Blood Physiology 1

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- Blood collection (venous, capillary)

Practical tasks
- Capillary blood collection
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- The count of erythrocytes
- Determination of haematocrit value
- Determination of haemoglobin content
- Teichmanns crystals
The body fluids

- Blood – „fluid tissue“
- Water comprises for about 60% of the human body

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

<table>
<thead>
<tr>
<th>Total body fluids</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. intracellular (ICF)</td>
<td>40%</td>
</tr>
<tr>
<td>2. extracellular (ECF)</td>
<td>20%</td>
</tr>
</tbody>
</table>
  - intravascular (blood plasma, lymph) | 4 - 5% |
  - interstitial (among cells in tissues) | 15% |
  - transcellular | 1% |

(product of secretory activity of cells - 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⁻
Blood – definition, composition and function

**Definition**
1. Red, opaque liquid that circulates in blood vessels, connective tissue
2. Suspension of the blood elements in blood plasma.

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

**Blood volume**
- 7% of body weight
  - male 6 l
  - female 4.5 l

**hypovolemia**
- bleeding
- dehydration

**normovolemia**

**hypervolemia**
- pregnancy
- in some diseases (e.g. kidney failure)
Blood – definition, composition and function

Functions of blood:
1. transportation of
   - \( \text{O}_2, \text{CO}_2 \)
   - nutrients
   - metabolic waste products
   - organic and inorganic substances
     (e.g. ions, clotting factors, hormones, vitamins)
   - components of immune system
   - heat - thermoregulation

2. haemostatic function
   - haemostasis = bleeding arrest
   - involves components of blood (platelets, clotting factors) that are activated in bleeding
   - as a result the vessel is sealed
3. takes part in maintenance of homeostasis

- **homeostasis** = maintenance of constant internal environment despite fluctuations in external environment
  - constant temperature – **isothermia**
  - constant pH = concentration of $H^+$ - **isohydria**
  - ion concentration and osmotic pressure - **isoosmia**
  - volume of water in the body – **isovolaemia**
  - etc.

4. maintenance of the blood pressure

- blood pressure – pressure of blood on the vessel wall
- normal blood volume is required for maintenance of normal blood pressure
- excessive bleeding $\rightarrow$ decreased blood volume $\rightarrow$ a decline in blood pressure
Capillary blood collection

Material:
- sterile needles, cotton swabs, disinfecting agent (alcohol/aether)

Procedure
- disinfect the tip of the fourth or third finger of the non-dominant hand (swabs soaked in alcohol/aether)
- after the skin has dried, make a skin puncture just off the centre of the finger pad (insert the needle 2 – 3 mm in depth)
- wipe off the first drop (usually contains excess of tissue liquid)
- collect next drops for biochemical analyses
- put a swab to the wound, press until the bleeding has stopped

- do not squeeze the finger – tissue fluid may alter the results
- if patient´s hands are cold – warm them in water
Venous blood collection

- ask the patient if he ever underwent a venous blood collection
- if yes, ask about possible problems (collapse, dizziness, etc.)
- if patent tends to collapse, decide for collection in lying position, otherwise patient is sitting opposite the doctor

- find a suitable vein - the most common site is the antecubital area of the arm - veins are most prominent
- look at both right and left cubital area – select the „better“ vein
- if no veins can be located, place the Esmarch tourniquet on patient´s arm, the blood congestion makes the veins better visible
- „pumping, exercising“ with the forearm may help
- if not successful, try to palpate a vein
- if not successful, attempt to locate a vein on the back of the hand
1. Preparation

- prepare all the equipment necessary for blood collection:
  1. cotton swabs (recommended 3 pieces)
  2. alcoholaether
  3. Esmarch tourniquet
  4. needles (or a lancet)
  5. syringes
- place the Esmarch tourniquet on the upper arm (the doctor pulls, the patient holds it tight)
- ask the patient „to make a fist“

- disinfect the area of the intended puncture site
- let the area dry
- hyperextend the arm
- stabilise the vein by stretching and holding the skin at both sides
- hold the syringe as shown in the picture

Source: http://t1.gstatic.com/images?q=tbn:ANd9GcTGctDFFbQuSdSv2b9z6EzGltJ66Zz44X5qS6lzQuolrpK5Y0&t=1&usg=__L6MkQE_8Pu-qqlEv2Py1hocLSzA=
2. Blood collection

• puncture the skin and insert the needle into the vein (see the photo)
• how to know that you are inside the vein???
  • when you will feel that the resistance became lower
  • when blood occurs in the tip of the syringe
• pull the syringe piston with your left hand and collect the required volume of blood

3. How to finish?

• release the tourniquet - always before you take out the needle
• put a cotton swab over the place of puncturing
• withdraw the needle
• ask the patient to press the cotton for about 5 min
• dispose the needle immediately into the designated container

Venous blood collection video (Fyzio HD)
https://www.youtube.com/watch?v=BdP3rnt-C24
Task: The count of erythrocytes
**Function** - transport (O₂, CO₂)

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

**Shape**

- biconcave disc
- advantages:

  1. *larger surface for gas diffusion* – surface of a 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 Ery through capillaries with diameter lower than their diameter
### The erythrocyte count

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

### Abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Causes</th>
</tr>
</thead>
</table>
| hypererythrocytosis (polycythemia, polyglobulia) | - in prolonged hypoxemia (e.g. long term stay in high altitudes, diseases)  
- prenatal period, newborn babies ($7-8 \times 10^{12} \text{L}^{-1}$) |
| erythrocytopenia                                 | - low production of RBC (e.g. lack of nutrients)  
- excess losses (e.g. bleeding)                   |

### Size

<table>
<thead>
<tr>
<th>Size</th>
<th>Diameter ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>microcytes</td>
<td>$&lt; 6.7$</td>
</tr>
<tr>
<td>normocytes</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>

### Lifespan

- formation in bone marrow  
- requires sufficient intake of nutrients: Fe, proteins, vit $B_{12}$, folate, Co, Cu  
- survive for 120 days, destruction in spleen
Procedure

• pipette 4975 µl of Hayem's solution into a flask (automatic pipette doser-volume preset) – work carefully, Hayem’s solution is toxic
• make a venous blood collection
• take 25 µl of blood with a pipette, clean the tip of the pipette
• put the blood into the flask with Hayem's solution
• rinse the pipette 2 -3 times with the Hayem's solution (=take into the pipette –then press out)
• mix

• place the coverslip on the Buerkers´s chamber, fix it with the clips
• take a small volume of the solution into a pipette
• place the pipette at the edges of the coverslip (1,2) – do not drop the blood not on the coverslip
• the liquid will flow to the chamber by capillary attraction
Procedure (cont.)

• place the chamber to the microscope
• use the objective $25 = 250 \times$ enlargement
• find the grid of the chamber
  – in case of problems – adjust the intensity of light
• find oblongs
• count the number of Er in 20 oblongs
  – count all the elements that are inside oblongs
  – if an element is touching a line (from inside or outside)
    • select two adjacent sides of each oblong, take into account Er touching the gridline
    • do not count Er touching opposite sides
• Result: make a sum of Er in 20 oblongs, divide it by 100 and multiply by $10^{12} = \text{Er count per liter}$
• Conclusion: Is the result normal? If not what may be the reason?
Determination of the haematocrit value
Haematocrit (PCV - packed cell volume)
- the proportion of blood volume that is occupied by the red blood cells

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

**Normal values**

<table>
<thead>
<tr>
<th></th>
<th>males</th>
<th>females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.39 – 0.49</td>
<td>0.35 – 0.43</td>
</tr>
<tr>
<td></td>
<td>(39 - 49%)</td>
<td>(35 - 43%)</td>
</tr>
</tbody>
</table>

**Abnormalities of haematocrit**

<table>
<thead>
<tr>
<th>causes</th>
<th>a change in the erythrocyte count</th>
<th>a change in the blood volume (plasma volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>decreased hematocrit</td>
<td>↓ - anaemias (less RBC, smaller RBC) - bleeding</td>
<td>↑ - kidney failure (oliguria, anuria) - after infusion (e.g.) saline</td>
</tr>
<tr>
<td>increased hematocrit</td>
<td>↑ - living in high altitudes - polycythaemia</td>
<td>↓ - dehydration</td>
</tr>
</tbody>
</table>
Determination of the haematocrit value
Procedure

• make a capillary blood collection
• suck a drop of blood into a microcapillary (2/3 - ⅓, avoid air bubbles)
• seal one end of the capillary by melting it over fire (the end without blood)
• place the capillary into the centrifuge (sealed end facing the margin)
• centrifuge 3 min at 14 000 rpm

suck a drop of blood into a microcapillary

avoid air bubbles

seal one end of the capillary

sealed end facing the margin
1. place the capillary into the groove of the vertical marker
2. bottom of the capillary matches the line on the bottom of the vertical marker
3. put the moveable arm in such position that the black line matches the upper level of the plasma column
4. move the vertical marker to the left until the black line on the moveable arm matches the Ery column
5. read the haematocrit value up on the scale = result
6. draw conclusions (is the result normal?)

Questions
1. Explain why in an acute blood loss the patient´s hematocrit may be normal
2. Give examples of clinical conditions associated with lower haematocrit
Determination of the haemoglobin content
Composition of Ery:
- water 60%
- dry matter 40 %, of that: 95 % is haemoglobin

Other important substances in cytoplasm
- ions
- carbonatdehydratase – enzyme important for transport of CO₂
  
  $\text{Catalyzes reaction: } H_2O + CO_2 \rightarrow H_2CO_3$

- 2,3 BPG
  - product of Ery metabolism, it affects the affinity of Hb to O₂
  - higher production leads to a decrease in affinity enhancing the ability of RBCs to release oxygen into the tissues (lower affinity)

Normal concentration of haemoglobin

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

Abnormalities
- anaemia - decreased haemoglobin concentration
  - usually associated with a decreased erythrocyte count and low haematocrit value
**Haemoglobin (Hb)**

*Function:* transport of the respiratory gases $O_2$, $CO_2$

*Composition:*

a molecule contains 4 subunits, each built of:

- **haem** - tetrapyrol ring (protoporphyrin IX) with centrally bound $Fe^{2+}$

- **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

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**HAEMOGLOBIN TYPES**

- **adult**  
  Hb A ($2\alpha 2\beta$) - 97.5%  
  Hb $A_2$ ($2\alpha 2\delta$) - 2.5%

- **fetal**  
  Hb F ($2\alpha 2\gamma$) combines easier with $O_2$ than Hb A (advantage for the foetus)

- **embryonic**

Source:  
http://www.chm.bris.ac.uk/motm/hemoglobin/heme.gif1
DERIVATIVES OF HAEMOGLOBIN

Normal

1. oxygenated haemoglobin
   – molecule of haemoglobin that carries $O_2$ - bound to $Fe^{2+}$
   (blood rich in oxy haemoglobin had bright red colour)

   - 1 molecule of Hb – maximum 4 molecules of $O_2$
   - 1 g Hb transports 1,34 ml $O_2$

Oxygen carrying capacity

– amount of oxygen that haemoglobin in 1 L of blood is able to transport

   - e.g. in a male with haemoglobin concentration 160 g.l$^{-1}$
   - $160 \times 1.34 = 214$ (ml of $O_2$ in 1 litre of blood)

2. reduced haemoglobin
   - after dissociation, no $O_2$ bound to haemoglobin
   - blood rich in reduced haemoglobin (veins) - dark red colour

3. carbaminohaemoglobin
   - a molecule of haemoglobin that carries $CO_2$ bound to – $NH_2$ group of globin chain
Abnormal

1. **carboxyhaemoglobin** – CO bound to the molecule of haemoglobin

- CO competes with O\textsubscript{2} for the same binding site (Fe\textsuperscript{2+})
- CO has high affinity to haemoglobin (200 times higher than O\textsubscript{2})

- atmosphere/ inspired air – contains 21 % of O\textsubscript{2}  
- if 0,1% of CO is present in the inspired air
  - saturates 50 % of Hb (50% transport capacity for O\textsubscript{2} left) - mild poisoning:
- if 0,3% of CO is present in the inspired air
  - saturates 75 % of Hb (only 25% transport capacity for O\textsubscript{2} left) - moderate poisoning
- above 1,2% - severe poisoning – lethal after a few minutes

CO is a product of combustion:
- it is produced by cars, gas heaters
- contained in cigarette smoke (smokers may have high concentrations, up to 20%)
2. **methaemoglobin** - Fe$^{2+}$ oxidized to Fe$^{3+}$

- Met Hb – unable to transport oxygen to the tissues

- in a healthy human 0,5 – 2,5 % of total haemoglobin

- **met Hb reductase** in erythrocytes converts metHb back to Hb
  (prevents excessive formation of met Hb)

- tissue hypoxia occurs

- main symptom - cyanosis (blue colour of the skin)

- babies up to 6 mo – low activity of the metHb reductase – therefore prone to formation of met Hb

- formula fed infants may develop **methaemoglobinaemia** – high concentration of metHb
  - formula prepared with well water may contain nitrites, which are an oxidizing agent, and may cause oxidation of Fe$^{2+}$ into Fe$^{3+}$
Determination of the haemoglobin content

Procedure

- pipette 7 ml of Drabkin solution into a tube (automatic doser)
- make a capillary blood collection – 25 µl of blood into a pipette
- add blood into the Drabkin solution in the tube
- rinse the pipette 2-3 times with the solution from the tube
- mix well and leave standing for 15 minutes
- into a spectrophotometer put a cuvette with Drabkin solution (blank)
- spectrophotometer gets preset to the optical density of the blank
- read the optical density of the sample (spectrophotometer will automatically subtract optical density of the Drabkin solution)

Report

- in the tables find the value of haemoglobin concentration corresponding to the obtained value of optical density
- is the value normal?
- if not, try to explain
Task: Teichmann´s crystals

- microscopic crystals of the chlorhaemin
- chlorhaemin – derivative od haemoglobin (formed from the haem in acidic environment after adding NaCl)
- rhomboid shape, dark brown colour

- Teichmann´s crystals
  - an old forensic method to prove blood
Teichmann’s crystals
Procedure

- make capillary blood collection
- put a drop of blood on a glass slide
- add 1-2 drops of acetic acid
- add salt (not more than a few crystals!!!)
- mix together, spread on the glass slide
  (make a fine, thin layer)
- heat mildly over a flame, until the sample dries

- observe in a microscope - use immersion oil and objective 100
  - Teichmann’s crystals are brown and rhomboid
  - transparent cubic crystals are NaCl !!

Report
A/ Draw a picture of the crystals observed in a microscope
B/ What is the difference between haem and haemin?
C/ What types of haemoglobin exist in humans?
**Topics to study**

- Blood – its composition and functions, normal volume
- Main functions of the blood components
- Homeostasis - definition and its main parameters (isoionia, isohydria, isosmia, isovolemia),
- Importance of homeostasis for the function of the body
- Body fluids – classification, main cations and anions in ECF, ICF.
- Haematocrit - definition, normal values in males and females and in newborns
- Main causes of abnormalities of haematocrit
- Haemoglobin – composition
- Haemoglobin concentration in blood of males/females/newborns – causes of the difference in normal values, anaemia – definition
- Types of haemoglobin,
- Main derivatives of haemoglobin

- Capillary blood collection – procedure
- Venous blood collection - procedure

- Medical terms related to blood physiology
- Procedures of the tasks, results and their interpretation

**Literature:**
- Physiology lectures
- Physiology textbooks (see the recommended literature)