

Biochemistry of Blood

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Overview

- Blood as an important diagnostic material
- Transport of blood gases
- Metabolism of RBC
- Iron metabolism
- Haematopoiesis from the biochemical point of view
- Anemias

Blood is...

- ...easily available material useful for a huge of various assays and measurements
- ... plazma and cells.

Gas transport

- Oxygen is a major e^- acceptor – indispensable for ATP production.
- CO_2 (and water as well) is a major byproduct of energy metabolism
- Gas transport is continuous interchange of CO_2 and O_2 between lungs and tissues.

Oxygen release helps to maintain pH in tissues

- Lungs: $H\text{Hb} + \text{O}_2 = \text{HbO}_2 + \text{H}^+$
- CO_2 is formed from plasmatic bicarbonate and proton released from Hb
- Tissues: CO_2 forms proton and bicarbonate:
 - Proton is bound to Hb, when O_2 is released
 - Bicarbonate leaves RBC
- Carboanhydrase plays a key role...
- $\text{Cl}^- / \text{HCO}_3^-$ interchange is Hamburger effect

Hemoglobin

- 4 peptide subunits ($2\alpha + 2\beta$),
4 molecules of hem (Fe ++)
- Each subunit in R or T state
- Hb dissociation curve is % sat. Hb
dependency on pO_2
- 1g of 100% sat. Hb contains 1.39 ml O_2
- 1g of 75% sat. Hb contains 1.00 ml O_2

Further forms of Hb

- HbA ($2\alpha 2\beta$): 90% of Hb in adult
- HbA₂ ($2\alpha 2\sigma$): 2-3% of Hb in adult
- HbA_{1C}: glycated Hb – important marker of long-term diabetes compensation
- HbF ($2\alpha 2\gamma$) - fetal Hb, high affinity to O₂
- Hemoglobinopathies: rare monogenic diseases (HbS –anemia).

Hemoglobine derivatives unable to transport CO₂

- Methemoglobine: contains Fe 3+ instead of Fe 2+ (e.g. nitrate/nitrite containing food or water)
- Carboxyhemoglobine – CO poisoning, smokers (cherry red colour)
- Sulfhemoglobine – green

Factors with influence on Hb affinity to O₂

- *Right shift* means higher ability of Hb to release O₂ , but lower ability to bind it.
- Is useful in tissues (site of O₂ release):
 - higher temperature
 - lower pH (Bohr effect)
 - higher 2,3 BPG level

2,3-Bisphosphoglycerate

- Is very important for long-term regulation of Hb affinity to O₂
- 2,3 BPG shunt is a pathway derived from glycolysis.
- Competition with oxygen for binding site on β -subunits
- Hypoxia stimulates 2,3 BPG synthesis, i.e. improve O₂ release.

There are 3 ways of CO₂ transport...

1. Bicarbonate formation within RBC (carboanhydrase) and Cl interchange...
2. CO₂ dissolved in blood plasma
3. Carbaminohemoglobine formation (reaction with amino groups of globine)

Clinical interpretation of Astrup assay

- Arterial (or capillary) blood sample
- Measurements of pH (7.35 – 7.45),
 $pO_2 = 9.9 - 13.6 \text{ kPa}$, $pCO_2 = 4.5 - 6.0 \text{ kPa}$
and calculation of further ABB parameters...
- Pulse oxymetry is noninvasive monitoring of Hb saturation.

Metabolic specialities of red blood cell

- No organelles – no mitochondria
- Anaerobic glycolysis (lactate formation) is the only one source of ATP!
- 2,3 BPG shunt is unique for RBC
- 20% of glucose is metabolised via pentosa phosphate pathway

Defense against oxygen radicals

- High tension of oxygen...
- GSH as a defense against harmful oxygen radicals

- Inactivation of $O\bullet$ is coupled with GSH oxidation, back reduction need NADPH



- Pentose phosphate pathway is a source of NADPH
- Glc-6-P deficiency – haemolytic anemia

Coffee break

Iron metabolism

- **Iron is indispensable for life**

(either in heme or non-heme form essential for oxygen transport, electron transfer, DNA synthesis, etc.)

- **Iron is insoluble**

($[Fe]$ cannot exceed 10^{-17})

- **Iron is potentially toxic**

(unless appropriately chelated, Fe plays a key role in the formation of oxygen radicals)

Iron storage - ferritin

- Protein, 24 subunits, up to 4 500 Fe atoms per ferritin molecule
- Ferritin is important for intracellular iron storage
- **Ferritin synthesis is stimulated by higher iron stores...**

Transferrin (Tf)

transports Fe in plasma

- Glycoprotein with 2 high affinity binding sites for Fe^{3+}
- Tf transports Fe between sites of absorption, storage and utilization
- Cells (esp. Erythroid precursors) strip Fe from Tf by expressing Tf-R
- **Tf synthesis is stimulated by lack of Fe in the body.**

When iron stores are sufficient...

- Ferritin expression in the enterocyte is stimulated. More Fe is then waist with stool.
- Transferrin synthesis is supressed, plasmatic Tf level is low, Tf is highly saturated...
- Only a small part of ingested iron is absorbed.

When iron is needed...

- Ferritin expression in the enterocyte is suppressed, only a small part of ingested iron is lost with stool.
- Transferrin synthesis is accelerated, plasmatic Tf level is high and Tf is unsaturated...
- However, iron is absorbed with high efficacy.

It is interesting, that...

- ...iron regulates ferritin and Tf-R synthesis at the level of translation (and not transcription)
- IRE of mRNA binds IRP in the presence of Fe and:
 - Activates ferritin translation
 - Block Tf-R translation

Heme synthesis

- 80% of body Fe is used for heme synthesis in developing erythroid cells
- The 1. step is ALA formation from Gly + sucCoA (ALA-S1 –regulatory in liver)
- The 8. step is heme synthesis from proto-IX, (ferrochelatase – regulatory in erythroid cells in the presence of ALA-S2)
- ALA-S2 mRNA contains IRE

Iron overload

- There is no physiological mechanism for the excretion of excess iron!
- Causes:
 - Hemochromatosis: congenital enhancement of iron absorption
 - Hemosiderosis: acquired, e.g. regular blood transfusion (aplastic anemias)
- Symptoms (over 28g Fe): diabetes, cirrhosis, hypoadrenalism, slow growth in childhood

Lack of iron causes anemia and microcytosis

- Causes: chronic bleeding (GIT, menstr.), malignancy, extreme diet
- Symptomatology :
 - low hemoglobine level
 - red blood cell count normal or high
 - RBC are small (vol. < 80 fl)

„WHY OUR BLOOD IS RED...”

- Iron stores in the body are regulated only at the level of iron absorption...
- Transferrin and ferritin play a key role in iron intake and delivery for tissues...
- Iron overload cause hemosiderosis, lack of iron is the main cause of microcytic anaemia.

Questions...?