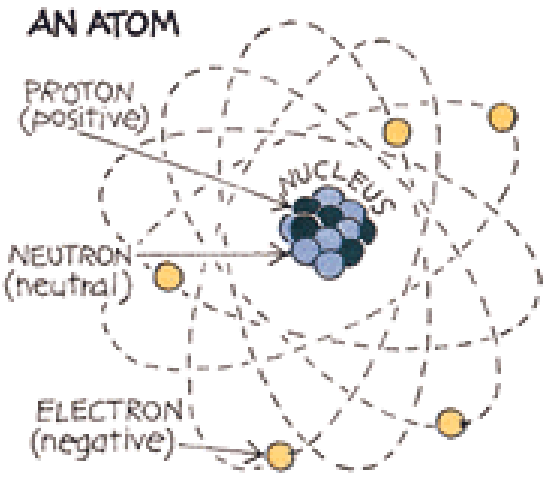
Experimentally induced radiation syndrome in laboratory animal



Aim of the practicals

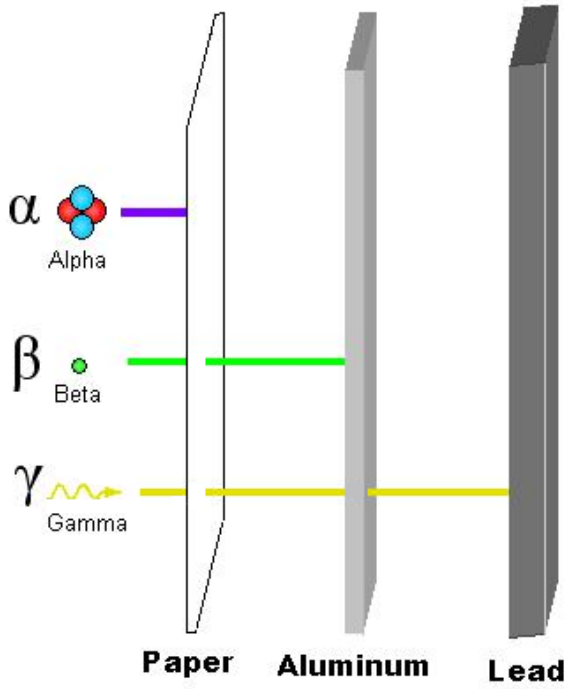
- Basics of radiobiology
- Model of acute radiation syndrome - blood form
- Presentation of experiment (data obtained during practice on a model of acute radiation syndrome)

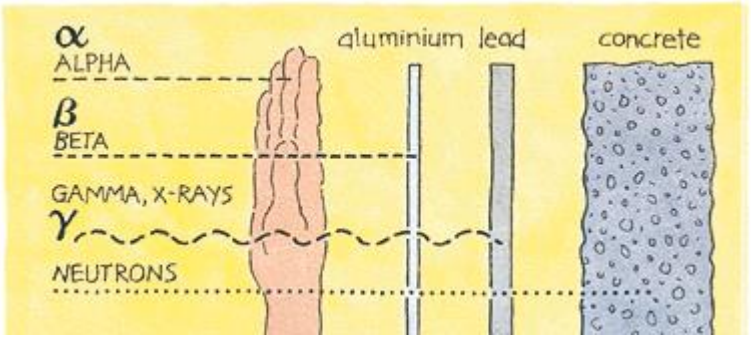
Ionizing radiation

- The radiation emitted by radioactive nuclides
- Electromagnetic or corpuscular radiation which, when penetrating mass, causes ionization (must have sufficiently high energy).
- Energy is in the range of keV-MeV
- At the same time, the atoms and molecules of the environment are excited

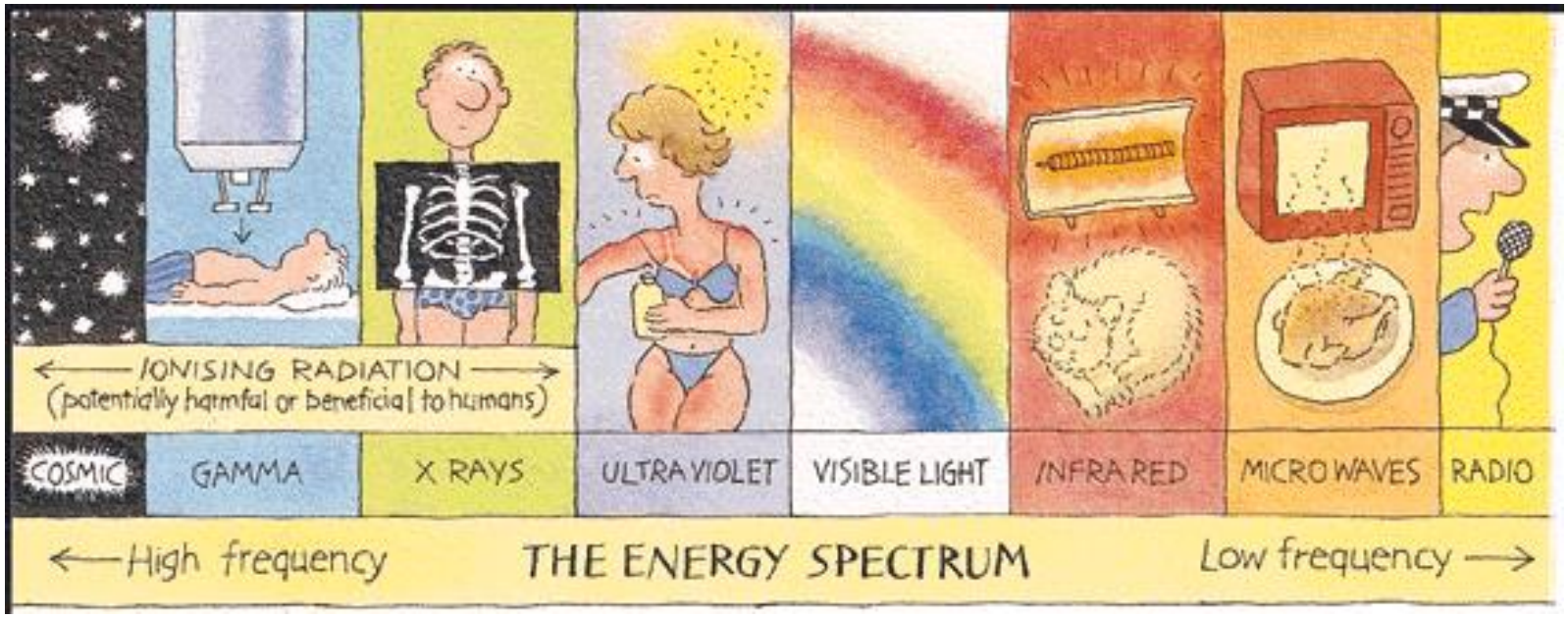
Types of ionizing radiation

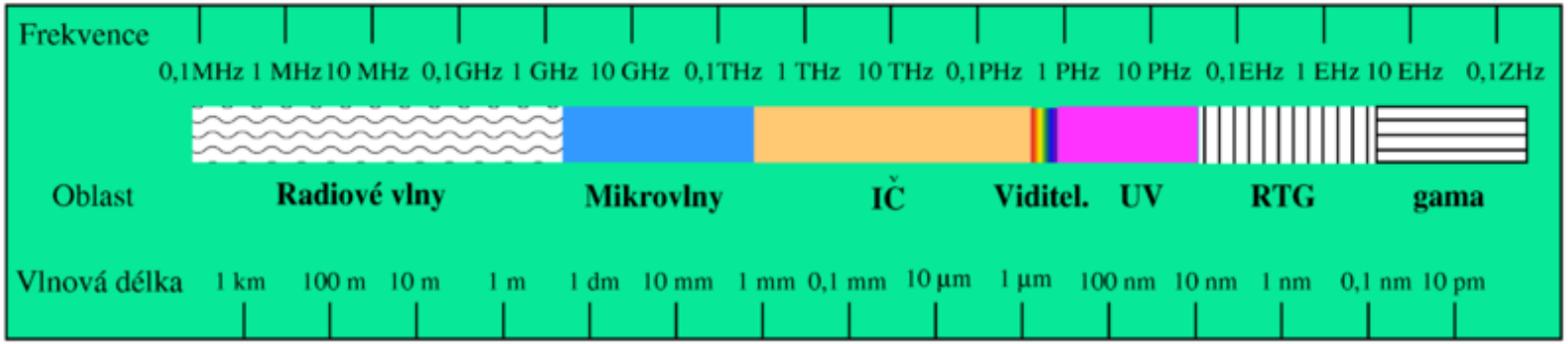
- Corpuscular α, β, neutrons
- electromagnetic γ





The energy spectrum of radiation





Ionization vs. excitation

$$M \longrightarrow M^{+} + e^{-}$$

$$M \longrightarrow M^{excit}$$

- Both types of interactions are very fast
- Formed in a ratio of 1:2
- Radiation is not limited to radioactive nuclides, but also behaves the same way with X-rays, particles from accelerators and cosmic rays

Units

D = dE / dm

(J.kg-1) - Gray (Gy)

Units

- MeV, a unit of energy
- Roentgen, a unit of exposure [C / kg dry air]
- Becquerel, activity [s-1] ≈ Curie
- Gray, dose [J / kg] ≈ rad
- Sievert, dose equivalent [J / kg] ≈ rem

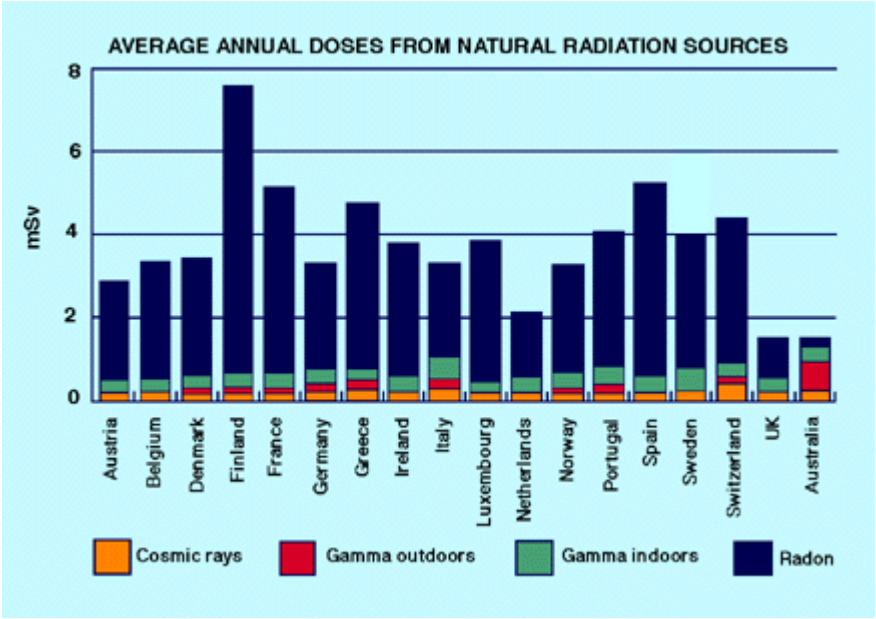
Units

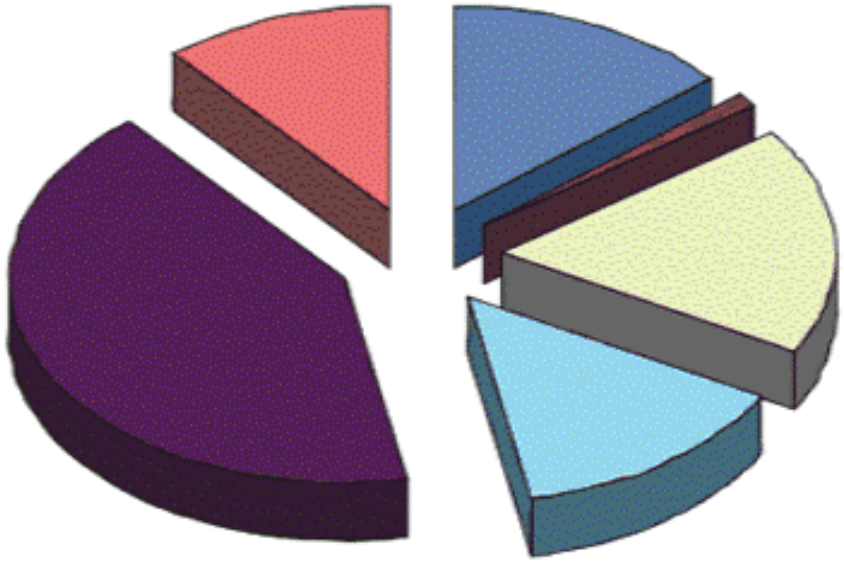
- Dose equivalent = dose * constant
- γ, β, X = 1
- neutrons = 10
- α = 20

Sources of ionizing radiation?

- Natural
  - space
    - exposure increases with altitude
  - solar
  - terrestrial sources
    - natural radioactive decay of radioisotopes (soil and rock)
  - Radon
    - gas, there is a decay of radium-226 (Uranium)
- Artificial
  - medicine
    - diagnosis, therapy, sterilization
  - industrial
    - nuclear energy

Sources of ionizing radiation





Medicine - 14%
Nuclear industry - 1%
Buildings/Soil - 18%
Cosmic - 14%
Radon - 42%
Food/Drinking Water - 11%

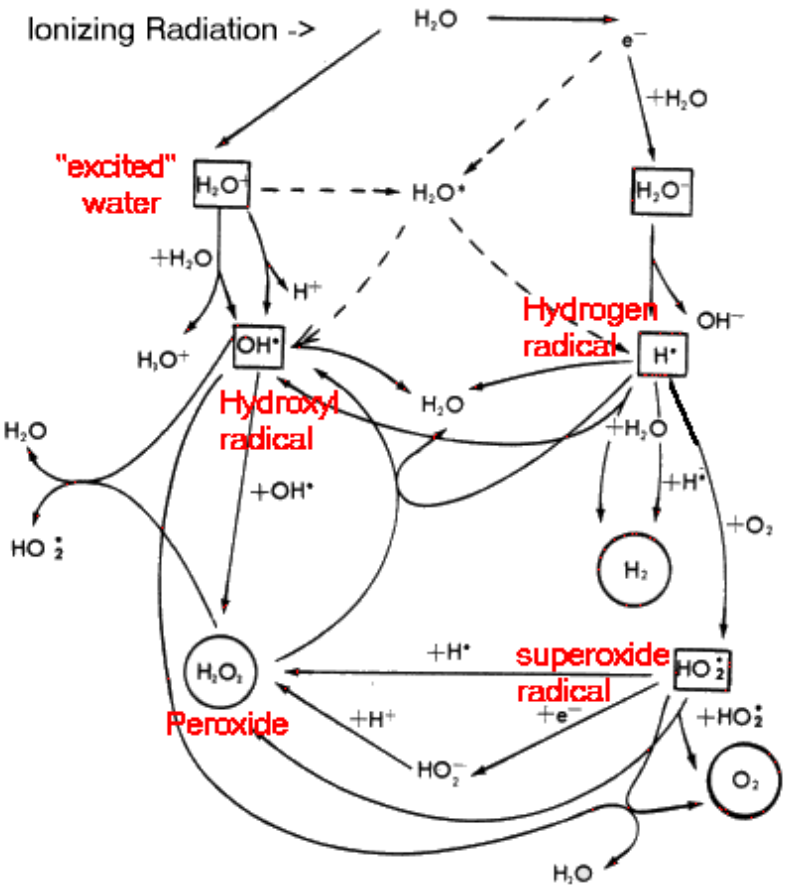
Natural Radiation 85%

Chemical effect of ionizing radiation

- Radiation-chemical reactions in liquids are best studied (less so in gases and solids)
- If water contains dissolved oxygen, the following reaction takes place:

$O_2 + H \quad HO_2$

$O_2 + e^- \quad O_2^{-\cdot}$



Oxygen effect!!

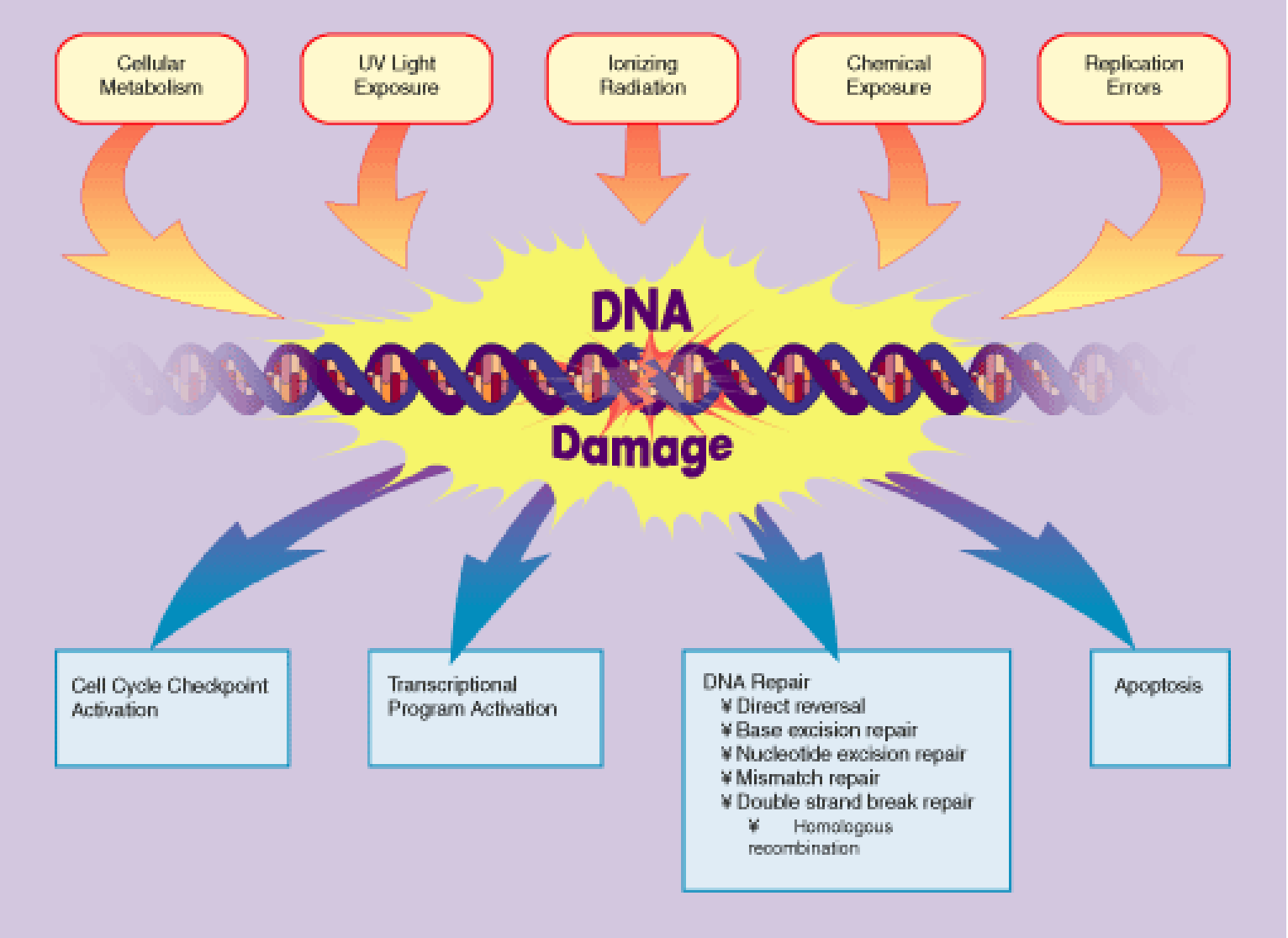
Biological effects of ionizing radiation

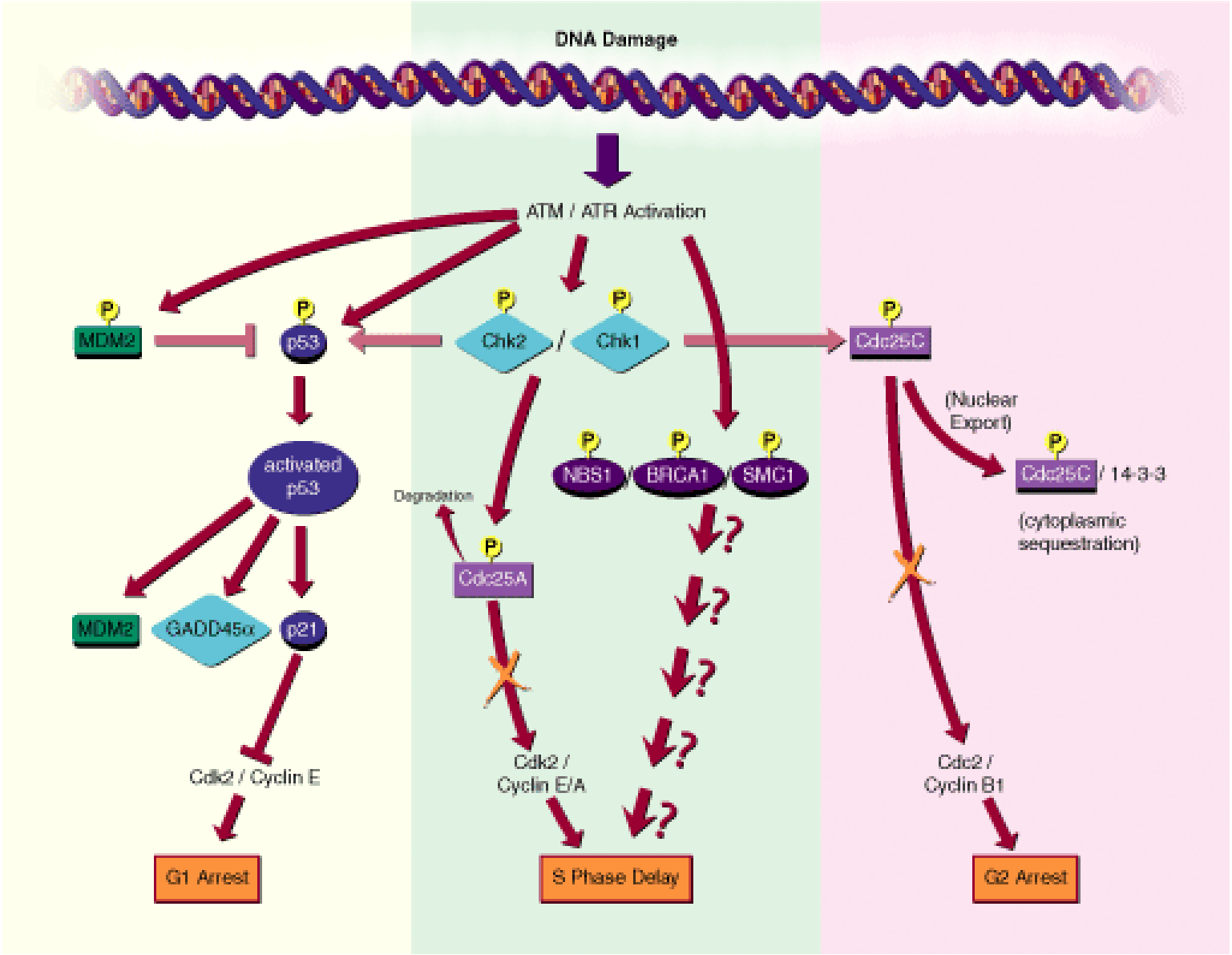
- Direct ionizing = direct destruction of biomacromolecules
- Indirectly (nondirect) effects = production of free radicals (radiolysis of water)

DNA damage

- Very serious process
- DNA damage is reflected in the synthesis of damaged proteins
- DNA repair mechanisms
- Reproductive ability of cells

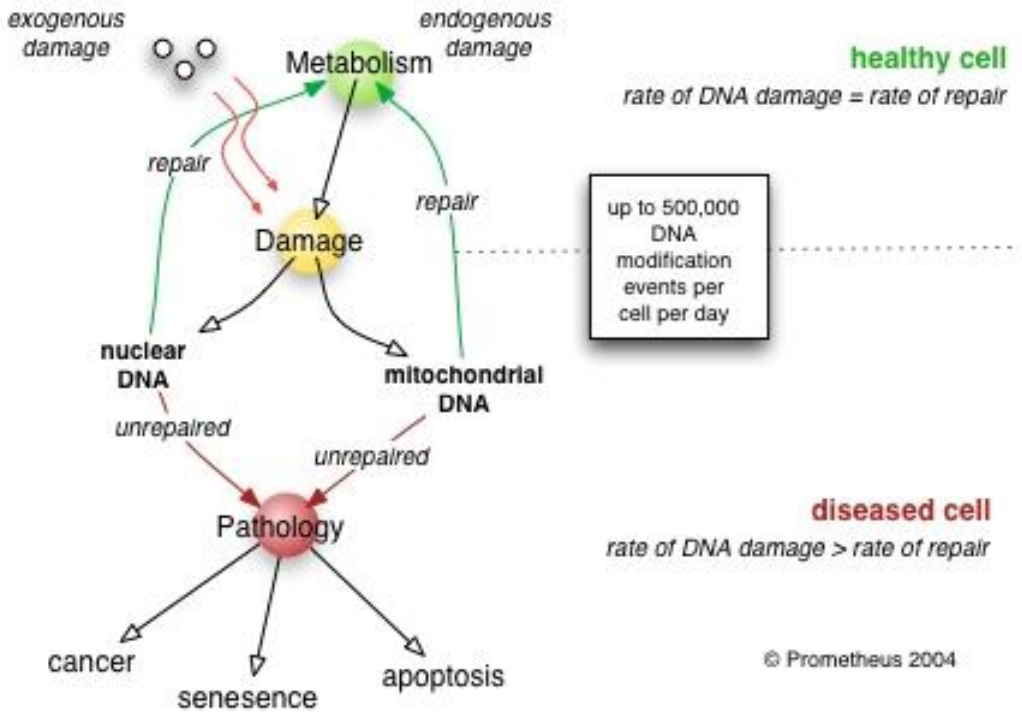
Mechanisms of DNA damage




DNA Damage
ATM / ATR Activation
MDM2
p53
Chk2
Chk1
Cdc25C
(Nuclear Export)
Cdc25C / 14-3-3
(cytoplasmic sequestration)
activated p53
NBS1
BRCA1
SMC1
Degradation
Cdc25A
MDM2
GADD45α
p21
Cdk2 / Cyclin E
Cdk2 / Cyclin E/A
Cdc2 / Cyclin B1
G1 Arrest
S Phase Delay
G2 Arrest

Repair mechanisms

- Direct repair
- Excision repair
- Mismatch repair
- SSB repair
- (DSB repair)



Effects of ionizing radiation on the human body

- **Deterministic effects**
- **Stochastic effects**

Deterministic effects

- There are those that will take effect after total body irradiation, or a tissue once
- The dependence of the probability of occurrence of damage to an equivalent dose have the sigmoid character

Deterministic effects

- Acute radiation syndrome (radiation syndrome)
- Local acute skin damage
- Damage to the fetus
- Infertility
- Lens damage

Stochastic effects

- They result from damage to a small number of cells
- They can occur after a single exposure of sub-threshold dose or chronic radiation tissue or whole body

Character of biological effects

- **Deterministic**
- **severity dependent** ("determined") **on the dose**
- manifestation is **specific**
- effect only when exposure **exceeded threshold**
- damage of **large amount of cells**
- onset rather close to the exposure (**short latency**)
- types:

  acute radiation syndrome

  chronic post-radiation syndrome

  cataract, radiation dermatitis,

  damage of the foetus *in utero*

  sterility

- **Stochastic**
- **probability increases with the dose** (not the severity!)
- manifestation **non-specific**
- gradual increase of the risk **without "safe" threshold**
- damage of the **single cell** enough to cause effect
- **manifestation delayed** (typically years)
- types:

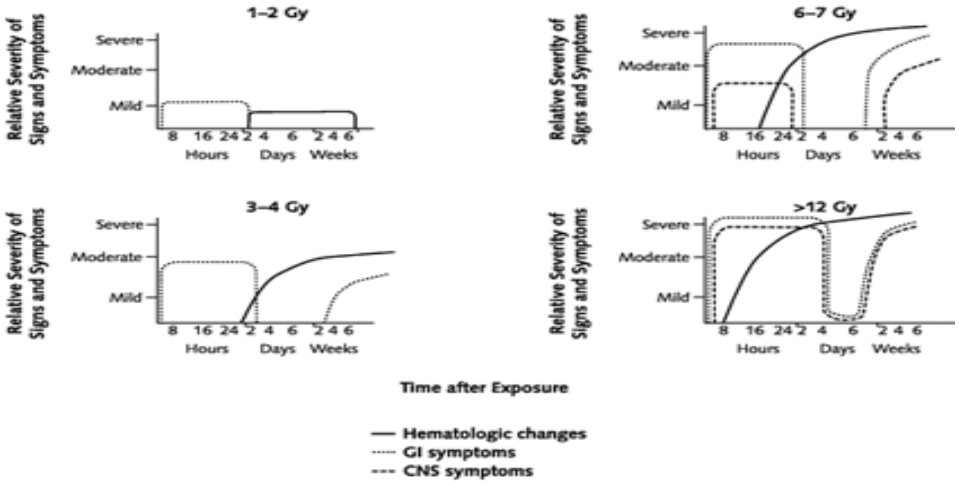
  somatic mutation - cancer

  leukemias, thyroid, lung, breast, bones

  germinative mutation (oocyte, sperm cell)

Acute radiation syndrome

- affecting the hematopoietic, gastrointestinal system and cerebrovascular
  - timing, extent and severity graded according to the dose - deterministic effect!!
- from several hours to several months after exposure

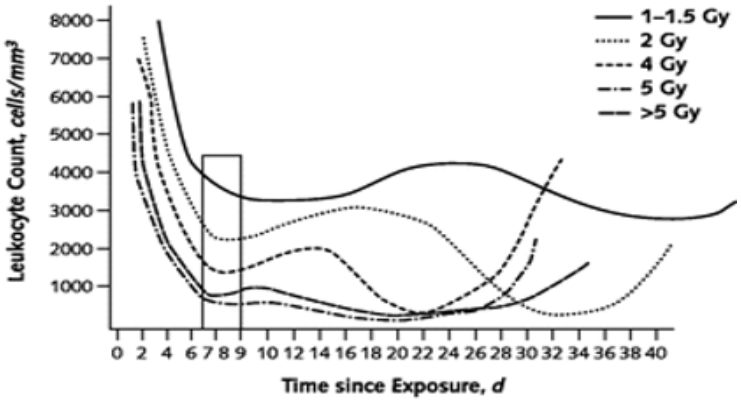


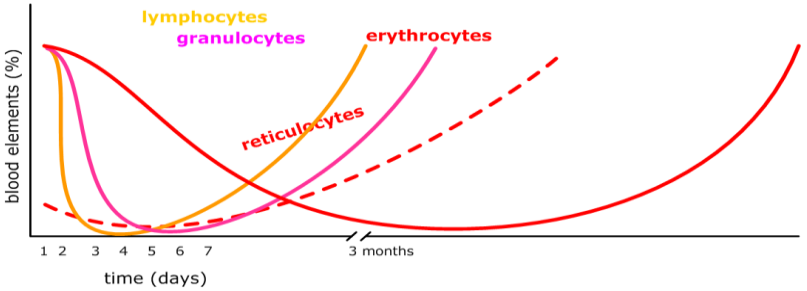
Acute radiation syndrome

- Haematopoetic syndrome (> 1GY)
- GI syndrome (> 10Gy)
  - early (hours) - nausea, vomiting, diarrhea
  - late (days) - loss of intestinal integrity
    - malabsorption, dehydration, toxemia / sepsis, ileus, bleeding
- Cerebrovascular syndrome (tens of Gy)
  - headache, cognitive impairment, disorientation, ataxia, convulsions, fatigue and hypotension
- Cutaneous
  - erythema, burns, edema, impaired wound healing

Hematopoetic syndrome

- bone marrow irradiation (> 1Gy) leads to an exponential cell death - haematological crisis
  - marrow hypoplasia to aplasia + peripheral pancytopenia (infection, bleeding)
- subpopulation of stem bb is selectively more radioresistant, (probably due to predominance bb. stage in Go)
  - necessary for regeneration
- anemia is the result of late (erythrocytes ~ 120 days)!
- massive stress response (glucocorticoids) contribute to lymphopenia (cytolytic effect) and paradoxically delay the onset of granulocytopenia (release stocks. granulocytes from the spleen)





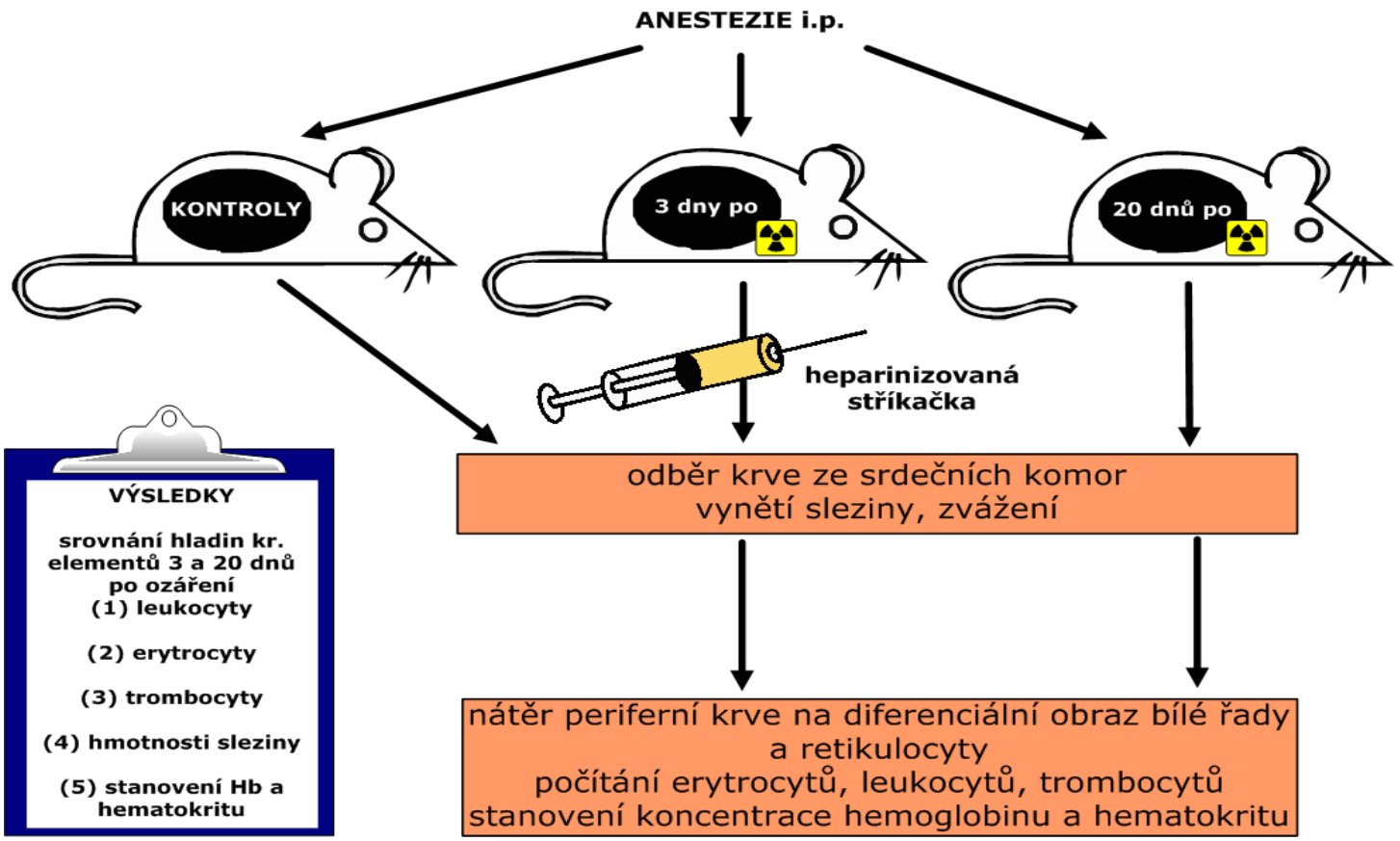
Therapeutic effects of ionizing radiation

- Teleterapy (^{60}Co)
- Contact therapy (^{32}P, ^{90}Sr)
- Brachytherapy (^{60}Co, ^{137}Cs)
- Endotherapy ($Na^{131}I$)
- Radioimunotherapy

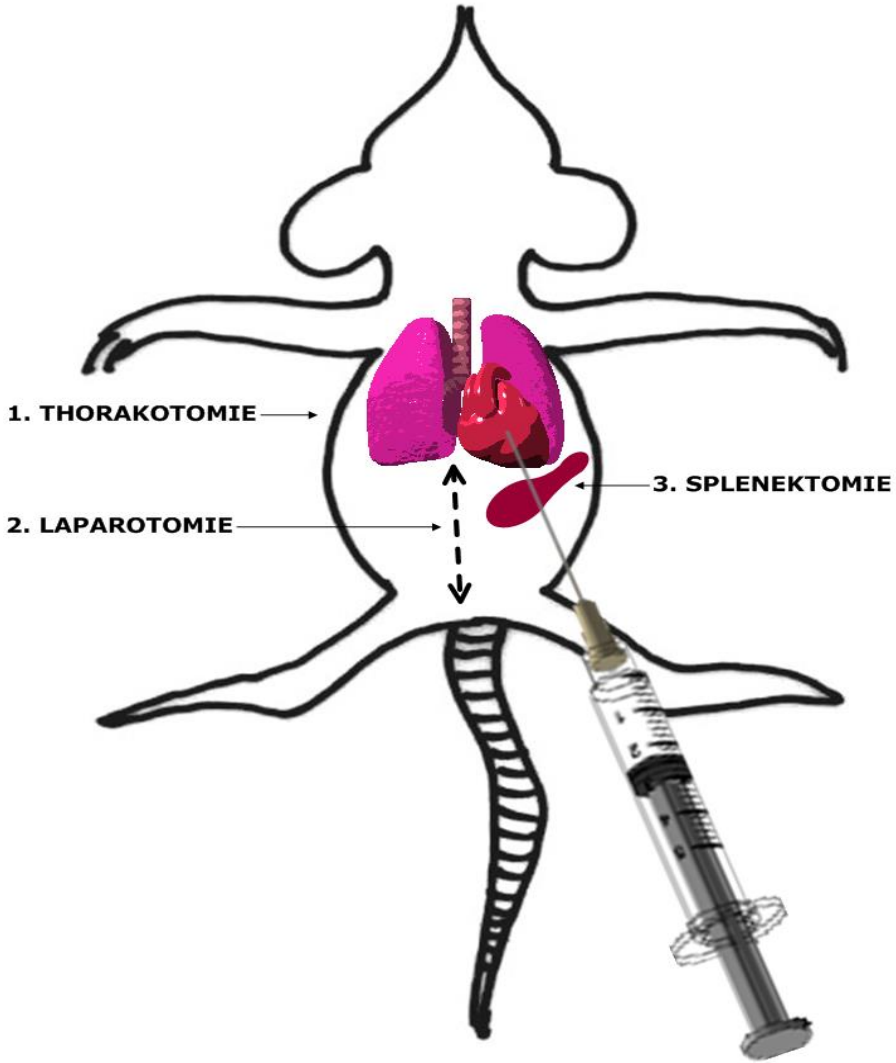
AIMS of experiment

- to demonstrate the deterministic effects of ionizing radiation on hematological parameters
- to observe the dynamics of peripheral blood count changes resulting from the changes in the blood marrow
- acute radiation syndrome is a model situation, helping us to understand the hematopoiesis

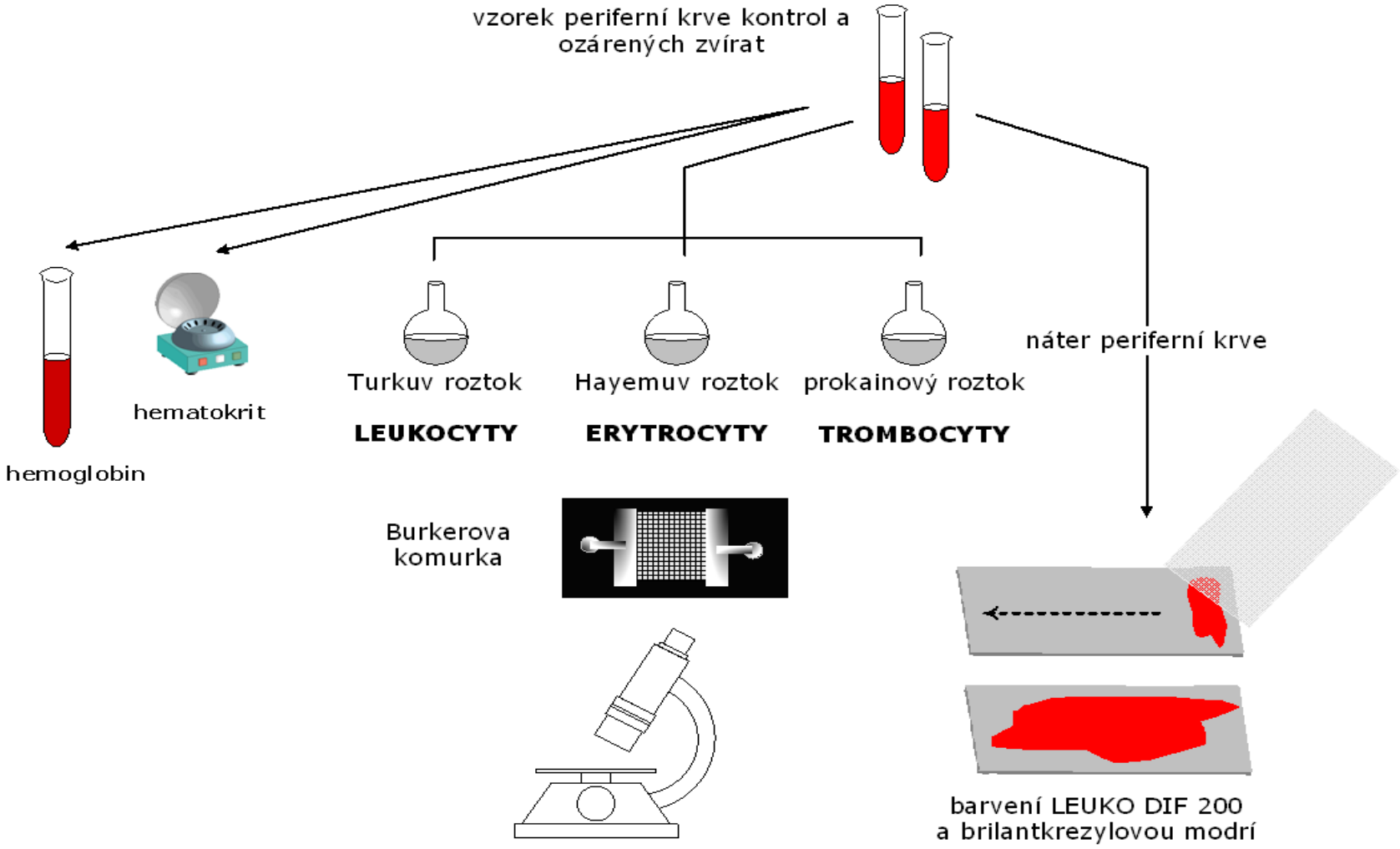
Practicals I - design



Praktikum I – operační postup

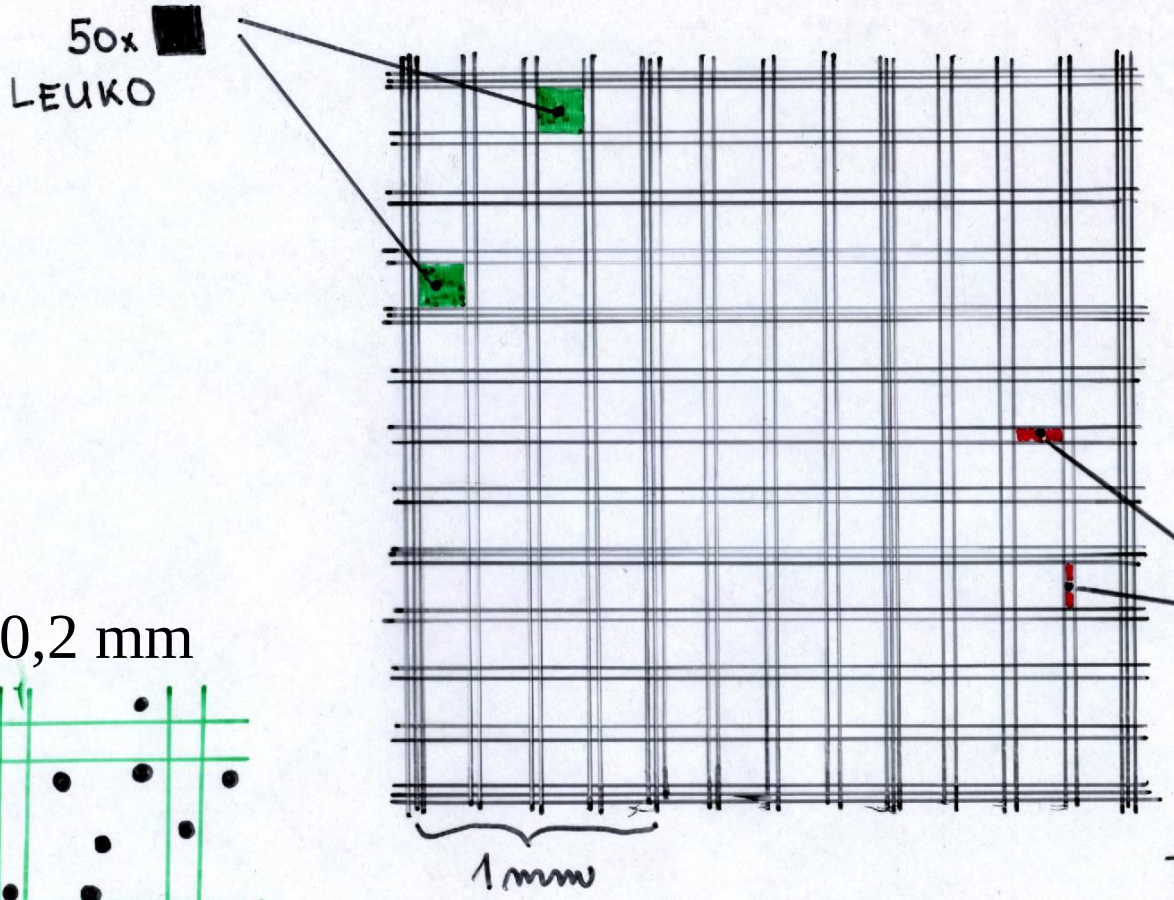


Praktikum I - hodnocení

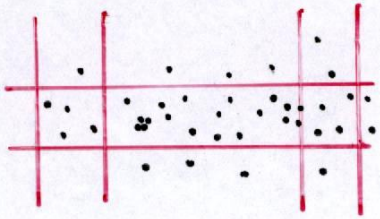


BŮRKEROVA KOMŮRKA

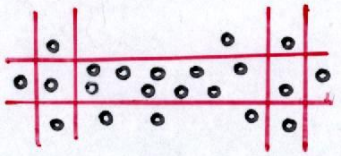
Výška = 0,1 mm



TROMBOCYTY



0,05 mm

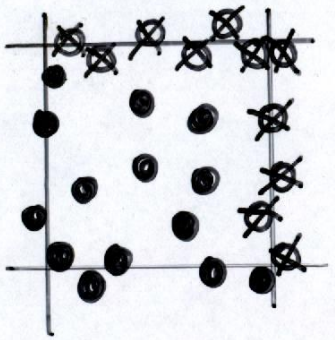


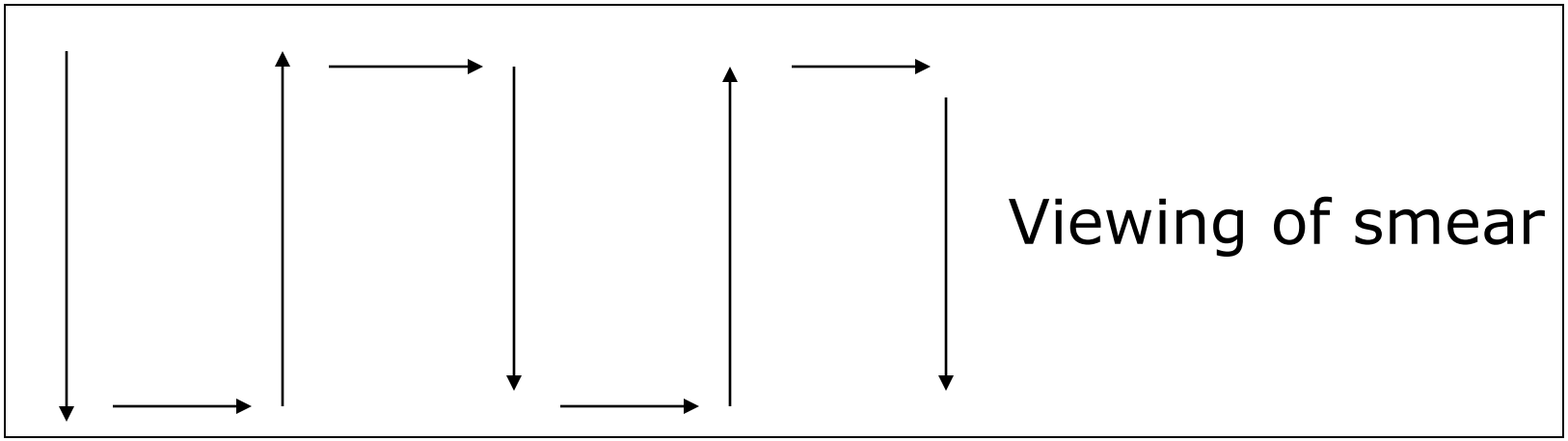
ERYTROCYTY

0,2 mm

LEUKOCYTY

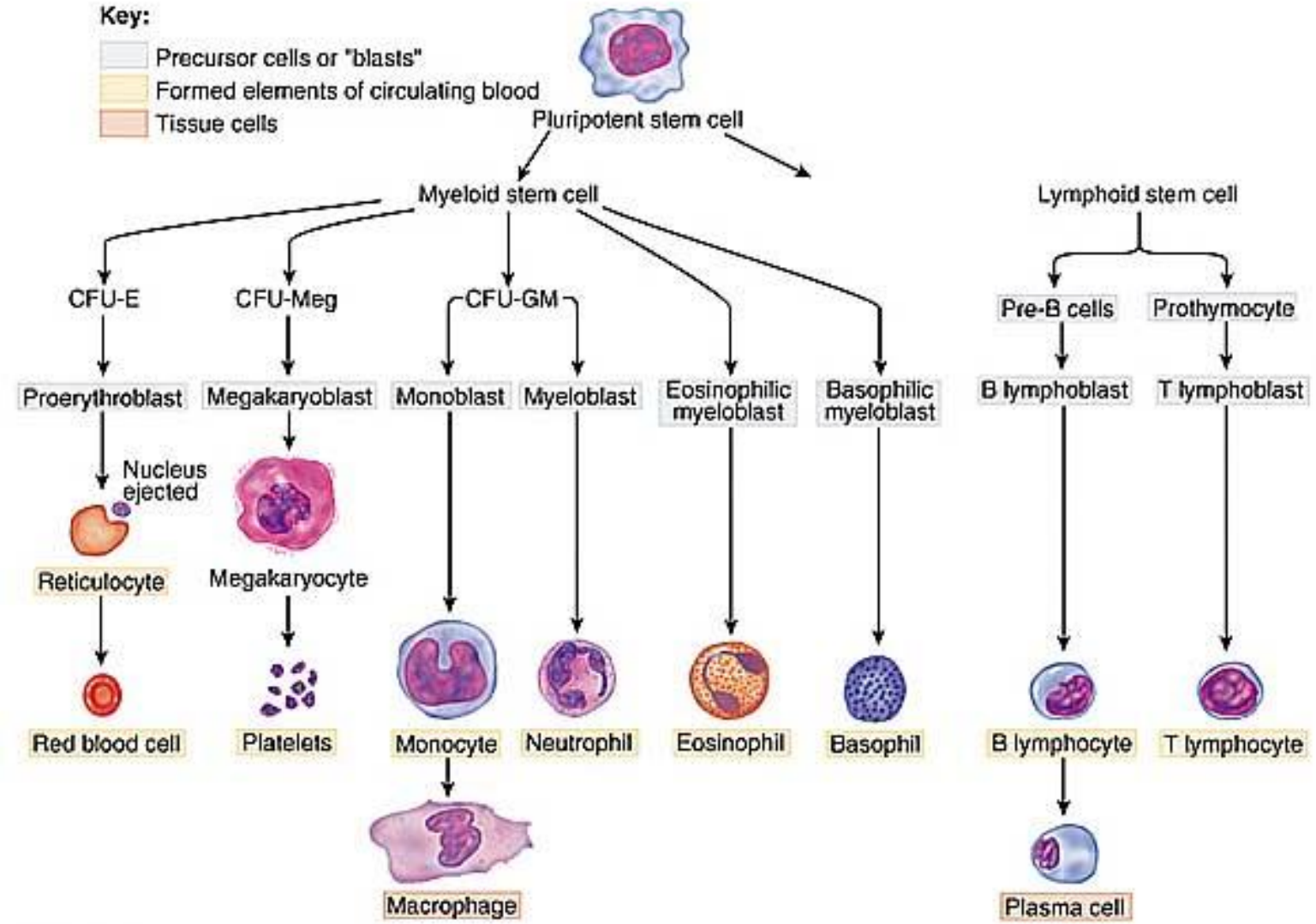
BŮRKEROVO PRAVIDLO

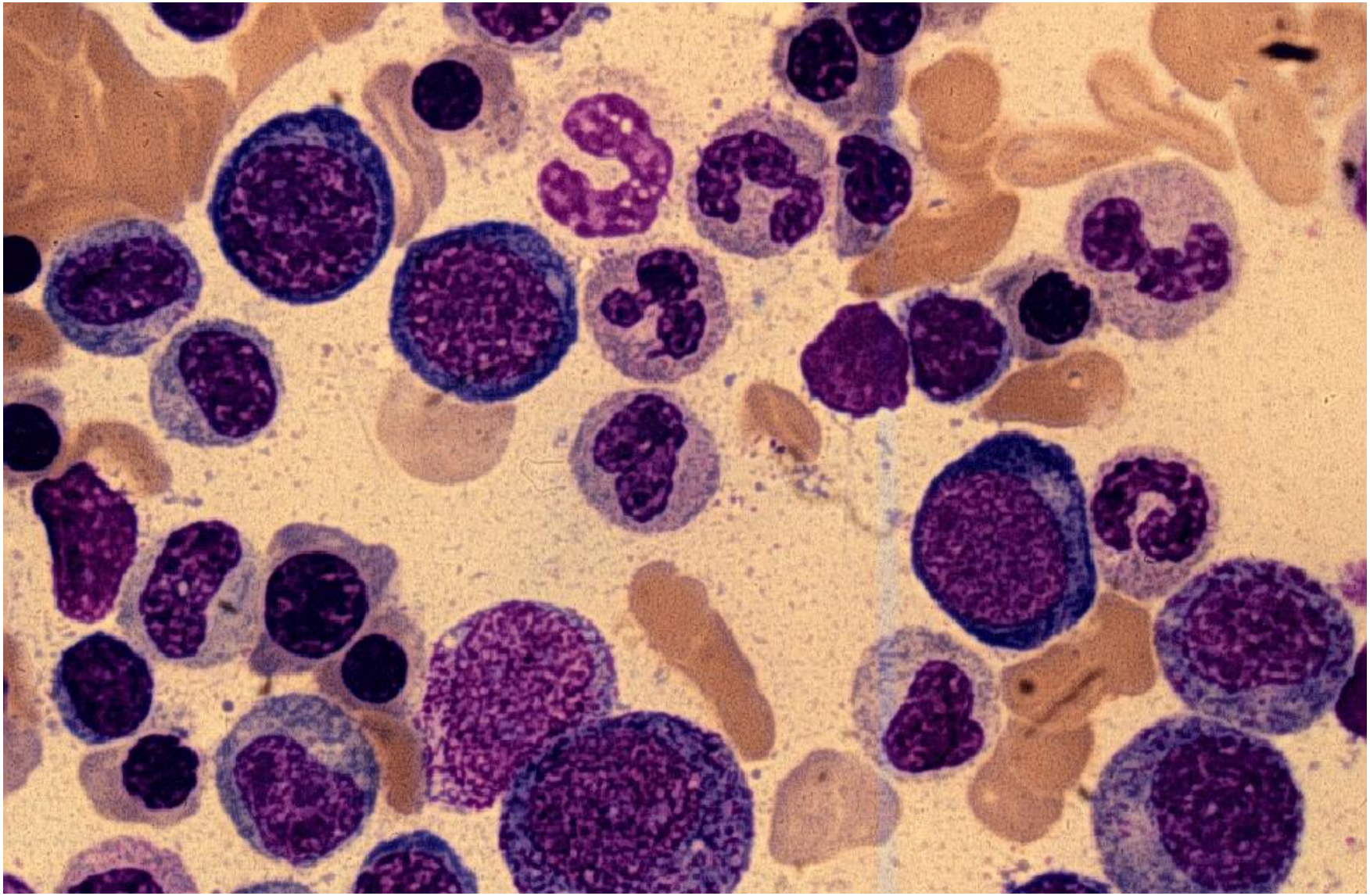




- The stained smear will first be viewed at a low magnification and an area where white cells are not overlaid by red cells will be selected.
- Using an immersion objective, a total of 100 WBC will be registered and identified as to their individual type.
- The viewing field will be moved in order to count the prescribed number of leucocytes.

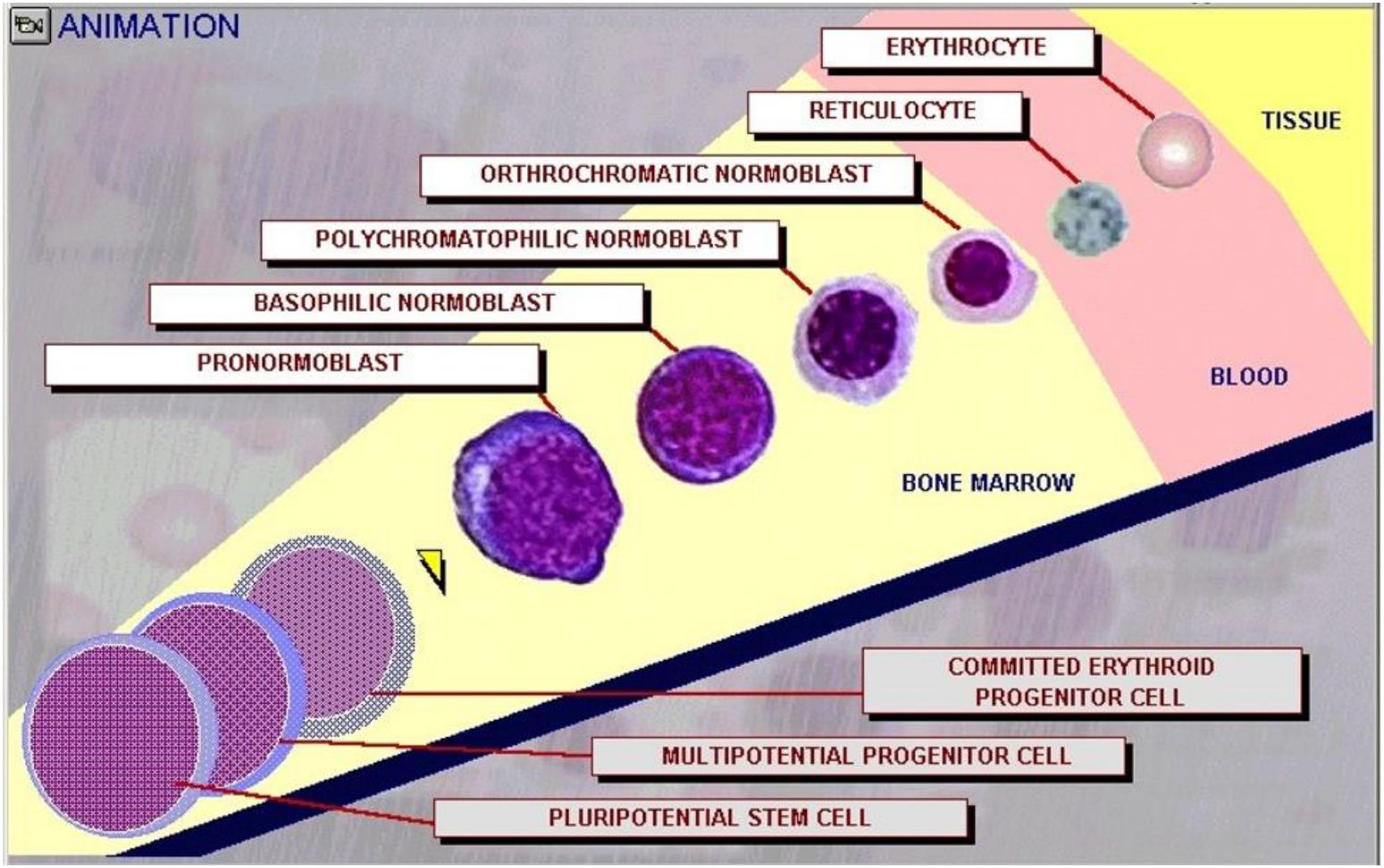
Hemopoesis


Key:
Precursor cells or "blasts"
Formed elements of circulating blood
Tissue cells
Pluripotent stem cell
Myeloid stem cell
Lymphoid stem cell
CFU-E
CFU-Meg
CFU-GM
Pre-B cells
Prothymocyte
Proerythroblast
Megakaryoblast
Monoblast
Myeloblast
Eosinophilic myeloblast
Basophilic myeloblast
B lymphoblast
T lymphoblast
Nucleus ejected
Reticulocyte
Megakaryocyte
Red blood cell
Platelets
Monocyte
Neutrophil
Eosinophil
Basophil
B lymphocyte
T lymphocyte
Macrophage
Plasma cell




Red Blood Cell Club
EXCHANGE RACE
Get Oxygen
& Nutrients Here!
Get Rid of
Wastes Here!
Oxygen

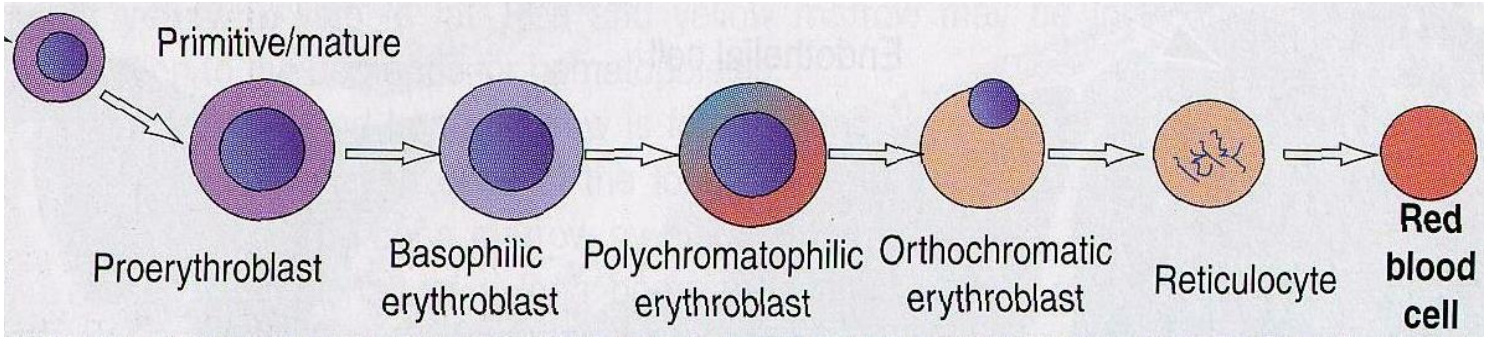
Erythropoiesis



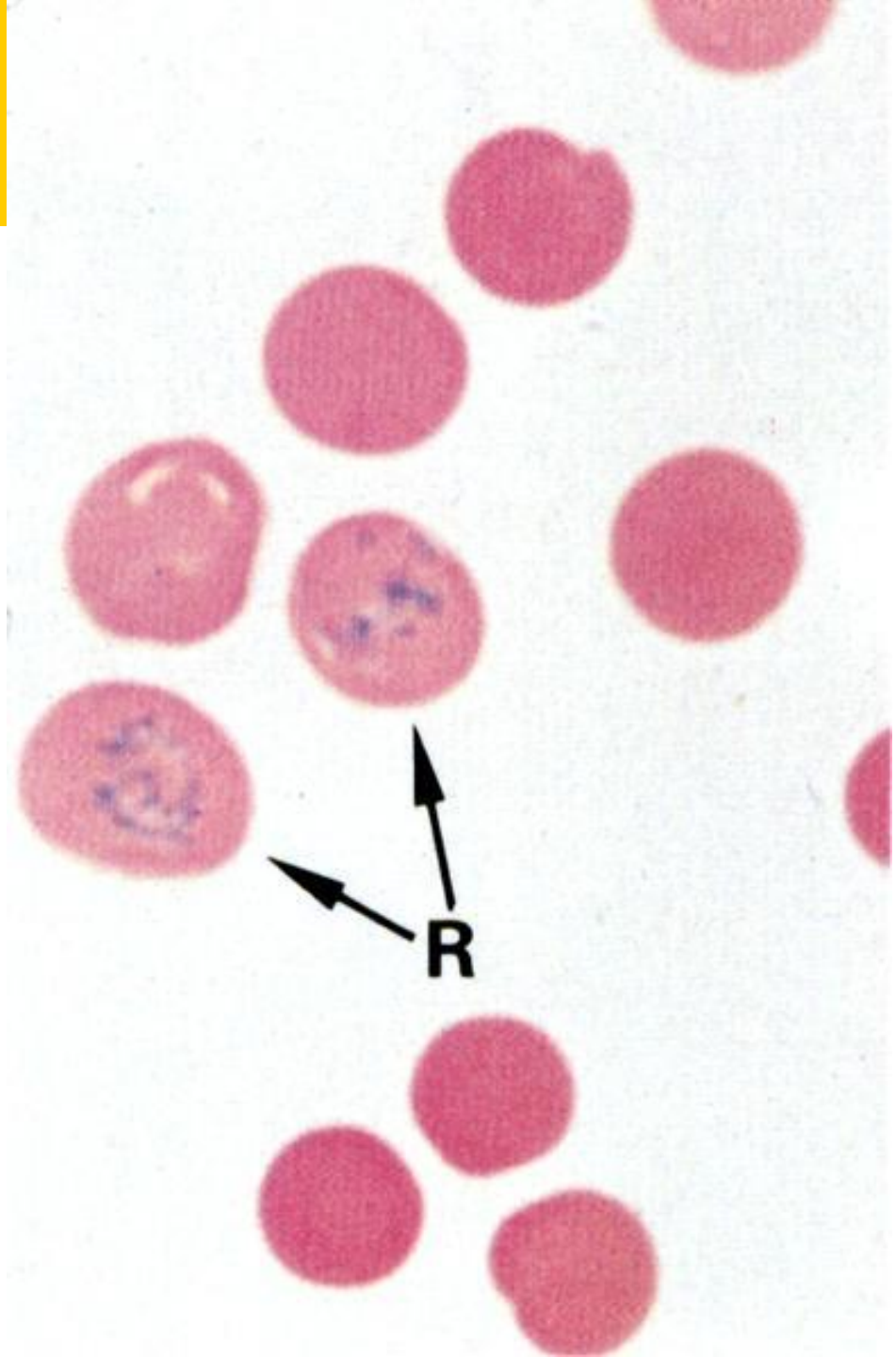
http://www.noblesmedart.com/morph.mov

Erythropoiesis

- Erythropoiesis is the development of mature red blood cells (erythrocytes).
- Like all blood cells, erythroid cells begin as **pluripotential stem cells**.
- The first cell that is recognizable as specifically leading down the red cell pathway is the **proerythroblast** .
- As development progresses, the nucleus becomes somewhat smaller and the cytoplasm becomes more basophilic, due to the presence of ribosomes. In this stage the cell is called a **basophilic erythroblast** .
- The cell will continue to become smaller throughout development. As the cell begins to produce hemoglobin, the cytoplasm attracts both basic and eosin stains, and is called a **polychromatophilic erythroblast** .
- The cytoplasm eventually becomes more eosinophilic, and the cell is called an **orthochromatic erythroblast** .
- This orthochromatic erythroblast will then extrude its nucleus and enter the circulation as a b . Reticulocytes are so named because these cells contain reticular networks of polyribosomes. As reticulocytes loose their polyribosomes they become **mature red blood cells**.



Reticulocyte



Reticulocytes

- **Reticulocytes** are immature red blood cells, typically composing about 1% of the red cells in the human body.
- Reticulocytes develop and mature in the red bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells.
- Like mature red blood cells, reticulocytes do not have a cell nucleus.
- They are called reticulocytes because of a reticular (mesh-like) network of ribosomal RNA that becomes visible under a microscope with certain stains such as new methylene blue.

Classification of reticulocyte counts

- Counting with imersion, magnification 100
- **Out of 1000 RBC in the moving viewing field, the number of RET will be counted**
- Normal counts RAT: app. 20 ‰ RET
- Normal counts MAN: app 0.5-1.5% (5-15‰) RET

↑ number: increasing bloodforming (regeneration)
↓ number: inhibition of erythropoiesis

Differential leucocyte counts

- Changes in WBC number (%)
  - Indication of infection, poisoning, leukemia, chemotherapy, alergic reaction

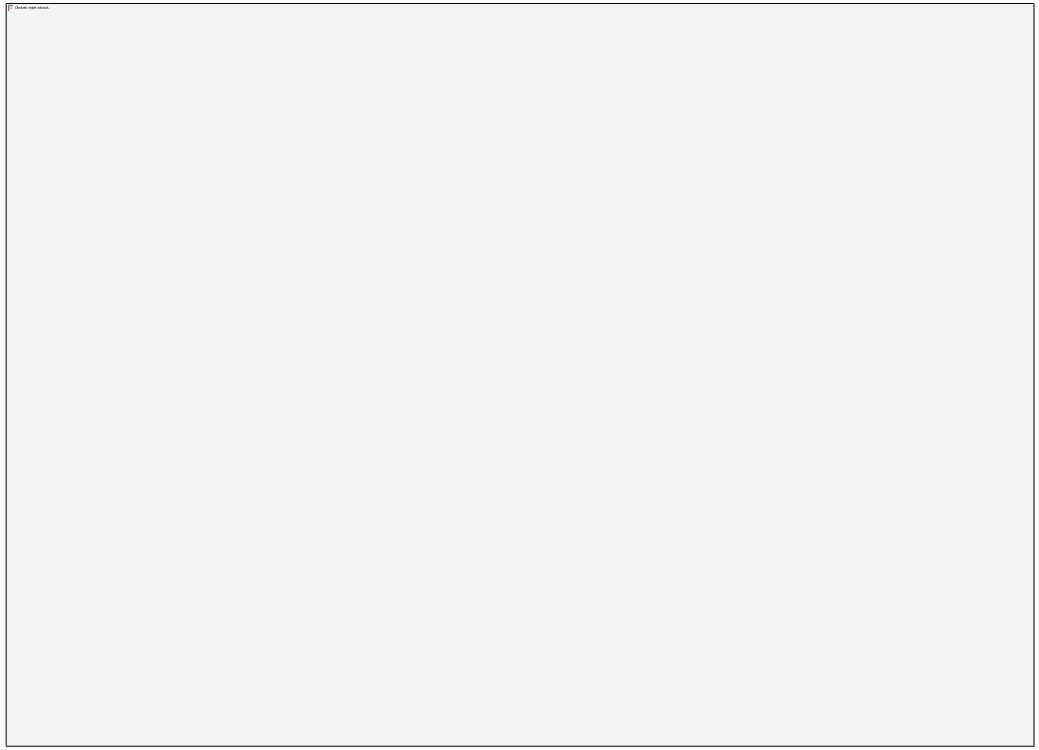
- Normal WBC counts in man:
  - neutrophils 60-70% (incr. in bacterial infection)
  - lymphocytes 20-25% (incr. in viral infection)
  - monocytes 3-8 % (incr. in fungal/viral infection)
  - eozinophils 2-4 % (incr. in alergic reaction and parasital infect.)
  - bazophils <1% (incr. in alergic reaction)

Normal counts of WBC in rat

- Number of WBC.....cca 12.5 tis.mm-3

- neutrophil granulocytes	18 - 36%
- eozinophil granulocytes	1 - 4%
- bazophil granulocytes	0 - 1%
- lymphocytes	62 - 75%
- monocytes	1 - 6%

Blood smear



- neutrophil „band“
- neutrophil „segment“
- monocyte
- lymphocyte
- eozinophil
- bazophil
- trombocyte