(I.) Red blood cell count. Estimation of haemoglobin concentration. Calculated parameters of red blood cells
(II.) Estimation of blood group by slide method

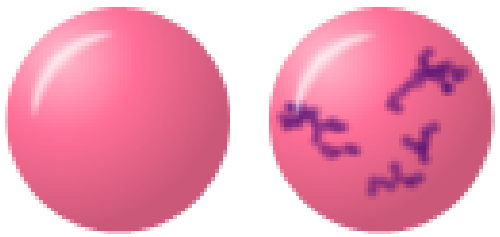
Physiology I – practicals

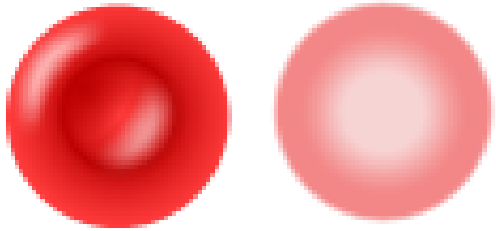
Red Blood Cell (RBC) – erytrocyte

- Anucleated cell, the most abundant blood cell
- Shape:
  - Biconcave disc
    - surface increased by 30%
    - spectrin
    - deformation inside the capillaries
- Functions:
  - Transport of oxygen (haemoglobin)
  - Role in acidobasic balance and CO_2 transport
- Size:
  - Normocyte: 7.5 µm
  - Microcyte: ≤ 7 µm
  - Macrocyte: ≥ 9 µm
  - Megalocyte: ≥ 20 µm

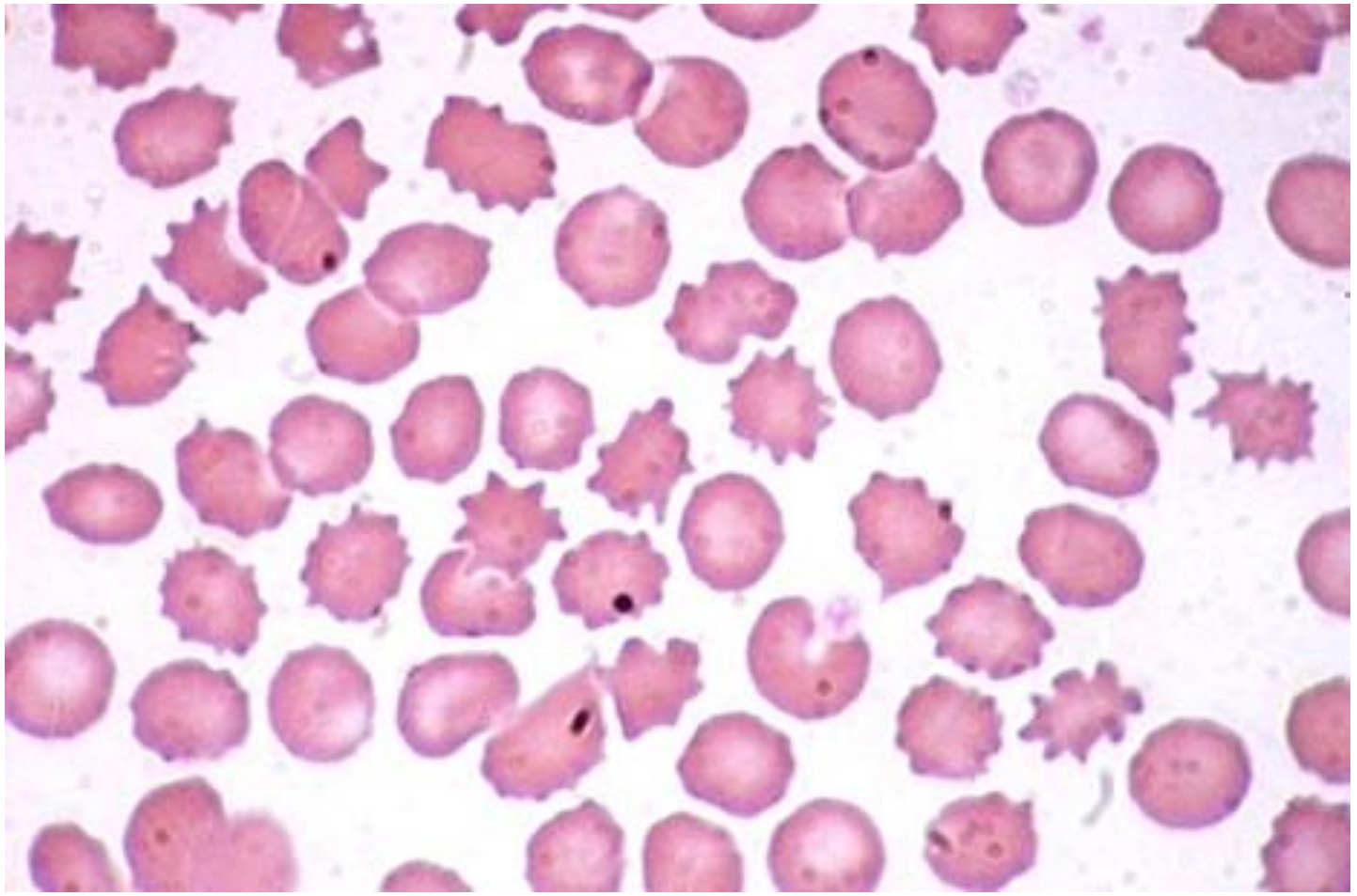
Reticulocyte

- Immature RBC
- In peripheral blood: 1% ± 0.5% of RBC
  - reticulocytosis: increased ratio of reticulocytes in peripheral blood
- No nucleus, but residues of membrane organelles in cytoplasm (substantia granulo-filamentosa)
- Within 48 hrs. maturation to RBC

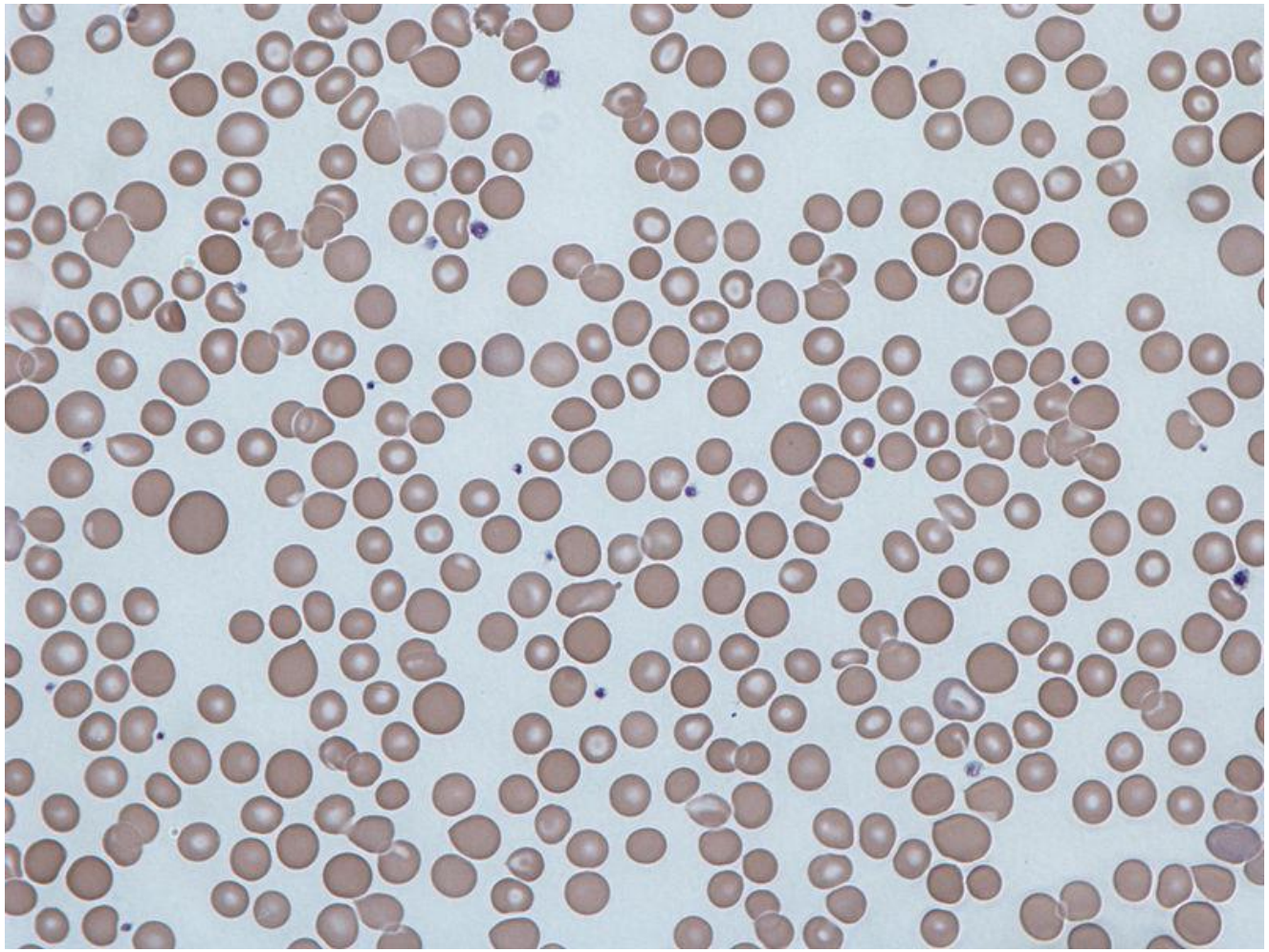




Alteration of shape: poicylocytosis



Anisocytosis: varying size of RBCs



RBC count

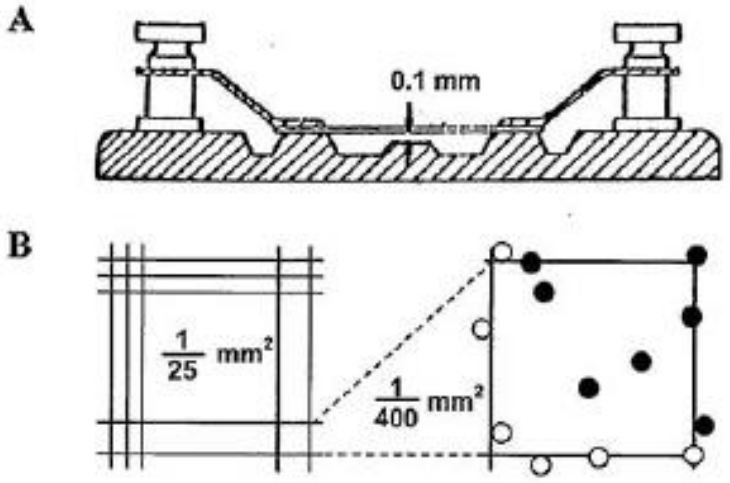
- Number of RBCs
  - Men: 4.3-5.3 * 10^{12} / l
  - Women: 3.8-4.8 * 10^{12} / l
  - Newborns: 4.4-7.0 *10^{12} / l

- Intersexual differences:
  - Men: testosterone (male sex hormone) stimulates releasing of erythropoietin
  - Women in fertile period: relative erythrocytopaenia due to menstruation

Alterations of RBC count

- polyglobulia – increased number of RBCs
- erythrocytopaenia – decreased number of RBCs

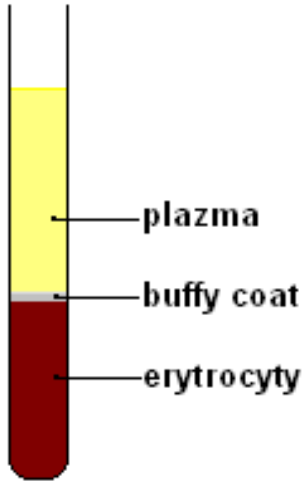
Estimation of RBC count



- Automatic methods

- Classical method: Bürker´s chamber + Hayem´s solution

Haematocrit



- Volume percentage of blood cells in blood sample (erythrocyte volume fraction)
- Centrifugation of **anti-coagulated** blood
  - Plasma
  - Buffy coat
  - RBC

- Hct (hematocrit)
  - Men: 42-52%
  - Women: 37-47%

Haemoglobin

- Haemoglobin concentration (HGB)
  - Men: 140-180 g/l
  - Women: 120-160 g/l
  - Newborns: 160-240 g/l

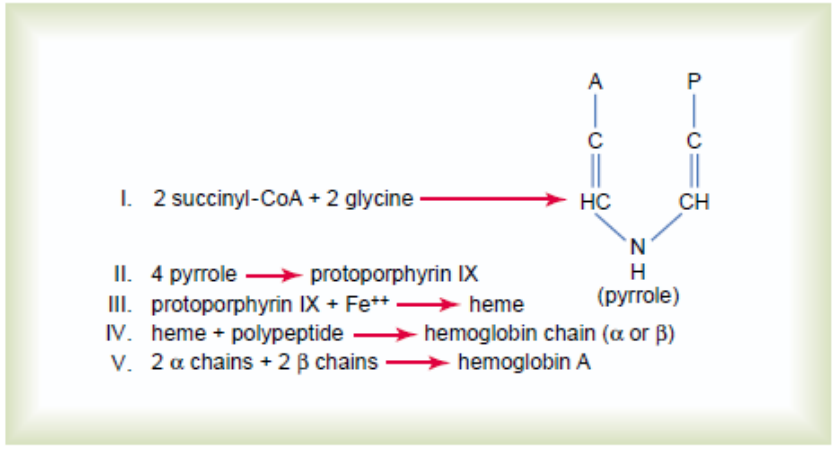


Figure 32–5

Formation of hemoglobin.

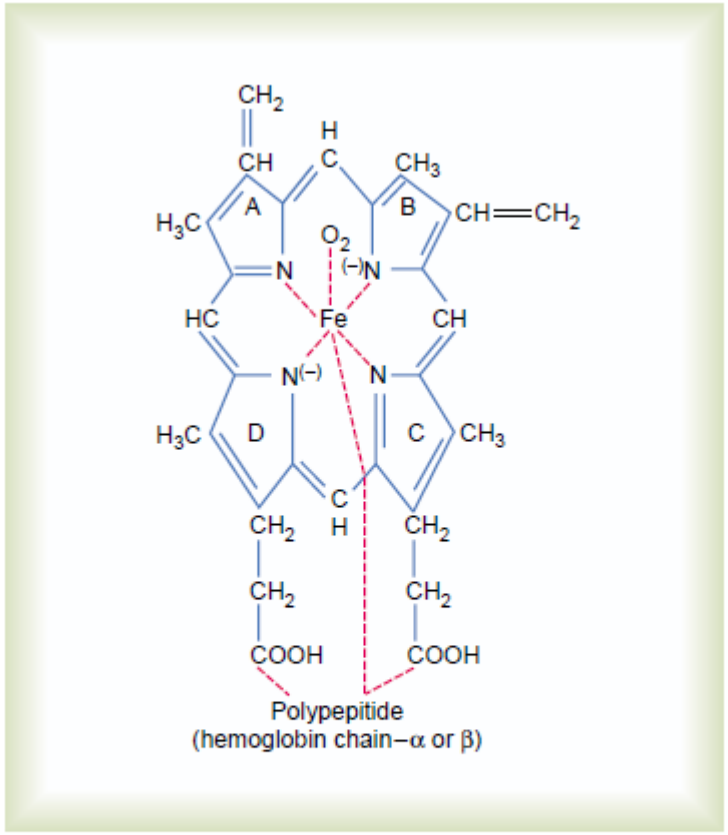


Figure 32–6

Basic structure of the hemoglobin molecule, showing one of the four heme chains that bind together to form the hemoglobin molecule.

Estimation of HGB

- Spectrophotometric method:
  - Lysis of RBC by transforming solution + stabilisation of haemoglobin (Hb-cyanide)
  - Measurement of light absorption

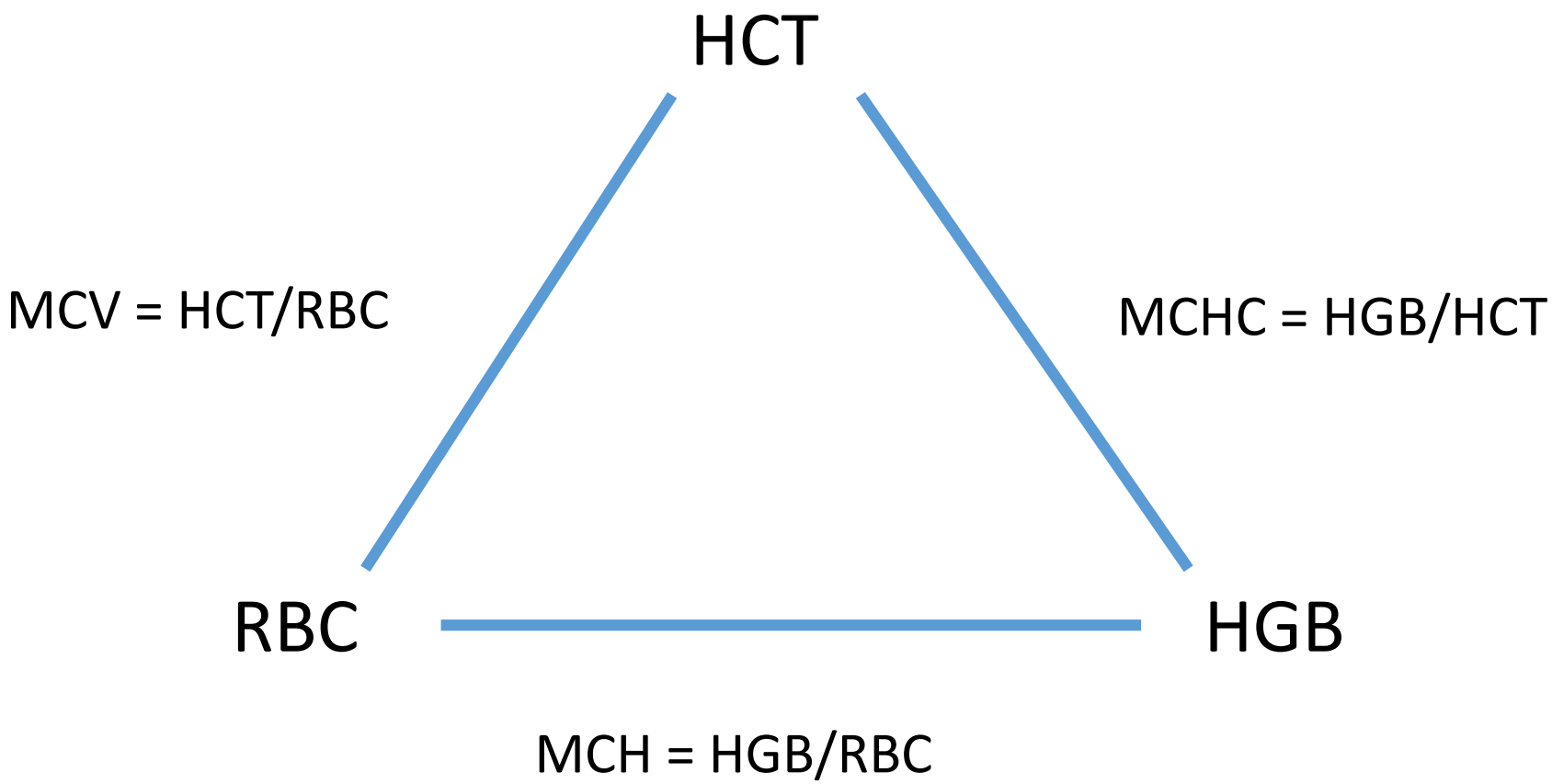
Haemoglobin - derivatives

- **Oxyhaemoglobin** – haemoglobin + O_2
- **Carboxyhaemoglobin** – haemoglobin + CO
- **Carbaminohaemoglobin** – haemoglobin + CO_2
- **Methaemoglobin** = haemoglobin with oxidized Fe (Fe^{3+})

Calculated parameters of RBCs

- Average volume of RBC (**MCV**, mean corpuscular volume)
  - MCV = HCT/RBC (hematocrit/ red blood count) = 80-95 fl
- Average weight of Hb in RBC (**MCH**, mean corpuscular hemoglobin)
  - MCH = HGB/RBC (haemoglobin/ red blood count) = 28-32 pg
- Average concentration of Hb in RBC (**MCHC**, mean corpuscular hemoglobin concentration)
  - MCHC = HGB/HCT (haemoglobin/ haematokrit) = 310-360 g/l
- Red cell distribution width (**RDW**) = 11,5-14,5%
  - Variation of RBCs size
  - ↑RDW – anisocytosis

Calculated parameters of RBCs



Anaemia

- **Decreased concentration of Hb** in blood

- Symptoms:
  - Pale mucose membrane
  - Fatigue
  - Tachycardia
  - Dyspnoe

Sideropenic anaemia

- Deficiency of Fe^{2+} → decreased production of RBCs→ hypoxia stimulates releasing of erythropoietin → increased production of RBCs with lack of Hb

- **Microcytic hypochromic anaemia**

Pernicious anaemia

- Deficiency of B_{12} or folic acid
- **Macrocytic, hyperchromic anaemia**

Blood groups

- Depend on antigens on the surface of RBC
- Various systems: AB0, Rh, MNs, Kell, Lewis

AB0 system

- Agglutinogens – glycoproteins:
  - 0 – no agglutinogen, incidence: 33%
  - A – agglutinogen A, incidence: 45%
  - B – agglutinogen B, incidence: 16%
  - AB – both of agglutinogens A and B, incidence: 6%

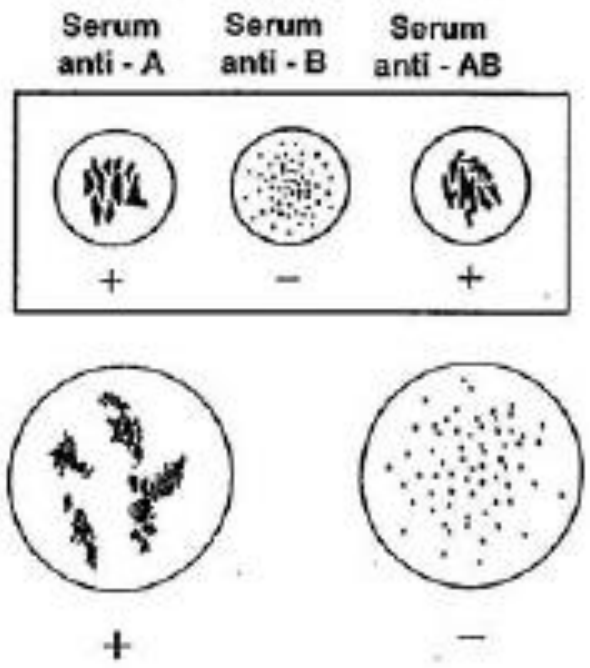
- Agglutinins – IgM antibodies:
  - 0 – anti-A + anti-B
  - A – anti-B
  - B – anti-A
  - AB – no agglutinin
  - Production of agglutinins starts in newborns

AB0 system: heredity

- Alleles: A, B, i
- Co-dominancy: A and B
- Allele i is recessive for both other alleles
- Genotypes:
  - Group A: $I^A I^A$, $I^A I^i$
  - Group B: $I^B I^B$, $I^B I^i$
  - Group AB: $I^A I^B$
  - Group 0: $I^i I^i$

Estimation of blood group by slide method

- Slide method:
  - sera + small amount of blood
  - agglutination



Agglutination

vs.

Coagulation

Rh factor

- Antigens C, c, D, d, E, e
  - D is determinative → Rh^+, dominant
  - Rh^- - recessive homozygote
- Antibodies IgG – production after immunization by antigen D
  - Transfusion of incompatible blood
  - Delivery (miscarriage, abortion) of Rh^+ fetus vs. Rh^- mother
    - **foetal erythroblastosis –** hemolysis of foetal RBCs due to production of anti-D antibodies by maternal immune system → hydrops foetalis
      - Prevention: anti-D serum for mother after delivery/abortion – avert the immunization

Picture sources

- Slide 3 - https://en.wikipedia.org/wiki/Reticulocyte [cited 30.8.2015]
- Slide 4 - http://medicaltreasure.com/poikilocytosis/ [cited 30.8.2015]
- Slide 5 - https://commons.wikimedia.org/wiki/File:Anisocytosis.jpg [cited 30.8.2015]
- Slide 7, 21 – Praktická cvičení z fyziologie, Masarykova univerzita 2011
- Slide 8 - http://www.wikiskripta.eu/index.php/Hematokrit [cited 30.8.2015]
- Slide 9 – Ganong‘s Review of Medical Physiology, Ganong, McGrow Hill, 2010
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