volume - T1824 (Evans Blue), I$^{125}$ albumin
    - blood volume - Cr$^{51}$ marked RBC

- Blood volume = plasma volume / (1 – Ht)

Ht = hematocrit = fraction of blood composed of blood cells, mainly RBC

Properties of ideal tracer
- to be nontoxic
- to rapidly and evenly distribute throughout the specific fluid compartment;
- not to be metabolized
- not to be excreted during the equilibration period
- to be easy to measure

*If the tracer is excreted in the urine, then the loss can be determined and corrections made in the calculation.
Blood specific gravity

$= 1.055-1.060$

-determined mainly by Hb and plasma protein concentration

→ quick screening test for overall quality of blood (e.g. blood donation)

-method of determination: a drop of blood dropped in a column of CuSO4 sol with specific gravity of 1.050 → drop should sink at least halfway down the column, and not float...

Blood color

The red color of whole blood stems from hemoglobin. Oxygenated iron in hemoglobin gives the blood a bright red color.

Deoxygenated venous blood is darker red.

Veins, when seen through the skin, typically appear blue in color as a result of the deflection of light when it penetrates the skin.
Plasma composition

- Plasma proteins
- Glucose - main energogenic substance
- Bilirubin - from hemoglobin
- Calcium
- Uric acid - from purines
- BUN (blood urea nitrogen) - assessment of kidney and liver function
- Total cholesterol
- HDL = ‘good cholesterol’
- VLDL and LDL cholesterol = ‘bad cholesterol’
- Triglycerides
- Many other compounds: salts, amino acids, etc.
Plasma composition

**PLASMA PROTEINS**

- Albumin
- Globulins - α, β, γ
- Fibrinogen
- Enzymes
  - *Alkaline Phosphatase AP*
  - *Lactate Dehydrogenase LDH*
  - *Creatine Phosphokinase CPK*
  - *Alanine aminotransferase ALT*
  - *Aspartate aminotransferase AST*
  - *Thrombin*
  - *Plasmin*
- others

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*The proteins are listed in the approximate order of decreasing electrophoretic mobility.*
Plasma vs serum

- Serum = plasma without fibrinogen and other coagulation factors, and with higher concentration of serotonin due to platelets’ breakdown. However, serum still contains albumin, antibodies, and other proteins.

- Serum is the most preferred part of blood used in checking blood groups and diagnosis of diseases whereas plasma is delivered to the patients for treatment purposes.
## Plasma composition:
comparison with other fluid compartments

<table>
<thead>
<tr>
<th></th>
<th>Plasma (mOsm/l H2O)</th>
<th>Interstitial (mOsm/l H2O)</th>
<th>Intracellular (mOsm/l H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na</strong></td>
<td>142</td>
<td>139</td>
<td>14</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>4.2</td>
<td>4</td>
<td>140</td>
</tr>
<tr>
<td><strong>Ca</strong></td>
<td>1.3</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Mg</strong></td>
<td>0.8</td>
<td>0.7</td>
<td>20</td>
</tr>
<tr>
<td><strong>Cl</strong></td>
<td>108</td>
<td>108</td>
<td>4</td>
</tr>
<tr>
<td><strong>HCO</strong>&lt;sub&gt;3&lt;/sub&gt;</td>
<td>24</td>
<td>28.3</td>
<td>10</td>
</tr>
<tr>
<td><strong>HPO</strong>&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, H&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td><strong>Creatine</strong></td>
<td>0.2</td>
<td>0.2</td>
<td>9</td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td>1.2</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>5.6</td>
<td>5.6</td>
<td>4</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>1.2</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Urea</strong></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>5.3</td>
<td>4.4</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total mOsm/l</strong></td>
<td>301.8</td>
<td>300.8</td>
<td>301.2</td>
</tr>
</tbody>
</table>
Plasma Proteins

Plasma proteins at a normal conc. of ~ 7.0 g/dL (from which 4 g/dl albumin), account for a colloid osmotic/oncotic pressure of ~ 25 mmHg

Plasma protein components and their function:
- albumin - osmotic pressure
- globulins (alpha1, alpha2, beta, gamma globulins= immunoglobulins/antibodies) - immune function
- fibrinogen and other coagulation and fibrinolytic factors-clotting
- lipoproteins - nutrients
- transferrin, transcobalamin, IGF-binding proteins, thyroid-binding globulin, corticosteroid-binding globulin, sex hormone-binding globulin - carriers
Plasma Proteins

-ceruloplasmin: copper-carrying protein, acute phase protein, exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe\(^{2+}\) (ferrous iron) into Fe\(^{3+}\) (ferric iron), therefore assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferric state.

-hormones: anterior pituitary hormones, angiotensin

-enzymes: amylase, alkaline phosphatase

-actin scavengers (important after cell necrosis and release of actin from cell cytoskeleton): gel-solin (depolymerizes F-actin), Gc protein or Vit D-binding protein (binds G-actin)
Synthesis of plasma proteins

**LIVER**: 30 g plasma proteins/day
- albumin
- fibrinogen
- 50 - 80% of the globulins in the plasma

**LYMPHOID TISSUE/PLASMA CELLS**
- the remainder of the globulins (mainly the gamma globulins)

**MACROPHAGES**
- complement factors,

**INTESTINAL CELLS**
- Apoproteins

**ENDOTHELIAL CELLS**
- coagulation factors.

### TABLE 46-3 Proteins Made by the Liver for Export

<table>
<thead>
<tr>
<th><strong>Major Plasma Proteins</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>α₁-fetoprotein</td>
</tr>
<tr>
<td>Plasma fibronectin (an α₂-glycoprotein)</td>
</tr>
<tr>
<td>C-reactive protein</td>
</tr>
<tr>
<td>α₂-microglobulin</td>
</tr>
<tr>
<td>Various other globulins</td>
</tr>
</tbody>
</table>

### Factors Involved in Hemostasis/Fibrinolysis

- **Coagulation**: fibrinogen and all others except for factor VIII
- **Inhibitors of coagulation**: α₁-antitrypsin and antithrombin III,
  - α₂-macroglobulin, protein S, protein C
- **Fibrinolysis**: plasminogen
- **Inhibitors of fibrinolysis**: α₂-antiplasmin
- Complement C3

### Carriage Proteins (Binding Proteins)

- Coruloplasmin (see pp. 970–971)
- Corticosteroid-binding globulin (CBG, also called transcortin; see p. 1021)
- Growth hormone-binding protein (low-affinity form; see p. 994)
- Haptoglobin
- Hemopexin
- Insulin-like growth factor 1–binding proteins (see p. 996)
- Retinol-binding protein (RBP; see p. 970)
- Sex hormone–binding globulin (SHBG; see p. 1099)
- Thyroxine-binding globulin (TBG; see pp. 1008–1009)
- Transferrin (see p. 941)
- Transthyretin (see pp. 1008–1009)
- Vitamin D–binding protein (see p. 1064)

### Prohormones

- Angiotensinogen (see p. 1028)

### Apolipoproteins

- Apo A-I
- Apo A-II
- Apo A-IV
- Apo B-100
- Apo C-II
- Apo D
- Apo E
Protein electrophoresis

The electrophoretic mobility of a protein depends on its molecular weight (size and shape) as well as its electrical charge.

Plasma proteins comprise, in decreasing order of electrophoretic mobility: albumin, α1-globulins, α2-globulins, β-globulins, fibrinogen, & γ-globulins (immunoglobulins / antibodies, which can be separated into IgA, IgD, IgE, IgG, IgM)
Visual mnemonics

- Right hand
Normal protein electrophoresis

- Albumin – major pl. protein
- Fibrinogen
- Globulins
  - Alpha1-globulins
    - Alpha1 antitrypsin
    - Thyroid binding globulin
    - Transcortin
  - Alpha2-globulins
    - Haptoglobin
    - Ceruloplasmin
    - Alpha2- macroglobulin
- Beta-globulins
  - Beta 1- transferrin
  - Beta 2- beta-lipoprotein, complement
  - C reactive protein- between beta and gamma
- Gamma-globulins
  - Immunoglobulins- IgG
Normal ranges are as follows:

- **Total protein**: 6 to 8 g/dL;
- **Albumin**: 3.1 to 5.4 g/dL;
- **α1-globulins**: 0.1 to 0.4 g/dL;
- **α2-globulins**: 0.4 to 1.1 g/dL;
- **β-globulins**: 0.5 to 1.2 g/dL;
- **γ-globulins**: 0.7 to 1.7 g/dL.

Note that the albumin fraction is more homogenous than globulin fraction ($\alpha_1$, $\alpha_2$, $\beta$, $\gamma$).

**Plasma fibrinogen**: 200 to 400 mg/dL

Proteins present in plasma at low concentrations are determined by immuno-logical techniques (radio-immuno-assay, enzyme-linked immunosorbent assay).
Plasma proteins

Plasma proteins as a source of amino acids for the tissues.

Protein tissues depletion – plasmatic proteins pinocytosis by macrophages → intracellular split into amino acids that are transported back into the blood (*plasma proteins function as a protein storage medium*)
Albumin

Plasma concentration: 3.5 - 5.5 g/dL (total plasma albumin pool of ~135 g).

Albumin half-life in the circulation of ~ 20 days; urinary losses normally negligible (<20 mg/day)- physiological albuminuria

High in dehydration
Low in malnutrition, liver failure, renal loss (nephrotic syndrome), pregnancy (dilution)

Hepatic synthesis of albumin is strongly enhanced by a low plasma colloid osmotic pressure!
Albumin

- Osmotic pressure of the plasma

- Transports thyroid hormones and other hormones, in particular, ones that are fat-soluble (steroids)

- Transports fatty acids ("free" fatty acids) to the liver and to heart muscle cells – energy

- Transports indirect bilirubin
Albumin

- Transports many drugs; serum albumin levels can affect the half-life of drugs
- Competitively binds calcium ions (Ca$^{2+}$)
- Buffers pH
- Serum albumin, as a negative acute-phase protein, is down-regulated in inflammatory states. As such, it is not a valid marker of nutritional status; rather, it is a marker in inflammatory states
Osmotic pressure of the plasma

- Relation between osmotic pressure – osmolarity: directly proportional

- ex: albumin, glucose, NaCl

- Van’t Hoff’s law for osmotic pressure ($P$)
  \[ P = CRT \]
  - $C$ = concentration of solutes (osmoles/L)
  - $R$ = ideal gas constant
  - $T$ = absolute temperature (Kelvin degrees)

  \[ P \text{ (mmHg)} = 1 \text{ osm/L} \times R \times (37+273) = 19,300 \text{ mmHg} \]
  \[ 1 \text{ mosm/L} \quad 19.3 \text{ mmHg} \]

  Quick calculation of body osmolarity:
  \( (2 \times \text{Na}^+ \text{ conc.}) + \text{glucose conc.} + \text{urea conc.} \)
Fibrinogen

Involved in blood coagulation = coagulation factor I: thrombin-induced fibrinogen cleavage into fibrin monomers that further assemble into a fibrin polymer.

Is a dimer of identical heterotrimerers, each composed of Aα-, Bβ-, and γ chains.

Is synthesized only by the liver and circulates in plasma at concentrations of 150 to 300 mg/dL.

The acute-phase response greatly enhances fibrinogen synthesis.
Globulins

- Subtraction of the albumin and fibrinogen moiety from total protein concentration yields the concentration of all the proteins grouped as **globulins**
  - **Alpha 1-globulins**: alpha-1 antitrypsin, thyroxine binding globulin (TBG) and transcortin (corticosteroid-binding globulin)
  - **Alpha 2-globulins**: haptoglobin, alpha-2 macroglobulin (protease inhibitor), ceruloplasmin
  - **Beta-globulins**: transferrin, betalipoprotein, complement
  - **Gamma-globulins**: immunoglobulins/antibodies M, G, A, E, D
Alpha 1 antitrypsin

- Protease inhibitor, produced in the liver
- Protects lungs from neutrophil elastase - that can disrupt connective tissue
- Acute phase reactant
- Abnormal enzyme - genetic disease - produces lung emphysema and liver damage
**Normal**

- Alpha-1 antitrypsin coats lungs, protecting them from neutrophil elastase.

**Alpha-1 Antitrypsin Deficiency**

- Lungs lack alpha-1 antitrypsin coating, leaving them open to damage by neutrophil elastase.

- Alpha-1 antitrypsin Trapped in liver, causing liver damage.

- Neutrophil elastase Uninhibited, causing lung damage.

**Neutrophil elastase**

Produced by white blood cells to break down harmful bacteria. Potentially damaging to lungs if exposed.
Alpha 2 globulins

- **Haptoglobin** binds *free hemoglobin* released from erythrocytes → removed by reticuloendothelial system

- used to monitor *hemolysis* → low levels/absent

- acute-phase reactant (sequestrates iron)

- In neonates, haptoglobin is absent

- Adult levels are reached by age 4 months.
RBC hemolysis

Hemoglobin

Hb dimers

Haptoglobin

Hp2-1:Hb complex (example)

Hp2-2:Hb complex (example)

Hp1-1:Hb: Small molecule

Hp2-1:Hb: Intermediate molecule

Hp2-2:Hb: Large molecule

Macrophage

Nucleus

Lysosome

Recycling

Degradation

Free heme
Alpha 2 globulins

- largest major nonimmunoglobulin protein in the plasma;
- roles:
  - **Antiprotease** - proteases inhibitor
    - plasmin, kallikrein - antifibrinlolytic
    - Thrombin - anticoagulating
    - Trypsin - released from immune cells
  - **Carrier** - growth factors & cytokines (PDGF, TGFβ, insulin)
Alpha 2 globulins

• **Ceruloplasmin**
  - Main copper carrying protein (70%); the rest of Cu\(^{2+}\) is carried by albumin and macroglobulins
  - Role in iron metabolism – copper-dependent oxidase activity
    - oxidation of Fe\(^{2+}\) to Fe\(^{3+}\); **helps transferrin transport iron**, as this protein can only carry the ferric (Fe\(^{3+}\)) state iron.

  \[
  \begin{array}{c}
  \text{Fe}^{2+} \quad \overset{\text{Ceruloplasmin-Cu}^{2+}}{\longrightarrow} \quad \text{Fe}^{3+} \\
  \end{array}
  \]

  \[
  \begin{array}{c}
  \text{Ceruloplasmin-Cu}^{2+} \quad \overset{\text{Ceruloplasmin-Cu}^{1+}}{\underset{\text{Fe}^{2+}}{\longrightarrow}} \quad \text{Fe}^{3+} \\
  \end{array}
  \]

  - Copper acts as an electron donor or acceptor as its oxidation state fluxes between Cu\(^{1+}\)(cuprous) and Cu\(^{2+}\)(cupric)

• Cuproenzymes: copper is involved in:
  - mitochondrial respiration
  - synthesis of melanin
  - cross-linking of collagen
  - iron homeostasis as a cofactor in ceruloplasmin
Beta globulins

- **Transferrin** - iron binding (Fe$^{3+}$) - tight binding but reversible; less than 0.1% of body iron - but highest turnover (25 mg/day)

- Lipoproteins

- Complement proteins

- C- reactive protein
Lipoproteins

- **Chylomicron** (largest; lowest in density due to high lipid/protein ratio)
- **VLDL** (very low density lipoprotein)
- **IDL** (intermediate density lipoprotein)
- **LDL** (low density lipoprotein)
- **HDL** (high density lipoprotein, highest in density due to high protein/lipid ratio).
Lipoproteins

- LDL and VLDL - transport cholesterol to the tissue
- HDL transports cholesterol to the liver
Complement system - 5% of the globulin fraction

- 25 proteins found in the blood; synthesis in liver
- Activated by antigens on the surface of the bacteria (polysaccharides) or by antigen-antibody interaction
- Factors activated in cascade manner; in the end → membrane attack complex (MAC) is formed → cell lysis
- Clears pathogens out of the body
- Other roles: opsonisation, chemotaxis, inflammation
C-reactive protein (CRP)

- Synthesized by the liver; normal range < 10 mg/l
- Acute-phase protein
- Binds to phosphocholine on the surface of dead/dying cells/some bacteria → activation of the complement system → promotes phagocytosis by the macrophages
- Rises in 4-6 h and peaks at 48 h after the onset of inflammation
- Has a constant half-life of 48 h → monitoring the severity and screening inflammation
- Mainly used as a nonspecific marker of inflammation
Acute - phase reactants

- When there is significant ongoing inflammation or tissue necrosis, the body usually responds with increased serum levels of several proteins

- High **C-Reactive Protein**, **fibrinogen**, alpha 1 antitrypsin, C3 complement fraction, ceruloplasmin, haptoglobin

- Low albumin

- **!!! Very important - albumin: globulin ratio - normal value = 1-2**
Hypoproteinemia - conditions that cause rapid loss of plasma proteins: severe burns that denude large surface areas of the skin; severe renal disease – nephrotic syndrome cirrhosis of the liver, large amounts of fibrous tissue develop among the liver parenchymal cells, causing a reduction in their ability to synthesize plasma proteins malabsorption, malnutrition, prolonged starvation chronic inflammation (inflammatory mediators inhibit albumin synthesis) → All these lead to decreased plasma colloid osmotic pressure, which causes generalized edema.

During mid-pregnancy the fall in total plasma protein concentration is largely due to hemodilution, despite increase in globulin’s hepatic synthesis.

Hyperproteinemia - occurs in acute inflammation, multiple myeloma Globulins increase sharply in any acute inflammation Acute phase proteins, important for non-specific immunity of the body: C-reactive proteins (CRP reacts with C-polysaccharide of pneumococci); a-antitrypsin, haptoglobin, von Willebrand factor, fibrinogen, ceruloplasmin. CRP also increases in chronic inflammation and malignancy g-globulins increase in large amounts in multiple myeloma
Hypoproteinemic edema - nephrotic syndrome
Hematocrit (packed red cell volume)

Methods of determination: centrifuging a sample of blood containing an anticoagulant in a calibrated ‘hematocrit tube’ for ~ 5 min at 1,000g (~3000 rpm in a small centrifuge)

→ the bottom fraction contains **formed elements:** RBCs (the highest density), WBCs (leukocytes) and platelets: whitish gray layer—the **buffy coat**—between the RBCs and plasma;

→ the top fraction is blood **plasma**;

*Figure 18-1*  Determination of the hematocrit.
Corrected hematocrit

- Corrected HT = Measured HT x 0.91 x 0.96 x 1.09

- 0.91 - correction factor for venous blood (HT in venous blood is 9% higher than total somatic HT)
- 0.96 - trapped plasma (4%)
- 1.09 - Na oxalate - anticoagulant with high osmotic pressure - lower erythrocyte volume (when heparin is used, this correction is not necessary)
Hematocrit (Ht): values and interpretation

- The Ht is a measure of concentration of RBCs, not of total body red cell mass:
  - expansion of plasma volume in a pregnant woman reduces the Ht, whereas her total red cell volume also increases but less than plasma volume;
  - immediately after hemorrhage, the Ht may be normal despite the loss of blood volume.

- The normal Ht is ~ 35% -40% for adult women
  ~ 45% for adult men

- The Ht in the newborn is ~ 55% and falls to ~ 35% at 2 months of age, from which time it rises during development to reach adult values at puberty.

- Values ~ up to 10% in severe anemia
  ~ up to 65% in polycytemia

- Total RBC volume is ~ 28 mL/kg body weight in the adult woman and
  ~ 36 mL/kg body weight in the adult man.
Hematocrit

Variations of hematocrit:

1. With red cell count:
   - decrease in anemia
   - increase in polyglobulia

2. With the place of sampling: venous blood has higher hematocrit than arterial blood, but lower than splenic blood ($\rightarrow 50\%$).

3. With hydration status – hemodilution vs hemoconcentration

4. With erythropoiesis/erythrolysis ratio
Venous vs arterial Ht

- Capillary changes: 90% of fluid is retaken by the venous end, but 10% goes to the lymphatics.
Venous vs arterial Ht

- Chloride shift
Hematocrit and blood viscosity

- Of the formed elements, red cells have the greatest effect on viscosity under normal conditions.
- An increase in red cell **hematocrit** leads to an increase in **relative viscosity**
- The increase is non-linear, so that doubling hematocrit more than doubles the relative viscosity.
Hematocrit and blood viscosity

- At a normal hematocrit of 40-45%, the relative viscosity of blood is 3-4.

- **Polycythemia** increases the resistance to blood flow and therefore increases the work of the heart and can impair organ perfusion.

- Some patients with **anemia** have low hematocrits, and therefore reduced blood viscosities.
Effect of hematocrit on blood viscosity

- Water viscosity (1)
- Plasma viscosity (1.5)
- Normal blood viscosity (~ 3)
- Polycythemia

Viscosity (water = 1) vs. Hematocrit (%RBC)

- 0 10 20 30 40 50 60 65 70 %RBC
ESR = erythrocyte sedimentation rate

- Measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity.

- The most satisfactory method of performing the test was introduced by Westergren in 1921
ESR

- ESR determination - simple and inexpensive laboratory test that is frequently ordered in clinical medicine

- the usefulness of this test has decreased as new methods of evaluating disease have been developed
Factors influencing ESR

a. Rouleaux formation
b. Blood viscosity
c. Shape of RBC
d. Size of RBC
e. No of RBC
Rouleaux formation

- RBC adhere to one another
- Their collective surface area is reduced
- They gravitate to the bottom at a much higher speed
- Thus, factors which increase rouleaux formation, increase ESR
- Normally, RBCs are negatively charged particles (adsorbed albumins on surface)- they repel each other, decreasing rouleaux formation
Factors influencing rouleaux formation

- Products of tissue damage and inflammation favour rouleaux formation.
- The above plasma factors affect rouleaux formation by changing the electrical charges on the red cells.
- Plasma proteins
  - **Albumin** reduces rouleaux formation
  - **Fibrinogen** favours rouleaux formation
  - **Globulins** are less negative than albumins, and changes in albumin/globulin ratio modify the ESR (e.g., increase of globulins in infectious process or inflammation leads to an increased ESR.
- Biconcave disk shape of RBCs facilitates rouleaux formation
Factors influencing ESR

- **Blood viscosity**
  - Low viscosity = high ESR
  - High viscosity = low ESR

- The ESR is directly proportional to the mass of the erythrocytes and inversely proportional to the surface area, which carries the negative charge that prevents aggregation.

- **Macrocytes sediment more rapidly than normal cells while microcytes sediment more slowly.**
Factors influencing ESR

- **RBC shape**
  Abnormally shaped RBC decrease ESR
  - Sickle cell disease
  - Anisocytosis
  - Spherocytosis
  - Acanthocytosis

- **RBC number**
  - ANEMIA = HIGH ESR
  - POLYCYTHEMIA = LOW ESR
Normal values

Reference Ranges for the ESR in Healthy Adults

<table>
<thead>
<tr>
<th>Adults</th>
<th>Upper limit of reference range (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 50 years</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0 to 15</td>
</tr>
<tr>
<td>Women</td>
<td>0 to 20</td>
</tr>
<tr>
<td>Age &gt; 50 years</td>
<td></td>
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</tr>
<tr>
<td>Women</td>
<td>0 to 30</td>
</tr>
</tbody>
</table>

ESR = erythrocyte sedimentation rate.

\[
ESR \ (mm/hr) \leq \frac{Age \ (in\ years) + 10 \ (if\ female)}{2}
\]
Physiological changes ESR

- Age
  - newborns have low ESR (high RBC count);
  - it raises with age

- Sex
  - females higher ESR (low RBC count)

- Pregnancy
  - high ESR (increased fibrinogen and globulin)

- Temperature
  - a rise in body temperature increases ESR (decreased viscosity)

- Exercise and after meals
  - ESR should be determined on empty stomach
Factors that increase ESR

- Red blood cell number
  - Anemia
- Red blood cell abnormalities
  - Macrocytosis
  - Cold agglutinins
- Technical factors
  - Dilutional problem
  - Increased temperature of specimen
  - Tilted ESR tube
- Elevated fibrinogen level
  - Infection
  - Inflammation
  - Malignancy
  - Diabets mellitus
- Fever
- Renal failure (anemia, hyperfibrinogenemia)
Factors that decrease ESR

- Extreme leukocytosis
- Polycythemia
- Red blood cell abnormalities
  - Sickle cell disease
  - Anisoctyosis
  - Spherocytosis
  - Acanthocytosis
  - Microcytosis
- Technical factors
  - Dilutional problem
  - Inadequate mixing
  - Clotting of blood sample
  - Vibration during testing
- Protein abnormalities
  - DIC (disseminated intravascular coagulation)
  - Hypogammaglobulinemia
  - Aspirin and other NSAIDs
  - Hypothermia
Sickle cell disease

- Genetic disorders resulting from the presence of a mutated form of molecular unstable hemoglobin, hemoglobin S (HbS).
- In deoxy state - forms polymers of Hb - tactoids $\rightarrow$ vaso-occlusion
Inflammation tests

- ALL INFLAMMATORY TESTS ARE UNSPECIFIC
- THEY SHOW ACUTE OR CHRONIC INFLAMMATION

**ESR**

- CHEAP, QUICK, SIMPLE
- BUT AFFECTED BY A VARIETY OF FACTORS

**C- REACTIVE PROTEIN**

- MOST RAPID RESPONSE TO ACUTE INFLAMMATION - h
- RETURNS TO NORMAL MORE RAPIDLY DUE TO THERAPY
- MORE EXPENSIVE

**FIBRINOGEN**

- clotting factor I
- also acute phase protein
- normal levels- 200-400 mg/dl
Using the ESR to Make a Diagnosis

- The ESR remains an important diagnostic criterion for only two diseases: polymyalgia rheumatica and temporal arteritis - HIGH VALUES >60 MM/H

- **Nonspecific inflammation**

- Diagnosis
  - Temporal arteritis
  - Polymyalgia rheumatica

- Monitoring disease activity or response to therapy
  - Rheumatoid arthritis (remission < 20 mm/ h)

- Oncologic disease
  - High ESR= poor prognosis
  - > 100 mm/ h usually metastatic disease

- Extremely high ESR > 100 mm/ h
  - Infection; Collagen vascular disease; Metastatic tumors; Renal disease
## Comparison of the ESR, C-reactive Protein and Plasma Viscosity Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>Inexpensive, quick, simple to perform</td>
<td>Affected by a variety of factors, including anemia and red blood cell size; not sensitive enough for screening</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Most rapid response to inflammation (complementary to ESR in this regard)</td>
<td>Wide reference range may necessitate sequential recording of values, expensive, batch processing may delay individual results</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>Unaffected by anemia or red blood cell size</td>
<td>Expensive, not widely available, technically cumbersome to perform</td>
</tr>
</tbody>
</table>

*ESR = erythrocyte sedimentation rate.*