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III. Erythrocyte sedimentation rate
IV. Estimation of osmotic resistence of red blood cells

Physiology I – practice

Autumn, weeks 4-6

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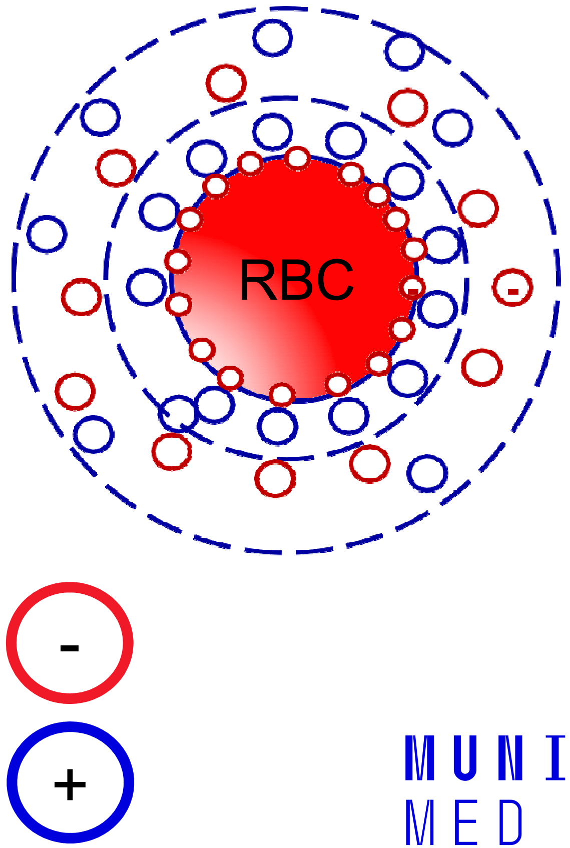
Erythrocyte sedimentation rate

Sedimentation of erythrocytes (RBCs)

- It is a physical process of settling (sedimentation) of RBCs in non-flowing non-coagulating blood.
- The estimation of erythrocyte sedimentation rate (ESR)
  - Non-specific laboratory method, low sensitivity
  - Speed of the erythrocyte sedimentation in the column of anti-coagulated blood in a glass tube
- The value of ESR is inversely related to the suspension stability of blood

Suspension stability of blood

- Helmholtz electrical double-layer
  - There is a negative charge on the membrane of red blood cells caused mainly by sialic acid residues of membrane proteins
  - Positively charged ions (primarily Na^+) are held in proximity to the membrane by electrostatic forces - they form the 1st layer of ions
  - Negatively charged ions are attracted to the positively charged layer, which makes up the majority of the 2nd ion layer
  - Due to their "electric envelope", the RBCs repel each other, which ensures that the non-flowing non-coagulating blood remains as a suspension of blood elements in the plasma for a certain time = **suspension stability**



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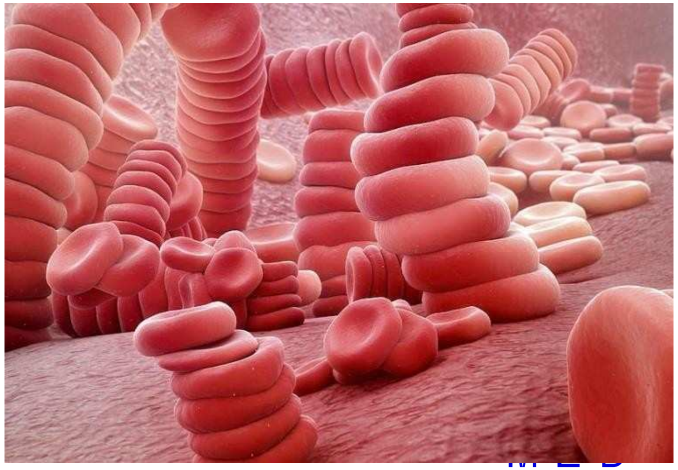
Mechanism of erythrocytes sedimentation

- Gravity – under its influence, erythrocytes in non-flowing non-clotting blood gradually settle down (sediment).
- Factors impairing the Helmholtz electrical double-layer cause aggregation of RBCs into cylindrical aggregates (rouleaux) with higher volume and relatively smaller surface (compared to the corresponding volume of separate RBCs)
  - Aggregates fall down (sedimentate) faster than separate RBCs = value of ESR is higher
  - Disruption of the electrical double layer will thus cause an increase in the sedimentation rate



Basic factors affecting ESR

- ESR depends on RBCs characteristics and plasma composition
- Plasmatic proteins
  - Albumin is charged negatively – it helps the suspension stability of blood
  - Immunoglobulins and fibrinogen – possess negative and positive charges which cause impairment of Helmholtz electric double-layer
  - Fatty foods should not be eaten before blood taking for sedimentation testing
  - Sedimentation is also affected by RBC's shape (spherocytosis, sickle cell anemia)

Effect on ESR	↑ value	↓ value
Erythrocytes		
Number of RBCs	decelerates	accelerates
Size of RBCs	accelerates	decelerates
Plasma		
Albumin	decelerates	accelerates
Imunoglobulins	accelerates	decelerates
Fibrinogen	accelerates	decelerates
Lipids	accelerates	decelerates

Methods of ESR estimation

- Fahraeus-Westergren (FW) – direct method
  - A glass tube in vertical position
  - Measured after 1 hour (2 hours)
- Wintrobe
  - 100 mm long, thin glass tube in oblique position (45°)
  - Measured after 15 minutes
  - Less sensitive than the FW

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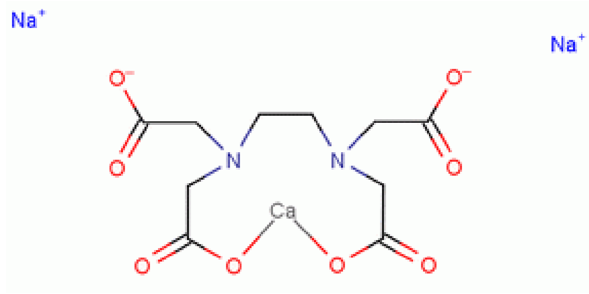
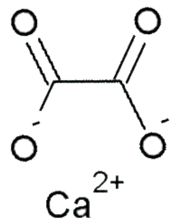
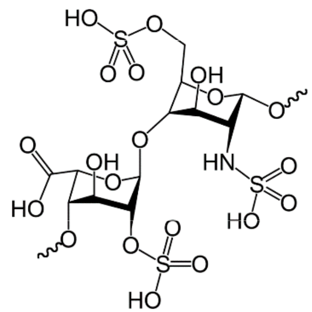
Anti-coagulated blood

- A blood sample with the coagulating system blocked
- Possible anticoagulant agents
  - Chelation of Ca2+ ions which are essential for coagulation (chelation anticoagulants)
    - Sodium citrate
    - EDTA (ethylenediaminetetraacetic acid)
    - Sodium oxalate
- Activation of antithrombin III – heparin and its low molecular weight derivatives (LMWHs)
  - Antithrombin III inactivates thrombin and some other coagulation factors, heparin enhances the effect of antithrombin III







Physiological values of ESR (FW)

– Men: 2-8 mm/h
– Women: 7-12 mm/h
– Newborns: 2 mm/h
– Infants: 4-8 mm/h

– Intersexual differences in adults are caused by different RBC count and by differences in concentration of plasmatic proteins (women have fewer RBCs and more fibrinogen)
  – Sedimentation accelerates with age

Changes in ESR (FW)

– Increased ESR (FW)
  – Pregnancy, menstruation
  – Macrocythemia
  – Inflammation
  – Infection
  – Necrosis (myocardial infarction, injury)
  – Cancer
  – Relative/absolute loss of albumin (e.g. renal disorders)
  – Hyperlipidemia

– Decreased ESR (FW)
  – Irregular RBC shape - spherocytosis
  – Polycythemia vera
  – Leukocytosis
  – Dysproteinemia – hypofibrinogenemia, hypogammaglobulinemia
  – Dehydration

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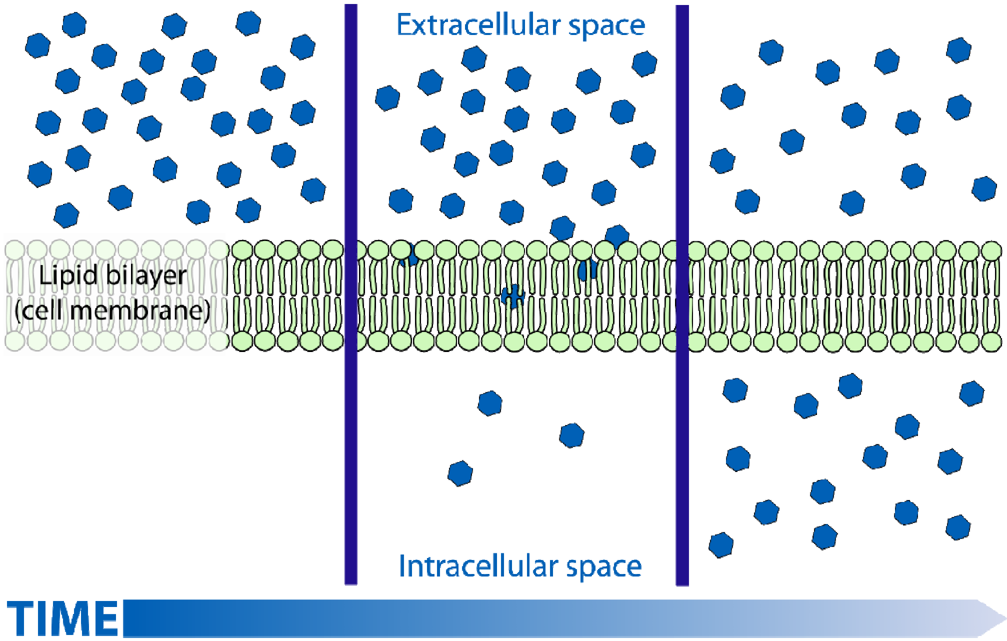
Types of blood in physiology practice

- Full human blood
  - RBCs in plasma (normal hematocrit) – sedimentation rate should be normal
- Human RBCs + physiological solution
  - Sedimentation will be slow – there are no plasma proteins
- Full horse blood
  - Large RBCs, more plasma proteins - rapid sedimentation
- Horse RBCs + physiological solution
  - Horse RBCs in saline solution sediment more slowly than in full horse blood
- Only theoretically:
- (Beef blood - A higher number of smaller RBCs - very slow sedimentation)
- (Anemic blood - RBCs in plasma (low hematocrit) – sedimentation should be faster)

Osmotic resistence of red blood cells

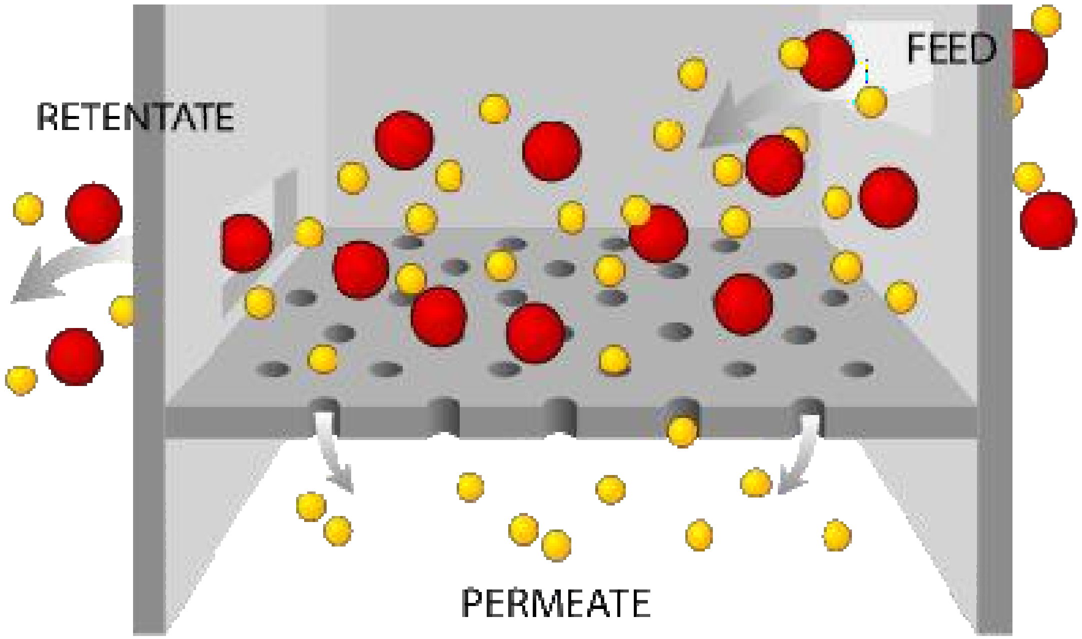
Diffusion

– Movement of molecules or atoms from a region of high concentration to a region of low concentration

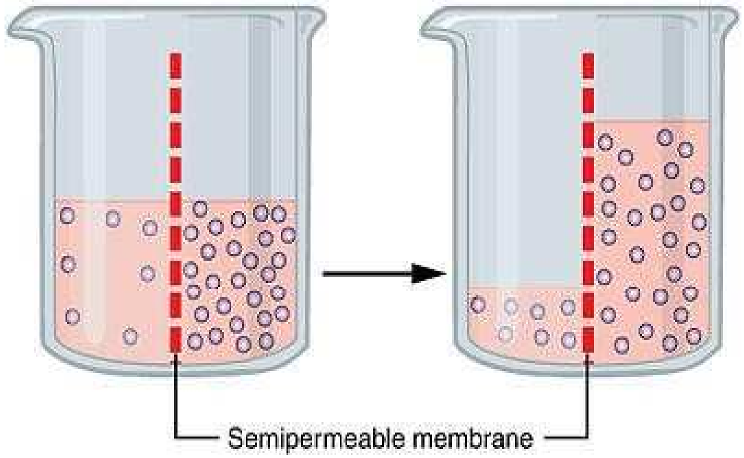


Filtration

– Separation of fluids from the „solid“ parts through the membrane powered by the pressure gradient



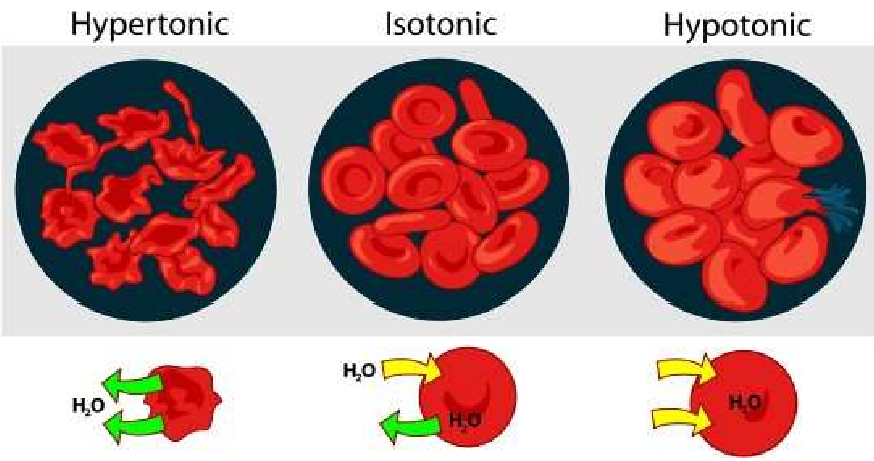
Osmosis



– A flow of solvent (water) through a semipermeable membrane according to an osmotic gradient
– **Osmotic pressure** – pressure necessary to stop the osmosis
– **Osmolarity** – concentration of osmotic active particles in 1 litre of solution
– **Osmolality** – concentration of osmotic active particles per 1 kg of solvent
– Osmolality of plasma = 2*[Na^+] + [glc] + [urea] =
= 275-295 mmol/kgH_2O

Tonicity

– The osmolality of a solution compared to the osmolality of intracellular fluid
  – **Hypotonic solutions** – the solution had lower osmolality than intracellular fluid → water flows into the cells
  – **Isotonic solutions** – same osmolality of solution and intracellular fluid – 0.9% NaCl = physiological solution (saline)
  – **Hypertonic solutions** – the solution has a higher osmolality than the intracellular fluid -> water leaves the cells.



Hemolysis

- Break-up of RBC due to disintegration of its membrane – hemoglobin and intracellular fluid are spilt into the solution (plasma)
  - Hemolysed blood – plasma is pink in colour thanks to hemoglobin
  - Old fragile RBCs are captured and processed in the red pulp of the spleen
  - After hemolysis in the circulation, hemoglobin is bound to haptoglobin, which prevents filtration of hemoglobin by the kidneys
  - In excessive intravascular hemolysis, there is not enough haptoglobin, and hemoglobin is filtered by the kidneys and lost in the urine (hemoglobinuria), together with iron (iron loss). It can lead to tubule blockage and kidney damage (hemoglobinuric nephrosis)

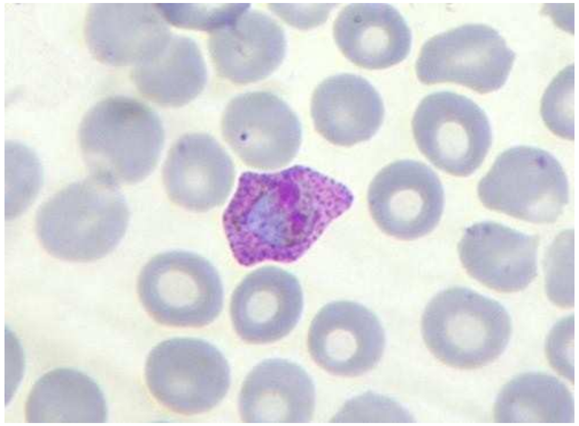
Blood with sediment and various levels of hemolysis

Urine with various levels of hemoglobinuria

Types of hemolysis

- Physical
  - Mechanical damage to the membrane, shaking, ultrasound, extreme temperature changes, UV radiation
- Osmotic
  - RBC in a hypotonic solution absorbs water and bursts
- Chemical
  - A chemical reaction of lipids in the membrane with a chemical substance – strong acids and bases, fat solvents, surfactants (detergents)
- Toxic
  - Bacterial toxins, poisons (plant, snake, insect, spider,...), parasites (Plasmodium spp. - malaria)
- Immunological
  - Transfusion of incompatible blood – immune system hemolyzes erythrocytes (via complement)

Malaria
(Plasmodium spp.)

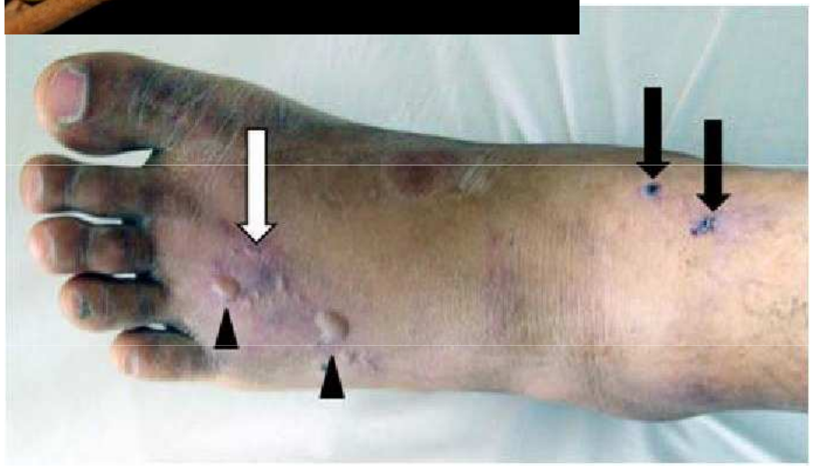


Hemolytic poisons

Brown recluse (*Loxosceles reclusa*) is native to America, and very rarely appears in Europe, at least for now

White-lipped tree viper (*Cryptelytrops albolabris*)

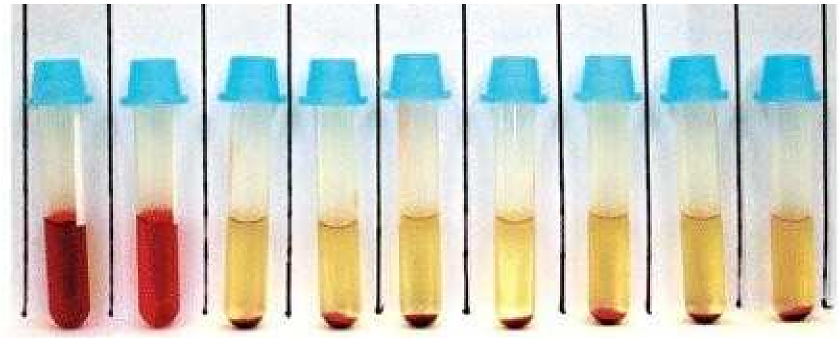
Vipers and rattlesnakes also have hemolytic poisons. Czech viper (adder) has a combination of hemolytic and neurotoxic poison. It is dangerous for weakened individuals, mainly due to an allergic reaction.



Chill, almost all Czech spiders are poisonous, but only some can bite through human skin.
Examples: Moravian steppe, Moravian weaver, poison ivy, rarely poison weaver (black widow). But apart from the black widow, they are rare, outdoor, shy spiders. The bite usually causes a local allergic reaction that disappears within two days

Osmotic resistance of RBCs

- Estimation of RBCs resistance in hypotonic solutions
  - A specific method used in the differential diagnosis of hemolytic anemia
- Minimal osmotic resistance (0.40-0.44%)
  - Concentration of hypotonic NaCl solution in which first RBCs are hemolysed – there is a pink coloured plasma above the sediment, the least resistant RBCs hemolyze
- Maximal osmotic resistance (0.30-0.33%)
  - Lowest concentration of hypotonic NaCl solution in which the small fraction of RBCs are still not hemolyzed – the last tube containing the sedimented RBCs, the most resistant ones
- Osmotic resistance range
  - The difference between minimal and maximal osmotic resistance



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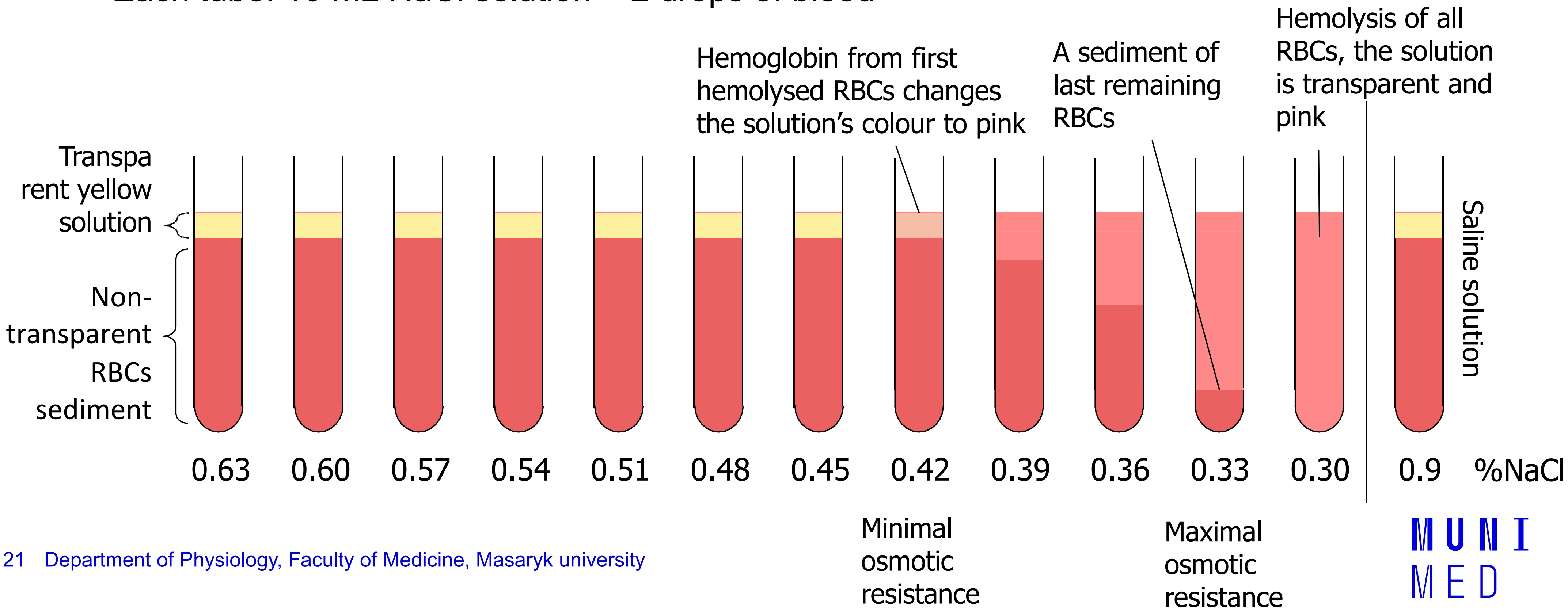
Osmotic resistance of RBCs – in practice

- Solution gradient from weakly hypotonic to strongly hypotonic
  - Each tube: 10 mL NaCl solution + 2 drops of blood



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Pathological values of osmotic resistance

- Higher values of minimal osmotic resistance
  - Congenital hemolytic anemia
- Lower values of maximal osmotic resistance
  - Polycythemia vera
  - Thalassemia
  - Sickle cell disease
  - Sideropenia
  - Status after splenectomy

Isotonic hemolysis

- *In vitro* conditions
- **Isotonic glucose solution**: RBCs intake and metabolize glucose, the solution becomes hypotonic and osmotic hemolysis occurs
- **Isotonic urea solution**: urea freely passes through the membrane into the RBCs (it diffuses along its concentration gradient) and the surrounding solution becomes hypotonic