

- (III.) Erythrocyte sedimentation rate
- (IV.) Estimation of osmotic resistance of red blood cells

Physiology I - practical

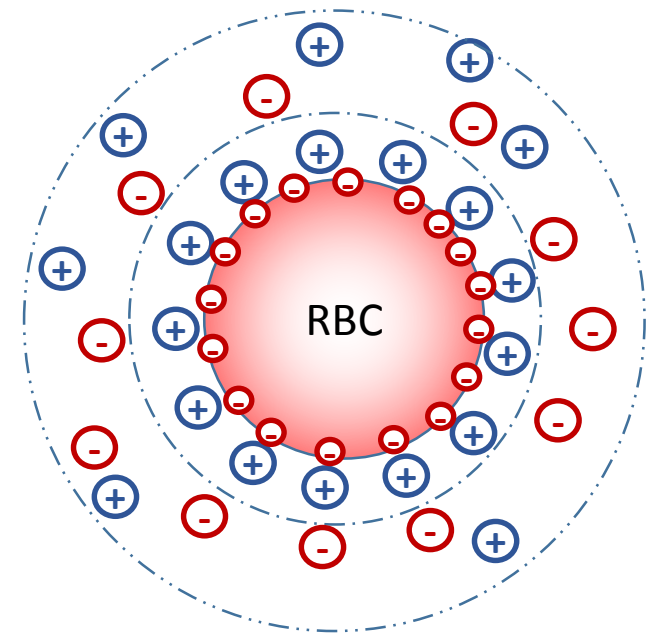
Sedimentation of erythrocytes (RBCs)

- 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 suspension stability of blood

Suspension stability of blood

Helmholtz electrical double-layer

- **Negative charge** on the membrane of red blood cell membrane
- Due to attractive electrostatic forces, positive charged ions are driven
- RBCs are driven away each from other



Mechanism of erythrocyte sedimentation

- Gravitational force
- Factors impairing the Helmholtz electrical double-layer cause aggregation of RBCs into cylindrical aggregates (rouleaux) with higher volume and relatively smaller surface (compared to corresponding volume of separate RBCs)
- Aggregates fall down (sedimentate) faster than separate RBCs = value of ESR is higher

Basic factors affected ESR

- Volume of RBC: bigger RBC falls down faster
- Number of RBCs: higher RBC count decreases ESR
- Plasmatic proteins
 - Albumin – is charged negatively
 - hypoalbuminemia = increased ESR
 - Immunoglobulins, fibrinogen – negative and positive charge, impairment of Helmholtz electric double-layer
 - Increased plasmatic concentration of immunoglobulins (e.g. due to systemic infection or inflammatory disease) = increased ESR

Estimation of ESR



- Fahraeus-Westergren (FW)
 - Glass tube in vertical position
 - Measured after 1 hour (2 hours)
- Wintrobe
 - 100 mm long, thin glass tube in upright or oblique position
 - Measured after 15 minutes
 - Less sensitive than the FW



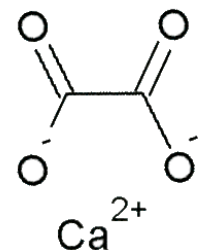
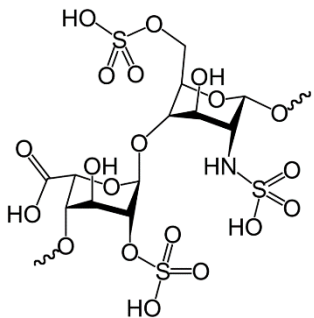
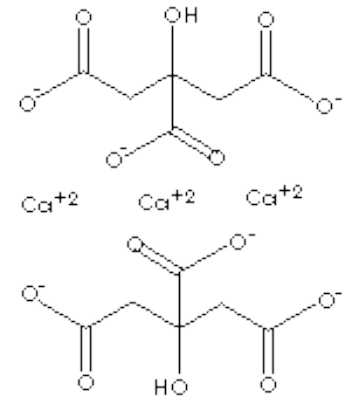
Robin Fåhræus (1888-1968)



Maxwell M. Wintrobe (1901-1986)

Anti-coagulated blood

- Blood sample with the coagulating system blocked
- Possible anticoagulant agents
 - Chelation of Ca^{2+} ions (chelation anticoagulants)
 - Sodium citrate
 - EDTA (ethylenediaminetetraacetic acid)
 - Sodium oxalate
 - Activation of antithrombin III – heparin and its low molecular weight derivatives (LMWHs)



Physiological values of ESR (FW)

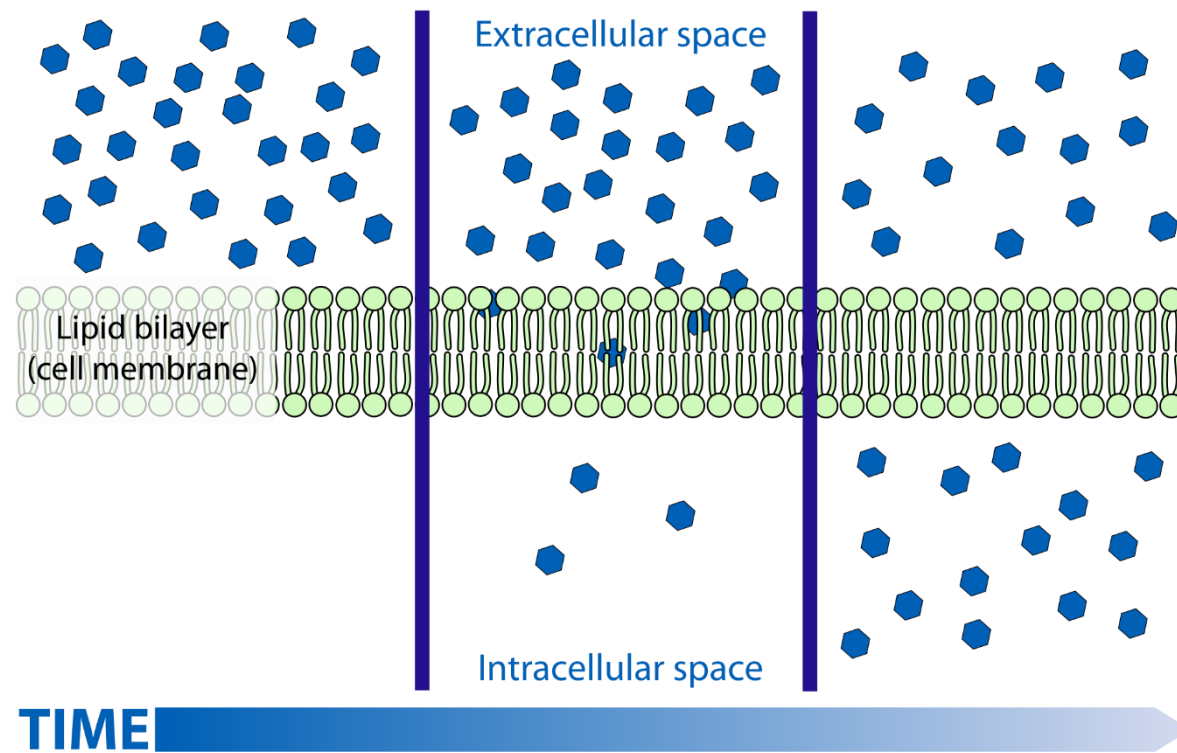
- Men: 2-8 mm/h
 - Women: 7-12 mm/h
 - Newborns: 2 mm/h
 - Infants: 4-8 mm/h
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- Intersexual differences in adults are caused by different RBC count and by differences in concentration of plasmatic proteins

Changes of ESR (FW)

- Increased ESR (FW)
 - **Pregnancy, menstruation**
 - Macrocythemia
 - Inflammation
 - Infection
 - Necrosis (myocardial infarction, injury)
 - Cancer
 - Relative/absolute loss of albumin (e.g. renal disorders)
- Decreased ESR (FW)
 - spherocytosis
 - Polycythemia vera
 - Leukocytosis
 - Dysproteinemia – hypofibrinogenemia, hypogammaglobulinemia
 - Dehydration

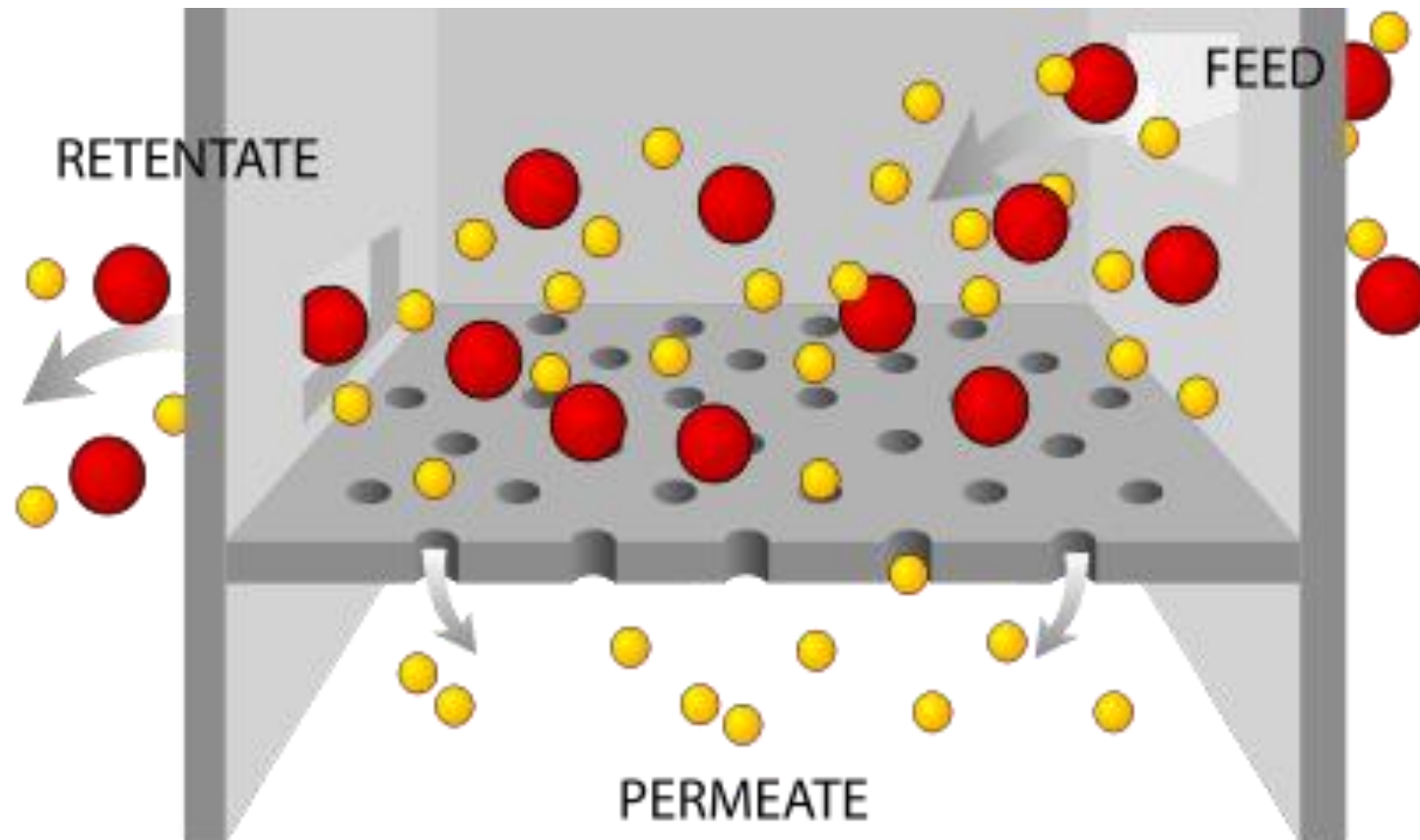
Diffusion

- movement of molecules or atoms from a region of high concentration to a region of low concentration



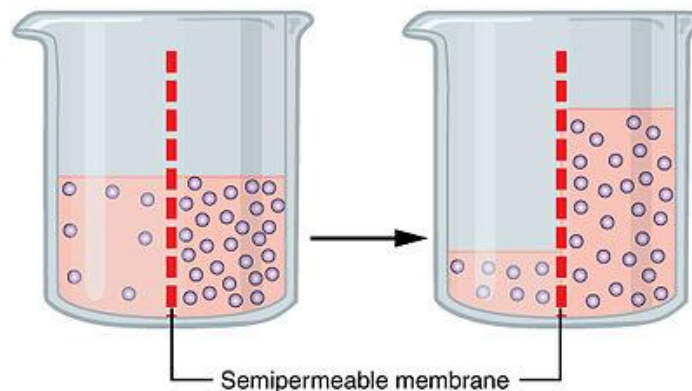
Filtration

- Separation of fluids from the „solid“ parts through the membrane



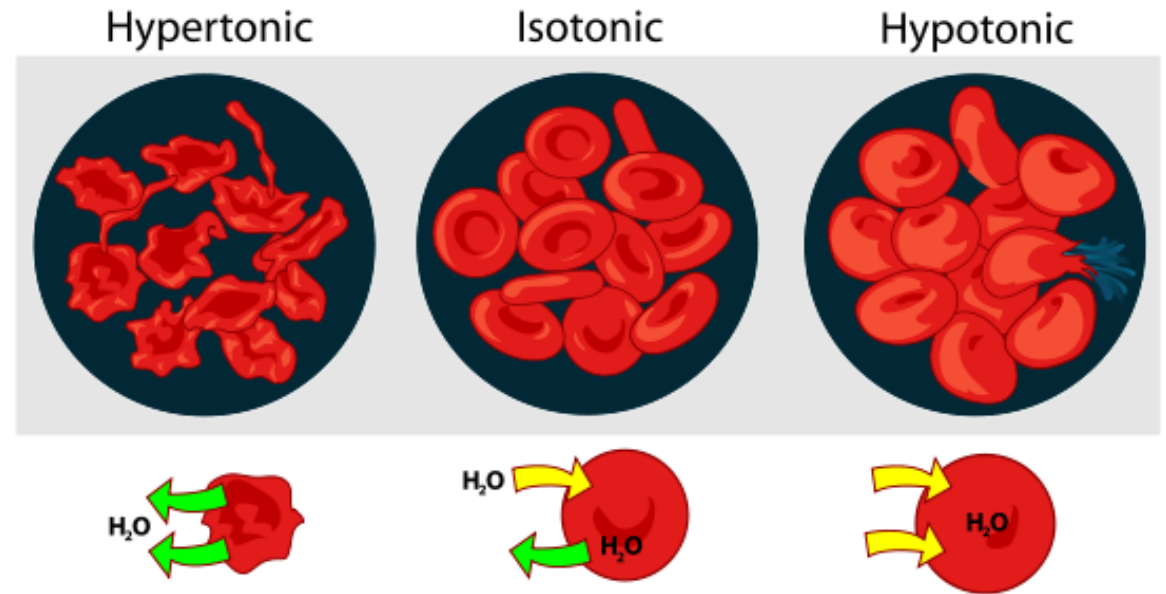
Osmosis

- Flow of solvent (water) through semipermeable membrane according to osmotic gradient
- **Osmotic pressure** – pressure necessary to stop the osmosis
- **Osmolarity** – concentration of osmotic active particles in 1 liter of solution
- **Osmolality** – concentration of osmotic active particles per 1 kg of solvent
- Osmolality of plasma = $2 * [Na^+] + [glc] + [urea] = 275-295 \text{ mmol/kgH}_2\text{O}$



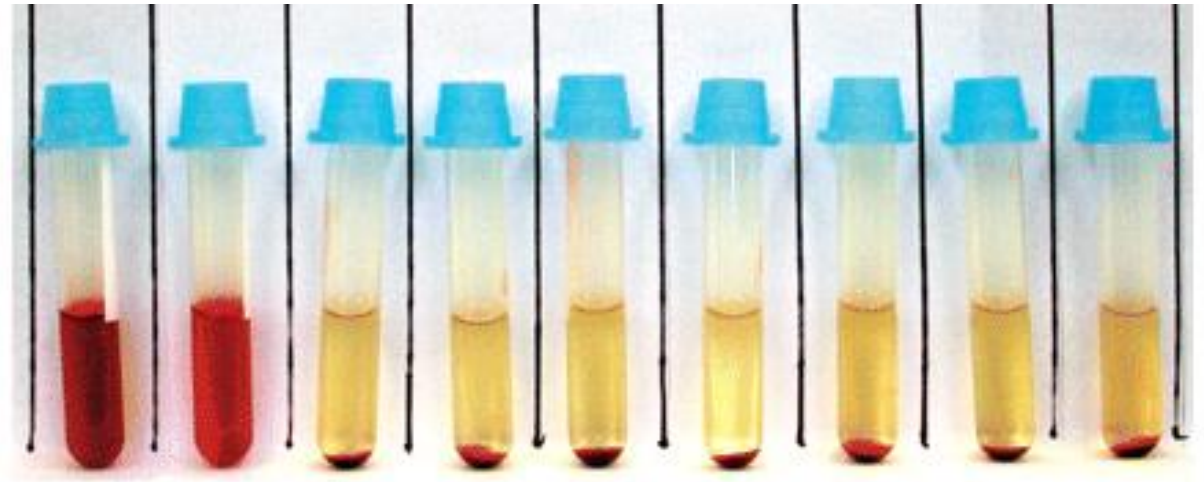
Tonicity

- Osmolality of solution compared to osmolality of intracellular fluid
- **Hypotonic** solutions
- **Isotonic** solutions
 - 0.9% NaCl = physiological solution (saline)
- **Hypertonic** solutions



Hemolysis

- Break-up of RBC due to disintegration of its membrane
- Hemoglobin is spilled into the solution (plasma)
- Mechanisms
 - Physical
 - Osmotic
 - Chemical
 - Immunological
 - Toxic



Osmotic resistance of RBCs

- Estimation of resistance of RBCs in hypotonic solutions
- Specific method
 - differential diagnosis of haemolytic anaemia
- **Minimal osmotic resistance** – concentration of hypotonic solution of NaCl, in which first RBCs (minimally resistant) are haemolysed
 - 0,4-0,44%
- **Maximal osmotic resistance** – lowest concentration of hypotonic solution of NaCl, in which the small fraction of RBCs (maximally resistant) are still not haemolysed
 - 0,3-0,33%
- **Osmotic resistance range** – difference between minimal and maximal osmotic resistance

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

Sources of the pictures

- Slide 6 - <http://www.medipos.cz/odberove-nadoby-pomucky/pipeta-zkumcitrat-jednorazph-dispette.html>, <http://www.coe.ou.edu/isb/awardees/1966.htm>, <http://www.hematology.org/Publications/Legends/3898.aspx>, [cited 30.8.2015]
- Slide 7 - <http://www.chemicaland21.com/lifescience/foco/CALCIUM%20CITRATE.htm> + https://cs.wikipedia.org/wiki/%C5%A0%C5%A5avelan_v%C3%A1penat%C3%BD#/media/File:Calcium_oxalate.png + <https://cs.wikipedia.org/wiki/Heparin#/media/File:Heparin-2D-skeletal.png> [cited 30.8.2015]
- Slide 10 - https://en.wikipedia.org/wiki/Passive_transport#/media/File:Scheme_simple_diffusion_in_cell_membrane-en.svg [cited 30.8.2015]
- Slide 11 - https://en.wikipedia.org/wiki/Passive_transport#/media/File:Filtration_diagram.svg [cited 30.8.2015]
- Slide 12 - https://en.wikipedia.org/wiki/Osmosis#/media/File:0307_Osmosis.jpg [cited 30.8.2015]
- Slide 13 - https://en.wikipedia.org/wiki/Passive_transport#/media/File:Osmotic_pressure_on_blood_cells_diagram.svg [cited 30.8.2015]
- Slide 14 - <http://labmed.ascpjournals.org/content/41/4/209/F2.expansion.html> [cited 30.8.2015]