Blood physiology

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Comenius University
Bratislava 2015
Blood tests
• a routine examination in medicine
• will help you to make diagnosis and to treat

Physiology
• studies the function of a healthy human body
• if you know what is normal, you can detect abnormalities/diseases and treat them
Blood

**Definition**
Red, opaque liquid that circulates in blood vessels, connective tissue.

**Blood components:**
- plasma
- blood elements (corpuscles):
  1. erythrocytes - red blood cells
  2. leukocytes - white blood corpuscles
  3. thrombocytes - platelets

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**SPECIFIC GRAVITY**
Informs about the weight of a blood volume

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>1,052 – 1,063 /ml (g.cm⁻³)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>blood elements</td>
<td>1,090 ml (g.cm⁻³)</td>
</tr>
<tr>
<td>plasma</td>
<td>1,026 – 1,031 / ml (g.cm⁻³)</td>
</tr>
</tbody>
</table>
NORMAL BLOOD VOLUME

- for normal body function constant blood volume is vital
- major blood loss is a life threatening event

7-8 % of body weight
male 6 l
female 4,5 l

= normovolaemia

hypovolaemia
e.g. in bleeding, dehydration

hypervolaemia
• e.g. in kidney disease
**Functions of blood:**

1. blood instantly circulates in blood vessels – ideal medium for **transportation**:
   - \( \text{O}_2 \) and \( \text{CO}_2 \) (lungs ↔ tissues)
   - **nutrients** (gut – liver/tissues)
   - cellular **waste products** to places of their elimination (kidney, liver)
   - **hormones** and physiologically active substances (e.g. clotting factors)
   - cells and molecules involved in **immune** functions
   - **heat** (liver, muscles → all over the body)
   - **medicaments**, etc.
2. blood helps to maintain homeostasis in the body
- **homeostasis** = constant internal environment (of the body) despite fluctuations in external environment (e.g. varying external temperature/constant body temperature)

main aspects of **homeostasis** (related to blood)
- **body temperature** (isothermia)
- **pH of body fluids** = concentration of \( H^+ \) (isohydria)
- **ion concentration** and osmotic pressure (isoosmia)
- **volume of blood** (isovolemia)
- (there are more aspects, e.g. blood glucose level, etc.)

Balance – constant temperature, pH, ion concentration, blood volume
- **homeostasis**
  - is vital for normal function/survival of the human body
  - is regulated by different control mechanisms (feedback mechanisms)

Imbalance (↑ ↓)
(temperature, blood volume, pH, ion concentration)

Balance reestablished

Mechanisms involved in homeostasis control
(kidneys, respiratory, CVS, endocrine, blood...)

3. **haemostatic function of blood**
- **haemostasis** = bleeding arrest
  - components of blood (platelets, clotting factors) are activated in case of bleeding in order to stop the bleeding
- the proportion of blood volume that is occupied by red blood cells

\[
\text{haematocrit} = \frac{\text{erythrocyte volume}}{\text{blood volume}}
\]

**Normal values**

- **males**
  (39 - 49%) 0.39 – 0.49

- **females**
  0.35 – 0.43 (35 - 43%)
causes a change in erythrocyte count

decreased hematocrit
- anaemias
- after chronic bleeding

increased hematocrit
- living in high altitudes
- polycytemia

Abnormalities of haematocrit

<table>
<thead>
<tr>
<th>causes</th>
<th>a change in erythrocyte count</th>
<th>a change in blood volume (plasma volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>decreased hematocrit</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>- anaemias</td>
<td>- pregnancy</td>
</tr>
<tr>
<td></td>
<td>- after chronic bleeding</td>
<td>- after infusion</td>
</tr>
<tr>
<td>increased hematocrit</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>- living in high altitudes</td>
<td>- dehydration</td>
</tr>
<tr>
<td></td>
<td>- polycytemia</td>
<td></td>
</tr>
</tbody>
</table>
ERYTHROCYTE SEDIMENTATION RATE (FW)

Blood is a suspension = heterogeneous fluid containing solid particles that are sufficiently large for sedimentation.

Blood sample in a tube (containing anticlotting agent)
-erythrocytes sink to the bottom (heavier than plasma - gravitation)
= Er sedimentation

-leave behind transparent upper layer of plasma
erythrocyte sedimentation rate = size of the plasma layer (mm)

- Er sedimentation depends on the electrically charged blood components
  - erythrocytes   - charged
  - plasma proteins + charged
Determination of sedimentation rate

- in tubes (e.g. Westergren tubes, Sedivettes)
- size of the plasma layer in the sample is measured

Normal values (normal FW)

<table>
<thead>
<tr>
<th></th>
<th>1st hour</th>
<th>2nd hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>2 – 5 mm (up to 15 mm)</td>
<td>two times the value in 1st hour or less (but not more !)</td>
</tr>
<tr>
<td>females</td>
<td>3 – 8 mm (up to 20 mm)</td>
<td></td>
</tr>
</tbody>
</table>

https://www.sarstedt.com/fileadmin/produkte/bilder/_processed_/csm_90.1090_2402_a3f8824e35.png
Abnormalities in sedimentation rate
(very much related to abnormalities in electrically charged components)

<table>
<thead>
<tr>
<th>Higher Sedimentation Rate</th>
<th>Concentration of Plasma Proteins - Globulins - Fibrinogen</th>
<th>Erythrocyte Count</th>
<th>Inflammatory Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td>Cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Some types – Lymphoma, Myeloma)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Periods</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Lower Sedimentation Rate</td>
<td>Erythrocyte Count</td>
<td>Plasma Protein Concentration</td>
<td>Polyglobulia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td>Abnormalities in Erythrocyte Shape</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Starvation</td>
</tr>
</tbody>
</table>

Females – lower sedimentation rate due to:
- Lower erythrocyte count
- Higher concentration of fibrinogen (plasma protein)

- Sedimentation rate – a non-specific marker of inflammation
VISCOSITY

- resistance of blood (liquid) to flow (due to internal friction of blood layers during blood flow + friction of blood and vessel walls)
- expressed in relation to distilled water (without units)
- viscosity of distilled water = 1

blood 4 – 5,3 (x higher than water)
plasma 1,5 – 2 (x higher than water)

- viscosity depends on:
  • *erythrocytes* – count, size, shape
  • *plasma protein* concentration
  • velocity of blood flow
  • diameter of the vessel

Hyperviscosity of blood (occurs in some conditions)
- excessive load for the heart
- aggregation of erythrocytes in small vessels - stops the blood flow – hypoxia
**Erythrocytes – red blood elements (corpuscles)**

**Function**
transport of the respiratory gasses $O_2$, $CO_2$

- erythrocytes lack nucleus and some other organelles - not true cells
- thus the capacity to transport oxygen is increased

**Shape**
- biconcave disc

Advantages of the biconcave shape:
1. **larger surface for gas diffusion** – a surface of biconcave disc is by 30% larger in comparison with a ball of the same diameter

2. **erythrocyte can change its shape** (deformability) – allows to pass through capillaries with diameter lower than diameter of erythrocyte

*(abnormal shapes: spherocytes, drepanocytes, anulocytes, etc. – results in abnormal function and faster destruction)*
### Erythrocyte count

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>males</strong></td>
<td>$4.3 - 5.3 \times 10^{12} \text{L}^{-1}$</td>
</tr>
<tr>
<td><strong>females</strong></td>
<td>$3.8 - 4.8 \times 10^{12} \text{L}^{-1}$</td>
</tr>
</tbody>
</table>

### Abnormalities

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypererythrocytosis (polycytemia, polyglobulia)</td>
<td>- conditions associated with hypoxia</td>
</tr>
<tr>
<td></td>
<td>- e.g. long term stay in high altitudes</td>
</tr>
<tr>
<td></td>
<td>- newborn babies ($7-8. \times 10^{12} \text{L}^{-1}$)</td>
</tr>
<tr>
<td>erythrocytopenia</td>
<td>- less RBCs - often in anaemias</td>
</tr>
</tbody>
</table>

### Size

<table>
<thead>
<tr>
<th>Type</th>
<th>Diameter ($\mu m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>microcytes</td>
<td>$&lt; 6.7$</td>
</tr>
<tr>
<td><strong>normocytes</strong></td>
<td>$7.2 \pm 0.5$</td>
</tr>
<tr>
<td>macrocytes</td>
<td>$7.7 - 9$</td>
</tr>
<tr>
<td>megalocytes</td>
<td>$&gt; 9$</td>
</tr>
</tbody>
</table>
Composition of erythrocytes

Cell membrane
- lipid bilayer
- protein skeleton - formed of **spectrin, actin** – allow to maintain the shape of Ery
- skeleton is attached to the cell membrane by the protein **ankyrin**
- integral membrane proteins (pass “through“ the membrane) involved in the function of Ery: receptors, ion channels etc.
- **antigens** („on the surface“ of the cell membrane)
**Blood groups (blood types)**

**Antigens**

- substances present in cell membranes (also of Ery)
- determine immunological identity of an individual (different people – different antigens)
- immune system is able to recognize cells with „own“ antigens and protect them
- if a foreign cell (with different antigens) enters the body it is recognized as non-self and potentially dangerous
- it starts an immune response, e.g.
  - production of antibodies against this antigen
  - or it reacts with antibodies already present in the body
- antibody is attached to antigen in cell membrane, subsequently the cell is destructed
- function: resistance against foreign agents

- strong antigens – fast and strong immune response
- weak antigens - weak or no response
Presence of antigens in the membrane of erythrocytes (blood group substances) determines the blood group.

Blood type must be considered in:
- transfusions
- transplantations
- gynecology and obstetrics

Major clinical importance (out of all existing blood systems):
1. ABO system
2. Rh system

- strong antigens (i.e. may cause a strong and rapid immune reaction)
- in case of mismatching transfusion – high risk of
  - serious health consequences
  - death

GENERAL RULE: USE MATCHING BLOOD (POSSIBLY THE SAME BLOOD TYPE)
- 4 blood types are recognized in ABO system: **A, B, AB, 0**
  (genetically determined, inherited)
- blood group in the ABO system - determined by:

1. Presence/absence of **antigen A and/or antigen B**
   in the membrane of erythrocytes
   - antigens – glycoproteins
   - antigens related to blood types are called **agglutinogens**

2. Presence/absence of **antibodies anti A and/or anti B** in plasma
   - immunoglobulins
   - produced after birth, maximum levels in the adults
   - antibodies related to blood groups are called **agglutinins**
**ABO – blood groups**

**Blood group**

- **A** (48%)
- **B** (9%)
- **AB** (4%)
- **0** (39%)

**Erythrocytes**

- **Agglutinogen**
- **Antigen**

**Plasma**

- **Agglutinins**
- **Antibodies**

- **A** (anti B)
- **B** (anti A)
- **A, B** (not present)
- **H** (anti A, B)

*substance H is not an antigen*

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**Blood groups and transfusion**

- **incompatible blood (mismatched)**

  - recipient (patient)  
    A / anti B

  - donor  
    B / anti A

- **compatible (matching) blood**

  - recipient (patient)  
    A / anti B

  - donor  
    A / anti B
<table>
<thead>
<tr>
<th>Donor</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>-</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Donor</td>
<td>Recipient</td>
<td>A</td>
<td>B</td>
<td>AB</td>
</tr>
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<tr>
<td>A</td>
<td>A</td>
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<tr>
<td>B</td>
<td>A</td>
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<tr>
<td>AB</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Reaction after transfusion of mismatching blood

• agglutinins get attached to agglutinogens in Er membranes – **agglutination** occurs
  – aggregates of Er are formed (2 – 10 Er attached to a molecule of an antibody)

• possible consequences - more or less serious:
  – immune reaction, **circulatory shock** (breathlessness, pain in the chest, nausea, sweating)
  – hemolysis, icterus, **kidney failure**, death

• symptoms usually occur soon after the transfusion has started – in this case immediately STOP the transfusion

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**ABO compatibility and transplantation**

• the donated organ should be ABO matching
Subgroups exist within blood groups:
A₁, A₂, A₃, Aₓ  B₁, B₂, B₃, Bₓ
• A₁, is stronger antigen than A₂
• subgroups are mismatching - posttransfusion reaction can occur between subgroups (e.g. A₁ and A₂)

Transfusion – general rule: give matching (compatible) blood

Blood derivatives
❌ full blood
  the same blood group
❌ erythrocytes
  - may be given also to some other blood groups
  - O: universal donor
  - AB: universal recipient
❌ plasma
  - may be given to some other blood groups
  - AB – universal donor,
  - O – universal recipient
Rh system

1. presence of **3 antigens** in the membrane of Er:
   (genetically determined)
   - C or c
   - D or d
   - E or e

   - Rh positivity (Rh⁺) – 85% of population
     - determined by the presence of **antigen D** in the erythrocyte membrane
     - CDE, CDe, cDe, cDE

   - Rh negativity (Rh⁻) – 15% of population
     - d antigen present: CdE, Cde, cde, cdE

   - sometimes the presence of E shows a weak positivity in subjects with d antigen

2. **antibodies** in Rh system - **normally not present**
   **However!!!**
   - D is a strong antigen (all the remaining are weak antigens)
   - if Rh⁺ Er enter blood of a Rh⁻ person, D is recognized as a „foreign“ antigen and production of antibodies is started
Rh factor and transfusion

Rh negat donor → Rh negat patient
- the same blood group - matching

Rh posit donor → Rh posit patient
- the same blood group - matching

Rh negat donor → Rh posit patient
- matching - „d“ does not trigger antibody production
Rh posit donor $\rightarrow$ Rh negat recipient
- their production can be triggered if Rh$^+$ erythrocytes enter the blood of a Rh$^-$ individual (e.g. transfusion of Rh incompatible blood)

A/ 1st transfusion – no posttransfusion reaction - no antibodies present in blood of recipient

B/ Rh$^+$ erythrocytes act as antigen and stimulate production of antibodies against antigen D (within weeks) – the individual becomes sensitized (i.e. antibodies are present in his blood)

C/ 2nd transfusion of incompatible Rh$^+$ blood – antibodies react with antigen D, posttransfusion reaction occurs

(„d“ does not induce production of antibodies)
Incompatibility of the blood systems of the mother and the fetus

\[ \text{Rh}^+ \text{ father } + \quad \text{Rh}^- \text{ mother } \rightarrow \]

- A/ \text{Rh}^- \text{ fetus (no problem)} or
- B/ \text{Rh}^+ \text{ fetus (may be a risk)}

1st pregnancy
- circulations of the mother and the fetus are separated by placenta that is a barrier for Er
- usually no problems with Rh incompatibility
- in case of complicated birth, accident, etc.
  the \text{Rh}^+ \text{ erythrocytes of the fetus may enter the blood of the Rh}^- \text{ mother}
- antibody production against baby’s Er is induced in the mother (even as little as 0,5 ml of blood may start the Ab production)
- antibodies remain in blood of a Rh^- mother
2nd pregnancy
- problems occur if the 2nd baby is also Rh⁺
- antibodies from mother’s blood enter blood of the fetus through the placenta, attach to baby’s Er
- agglutination and hemolysis of Er of the fetus

Consequences
- hemolytic disease of the newborn: anaemia, hypoxia, icterus-risk of brain damage, death in utero

Next pregnancies – production of antibodies is even more higher (problems in about 3% of 2nd and 10% of 3rd pregnancies)

Treatment and prevention
- anti-D serum latest until 72 hours after termination of the pregnancy (birth, abortion) is given to the mother
- antibodies anti-D from the serum are attached to the Er of baby (in mother’s blood)
- the Er marked by anti-D are destroyed, thus antibody production by the mother’s body is prevented
Other blood systems

- About 30 blood systems exist
- Clinically significant:

<table>
<thead>
<tr>
<th>Blood System</th>
<th>Blood Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kell (K, k)</td>
<td>MNSs</td>
</tr>
<tr>
<td>Kidd</td>
<td>P</td>
</tr>
<tr>
<td>Lewis (Lewis\textsuperscript{a}, Lewis\textsuperscript{b})</td>
<td>Diego</td>
</tr>
<tr>
<td>Lutheran, etc.</td>
<td></td>
</tr>
</tbody>
</table>

- may cause incompatibility of donor´s and recipient´s blood despite compatibility in ABO and Rh system
- may cause mother/fetus incompatibility
- may cause posttransfusion reaction in individuals who often receive transfusion
Crossmatching test

- assessment of compatibility between blood of donor and recipient
- blood of both donor and recipient is centrifuged, serum is separated from erythrocytes
- test is done in 2 steps:
  1. **major crossmatching test:**
     serum of recipient is mixed with erythrocytes of donor
  2. **minor crossmatching test:**
     serum of donor and erythrocytes of recipient

Result:
- no agglutination = blood compatible
- agglutination = mismatching blood

Biological test
- when transfusion starts
- give 20 ml of blood, then wait about 2-3 minutes
- repeat 2 more times
- check for symptoms of transfusion reaction
- dyspnea, tachycardia, sweating, low blood pressure, dizziness, etc.
Composizione Erythrocyte:
- Acqua 60%
- Materia secca 40%, di cui 95% è hemoglobina

Altri importanti sostanze in citoplasma
- Ioni
- Carbonatdehydratase – importante per il trasporto di CO₂
  \[ H₂O + CO₂ \rightarrow H₂CO₃ \]
- 2,3 BPG – prodotto della metabolismo Erythrocyte, influenza la affinità di Hb a O₂

Erythrocyte metabolism
Gliakolisi: 10% aerobico, 90% anaerobico

Main products:
- **ATP** – mantenimento della forma e elasticità della membrana eritrócita
- **2,3 BPG (2,3 - biphosphoglycerate)**
  - combina con le β catene di deossi-hemoglobina
  - riduce l'affinità del globina sanguigna per O₂ – supporta la sua dissoziazione da emoglobina e diffusione nelle tessuti
  - se l’affidabilità dell’ossigeno è inferiore, la concentrazione di 2,3 BPG aumenta
Haemoglobin (Hb)

Function:
- transport of the respiratory gases $O_2$, $CO_2$
- maintenance of the constant pH of blood

Composition:
4 subunits, each built of:
- **haem** - tetrapyrolic ring (protoporphyrin IX) with centrally bound $Fe^{2+}$
  
  - $2\text{ succinyl Co A} + 2\text{ glycine} = \text{ pyrol}$
  - $4\text{ pyrols} = \text{ tetrapyrolic ring (protoporphyrin IX)}$
  - $\text{ protoporphyrin IX} + Fe = \text{ haem}$

- **globin** (96% of the molecule)
  - chain of amino acids (approx. 140)
  - according to sequence of amino acids 6 types of Hb are distinguished: $\alpha,\beta,\gamma,\delta,\varepsilon,\zeta$
  - in a molecule of haemoglobin always 2 types of chains are present - in pairs
    
    haem + globin = haemoglobin
HEMOGLOBIN TYPES

Adult Hb A
(2α 2β) - 97.5%  Hb A₂ (2α 2δ) - 2.5%

Foetal Hb F
(2α 2γ) - easier combines with O₂ than Hb A

Embryonic Hb E
Gower I (2ζ 2ε), Gower II (2a 2ε), Portland (2g 2ζ)

Fetal haemoglobin
• main form of haemoglobin
  - in foetus
  - and in the newborn until about 6 mo old

• higher affinity to O₂ = binds O₂ more tightly than the adult form, giving the developing fetus better access to O₂ from the mother's bloodstream

• in newborns, fetal hemoglobin is nearly completely replaced by adult hemoglobin by approximately the 6-12 month of postnatal life
Abnormal hemoglobin

- abnormal sequence of amino acids, less than 4 chains in molecule
- abnormal erythrocyte shape, function, life span
- e.g. sickle cell anemia
  - one amino acid in β-chain is changed (glutamic acid → valine)
  - cells have sickle shape
  - become trapped in capillaries – hemolyze, anemia, hypoxia

Normal concentration of hemoglobin

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>140 – 180 g.l⁻¹</td>
<td>120 – 160 g.l⁻¹</td>
</tr>
</tbody>
</table>

Abnormalities

- **anaemia** - decreased haemoglobin concentration
  - usually associated with decreased erythrocyte count and low hematocrit value
DERIVATIVES OF HEMOGLOBIN

Normal

1. oxyhemoglobin - O₂ is bound to Fe²⁺
   (bright red colour)
   - 1 molecule of Hb – maximum 4 molecules of O₂
   - 1 g Hb transports 1,34 ml O₂

   **oxygen carrying capacity**
   - amount of oxygen that hemoglobin in 1 liter of blood is capable of transporting
   e.g. in a male with haemoglobin concentration 160 g.l⁻¹
   160 x 1,34 = 214 (ml)

**Saturation of Hb** = % oxy Hb from total Hb in blood that carries O₂

   arterial blood: 96 – 100 %
   venous blood          75 %
**Oxygen association – dissociation curve**

- indicates saturation of Hb in relation to partial pressure of $O_2$ ($pO_2$)

- sigmoidal shape

- the higher $pO_2$, the more $O_2$ is bound to Hb

- beginning is steep – i.e. already under low $pO_2$ is blood well saturated with $O_2$ (high affinity of Hb to $O_2$)

- after the first molecule of $O_2$ is bound, the spatioal configuration of Hb is changed and next molecules of $O_2$ are more readily bound

High altitudes:

- 3000 m – 60 mm Hg
- 4500 m – 44 mm Hg
Factors affecting combination of O₂ and Hb (oxygen dissociation curve):
1. pCO₂
2. pH
3. temperature
4. content of 2,3 DPG in erythrocytes

(2,3-difosfoglycerát – produkt metabolizmu Er, viaže sa na Hb)

**Affinity of Hb to O₂ is decreased**

= O₂ is more easily released from the bound to Hb

- pCO₂ ↑ (Bohr effect)
- pH ↓
- temperature ↑
- 2,3 DPG ↑

= shift to right and down (e.g. in tissues)

**Affinity of Hb to O₂ is increased**

= O₂ is released from bound with Hb less easily

- pCO₂ ↓
- pH ↑
- temperature ↓
- 2,3 DPG ↓

= shift to left and up (e.g. in lungs)
2. **reduced hemoglobin**
   - after dissociation, no O₂ in hemoglobin (dark red colour)

3. **carbaminohemoglobin**
   - carries CO₂ bound to – NH₂ group of globin chain

**Abnormal**

1. **carboxyhemoglobin**: CO bound to Fe²⁺
   - strongly attracted to hemoglobin (high affinity, 200 times higher than O₂)
     - 0.1% of CO in atmosphere - saturates 50% of hemoglobin
     - 0.3% of CO – saturates 75% of hemoglobin
     - present in higher concentration blood smokers
     - gas produced by cars – accidents (gas heating, car repair in closed garage)

2. **methemoglobin** (met Hb): Fe²⁺ oxidized to Fe³⁺
   - O₂ strongly bound, unable to dissociate from methemoglobin
     - in a healthy human: 0.5 – 2.5% of hemoglobin
     - further increase is prohibited by **met Hb reductase**
     - **babies up to 6 mo** are prone to formation of met Hb (immature body functions)
     - nitrites in drinking water (for milk preparation) cause oxidation of Fe²⁺
HAEMOLYSIS

destruction of the erythrocyte membrane, hemoglobin is released from erythrocyte (e.g. into plasma) (opaque suspension ⇒ transparent solution)

- osmotic
  - hypertonic solution
  - hypotonic solution
    - minimal osmotic resistance: \(0.44 - 0.40 \text{ g } \text{l}^{-1} \text{ NaCl}\)
    - maximal osmotic resistance: \(0.34 - 0.30 \text{ g } \text{l}^{-1} \text{ NaCl}\)

- chemical
  - acids, bases, tensides

- physical
  - thermic energy, irradiation, mechanic energy
    - (e.g. artificial heart valves)

- immunologic
  - transfusion of incompatible blood

- toxic
  - cell lysis caused by enzymes in poison of snakes, wasps, spiders, plants

- daily approx 1% of Ery do hemolyze – old elements
- hemolytic anaemia – decreased Hb concentration due to excessive hemolysis
• **production of Ery**: bone marrow

• **life span**: 120 days

• **destruction of Ery**: spleen

  – **iron and protein** is recycled and used for formation of new Ery
  – **bilirubin** = product of breakdown of Hb, normally present in blood in low concentration – excreted in bile
  – **icterus** (jaundice) – caused by excess of unconjugated bilirubin in blood
    - yellow coloration of the skin and sclera
**Neonatal icterus**

- elevated bilirubin production because of increased breakdown of fetal erythrocytes (and low capacity of newborn’s liver to conjugate bilirubin with glucuronic acid)

- usually not a serious condition, spontaneously disappears in 1-2 weeks

- Bi is neurotoxic – prolonged excess in blood may cause serious consequences:
  - kernicterus = bilirubin induced brain dysfunction
  - permanent mental and motor disability

- serious jaundice may occur in Rhesus incompatibility of mother and baby
Thrombocytes – blood platelets

- cell fragments split from megakaryocytes
- do not contain nucleus
- shape of disc, diameter 2 – 4 mm

Function

- **haemostasis** - formation of the platelet plug
  - blocks the „hole“ in the injured vessel

**Normal count**

150 – 350.10^9 . l^-1
• **cell membrane of platelets**
  - **invaginations** – channel system communicating with the surface of a platelet
  - **receptors** – make the platelets „sticky“ when bleeding occurs

• **cytoplasm of the platelets**
  - **vesicles** (granules: α, β, δ) - contain substances necessary for blood clotting: ADP, ATP, Ca++, platelet clotting factors, enzymes
  - **fibres** = microfilaments – allow contractility of the platelets
  - **dense tubular system** – a store of calcium (without calcium the blood clotting does not proceed)
Haemostasis – bleeding arrest

- a complex process which makes a bleeding to stop
  
  • *maintenance of normal blood volume* – vital
  
  • *massive bleeding may lead to cardiovascular collapse and death*

- haemostasis includes 3 simultaneous interrelated processes

  1/ reaction of the **injured vessel**
  
  2/ activity of the **platelets** (platelet plug formation)
  
  3/ **blood clotting** (haemocoagulation)
1. Vascular constriction

- contraction of the smooth muscle in the vessel wall (circular muscle)

Effects

- a decrease of the vessel diameter
- diminished blood flow through the ruptured vessel
- a decrease of the blood loss
2. Activation of the platelets - formation of the platelet plug

- includes several steps:

A/ ADHESION OF PLATELETS
- endothelial lining of vessels - repels the platelets

- vessel trauma
  - endothelial lining is damaged
  - exposure of subendothelial collagen tissue

- platelets stick to collagen
  - collagen has receptors for thrombocyte receptors

Q: What is the stimulus for platelet activation?
A: Contact of blood with collagen (due to damage of the endothium)

http://asheducationbook.hematologylibrary.org/content/2010/1/387/F1.expansion
B/ CHANGE OF THE SHAPE

- **platelets swell and become spherical**
  - caused by relaxation of the contractile fibres in cytoplasm (actin and myosin)

- **formation of pseudopods** protruding from the surface
  - for easier contact with
    - other platelets
    - collagen (vessel)
    - fibrin threads
  (produced in blood clotting)

C/ THE GRANULE RELEASE

- **releasing reaction – degranulation**
  - active substances are released from platelets into the blood
  where they support haemostasis
  - e.g. serotonin, ADP, thromboxane A$_2$ (TXA$_2$), platelet factors, etc.
**d/ AGGREGATION OF THROMBOCYTES**

- platelets stuck to the collagen stimulate sticking of their further layers

Platelet activity results in formation of the **platelet plug**

- it does not contain fibrin threads, therefore **loose, fragile**
- it is sufficient for **temporary blocking of the bleeding**, especially in small vessels
3. Blood coagulation (haemocoagulation, blood clotting)

- cascade of enzyme reactions following in definite and rapid sequence
- blood contains more than 50 substances related to blood clotting
- major role - plasma **clotting factors** (12 substances)

**Result of haemocoagulation:**

formation of **fibrin threads** - strengthen and stabilize the platelet plug

net of fibrin threads + platelet plug + trapped erythrocytes = **blood clot**

- **blood clot** - seals the broken vessel until the tissue is repaired
# Blood clotting factors

| I. | fibrinogen |
| II. | prothrombin |
| III. | tissue thromboplastin |
| IV. | Ca^{2+} ions |
| V. | proaccelerin |
| VII. | proconvertin |
| VIII. | antihemophilic factor |
| VIII. C | antihemophilic globulin |
| VIII. A | von Willebrand factor |
| IX. | Plasma thrombopastin component |
| | - Christmas factor |
| X. | Stuart – Prower factor |
| XI. | PTA – Plasma thromboplastin antecedent |
| XII. | Hageman factor |
| XIII. | fibrin stabilising factor |

- present in blood
- inactive forms of protheolytic enzymes (majority)
- blood clotting = a cascade of chemical reactions leading to conversion to active forms

\[
\begin{align*}
F_1 & \rightarrow F_{1A} \\
F_2 & \rightarrow F_{2A}
\end{align*}
\]

- synthesized in liver
- vitamin K – required for synthesis of factor II, VII, IX, X

- HK - High molecular weight kininogen
- PK - Prekallikrein
Blood clotting
- can be activated by 2 events (stimuli)

1. exposure of collagen in vessel wall (when endothelium is damaged)
   activates a sequence of chemical reactions referred to as
   intrinsic pathway of clotting

2. release of tissue thromboplastin from the damaged tissue
   activates a sequence of chemical reactions referred to as
   extrinsic pathway of clotting

final reactions of both intrinsic and extrinsic pathway are the same
and they are referred to as
=common pathway

- result: formation of fibrin thread
**INTRINSIC PATHWAY**
- activated by damage of endothelial layer
- f. XII

**EXTRINSIC PATHWAY**
- activated by damage of vessel wall and extravascular tissue
- f. III

**COMMON PATHWAY**

- Prothrombin
- Thrombin
- Fibrinogen
- Fibrin monomer
- Fibrin polymer
- Cross-linked fibrin polymer
INTRINSIC PATHWAY
- activated by damage of endothelial layer - f. XII

EXTRINSIC PATHWAY
- activated by damage of vesel wall and extravascular tissue - f. III

1. fibrin monomer
2. fibrin polymer
3. cross-linked fibrin polymer
4. stabilization of the cross linked fibrin polymer
EXTRINSIC PATHWAY
- activated by
damage of vesel wall and extravascular tissue
- f. III
INTRINSIC PATHWAY
- activated by damage of endothelial layer
- f. XII

EXTRINSIC PATHWAY
- activated by damage of vessel wall and extravascular tissue
- f. III

COMMON PATHWAY
What are the stimuli for haemocoagulation?

1. **exposure of collagen in vessel wall** (when endothelium is damaged)
   - activates a sequence of chemical reactions referred to as **intrinsic pathway of clotting**

2. **release of tissue thromboplastin from the damaged tissue**
   - activates a sequence of chemical reactions referred to as **extrinsic pathway of clotting**

http://ahdc.vet.cornell.edu/clinpath/modules/coags/images/primary.gif
In bleeding both pathways are activated

**Intrinsic pathway** - slow (fibrin threads are formed in 6-10 min)

**Extrinsic pathway** – faster (seconds)

**Blood clot**

- network of fibrin threads running in all directions
  - adhere to damaged surfaces of vessels
- contains trapped plasma, blood elements, coagulation factors

- in 20 -60 minutes after formation the clot retraction takes place
  - caused by the contraction of thrombocytes (contractile fibres – actin, myosin)
  - liquid (serum) is extruded from the clot
  - wound surfaces are drawn together, tissue repair is promoted
Deficiency of clotting factors

**VIII C** – haemophilia A (classical) – bleeding tendency
- blood clotting is slowed down (various degrees of severity)
  - **prolonged** spontaneous or traumatic bleeding, blood in urine, within joints, etc.
  - even though platelets function normally!!
- genetically transmitted deficiency (X chromosome)
- affects only males, females carry the gene but do not show symptoms

Other types of haemophilia

**VIII A** – von Willebrand disease

**IX** – haemophilia B (Christmas disease - rare)
4. **Fibrinolysis** (dissolution of the clot)

The clot can follow one of two courses:

**A/**
- it is invaded by fibroblasts
- connective tissue is formed (in 1-2 weeks)

**B/**
- dissolution of the clot (if the clot is „too large“, excess is dissolved – allows for re-opening of clotted vessels)
- starts approx. in 24 h after bleeding has been checked and tissue repair is underway
plasmin - active component of fibrinolytic system
- formed from inactive plasminogen (plasma protein) by plasminogen activators
- plasminogen circulates in blood and gets trapped in the clot and then activated
- breaks down fibrin, fibrinogen, prothrombin, f. V, VII, VIII

activators of plasminogen
- tissue activators – from damaged endothelium
- plasma activators – e.g. thrombin, kallikrein, HMW kinin
- exogenous activators – streptokinase, urokinase (treatment of haematomas)

plasminogen inhibitors (alpha2-antiplasmin)
- tie plasmin and make it inactive (e.g. after the clot has been dissolved)

- products of the clot degradation – removed by phagocytosis
IN VIVO – Intravascular anticoagulants

- endothelial factors
  - smooth surface of the endothelial layer – non-wettable surface
  - glycocalyx - layer of mucopolysaccharides on the surface of endothelium
    - repels clotting factors and thrombocytes
  - thrombomodulin – protein bound to endothelial cells, binds thrombin

- blood flow – homogenously dispels clotting factors, prevents their local concentration

- anticoagulant substances
  - antithrombin III – binds thrombin, inactivates clotting factors
  - heparin – produced in basophils and mast cells
    - a weak anti-clotting agent
    - its anticoagulant effect increases in complex with antithrombin III
      (100 – 1000 x) – removes thrombin and f. XII, XI, IX, X
  - heparin and its derivatives - used for anticoagulant therapy
  - fibrin – adsorbs thrombin, prevents further conversion of fibrinogen to fibrin
Anticoagulants for clinical use

- tubes from special „non-wettable“ materials (silicone)
  - non-wettable surface = a surface that does not start the blood clotting
- decalcification – binding of Ca\(^{2+}\) ions - oxalate, citrate
- defibrination – removal of fibrin (e.g. snake toxins)
- coumarin derivates (e.g. warfarin)
  - block the effect of vitamin K in liver (long-term effect)
  - production of vitamin K dependent factors is affected – lack of Ca\(^{2+}\) receptor in their molecule
  - are used also as medicaments in anticoagulation therapy
  - effective within 12 hours
- heparin

*In plasma both procoagulant and anticoagulant substances are present.*

*In normal conditions anticoagulants predominate.*

*After the vessel injury procoagulants become activated and override the anticoagulants.*
Leukocytes – White blood cells

- **real cells** – contain nuclei and organelles
- **largest** formed elements in blood
- **lack colour** („white“), become visible after staining (e.g. the Pappenheim method)

**Function**

– **defence against foreign material** - „seek out and destroy“
– **main cells of the immune system** - „mobile units“
  - transported by blood to all parts of the body
  - from blood move into **tissues**, where they spend most of their lives

**Normal count**

- adults, children 4 - 10.10⁹.l⁻¹
- newborns 18 - 20.10⁹.l⁻¹
**Leukocyte count**
- varies throughout the day
  - minimum in the morning
  - maximum in the afternoon

**Leukocytosis** – increased Le count
- normal (Le released from stores)
  - after meal (postprandial)
  - heavy physical activity
  - emotional stress
  - hot environment
  - pregnancy
- abnormal (production of new Le)
  - infectious diseases
  - intoxication
  - cancer

**Leukopenia** – decreased Le count
- some diseases (e.g. influenza, tuberculosis)
- some medicaments

**Leukocyte count**
- varies throughout the day
- minimum in the morning
- maximum in the afternoon

**adults, children** 4 - 10.10^9.l^-1
**Types of leukocytes**

- **granulocytes**
  - specific granules (vesicles)
  - lobulated nucleus - polymorphonuclears
    1. **neutrophilic** 56-64%
    2. **eosinophilic** 1-3%
    3. **basophilic** 0.5-1%

- **agranulocytes**
  - do not contain specific granules
  - mononuclear – simple shape nucleus
    4. **monocytes** 3-8%
    5. **lymphocytes** 24-40%

**differential white blood cell count (leukogram)**
- examination of the % of individual types of leukocytes in %
- helps to make diagnosis - individual types of Le are involved in different functions

!!! in children – the most prevalent type of Le are lymphocytes
Neutrophilic granulocytes (56 – 64 % Le)

Properties
• nucleus 1 – 5 segments (lobes)
  - number of segments indicates age of the cell
  - young cells – one segment („stick“)
  - by maturation the number of segments increases
• cytoplasmic granules - purple colour (lysosomes with hydrolytic enzymes)

Function
• professional phagocytes (microphages) – ingest and destroy foreign material
• involved in non-specific immune reactions
• high motility, first line defence – first arrive to the place of invasion (of all WBC)

Eosinophilic granulocytes (1-3% Le)
• dense purple cytoplasmic granules, 2-segment nucleus
• weak ability of phagocytosis
• involved in:
  - the defence against parasites
  - allergic reactions
Granulocyte kinetics and life span

- formation in the bone marrow, mature elements released into blood
- if stimulated (e.g. inflammatory stimuli), they can pass from blood into tissues through the capillary wall
- life span: 4 - 5 days, then die
- if involved into phagocytosis, they die soon afterwards (i.e. earlier than in 4-5 d)

Basophilic granulocytes (0,5 – 1% Le)

- dark blue granules in cytoplasm
- two segment nucleus
- release active substances:
  - **histamin** – causes vascular dilatation – increases blood flow into areas of tissue damage, facilitates the movement of leucocytes into tissues
  - **heparin** – anticoagulant (useful in immune reactions)
Monocytes (3 – 8% Le)

- largest blood elements, kidney-shaped nucleus

Life fate

- in blood 10-20 h
- from blood - migrate into tissues → here maturate and transform to macrophages
  - free macrophages – actively move in tissues
  - fixed macrophages – in the sites of potential invasion of the pathogens
    - e.g. skin (histiocytes), lungs, liver, lymph nodes

Function: macrophages = professional phagocytes (non-specific immunity)

- antigen presenting cells (process foreign material and present to lymphocytes)

Lymphocytes (24 – 40% Le)

- large round nucleus, narrow cytoplasm
- recirculate

Types

- T-Ly (produced in thymus)
- B-Ly (produced in the bone marrow)
- NK cells (natural killers)
- K (killer) cells, LAK cells

Function: involved mainly in the acquired (specific) type of immunity

Life span: years
Defensive properties of leukocytes

- **chemotaxis** – direction and speed of movement influenced by chemical substances (i.e. produced in the focus of infection)
- **diapedesis** – ability to squeeze and pass through the capillary wall
- **amoeboid motion** – active movement in tissues, the cell projects protoplasmic extensions and follows them
- **adhesivity** – ability to stick to solid surfaces (to receptors in endothelium, bacteria, cells, etc.)
- **phagocytosis** (especially neutrophils and macrophages)
White blood cells and immunity

**Immunity**
- capacity to resist foreign substances that tend to damage tissues and organs
  - microorganisms
  - molecules
  - own abnormal cells (cancer cells, infected and old cells)
- function performed by the **immune system**

**Immune system**
- organs positioned throughout the body
  (thymus, lymph nodes, lymphoid tissue in gut, spleen, etc.)
- **white blood cells** - main cells of the immune system

**Immunity**
1. innate
2. acquired (adaptive)
- develops after birth when the body is first attacked by a foreign substances
1. **Innate immunity** (defense mechanisms present from birth)

**Characteristics**
- the immune response is non-specific
  (it is not targeted at a specific antigen, but is rather equal to different antigens)
- rapid immune response

**Mechanisms** (only those performed by the white blood cells are mentioned)

**a) phagocytosis** (ingestion and destruction of foreign material)
  - professional phagocytes
    - **neutrophils**
    - **macrophages** (more potent phagocytes)
      - (eosinophils – waker ability to perform phagocytosis)

**b) action of some types of lymphocytes (other than B or T):**
- recognize absence of normal “self” antigens in the body’s infected and tumour cells and destroy them
- recognize and kill cells coated with antibodies
**Phagocytosis**
- ingestion and destruction of foreign material (viruses, bacteria, own changed cells, foreign molecules etc.)

**Cells specialized on phagocytosis (professional phagocytes)**
- microphages – *neutrophilic* granulocytes
  - die after phagocytosis

- macrophages (transformed monocytes-operate in tissues)
  - higher capacity of phagocytosis than microphages (can ingest more and larger particles)
  - survive after phagocytosis
2. Acquired immunity (developed throughout the life)

A/ Active immunity
- developed in the body after exposure to a foreign antigen (by an infection, vaccination)
  – after first contact with foreign substance a weak immune reaction occurs
  – in next response is strengthened (principle of vaccination)
- active response of a host
- this immunity is permanent

Active immunization - vaccination
- vaccines contain weakened or dead microbes, that trigger an immune response and active immunity develops

B/ Passive immunization
- transfer of antibodies from exogenous source (e.g. from an immunized donor to a patient, from mother to a newborn via the breastmilk)
- temporary protection (weeks) - no active response of the immune system
Acquired immunity

• **specific**
  = targeted at foreign material that triggered the response

• therefore **highly effective**

• exhibits **immunological memory** (permanent immunity)

• mediated by **B and T lymphocytes**

---

**Naive (virgin) cells**

• B and T lymphocytes before they „meet“ the antigen

---

**Effector cells**

• lymphocytes, that were activated by an antigen (who carry receptors for that specific antigen)

---

**Memory cells**

• lymphocytes that were once activated can „remember“ the foreign agent

• after repeated contact with that particular antigen they can produce clones directed against the antigen

---

[Image links to additional resources]
**Effector cells of acquired immunity**

**B-Lymphocytes**
- formation and maturation in bone marrow
- mediate **humoral type** of immunity

- B-Ly after recognizing the foreign agent - transform into **plasma cells**
  - macrophages – antigen presenting cells (phagocytosis of the foreign material and exposure of the antigens into their cell membranes)
  - activation requires cooperation with T-lymphocytes

- plasma cells produce specific molecules of **antibodies** (immunoglobulins):
  - Ig M  Ig A  Ig G  Ig D  Ig E

- antibodies bind to the foreign agent (e.g. the bacteria) and mark it for destruction (by phagocytosis or by other mechanisms)
**T-lymphocytes**

- formation in bone marrow, maturation in the thymus
- exhibit cell mediated immunity - directly destroy the target cells (mainly virus infected cells)

*• T_C (cytotoxic) – directly kill* foreign cells by releasing substances that attack their cell membranes (make „a hole“ in the membrane)

*• T_H (helper)*

- required for activation of B-Ly (without their cooperation the B-Ly cannot recognize majority of antigens – failure of the immune system)
- produce *interleukins – regulate* the immune response

*• T_S (suppressor)* - close down the immune response after invading organisms are destroyed and the immune response has achieved its goal
**Hematopoiesis**
- development of blood cells

**Life span of the blood elements**

<table>
<thead>
<tr>
<th>Blood Element</th>
<th>Life Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>120 days</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>4 - 5 days</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Weeks/Months</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Months/Years</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>8 – 10 days</td>
</tr>
</tbody>
</table>

**Hematopoiesis**
- production, differentiation and maturation of the blood elements
- maintenance of normal count of formed elements in blood
Development of hematopoiesis

Mesenchymal period
- 2 - 12 weeks prenatally
- yolk sac
  - early forms of erythrocytes

Hepato-lienal period
- from 6. week prenatally
- liver, spleen
  - early forms of Er, Le, Tr

Medullary period
- from 20. week of fetal life – bone marrow is the chief organ of hematopoiesis
- production in bone marrow starts
  - erythro-, thrombo- and leukopoiesis
## Bone marrow

- **a neonate, small child**
  - hematopoiesis in all bones

- **an adult**
  - flat bones
  - vertebrae
  - epiphyses of humerus, femur

- **red** – active hematopoiesis
- **yellow**
  - infiltrated by fat cells
  - in time of great demand, may become hematopoietic tissue again
- **grey** – no hematopoiesis
Haematopoietic stroma - stromal cells
- microenvironment that induces the differentiation of stem cells into the several blood-cell lines
- support the hematopoietic tissues
**Development of blood elements – blood cell lines**

- **Multipotent stem cell**
- **Common progenitor stem cells**
- **Precursor cells (unipotential)**

**Series**

Multipotential haematopoietic stem cell

- **Hematopoietic (myeloid)**
  - Erythroid
  - Megakaryocytic
  - Myelomonocytic

- **Lymphopoietic**
  - B-Ly
  - T-Ly
  - NK

- **Myeloblast**
  - Monoblast

- **Neutrophils (Ne)**
- **Basophils (Ba)**
- **Eosinophils (Eo)**

- **Granulocytes**
- **Monocytes**

- **Red blood cells**
- **Thrombocytes**

**Stem cells** – able to divide by mitosis, stores are continuously replenished

**Unipotential cells** – maturate into predetermined cells (colony forming units)
ERYTHROPOIESIS

Formation of red blood cells
• pronormoblast
• normoblast
  - basophilic
  - polychromatic
  - ortochromatic – expells the nucleus
• reticulocyte
  - lacks nucleus
  - larger than erythrocyte
  - contains small amount of RNA (i.e. ability to synthetize Hb)
  - in small counts present in blood (in 2 days in blood mature into Er)
• erythrocyte – mature element without nucleus and organelles

Maturation of the cells
- decrease in size
- decrease of RNA content
- increase in hemo – globin concentration
- blue colour is changing into pink
- expells the nucleus
Substances for erythropoiesis
- provided by diet - nutrients
  ✗ amino - acids
  ✗ iron
    A/ food derived - \( \text{Fe}^{3+} \)
    B/ recycled – from destroyed erythrocytes
    * deficiency – hypochromic anemia
  ✗ copper (plasma ceruloplasmin – stores)
    - helps in utilisation of iron stores
    * deficiency – hypochromic anemia
  ✗ cobalt
  ✗ vitamin B12 – extrinsic factor
    - it can be absorbed in small intestine only if bound to intrinsic factor
      produced in gastric mucosa
  ✗ folic acid
    - synthesis of heme (tetrapyrrol ring)
    * deficiency of B12 or folate – pernicious anaemia (megalocytic)
Regulation of erythropoiesis

erythropoietin
– a hormone produced in kidneys – glomerulus (mesangium)

effects of erythropoietin
• stimulates the stem cells – formation and release of erythrocytes into blood

erthropoietin production
- stimulated by hypoxia
(anaemia, staying in high altitudes, lung diseases, etc.)

source: http://wdict.net/img/erythropoietin.jpg
THROMBOPOIESIS

• megacaryoblast
• basophilic megakaryocyte
• granulated megakaryocyte

- polyploid cells (do not divide after replication, only nucleus is divided)

- extensions into capillaries in bone marrow

- platelets formed by fragmentation of cytoplasm

• thrombocyte
LEUKOPOIESIS

Granulocytes

- myeloblast
- promyelocyt
- myelocyt - Ne, Ba, Eo
- metamyelocyt - Ne, Ba, Eo
- stick - Ne, Ba, Eo
- granulocyte - Ne, Ba, Eo

Maturation of cells
- decrease in size
- granules are formed
- nucleus becomes smaller and lobulated
**LEUKOPOIESIS**

**Monocytes**
- monoblast
- promonocyt
- monocyct
- macrofage

**Lymfocytes**
- antigen independent cells
- immunocompetent cells (immunoblasts)
- lymphocytes
  - activated T-lymphocytes
  - plasma cells (from B lymphocytes) – after contact with antigen
Leukopoiesis

Granulocytes and monocytes
• granulopoietin – colony stimulating factor
produced in – monocytes, macrophages, activated T-Ly, endothelial cells
effects:
- stimulates proliferation and differentiation of granulocytes and monocytes
• growth factors stimulating granulocytes and macrophages
• exogenous factors – bacterial toxins
• lymphocytes – produce growth factors (e.g. lymphokines)

Lymphocytes
immunohormones (e.g. thymosin, thymopoietin), interleukins, growth factors

Thrombopoiesis
• thrombopoietin – humoral factor produced by kidney
- negative feedback
• factors stimulating colonies of megakaryocytes
• interleukins
Blood plasma

- liquid part of blood, component of the body fluids

**Body fluid compartments** (as % of body weight)

Total body fluids 60%
1. intracellular (ICF) 40%
2. extracellular (ECF) 20%
   - intravascular (plasma, lymph) 4 - 5%
   - interstitial (among cells in tissues) 15%
   - transcellular 1%
   (intraocular, synovial, pericardial, peritoneal, cerebrospinal fluid, etc.)

- ECF and ICF differ in ion composition
  
  **Main ions in**
  - extracellular fluid: Na⁺, Cl⁻, HCO₃⁻
  - intracellular fluid: K⁺, PO₄⁻
Plasma
transparent yellow fluid (separated from blood by standing or by centrifugation)

**Constituents:**
1. water 90%
2. dissolved substances – solutes 10%
   A/ organic – plasma proteins
   – other organic substances
   B/ inorganic

**PLASMA PROTEINS**
- produced in liver (except gamma globulins produced by the lymphocytes)

**Composition and amount**

<table>
<thead>
<tr>
<th>total protein</th>
<th>proteinemia</th>
<th>60 - 80 g .l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>fractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>albumin</td>
<td></td>
<td>35 - 50 g .l⁻¹</td>
</tr>
<tr>
<td>globulins (α₁, α₂, β, γ)</td>
<td>25 - 40 g .l⁻¹</td>
<td></td>
</tr>
<tr>
<td>fibrinogen</td>
<td></td>
<td>1,5 - 3,5 g .l⁻¹</td>
</tr>
<tr>
<td>albumin - globulin ratio</td>
<td>1,5 - 2</td>
<td></td>
</tr>
</tbody>
</table>
Functions:

- **Carriers** for other molecules
  - bind and transport substances (e.g. lipids, Fe, hormones, drugs, etc.)
  - if bound to proteins:
    - substances insoluble in water - become soluble
    - too fast filtration of substances with low molecules by kidneys is prevented
    - the effect of substances, e.g. hormones is slower and prolonged

- stability of blood suspension – affect the *sedimentation rate*

- influence the **blood viscosity** and normal blood flow

- maintenance of **constant pH** - protein buffer
  - proteins bind excess acids/bases, thus balance the pH of blood

- **Nutritional** function – rapid supply of amino-acids for tissues

- generate the **colloid – osmotic pressure** 3.3-4 kPa

- **Blood clotting** - plasma clotting factors

- **Immune functions** – immunoglobulins, complement
OTHER ORGANIC SUBSTANCES IN PLASMA

- include many different substances
  - glucose
  - lipids, cholesterol, triglycerides
  - nitrogen containing non-protein substances - creatin, creatinin, urea, uric acid
  - bilirubin, hormones, vitamins, etc.

- constant plasma concentration, e.g.
  - glycaemia 3,05 – 5,6 mmol.l⁻¹
  - cholesterolamaemia 2,8 – 5,0 mmol.l⁻¹

- their plasma levels indicate function of various organs or systems
  - hormone levels-endocrine system
  - creatine-kidneys
  - bilirubin-liver
  - glucose-pancreas, etc.

- affect plasma properties only to a small extent
INORGANIC SUBSTANCES IN PLASMA (IONS)

Main cations:
- sodium, calcium, potassium, iron, magnesium, copper, iodine

Main anions:
- chlorides, bicarbonates, phosphates

Function
- influence physical and chemical properties of plasma, e.g.
  - pH
  - osmotic pressure,
  - constant volume etc.
- participation in various biologic processes, e.g.
  - buffering
  - excitability of cells
  - permeability
  - blood clotting, etc.

Serum = plasma without fibrinogen and some other clotting factors
(when standing in a tube - plasma will form a clot, serum will remain liquid)
-plasma (but also all body fluids) contains dissolved substances that are osmotically active and give rise to osmotic pressure.

**Osmosis** - diffusion of solvent through semipermeable membrane from space with lower concentration of solute into the space with higher concentration.

- semipermeable membrane - permeable only for solvent, not for dissolved substances.

**Osmotic pressure** – water (solvent) passes the semipermeable membrane under pressure called osmotic pressure.
- the bigger the difference in concentration, the higher is the osmotic pressure.
Osmotic pressure of plasma

- the pressure that plasma (or any of the body fluids) would exert if separated from pure water by a membrane permeable only to water
- normal value 690 kPa

- osmotic forces are generated by
  96 % - electrolytes (of them 70 % NaCl),
  4% non – electrolytes (glucose, albumin)

- osmolarity of plasma (concentration of osmotically active substances):
  290 - 300 mmol.l⁻¹

Some functions in human body are based on osmotic pressure, e.g.:
  • regulation of water balance - hypothalamus monitors osmolarity of plasma
  • absorption in gut
  • water reabsorption in kidney
  • osmotic pressure needs to be considered when patient is given an infusion, or in laboratory experiments with blood

- blood plasma and blood elements – are isoosmotic (isotonic)
  = osmotic equilibrium – no water gain/loss
A/ Isotonic solutions
– the same osmotic pressure as plasma, optimum for function of Ery

B/ Hypertonic solutions
– higher osmotic pressure
– if a cell ("isotonic solution") is put into a hypertonic solution, it loses water, shrinks and may malfunction or die due to rupture of its membrane (haemolysis)

C/ Hypotonic solutions
– lower osmotic pressure
– water flow is directed into the erythrocyte
– cause expansion of cells, their malfunction and eventually destruction and death (haemolysis)

osmotic equilibrium can be broken in dehydration, after infusion of non-isotonic solution
In intravenous administration of solutions (fluids, nutrients, drugs)

• their concentration of osmotically active substances is adjusted to isotonicity

• isotonic solutions:
  – 0,9 % NaCl (physiological solution)
  – 5 % glucose
  – they can be infused without danger of disturbing osmotic equilibrium

• non – isotonic solutions may be used in special circumstances
  – e.g. hypertonic solution in cerebral oedema – water is attracted from brain tissue into the circulation
**Oncotic pressure (colloid-osmotic pressure of plasma proteins)**

- a component of osmotic pressure
- exerted by plasma albumins
- normal value: 3.7 – 4 kPa

(out of 690 kPa of the total osmotic pressure)

**Function:**
- plays role in water exchange in capillaries
- it exerts resorption pressure in capillaries – that allows the return of water from tissues into capillaries
- it prohibits water loss from circulation
- main factor for maintenance of constant blood volume
Blood capillaries

1. are permeable for low molecular weight substances (e.g. ions)
   - ions can freely cross the capillary membrane in both directions (tissue - capillary)
     therefore
   - the osmotic pressure of low-molecular weight substances in capillaries = 0
   - no net changes in water volume

2. are impermeable for plasma proteins (macromolecules)
   - plasma proteins exert oncotic (colloid-osmotic) pressure on capillary wall
   - concentration of proteins in plasma >> concentration of proteins in tissue fluid
   - water moves from tissues (interstitial fluid) into capillaries
**Capillary filtration and reabsorption** (effect of blood and oncotic pressures)

- **arterial end of capillary**
  
  blood pressure + oncotic pressure of interstitial fluid > **oncotic pressure of plasma**
  
  - filtration - liquid passes from capillary into interstitial space (4 + 0.7 > 3.7 kPa)

- **venous end of capillary** (blood pressure here lower than at the arterial end)
  
  blood pressure + oncotic pressure of interstitial fluid < **oncotic pressure of plasma**
  
  - resorption – water passes from interstitium into capillaries (2 + 0.7 < 3.7 kPa)

- normal concentration of plasma protein level – **maintenance of constant blood volume**
- hypoproteinemia (e.g. due to starvation, liver diseases, kidney disease) - oedema
Regulation of acid–base balance

- maintenance of constant pH value

- normal pH (arterial blood): $7,40 \pm 0,04$ (i.e. $7,36 - 7,44$)

- essential, because pH significantly affects enzyme systems, metabolism, membrane permeability, etc.

  - $pH$ – concentration of $H^+$ - $[H^+]$
  - normal $[H^+] = 40 \text{ nmol} = 0,00000004 \text{ mol/l} = 4 \times 10^{-7} \text{ mol/l}$
  - $pH = - \log [H^+]$
  - $pH = 7,4$

- Internal environment = body fluids and substances dissolved in them
- Normal function of the body requires constant composition of body fluids
  - volume, osmolarity, concentration of ions, pH

- **homeostasis**
  - includes maintenance of constant conditions in the internal environment
  - strictly controlled by regulatory mechanisms

Regulation of acid–base balance = maintenance of constant pH value
Disorders of acid – base balance are caused by excess of

- acids - substances that release $H^+$, thus cause:
  - an increase concentration of $H^+$
  - a decrease of pH

- bases (alkalis) - substances that accept $H^+$, thus cause
  - a decrease in concentration of $H^+$
  - and an increase in pH

normal pH $7,40 \pm 0,04 (7,36 – 7,44)$

- increased concentration of $H^+$
  pH $< 7,36$ acidosis
  pH $< 7,0$ leads to death

- decreased concentration of $H^+$
  pH $> 7,44$ alkalosis
  pH $> 7,8$ leads to death
- Abnormalities in pH may be caused by
  
  - **metabolic** disorders (metabolic acidosis, metabolic alkalosis)
  - **respiratory** disorders (respiratory acidosis, respiratory alkalosis)

- **Normal metabolism – production of acids predominates**

- In normal metabolic processes in the body 12,000 mmol/l $\text{H}^+$ are produced daily, that must be eliminated from the body

- Physical activity, diseases - even higher production of acids

- Exposure to alkaline substances is less frequent
  
  - Sources: diet (fruit, vegetables)
Chemical buffer systems
- substances present in body fluids that bind acids or bases, thus neutralising them
- react immediately (seconds) and correct changes of pH
- keep the ions tied temporarily until the balance is reestablished
- do not eliminate $\text{H}^+$ from the body
- limited capacity – can be exhausted
- can be reestablished (e.g. by involving of physiological mechanisms)

Regulatory mechanisms of acid-base balance

1. Chemical buffer systems

2. Physiological mechanisms
- activated in increased load (of acids / bases)
- eliminate excess acids/ bases from the body
  A/ respiratory system – quick response
  B/ kidney – delayed response (hours, days)
  - the most powerful system
- allow for regeneration of chemical buffers
1. The bicarbonate buffer system

- system consisting of a weak acid + its basic salt
- ability to tie H⁺ (if its concentration increases) or release H⁺ (if its concentration decreases) and thus minimize changes of pH
- total buffering capacity in human body 48 mmol/l

**Chemical buffer system**

- system consisting of a weak acid + its basic salt
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**1. The bicarbonate buffer system**

consists of 2 components (ratio 1:20)
- a weak acid
- bicarbonate salt

\[
\text{excess of } H^+ : \\
H_2CO_3 \leftrightarrow H^+ + HCO_3^- \\
\text{deficit of } H^+ : \\
H_2CO_3 \rightarrow H^+ + HCO_3^- \\
\]

- the most important buffering system
- half of the total buffering capacity (24 mmol/l)
- tightly cooperates with lungs and kidneys that eliminate excess of acids/bases (H⁺, HCO₃⁻, CO₂)
- main extracellular buffer, operates in erythrocytes
2. **Protein buffer** (buffering capacity 15 mmol/l)
- plasma proteins - amphoteric
  = ability to release or to bind H^+ ions according to pH
- acidic environment (i.e. excess of H^+ ) – combine with H^+
- alkaline environment (i.e. deficiency of H^+) – release H^+

3. **Hemoglobin buffer** (buffering capacity 7 mmol/l)
  deoxygenated Hb (basic) / oxygenated Hb (acidic)
  
  **tissues**
  • O₂ is released from oxy Hb, H^+ combines with deoxy Hb
  
  **lung**
  • O₂ combines with deoxy Hb, H^+ is released from oxy Hb
  • H^+ + HCO₃⁻ → H₂CO₃
  • H₂CO₃ → H₂O + CO₂ - eliminated in lungs

4. **The phosphate buffer system** (buffering capacity 2 mmol/l)
  HPO₄²⁻ + H⁺ → H₂PO₄⁻
  - low concentrations in plasma
  - main intracellular buffer, participates in regulation of pH of urine