Blood Physiology
Lecture 1
Blood Physiology - Syllabus

Lecture 1
- Functions of the blood. Blood volume. Composition and physical-chemical properties of blood. The hematocrit. ESR.

Lecture 2

Lecture 3

Lecture 4
Homeostasis

- Maintainance of nearly constant conditions in the internal environment

- Virtually all body components are homeostatic effectors

- BLOOD- links everything together!!!
Body compartments

- Total body water
  - 65% Intracellular fluid compartment
  - 35% Extracellular fluid compartment
    - Plasma
    - Interstitial fluid
    - Lymph
    - Transcellular fluid (cerebrospinal, synovial, serous fluids, etc.)
Internal environment

- Term introduced by Claude Bernard (milieu interieur)
- Extracellular fluid
How do body compartments communicate?
Output:
- Kidneys
- Lungs
- Feces
- Sweat
- Skin

Intake:
- Metabolism
- Ingestion

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

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

Intracellular (ICF):
- 40% of body weight
- 28 L

Starling hypothesis

Lymphatics

Osmotic gradient
Plasma- interstitial fluid exchange

- Fluid, electrolytes, gases, small and large molecular weight substances can transverse the capillary endothelium by several different mechanisms:
  - diffusion
  - bulk flow (convection)
  - vesicular transport
  - active transport
• Diffusion- important for gases, fluid and electrolytes in part (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)
Starling forces - movement of fluids

Arterial end of capillary

Tissue cells

Net HP (35-0) - Net OP (26-1)

Net HP (17-0) - Net OP (26-1)

Blood flow

NFP 10 mm

Net pressure out

Net pressure in

Key to pressure values:

\( HP_c \) at arterial end = 35 mm Hg
\( HP_c \) at venous end = 17 mm Hg
\( HP_{if} = 0 \) mm Hg
\( OP_{if} = 1 \) mm Hg
\( OP_{c} = 26 \) mm Hg
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

- 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).

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

1. Withdraw blood and place in tube
2. Centrifuge

Plasma (55% of whole blood)
Buffy coat: leukocytes and platelets (<1% of whole blood)
Erythrocytes (45% of whole blood)

Formed elements
Blood-connective tissue

Blood is considered a connective tissue for two basic 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 l

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

2 – blood cells: volume ~ 2 l

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
Composition of Blood

Plasma

White blood cells, the “buffy coat”

Red blood cells

Plasma

- Water
- Ions
- Organic molecules such as:
  - Proteins
  - Glucose
  - Lipids
  - Nitrogenous waste
  - Amino acids
  - Albumins
  - Globulins
  - Fibrinogen

Gases such as:
- CO₂
- O₂

Blood cells:
- Red blood cells
- White blood cells include:
  - Platelets
- Cellular elements

Cell types:
- 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, complement system proteins
Blood functions

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

- **Homeothermy** by distributing heat throughout the body
Maintainance of AB balance

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.
# Buffer systems

<table>
<thead>
<tr>
<th>Weak Acid</th>
<th>Weak Base</th>
<th>% Total Buffer Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic acid (H₂CO₃)</td>
<td>Sodium bicarbonate (NaHCO₃)</td>
<td>53</td>
</tr>
<tr>
<td>Hemoglobin (Hb)</td>
<td>Potassium hemoglobinate (KHB)</td>
<td>35</td>
</tr>
<tr>
<td>Oxyhemoglobin (Hbo₂)</td>
<td>Potassium oxyhemoglobinate (KHbo₂)</td>
<td>35</td>
</tr>
<tr>
<td>Plasma protein (HPr)</td>
<td>Proteinate (NaPr)</td>
<td>7</td>
</tr>
<tr>
<td>Acid organic phosphate (NaRHPO₄)</td>
<td>Alkaline organic phosphate (Na₂PO₄)</td>
<td>3</td>
</tr>
<tr>
<td>Acid inorganic phosphate (NaH₂PO₄)</td>
<td>Alkaline inorganic phosphate (NaHPO₄)</td>
<td>2</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 l; 65-80 ml/body weight in the adult; 7-8% body weight
- Changes:
  1. physiological: pregnancy, water loss/gain
  2. pathological: hemorrhage...
- Regulation mechanisms: RAgaAldo axis, ADH, ANP, pressure natriuresis
Physiological volume variations

-sex
Male> female- difference 1 l (androgens)
-effort- first 10-15 min- lowers (liquid extravasation in interstitial space- higher no 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** decreases in renal perfusion (juxtaglomerular apparatus)
- **Kidney** secretes Renin
- **Liver** secretes Angiotensinogen
- **Surface of pulmonary and renal endothelium: ACE**
- **Lungs**
- **Kidney**

**Sympathetic activity**

- **Tubular Na⁺ Cl⁻ reabsorption and K⁺ excretion. H₂O retention**
- **Adrenal gland: cortex**
- **Aldosterone secretion**
- **Arteriolar vasoconstriction. Increase in blood pressure**
- **Pituitary gland: posterior lobe**
- **ADH secretion**

**Collecting duct: H₂O absorption**

**Water and salt retention. Effective circulating volume increases. Perfusion of the juxtaglomerular apparatus increases.**

**Legend**
- Blue line: Secretion from an organ
- Yellow line: Stimulatory signal
- Red line: Inhibitory signal
- Black line: Reaction
- Black solid line: Active transport
- Black dashed line: 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

* Discuss criteria for indicator substances
Properties of ideal tracer

- be nontoxic
- be rapidly and evenly distribute throughout the nominated compartment; not enter any other compartment
- not be metabolised
- not be excreted (or excretion is able to be corrected for) during the equilibration period
- be easy to measure
- not interfere with body fluid distribution

*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 blood is darker red, which can be seen when venous blood samples are taken. 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

• Albumin
• Globulins, $\alpha$, $\beta$, $\gamma$
• Fibrinogen
• Enzymes
  • Alkaline Phosphatase AP
  • Lactate Dehydrogenase LDH
  • Creatine Phosphokinase CPK
  • Alanine aminotransferase ALT
  • Aspartate aminotransferase AST
  • Thrombin
  • Plasmin
• others
Plasma composition

- 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'
- LDL = TC - HDL - 0.2 TG
- Triglycerides
- Many other compounds, salts, amino acids, etc.
Plasma vs serum

- Serum = plasma without fibrinogen and other coagulation factors, and with higher conc. 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

## 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>₃</td>
<td>24</td>
<td>28.3</td>
<td>10</td>
</tr>
<tr>
<td><strong>HPO</strong>₄⁻, H₂PO₄⁻</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Amino acids</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.2</td>
<td>0.2</td>
<td>9</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.2</td>
<td>1.2</td>
<td>1.5</td>
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<tr>
<td>Glucose</td>
<td>5.6</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>1.2</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td>Urea</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>5.3</td>
<td>4.4</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total mOsm/l</strong></td>
<td><strong>301.8</strong></td>
<td><strong>300.8</strong></td>
<td><strong>301.2</strong></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 or 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 (II)

- 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.

- anterior pituitary hormones, angiotensin (hormones)

- amylase, alkaline phosphatase (enzymes)

- 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.
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 - largest protein component
- Globulins
  - Alpha1
    - Alpha1 antitrypsin
    - Thyroid binding globulin
    - Transcortin
  - Alpha2
    - Haptoglobin
    - Ceruloplasmin
    - Alpha2- macroglobulin
Normal protein electrophoresis

- Beta
  - Beta 1 - transferrin
  - Beta 2 - beta-lipoprotein, complement
  - C reactive protein - between beta and gamma

- Gamma
  - 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 immunological techniques (radio-immunoassay, 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 $\rightarrow$ intracellular split into amino acids that are transported back into the blood (plasma proteins function as a labile protein storage medium)
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
- 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

- The pressure which needs to be applied to a solution to prevent the inward flow of water across a semipermeable membrane.

- The osmotic pressure of a solution depends on the number of particles in solution. For an ideal solution the osmotic pressure is directly proportional to its molality (number of moles of solute per kilogram of the solvent).

- It is also defined as the minimum pressure needed to nullify osmosis.
**Osmolarity**

- Solute concentration - the no of osmoles (Osm) of solute/l

- Different from molarity - some components dissociate, some don’t

- Example - NaCl - dissociates into Na+ and Cl-, so in this case for every 1 mole of NaCl in solution, there will be 2 Osm/l
In osmosis, there is net movement of solvent from the area of higher solvent concentration (lower solute concentration) to the area of lower solvent concentration (higher solute concentration).
Osmotic pressure of the plasma

- Relation between osmotic pressure – osmolarity: **directly proportional**

- ex: **albumin**, glucose, NaCl

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

  $$\Pi \ (\text{mmHg}) = 1 \text{ osm/L} \times R \times (37+273) = 19,300 \text{ mmHg}$$

  $$1 \text{ mosm/L}$$

  $$19.3 \text{ mmHg}$$

Quick calculation of body osmolarity:

$$\text{(2 x Na}^+ \text{ conc.)} + \text{glucose conc.} + \text{urea conc.}.$$
Globulins

- Alpha 1 - alpha 1 antitrypsin, thyroxine binding globulin (TBG) and transcortin
- Alpha 2 - haptoglobin, alpha 2 macroglobulin, ceruloplasmin
- Beta - transferrin, betalipoprotein, complement
- Gamma - immunoglobulins/ antibodies M, G, A, E, D
Alpha 1 antitrypsin

- Antiprotease, produced in the liver
- Protects lungs form 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

Free heme

Haptoglobin

Hp-Hb

CD163 receptor

Lysosome

Degradation

Recycling

Heme

Nucleous

Macrophage

Hp1-1:Hb: Small molecule
Hp2-1:Hb: Intermediate molecule
Hp2-2:Hb: Large molecule

Hp2-1:Hb: complex (example)
Hp2-2:Hb: complex (example)
Alpha 2 globulins

- **Alpha 2 macroglobulin**
- largest major nonimmunoglobulin protein in the plasma;
  roles:
  - antiprotease - may inhibit a lot of proteases
    - plasmin, kallikrein - antifibrinolytic
    - thrombin - anticoagulating
    - trypsin - released from immune cells
  - carrier - growth factors and cytokines (PDGF, TGF beta, insulin)
  - high plasma values in nephrotic syndrome
Alpha 2 globulins

- **Ceruloplasmin**
  - Main copper carrying protein (70%; the rest of it - 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 state iron.
Dcyt b: duodenal cytochrome b  
HCP1: haem carrier protein 1  
DMT1: divalent metal transporter 1  
Hox: haem oxygenase  
Ft: ferritin
Copper

- ability to act as an electron donor or acceptor as its oxidation state fluxes between $\text{Cu}^{1+}$ (cuprous) and $\text{Cu}^{2+}$ (cupric)
- cuproenzymes, copper is involved in key redox
  - 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 (25mg/day)

- Lipoproteins

- Complement proteins

- C- reactive protein
Lipoproteins

- **Chylomicron** (largest; lowest in density due to high lipid/protein ratio; highest in triacylglycerols as % of weight)
- **VLDL** (very low density lipoprotein; 2nd highest in triacylglycerols as % of weight)
- **IDL** (intermediate density lipoprotein)
- **LDL** (low density lipoprotein, highest in cholesteryl esters as % of weight)
- **HDL** (high density lipoprotein, highest in density due to high protein/lipid ratio).

* Free fatty acids are transported bound with albumin
Lipoproteins

- The general **structure** of a lipoprotein includes:
  - a **core** consisting of a droplet of triacylglycerols and/or cholesterol esters
  - a **surface monolayer** of phospholipid, unesterified cholesterol and specific proteins (apolipoproteins)
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
- Activated by Ag on the surface of the bacteria (polysaccharides) or by antigen-antibody interaction
- Clears pathogens out of the body
- Synthesis in liver
- Factors activated in cascade manner
- In the end- membrane attack complex (MAC) is formed → cell lysis
- Other roles- opsonisation, chemotaxis, inflammation
certain bacterial polysaccharides, etc

IgM or IgG antibodies

C3  →  C3b

C5  →  C5b

C5b, C7, C8, C9  →  assembly of membrane attack complex (MAC)

C5a, C3a  →  increased phagocytosis, chemotaxis, inflammation

cell lysis

C5b, C6, C7, C8, C9  →  K and Cl ions

water and Na ions
### Table 18.3: Antibody Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>General Structure</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Monomer</td>
<td>Free in blood plasma; about 80% of circulating antibodies</td>
<td>Most abundant antibody in primary and secondary immune responses; crosses placenta and provides passive immunization to fetus</td>
</tr>
<tr>
<td>IgM</td>
<td>Pentamer</td>
<td>Surface of B cell; free in blood plasma</td>
<td>Antigen receptor on B cell membrane; first class of antibodies released by B cells during primary response</td>
</tr>
<tr>
<td>IgD</td>
<td>Monomer</td>
<td>Surface of B cell</td>
<td>Cell surface receptor of mature B cell; important in B cell activation</td>
</tr>
<tr>
<td>IgA</td>
<td>Dimer</td>
<td>Saliva, tears, milk, and other body secretions</td>
<td>Protects mucosal surfaces; prevents attachment of pathogens to epithelial cells</td>
</tr>
<tr>
<td>IgE</td>
<td>Monomer</td>
<td>Secreted by plasma cells in skin and tissues lining gastrointestinal and respiratory tracts</td>
<td>When bound to antigens, binds to mast cells and basophils to trigger release of histamine that contributes to inflammation and some allergic responses</td>
</tr>
</tbody>
</table>
C- reactive protein

- Synthetisized by the liver

- Acute- phase protein

- Binds to phosphocholine on the surface of dead/dying cells/ some bacteria $\rightarrow$ activation of the complement system $\rightarrow$ promotes phagocytosis by the macrophages

- Rises in 4-6 h after the onset of inflammation
C- reactive protein

- Peaks at 48 h

- Has a constant half-life of 48 h → monitoring the severity and screening inflammation

- Normal range < 10 mg/l

- Mainly used as a nonspecific marker of inflammation
Fibrinogen

- is a soluble, 340 kDa glycoprotein, coagulation factor I
- The purpose of the coagulation cascade is to transform fibrinogen into fibrin, by activation of an enzyme named thrombin
- Synthesized by liver
- Normal levels in venous blood- 200-400 mg/dl
- Acute-phase protein
Acute-phase reactants

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

- High CRP, 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.

Obs: during mid-pregnancy the fall in total plasma protein conc. 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);
- α-antitrypsin, haptoglobin, von Willebrand factor, fibrinogen, ceruloplasmin.

CRP also increases in chronic inflammation and malignancy

**γ-globulins** increase in large amounts in multiple myeloma
Hypoproteinemic edema-nephrotic syndrome
Hyperproteinemia

- Multiple myeloma
  - Cancer of plasma cells
  - Abnormal plasma cells in bone marrow
  - Produce paraprotein - abnormal antibody - renal blockage - MONOCLONAL GAMMOPATHY
A mnemonic sometimes used to remember some of the common symptoms of multiple myeloma is **CRAEB**:

- **C** = Calcium (elevated),
- **R** = Renal failure,
- **A** = Anemia,
- **B** = Bone lesions.
Hematocrit
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**;
Corrected hematocrit

- Corrected HT = Meas 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 - no correction is 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

- Discuss venous vs. arterial hematocrit ...

- 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 (→ 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
Figure 14-11

Hematocrits in a healthy (normal) person and in patients with anemia and polycythemia.

From Guyton, 2006
Effect of hematocrit on blood viscosity

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

Polycythemia
Hematocrit and blood viscosity

- Of the formed elements, red cells have the greatest effect on viscosity under normal conditions.
- Whole blood viscosity is determined in vitro using a viscometer.
- 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.
Fahraeus-Lindqvist effect

• effect where the viscosity of a fluid, in this case blood, changes with the diameter of the tube it travels through; in particular there's a decrease of viscosity as the tube's diameter decreases (only if the vessel diameter is between 10 and 300 micrometers).

• this is because erythrocytes move over the center of the vessel, leaving plasma at the wall of the vessel.
Fahraeus-Lindqvist effect

- This decrease in hematocrit in these flow vessels reduces the relative blood viscosity in the small vessels, which helps to offset the increase in viscosity that can occur because of reduced velocity in these same vessels.
ESR

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
A little bit of Physics...

Mathematically, terminal velocity—without considering buoyancy effects—is given by

\[ V_t = \sqrt{\frac{2mg}{\rho AC_d}} \]

where

- \( V_t \) = terminal velocity,
- \( m \) = mass of the falling object,
- \( g \) = acceleration due to gravity,
- \( C_d \) = drag coefficient,
- \( \rho \) = density of the fluid through which the object is falling, and
- \( A \) = projected area of the object.
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
  - Anisoctyosis
  - Spherocytosis
  - Acanthocytosis

- **RBC number**
  - ANEMIA = HIGH ESR
  - POLYCYTEMIA = LOW ESR
### Reference Ranges for the ESR in Healthy Adults

<table>
<thead>
<tr>
<th>Age</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>
</tr>
<tr>
<td>Men</td>
<td>0 to 20</td>
</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
  - Diabetus mellitus
- Fever
- Renal failure (anemia, hyperfibrinogenemia)
Cold agglutinin disease

- autoimmune disease
- presence of high concentrations of circulating antibodies, usually IgM, directed against red blood cells
- It is a form of autoimmune hemolytic anemia, specifically one in which antibodies only bind red blood cells at low body temperatures, typically 28-31°C.
Cold agglutinin disease

2 forms:
- primary - idiopathic
- secondary - several diseases:
  - TB
  - Lymphoproliferative diseases (leukemia, lymphoma)
Quinine use
Factors that decrease ESR

- Extreme leukocytosis
- Polycythemia
- Red blood cell abnormalities
  - Sickle cell disease
  - Anisocytosis
  - 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 hemoglobin, hemoglobin S (HbS).
- Moleculary instable
- In deoxy state- forms polimers of Hb- tactoids- vaso- occlusion
Acanthocytes

- RBC - spiked shape

- Occur when erythrocyte membranes contain excess cholesterol compared to phospholipid content

- Small surface

- Not elastic - hemolysis

- Liver disease, diseases of bone marrow etc
DIC

- disseminated intravascular coagulopathy or consumptive coagulopathy
- pathological activation of coagulation in response to a variety of diseases (major trauma, septic shock)
- formation of small blood clots inside the blood vessels throughout the body
- Consumption of coagulation proteins and platelets → normal coagulation is disrupted and abnormal bleeding occurs
Hypogammaglobulinemia

- Primary - congenital defect involving B cells - very rare
- Secondary
  - Extensive burns
  - Renal loss
  - Gastrointestinal loss
  - B-cell lineage malignancy
Inflammation TESTS

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
ALL INFLAMMATORY TESTS ARE UNSPECIFIC

THEY SHOW ACUTE OR CHRONIC INFLAMMATION
ESR clinical utility

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
Utility of the ESR: Key Considerations

The ESR is an inexpensive, simple test of chronic inflammatory activity.

Indications for the ESR have decreased as the sophistication of laboratory testing has increased.

The ESR rises with age, but this increase may simply reflect a higher disease prevalence in the elderly.

The use of the ESR as a screening test in asymptomatic persons is limited by its low sensitivity and specificity.

An elevated ESR is a key diagnostic criterion for polymyalgia rheumatica and temporal arteritis, but normal values do not preclude these conditions.

When there is a moderate suspicion of disease, the ESR may have some value as a “sickness index.”

An extremely elevated ESR (>100 mm/hr) will usually have an apparent cause—most commonly infection, malignancy or temporal arteritis.

A mild to moderately elevated ESR without obvious etiology should prompt repeat testing after several months rather than an expensive search for occult disease.
Polymyalgia rheumatica

Polymyalgia rheumatica is characterized by severe aching and stiffness in the neck, shoulder girdle or pelvic girdle areas. In some patients, systemic symptoms may predominate, with initial manifestations including anemia, fever of unknown origin or a nonspecific systemic illness accompanied by anorexia, malaise and weight loss.

Autoimmune disease
Temporal arteritis (Horton)

- Inflammation of blood vessels supplying the head-vasculitis
- Biopsy- giant cells- giant cell arteritis
- headaches, visual disturbances such as blindness, a tender, reddened or nodular temporal artery, facial pain and jaw claudication
ESR clinical utility

- 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
<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.*