Hematopoiesis

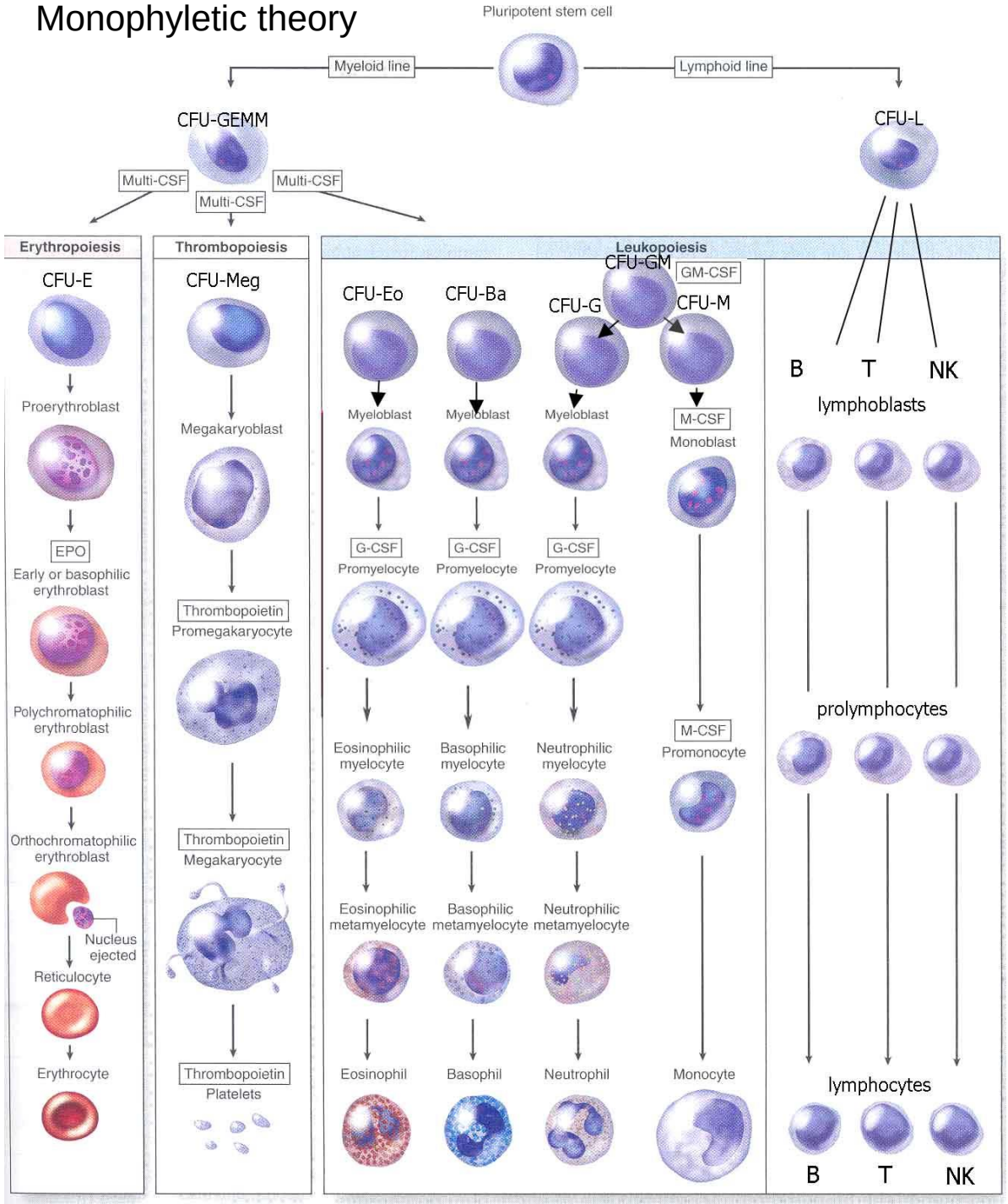
MUDr. Pavel Roštok

Stem cells

Progenitor cells (CFU)

Prekursor cells (blasts)

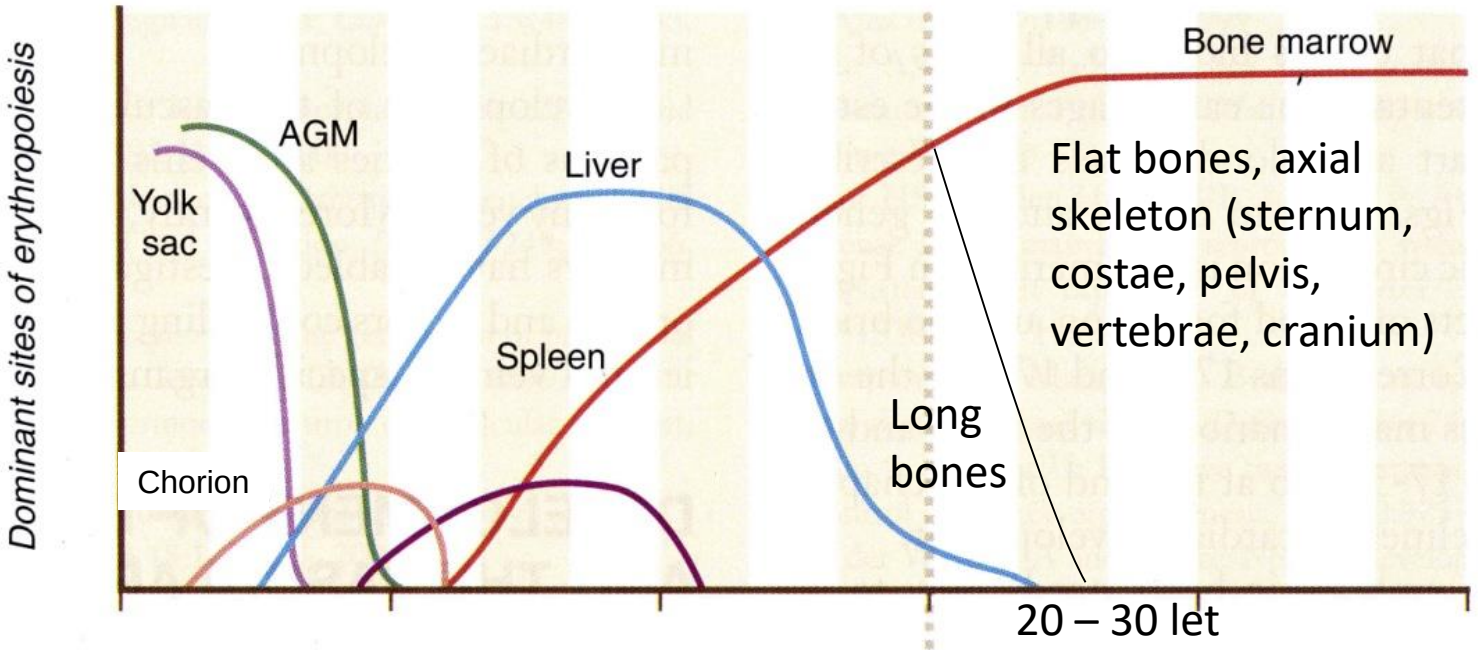
Mature cells



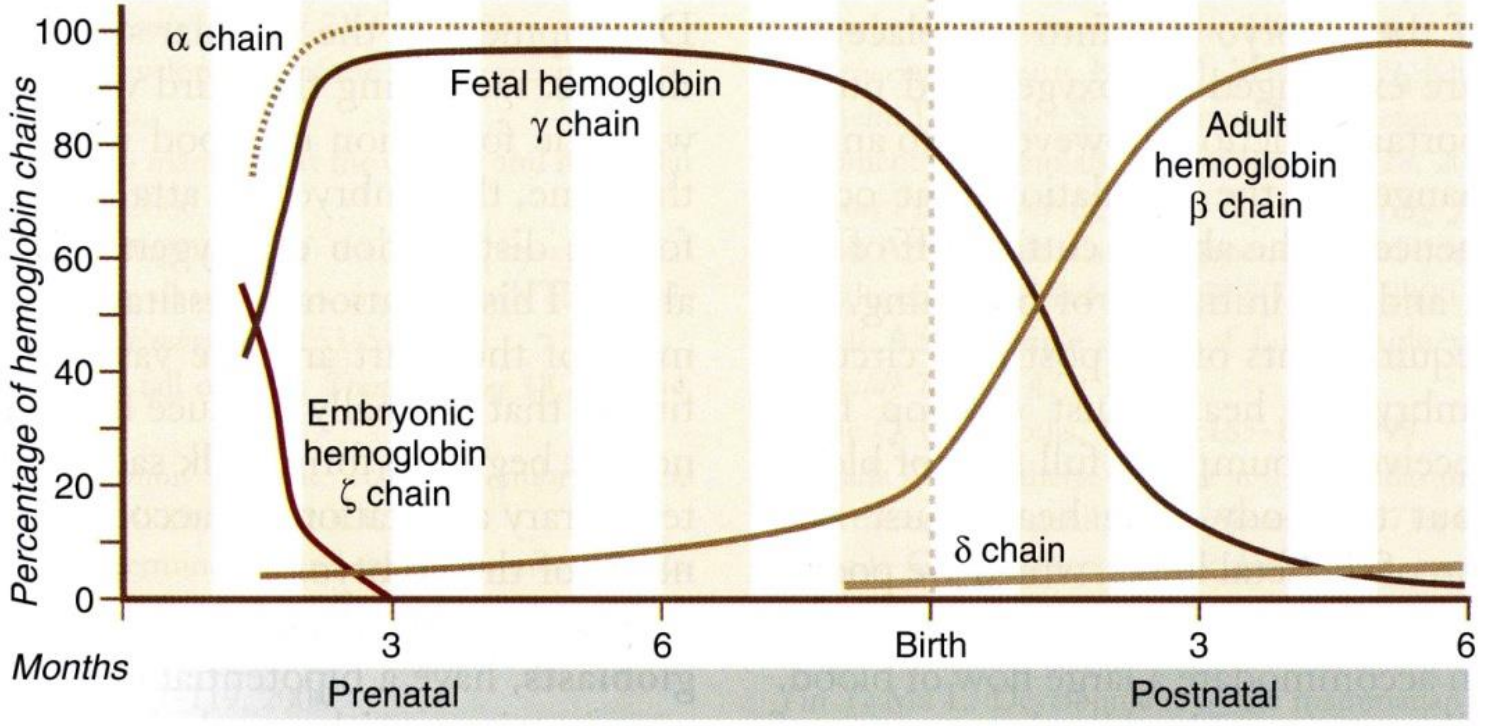
Hematopoiesis - introduction

- Monophyletic theory - leukocytes, erythrocytes and platelets originate from HSCs
- Next level of differentiation is 2 common progenitors - lymphoid CLP and myeloid CMP
- The progenitors give rise to CFUs (colony forming units) and then blasts (precursor cells of a particular type)
- Determining which type a cell will belong to (commitment) is a complex and not yet fully explored process
- Several stages of development follow, where they progressively acquire the characteristics of the respective differentiated cells

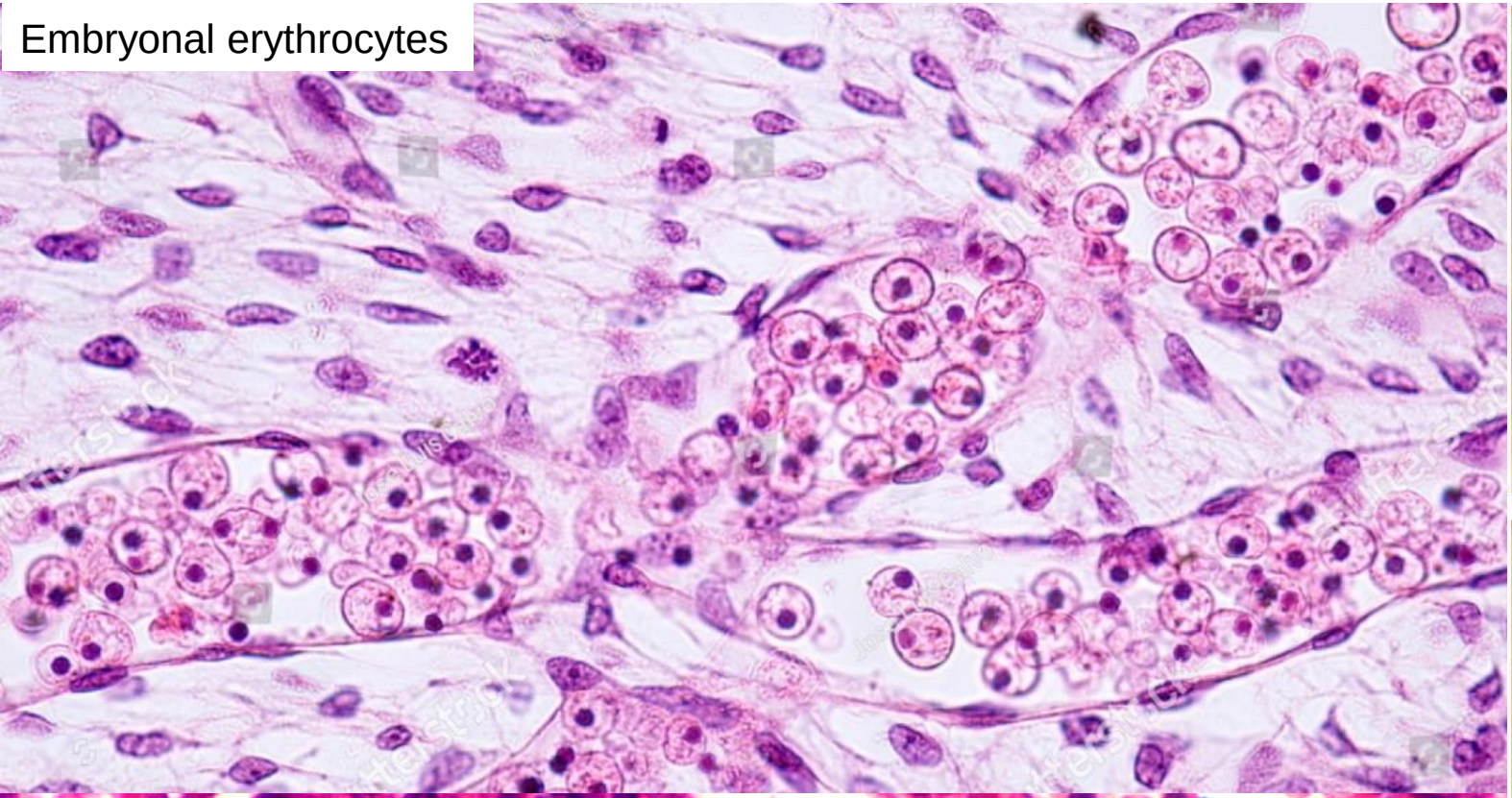
Development of hematopoiesis



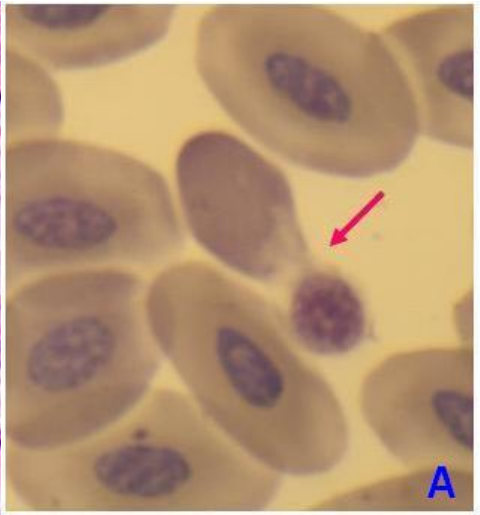
Hemoglobin types in development

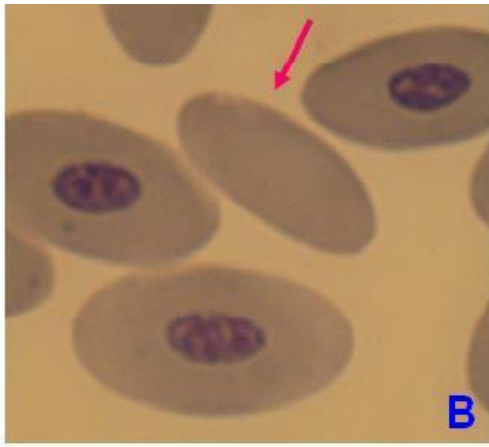


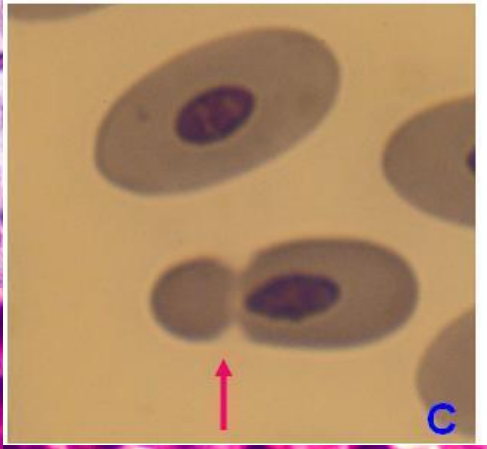
Embryonal erythrocytes

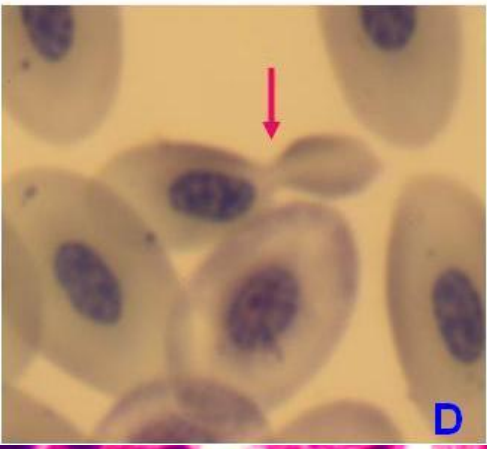


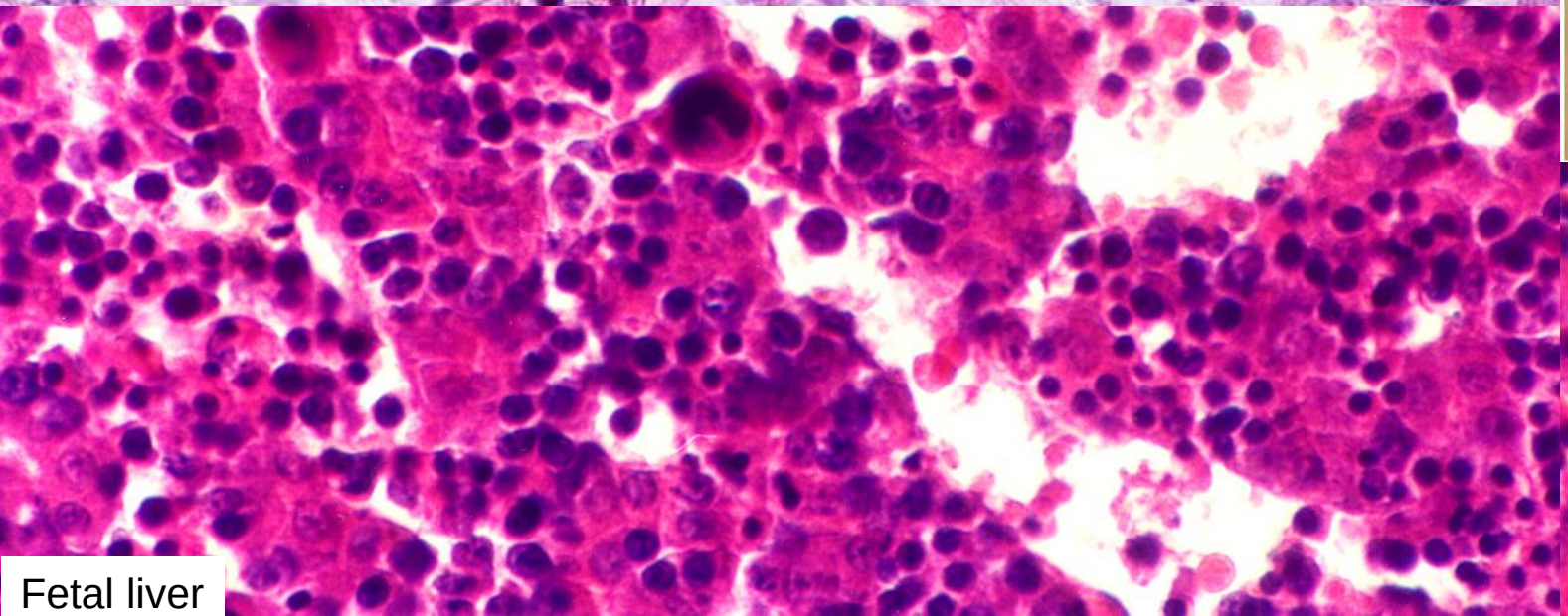
Erytrocytes of a penguin



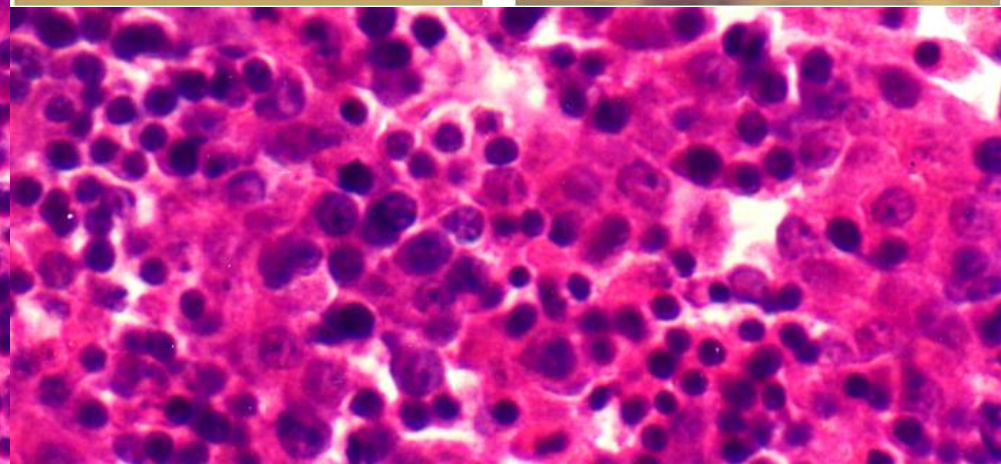


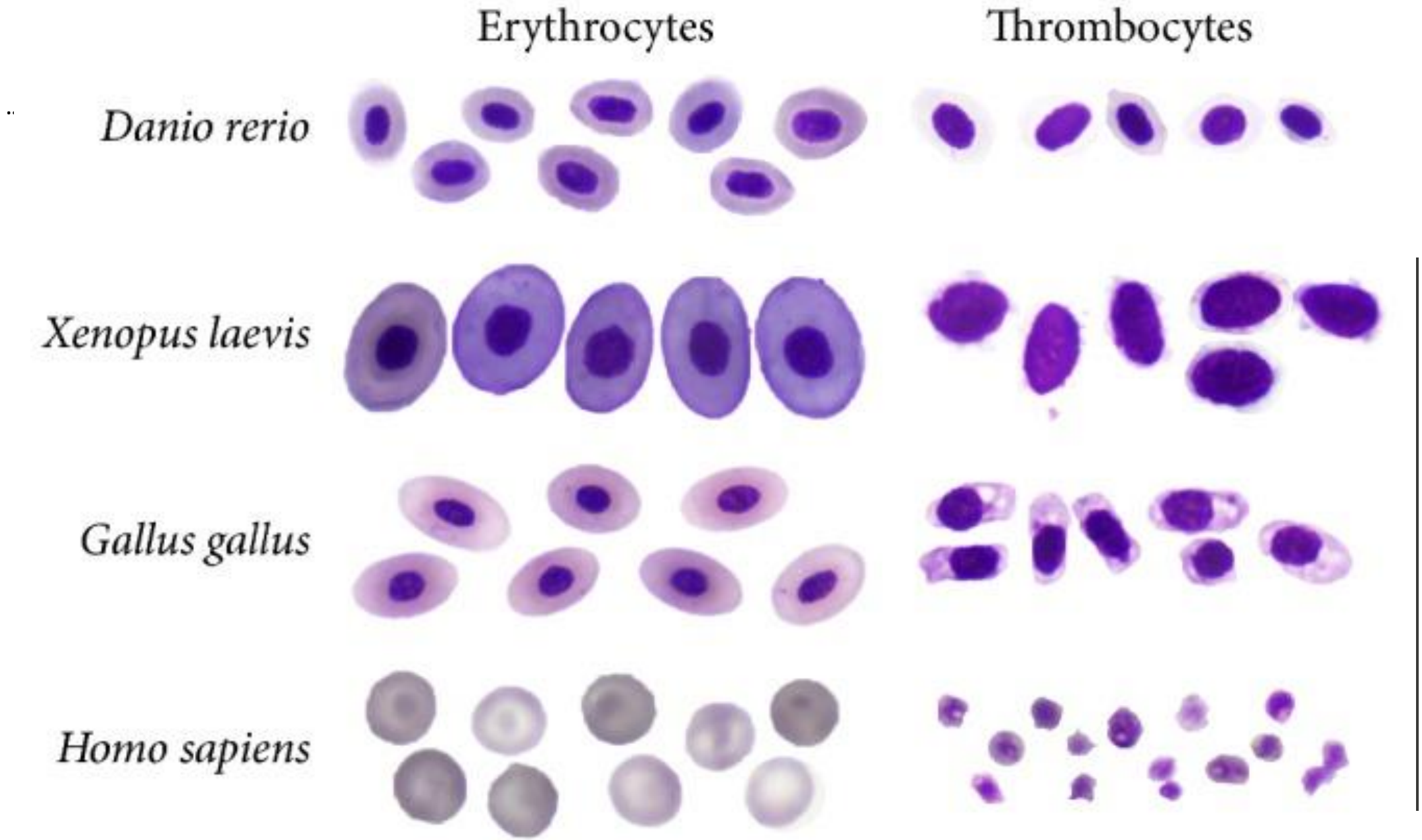






Fetal liver



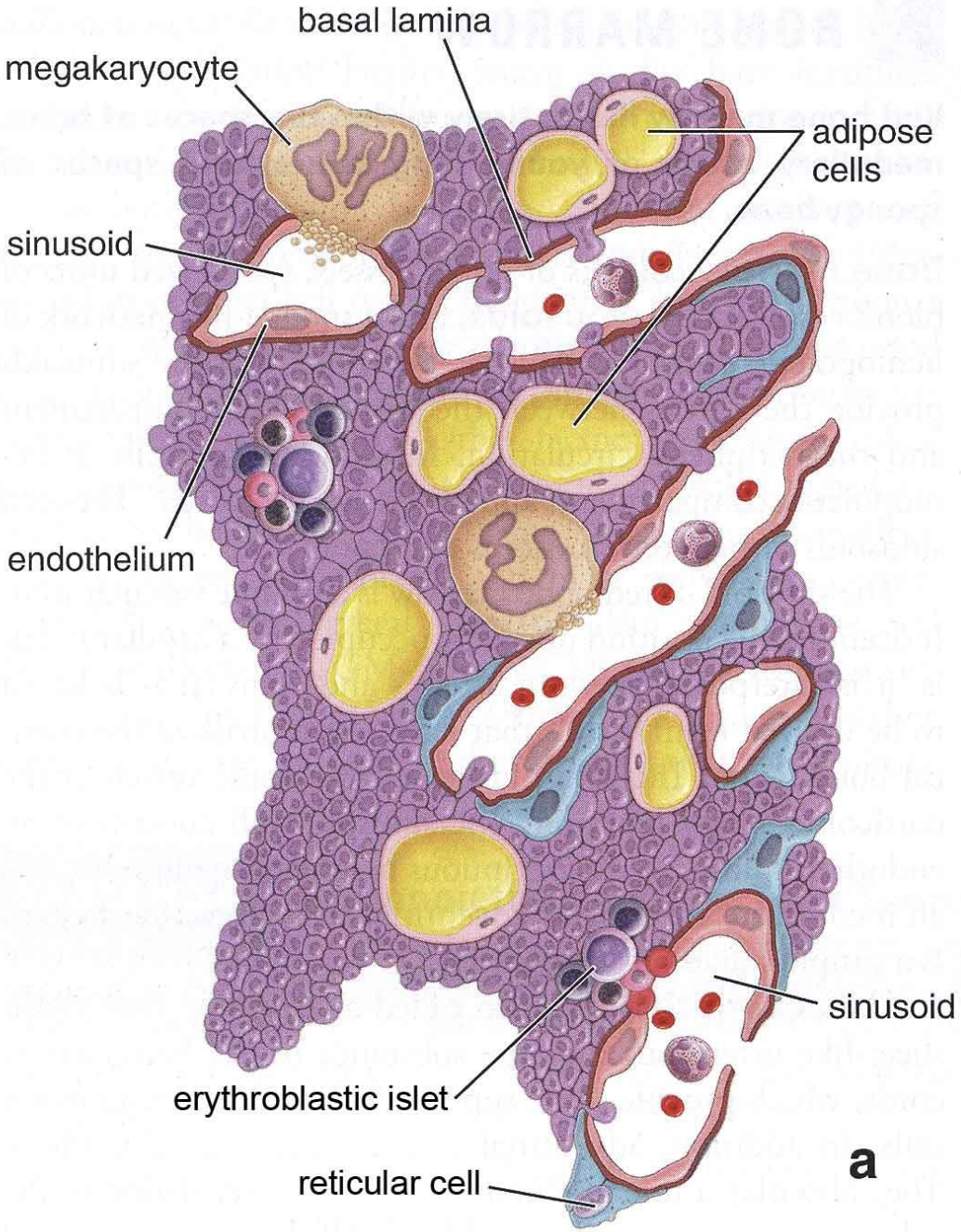


Svoboda O, Bartunek P. Origins of the Vertebrate Erythro/Megakaryocytic System. Biomed Res Int. 2015;2015:632171. doi: 10.1155/2015/632171. Epub 2015 Oct 18. PMID: 26557683; PMCID: PMC4628740.

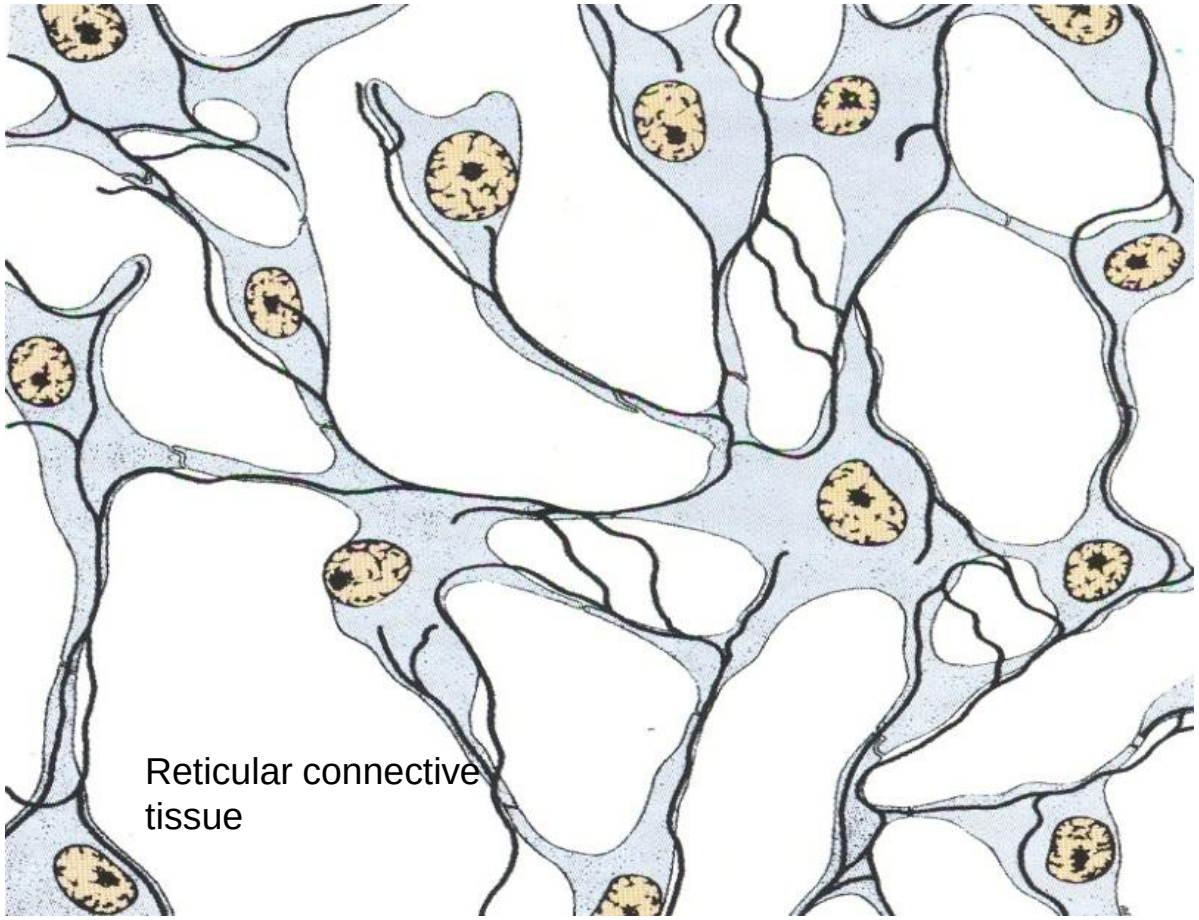
Prenatal development of hematopoiesis

- The first generation of erythrocytes is formed in the yolk sac (called blood islands), these erythrocytes are nucleated and have a special type of hemoglobin (ε, ζ), this phase is less studied
- This embryonic hematopoiesis is replaced by fetal hematopoiesis, originating from HSCs, concentrated mainly in the liver and to a lesser extent in the spleen, enucleated erythrocytes with fetal type hemoglobin are produced (α, γ)
- From the second trimester of pregnancy onwards, the importance of the bone marrow increases, which becomes the only physiological site of haematopoiesis after birth (lymphopoiesis partly in the lymphoid organs); after birth, the γ chain is replaced by the β chain

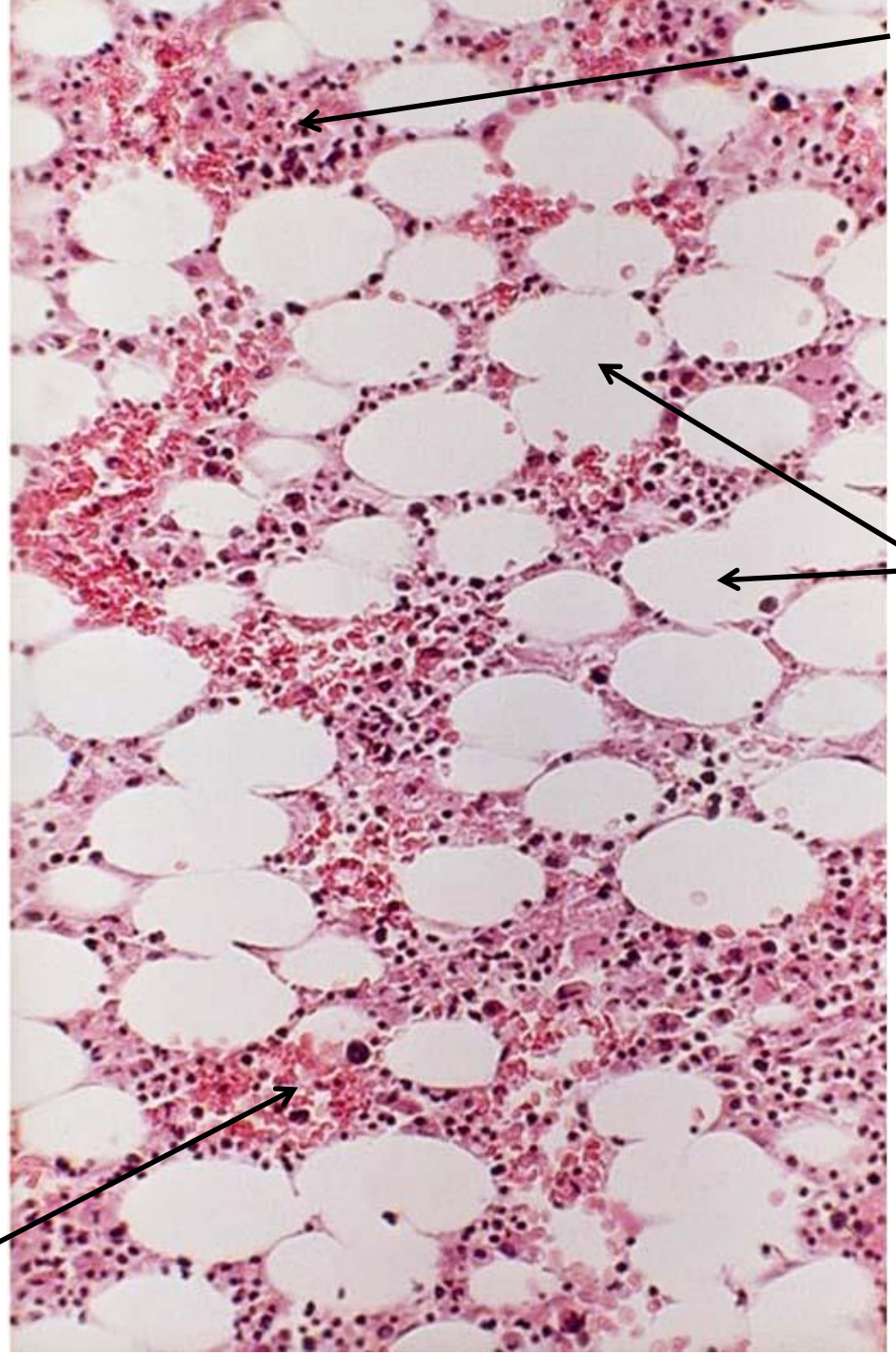
Bone marrow



Pawlina W.: Histology, a Text and Atlas, Wolters Kluwer 2016



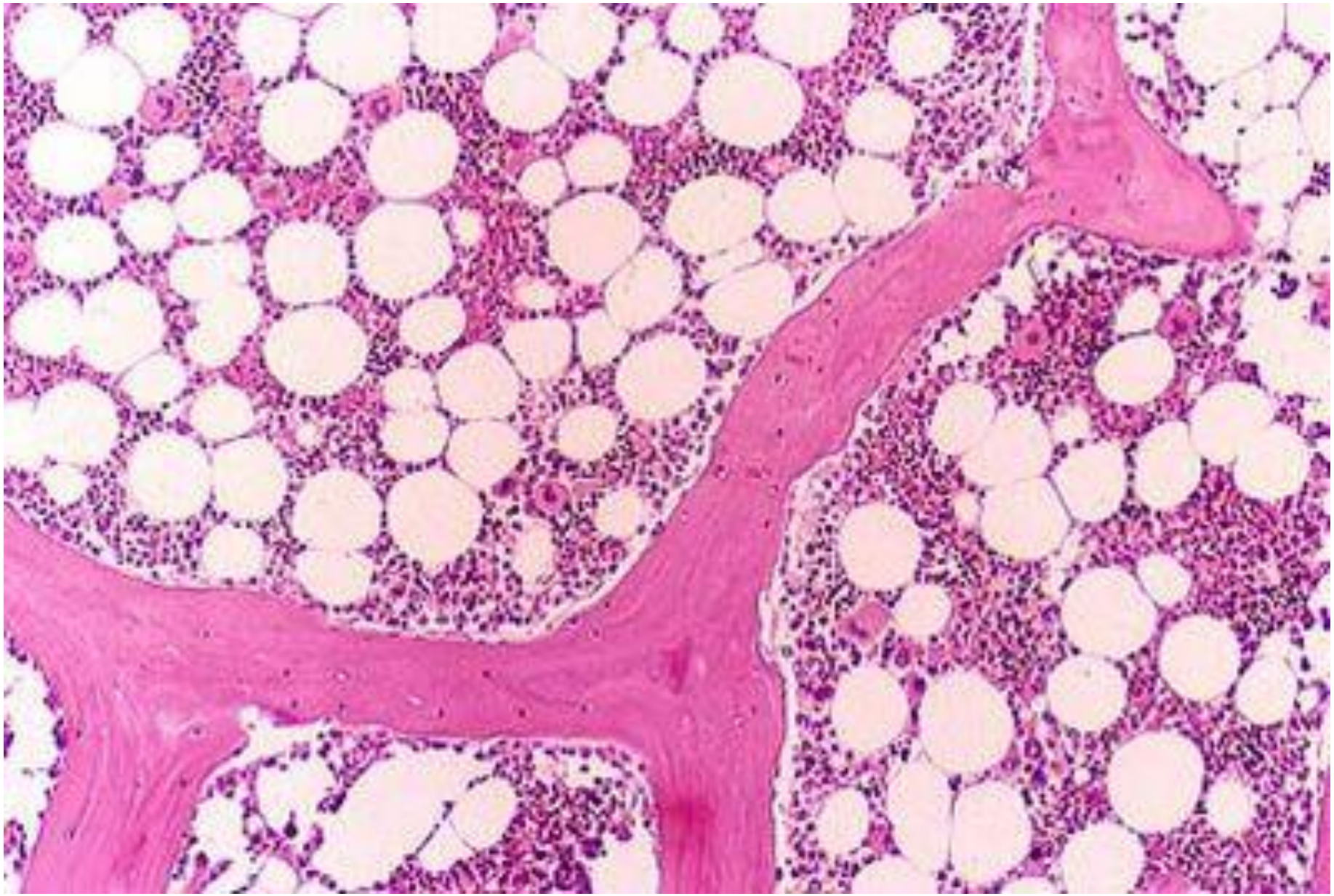
Reticular connective tissue

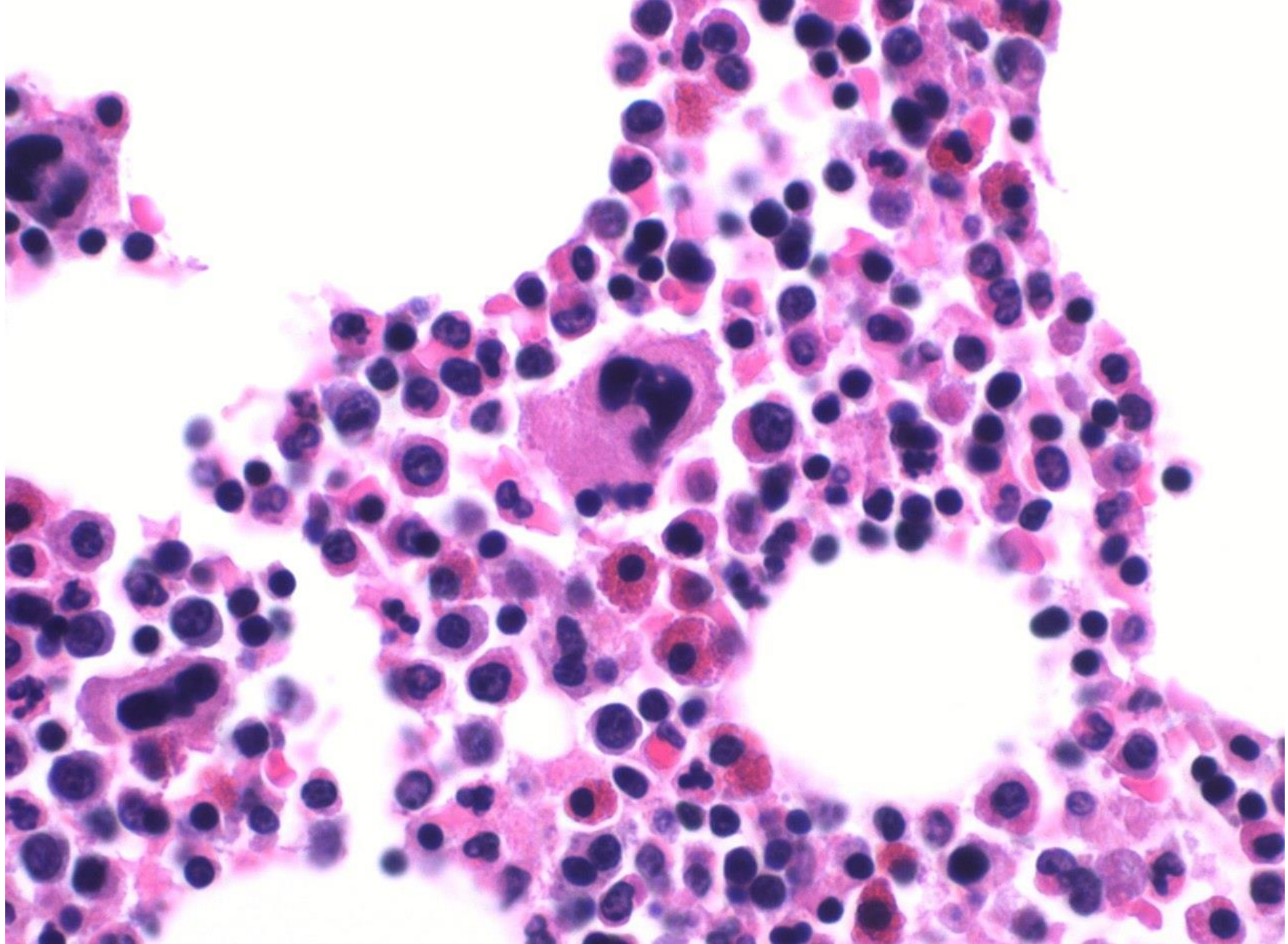


Hemopoetic area

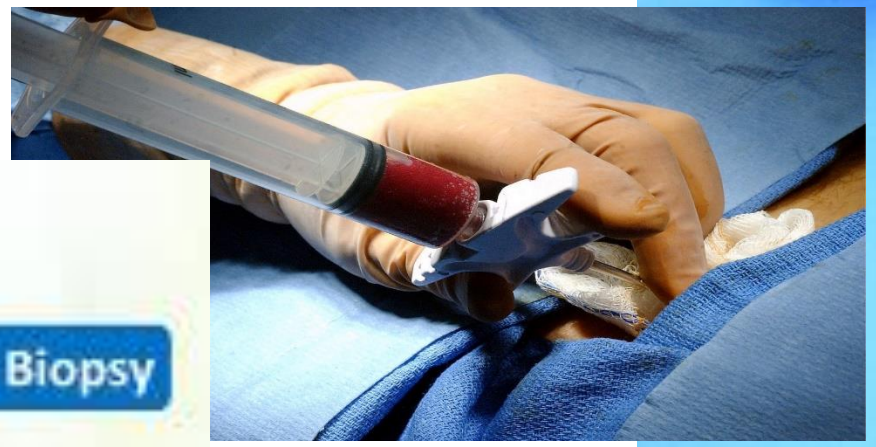
adipocytes

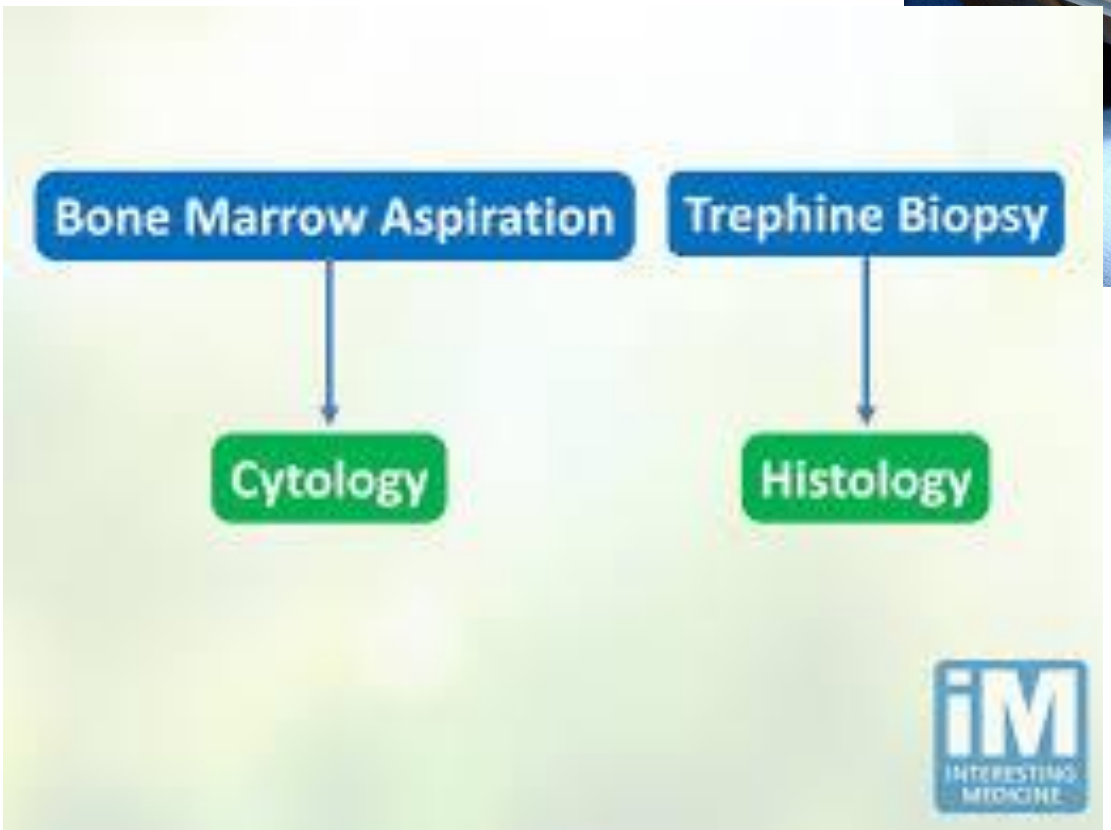
sinusoid





Bone marrow aspiration vs trephine biopsy

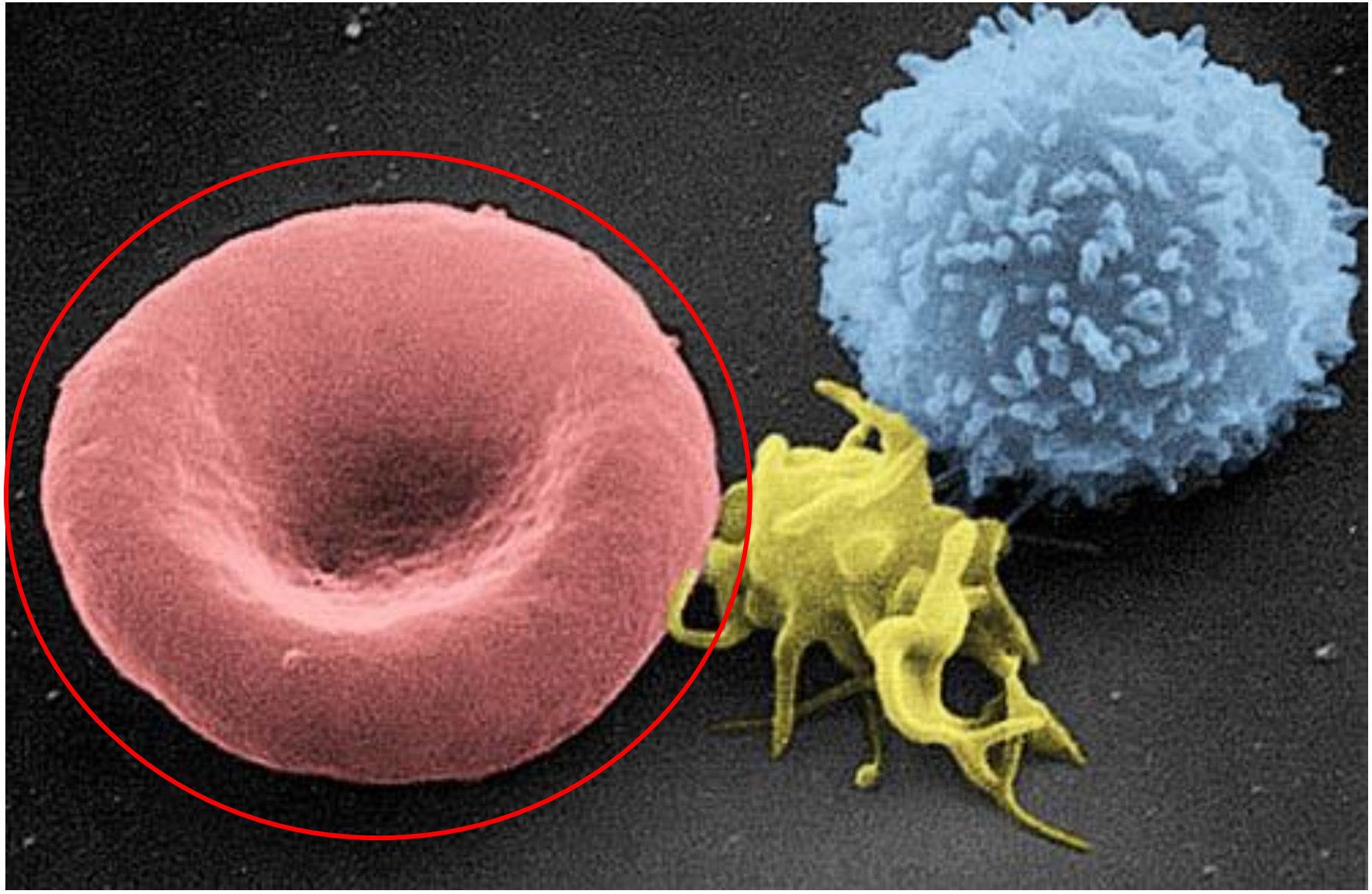




Bone marrow

- The red bone marrow is the site of hematopoiesis (mainly the axial skeleton), the yellow bone marrow is adipose tissue
- The basis is reticular connective tissue, which is colonized by HSCs and cell types derived from it
- Contains sinusoids (capillaries with large lumen)

Erytropoiesis

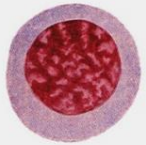


Development of red blood cells, erythropoiesis

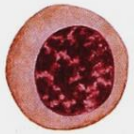
- Cell size ↓
- Nucleus size ↓
- Nuclear condensation ↑
- Basophilia (ribosomes) ↓
- Eosinophilia (hemoglobin) ↑


Proerythroblast

12 – 20 μm
24 h


Basophilic erythroblast

10 – 17 μm
24 h


Polychromatophilic erythroblast

10 – 15 μm
30 h

mitosis


Orthochromatophilic erythroblast

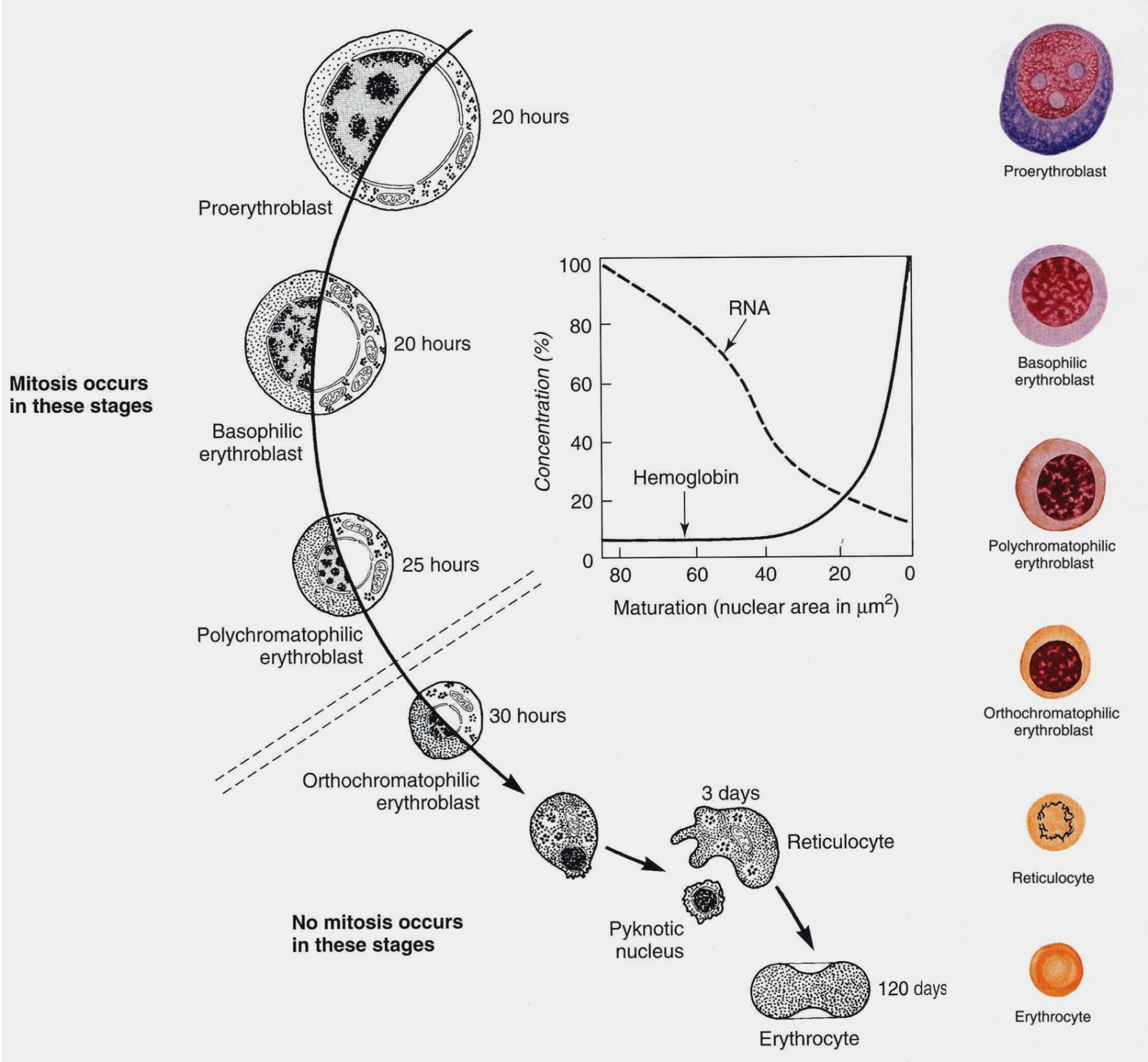
8 – 12 μm
48 h


Reticulocyte

Cca 8 μm
2 – 3 days


Erythrocyte

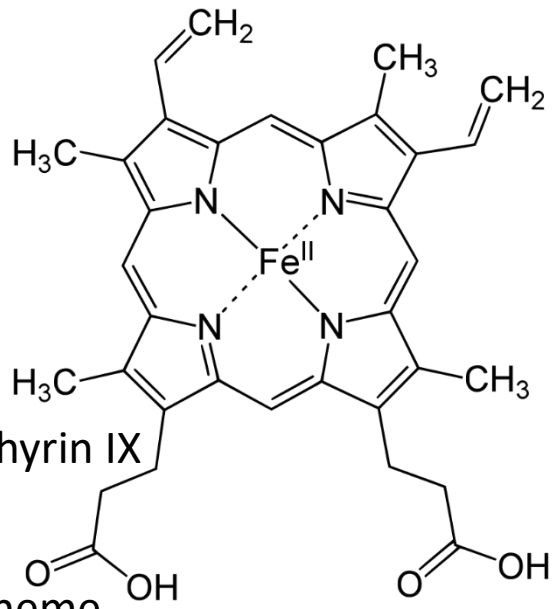
7,8 μm
120 days


20 hours
Proerythroblast
20 hours
Basophilic erythroblast
Mitosis occurs in these stages
25 hours
Polychromatophilic erythroblast
30 hours
Orthochromatophilic erythroblast
No mitosis occurs in these stages
3 days
Reticulocyte
Pyknotic nucleus
120 days
Erythrocyte
Concentration (%)
100
80
60
40
20
0
RNA
Hemoglobin
80
60
40
20
0
Maturation (nuclear area in μm²)
Proerythroblast
Basophilic erythroblast
Polychromatophilic erythroblast
Orthochromatophilic erythroblast
Reticulocyte
Erythrocyte

Synthesis and degradation of heme


Porfyr

Precursor – aminolevulic acid



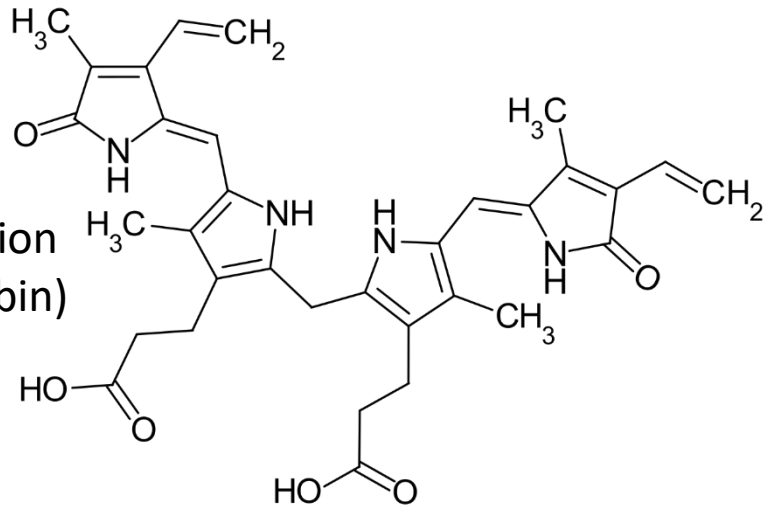
Protoporphyrin IX

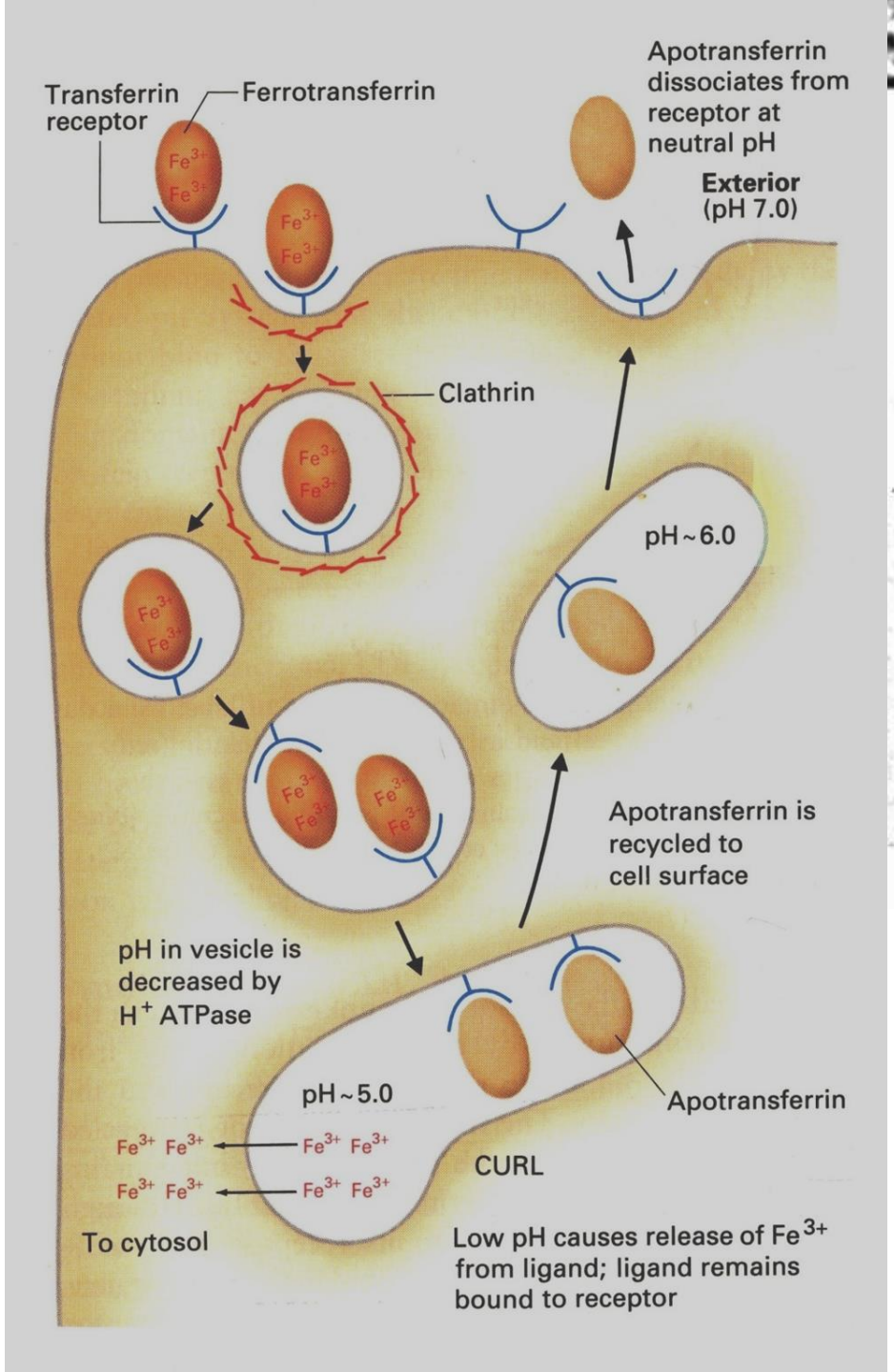
iron

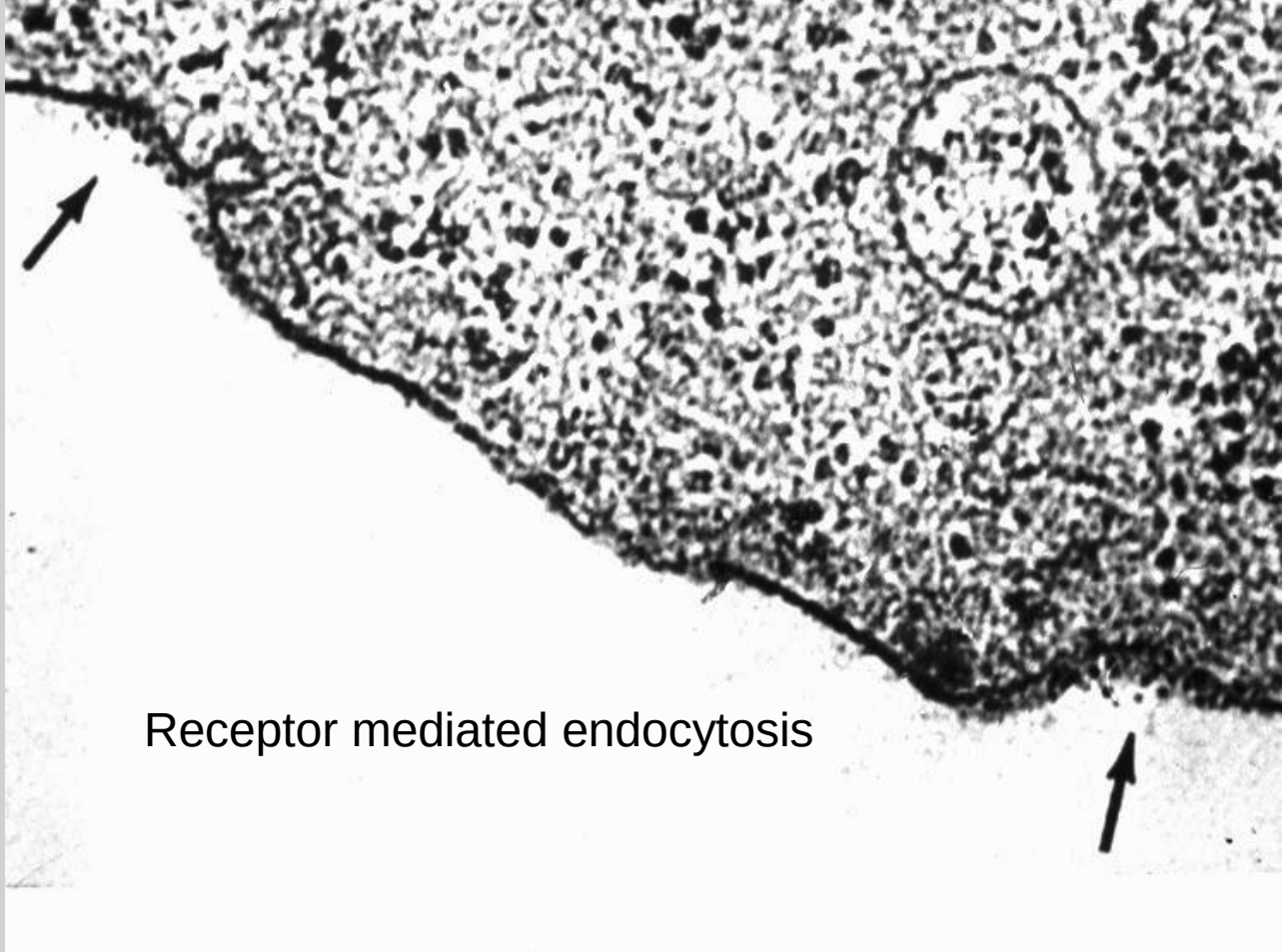
heme

Hem + protein globin = hemoglobin

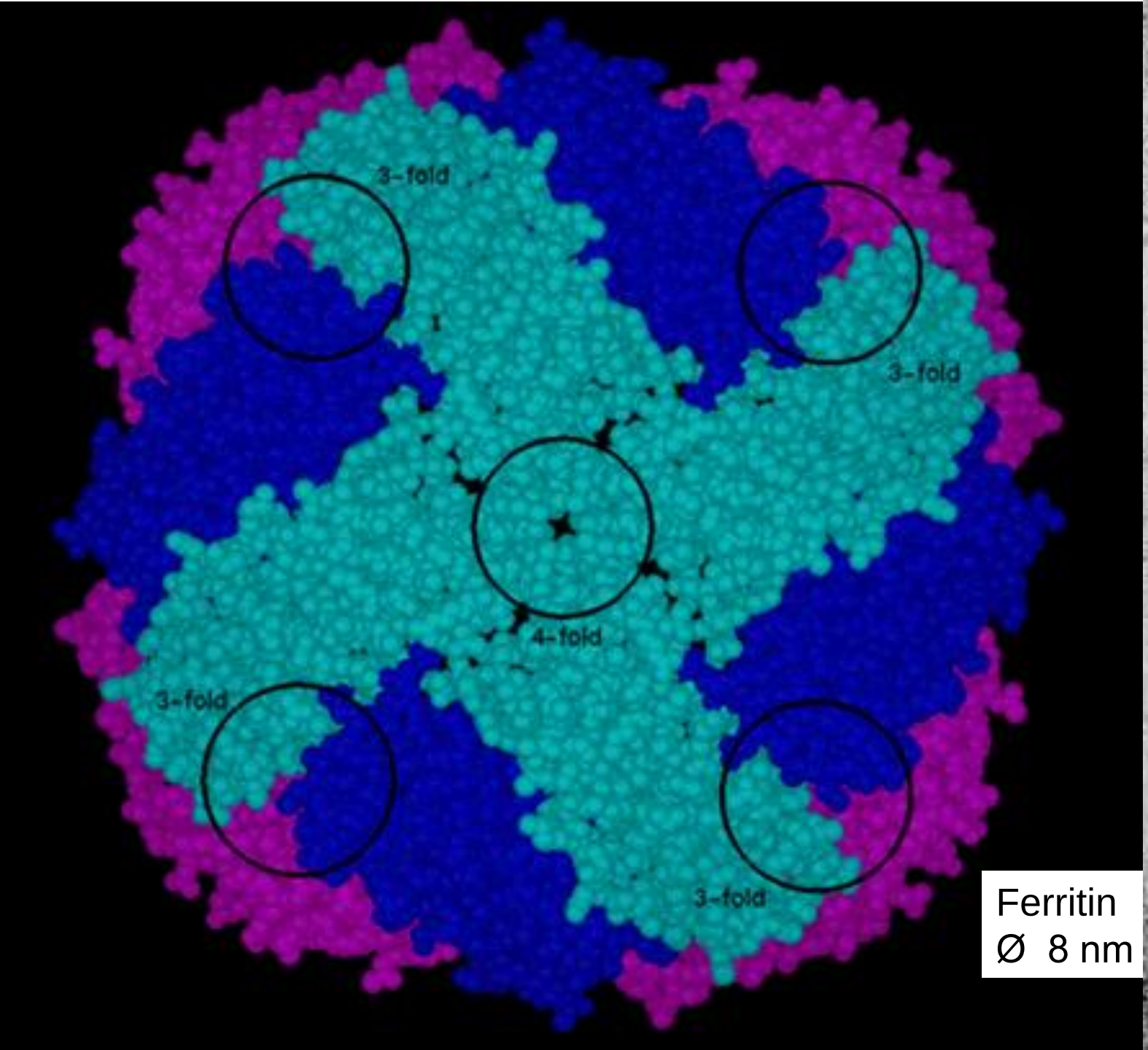
Heme degradation products (bilirubin)



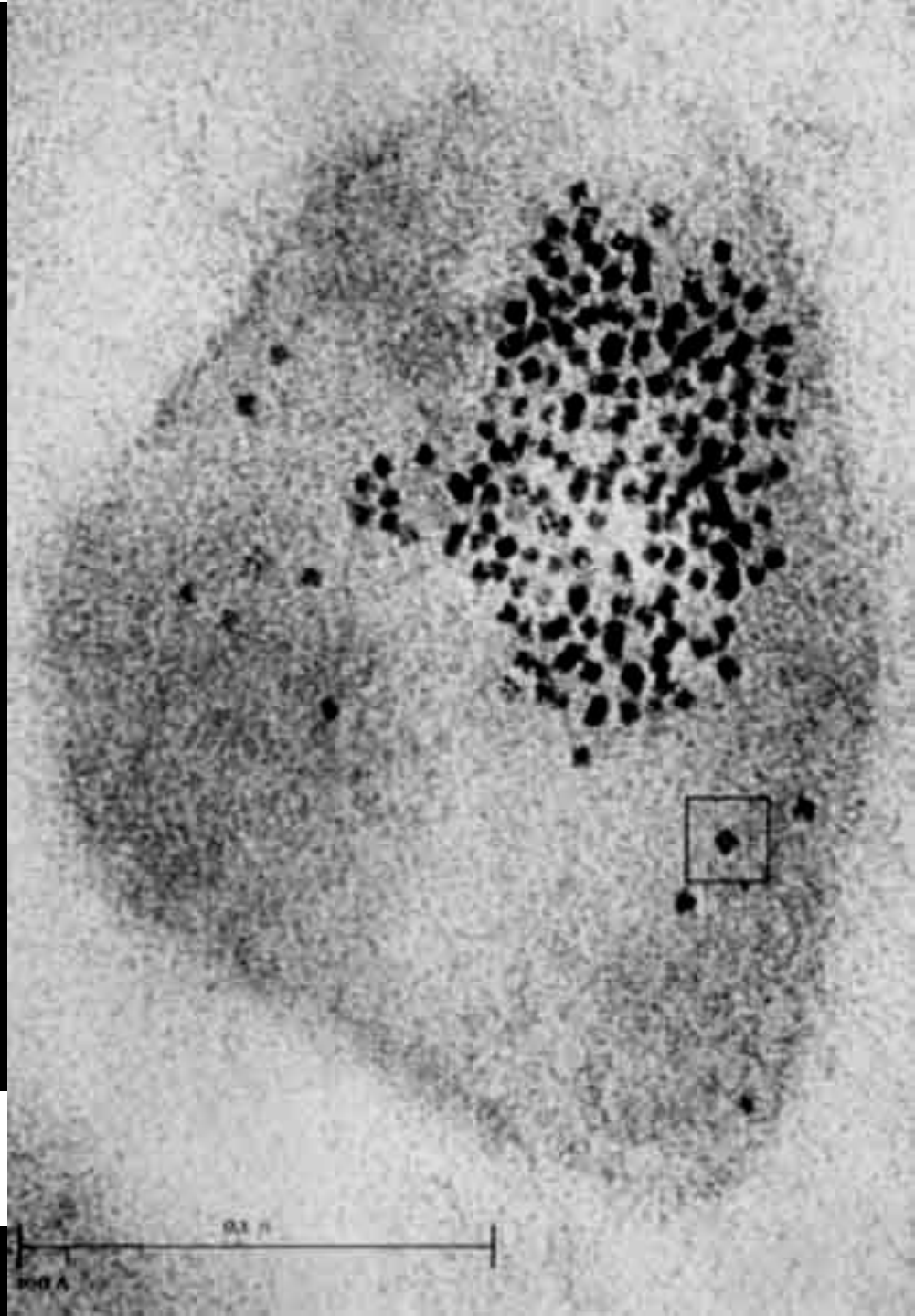




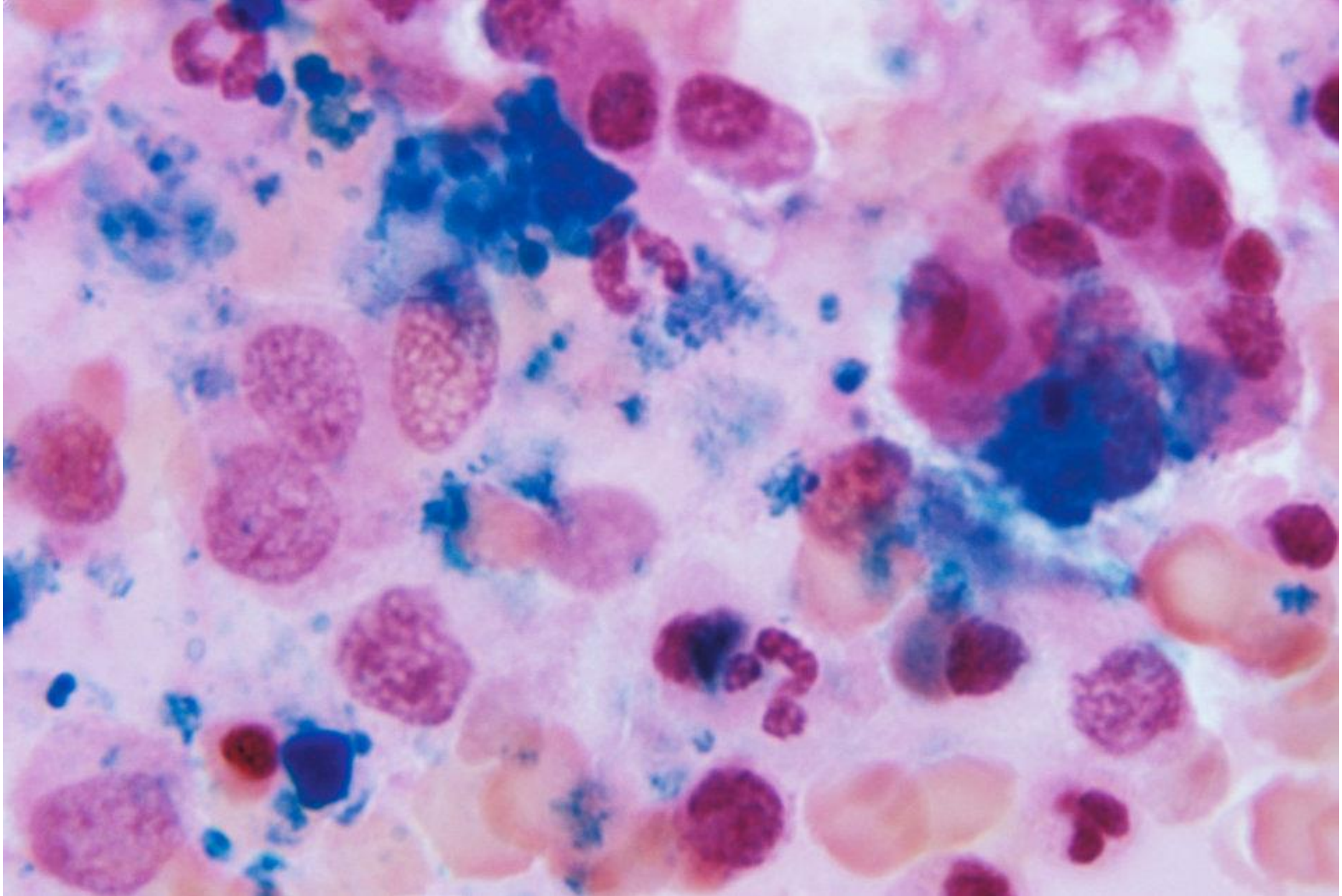
Receptor mediated endocytosis



Ferritin
Ø 8 nm



Perls reaction

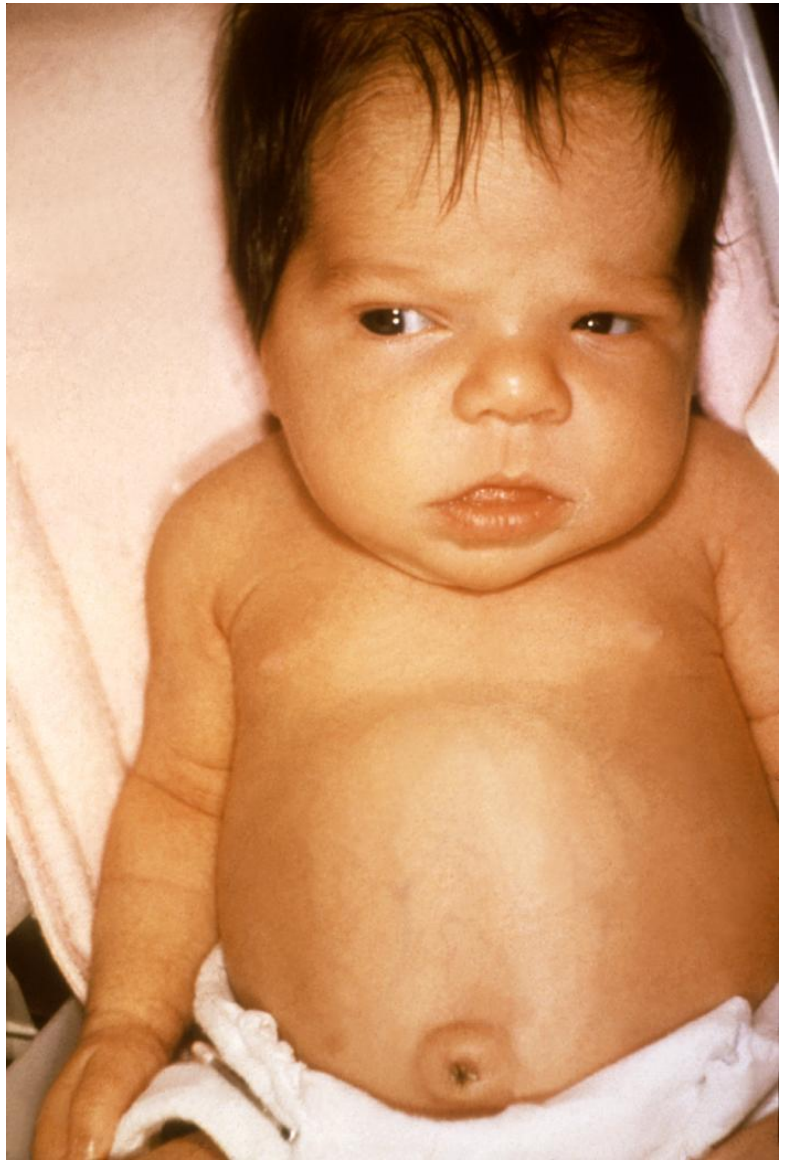


Hemoglobin and iron metabolism

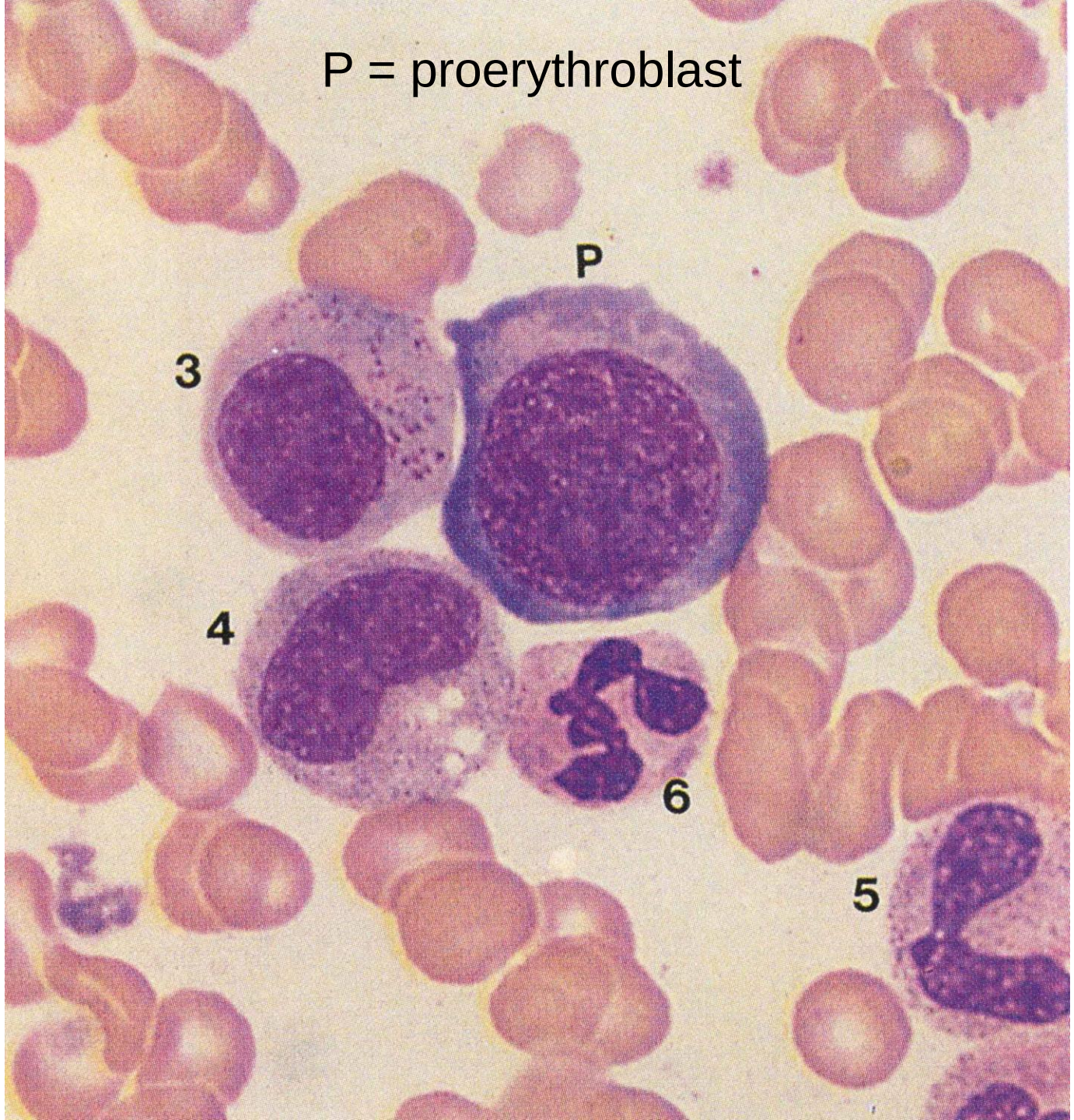
- Haemoglobin is formed from the protein globin (synthesis on polyribosomes) and heme, which contains a porphyrin nucleus with a centrally "embedded" iron atom
- After erythrocyte death, globin is metabolized by proteases, heme is broken down into waste products (biliverdin, bilirubin) and iron atoms, which are subsequently stored in ferritin
- Transferrin is used to transport iron through the circulation and is taken up by receptors on erythroblasts
- Hemosiderin is an iron-containing cellular inclusion contained e.g. in some macrophages
- The Perls reaction can be used to display iron in histology (Fe in blue)

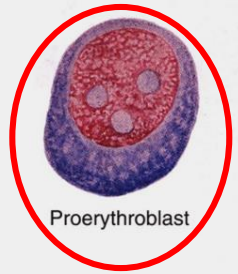
Clinical correlation – neonatal icterus

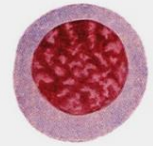
- The hemoglobin type switch is associated with increased erythrocyte breakdown
- Bilirubin gives the skin and sclera a yellow color
- The danger is damage to the brain (basal ganglia) when bilirubin passes through the not yet fully developed blood-brain barrier, called kernicterus
- Phototherapy accelerates the breakdown of bilirubin and reduces its concentration in the blood

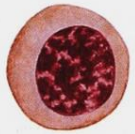


P = proerythroblast




Proerythroblast


Basophilic erythroblast

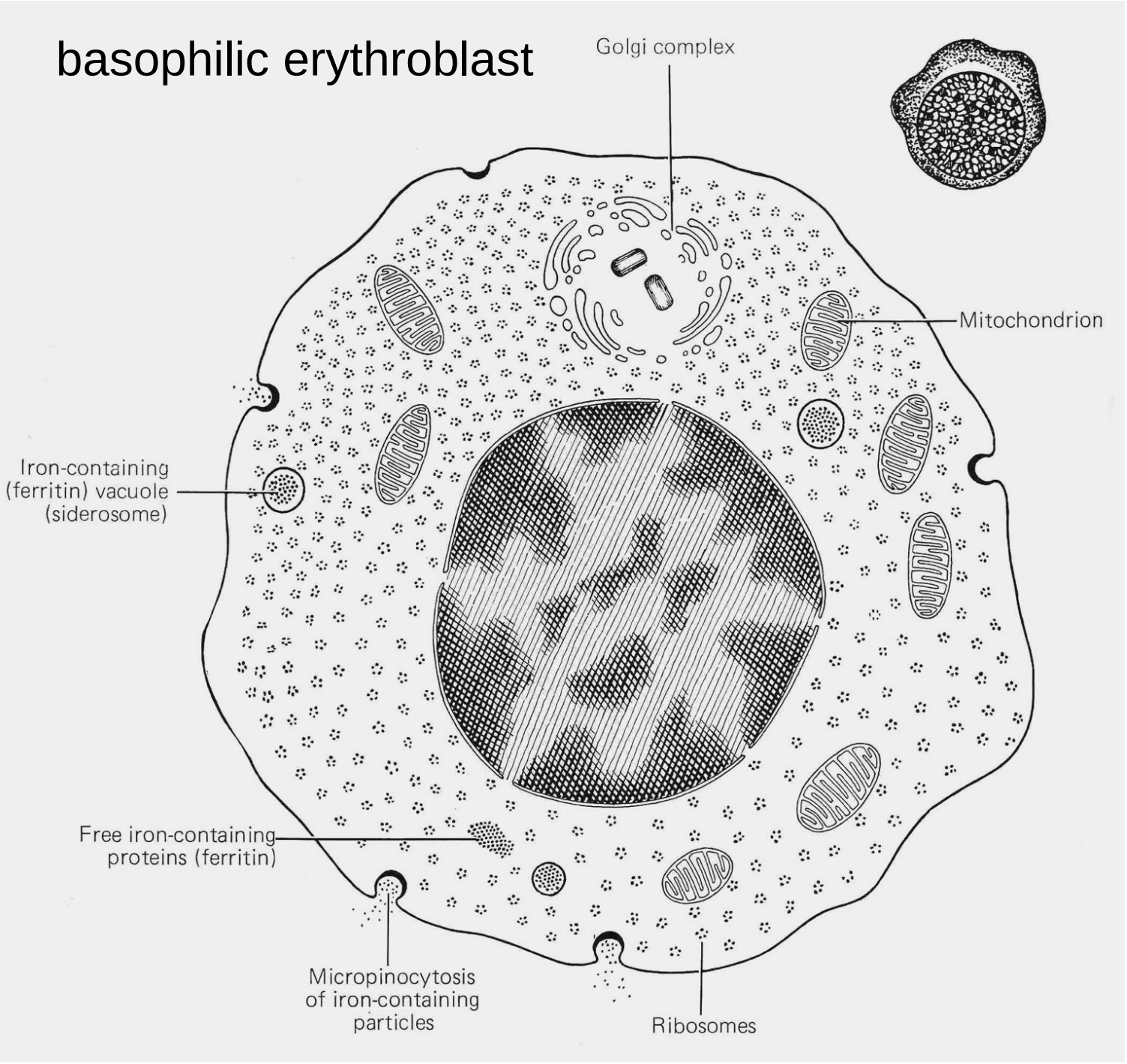

Polychromatophilic erythroblast


Orthochromatophilic erythroblast

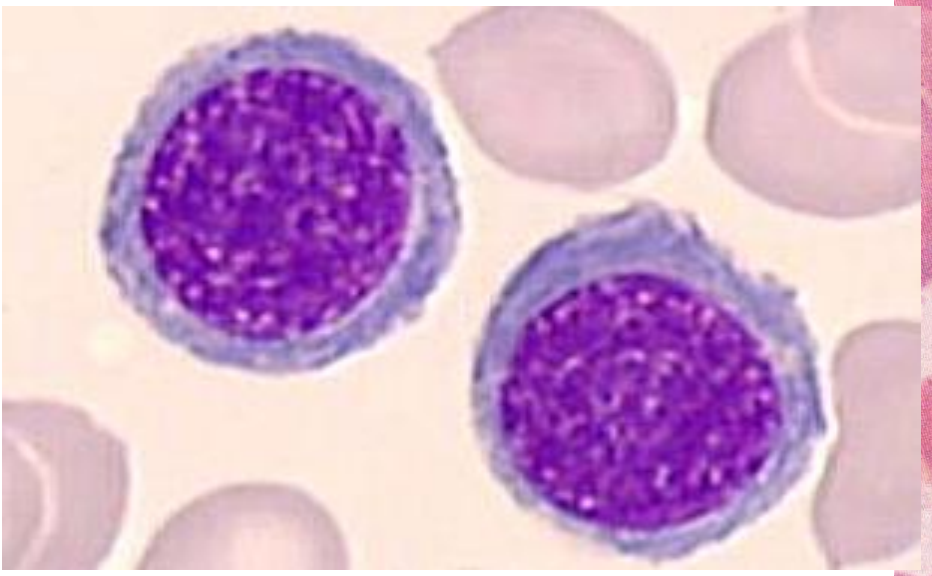

Reticulocyte


Erythrocyte

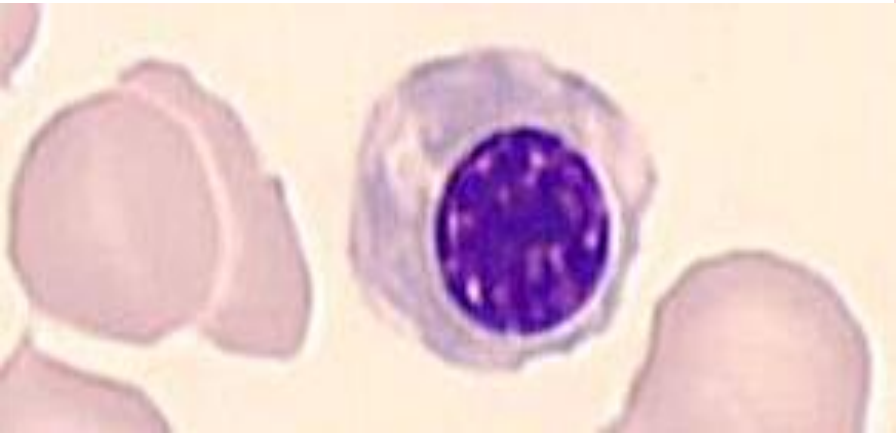
basophilic erythroblast

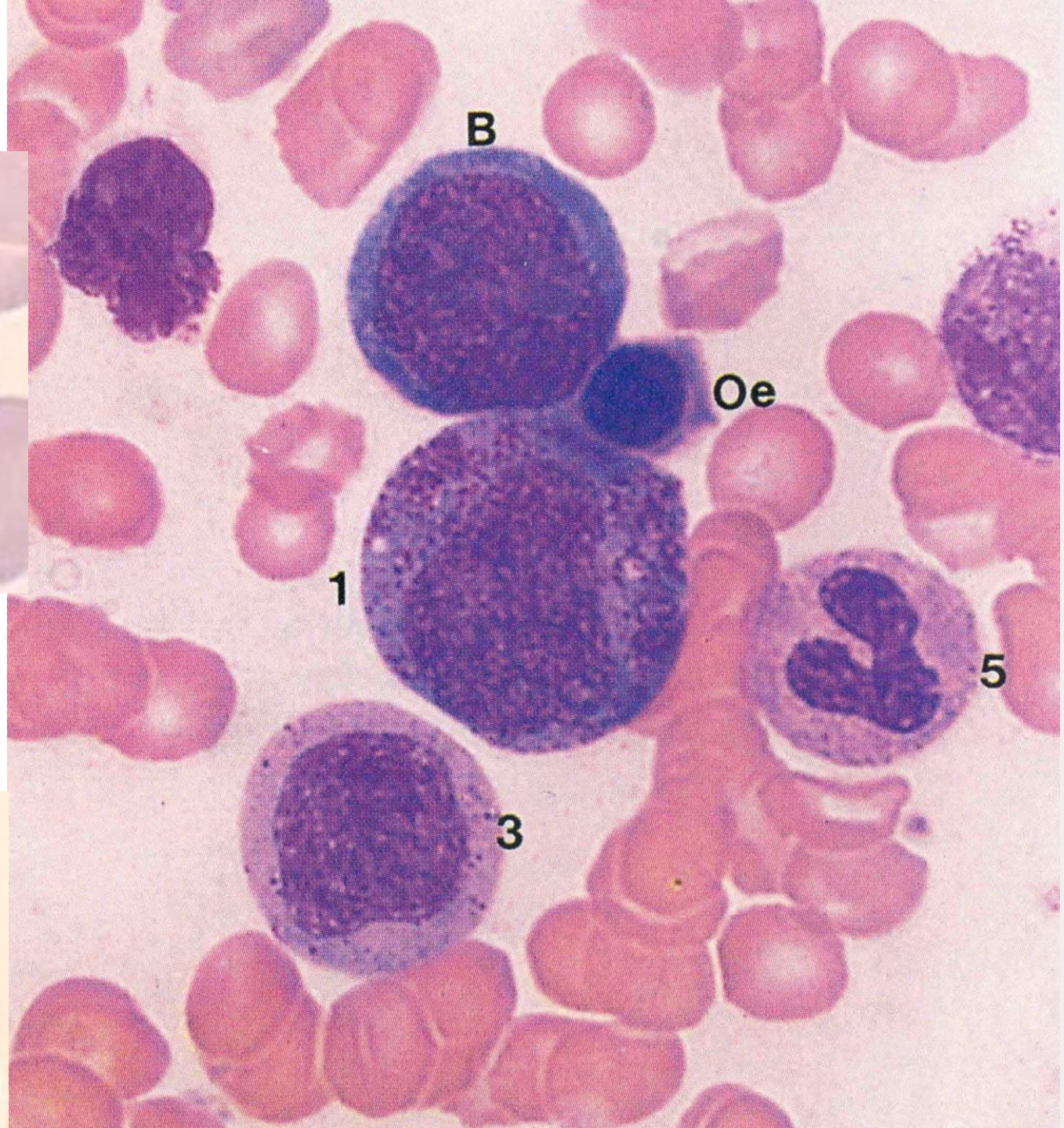


B = basophilic erythroblast
10 – 17 µm

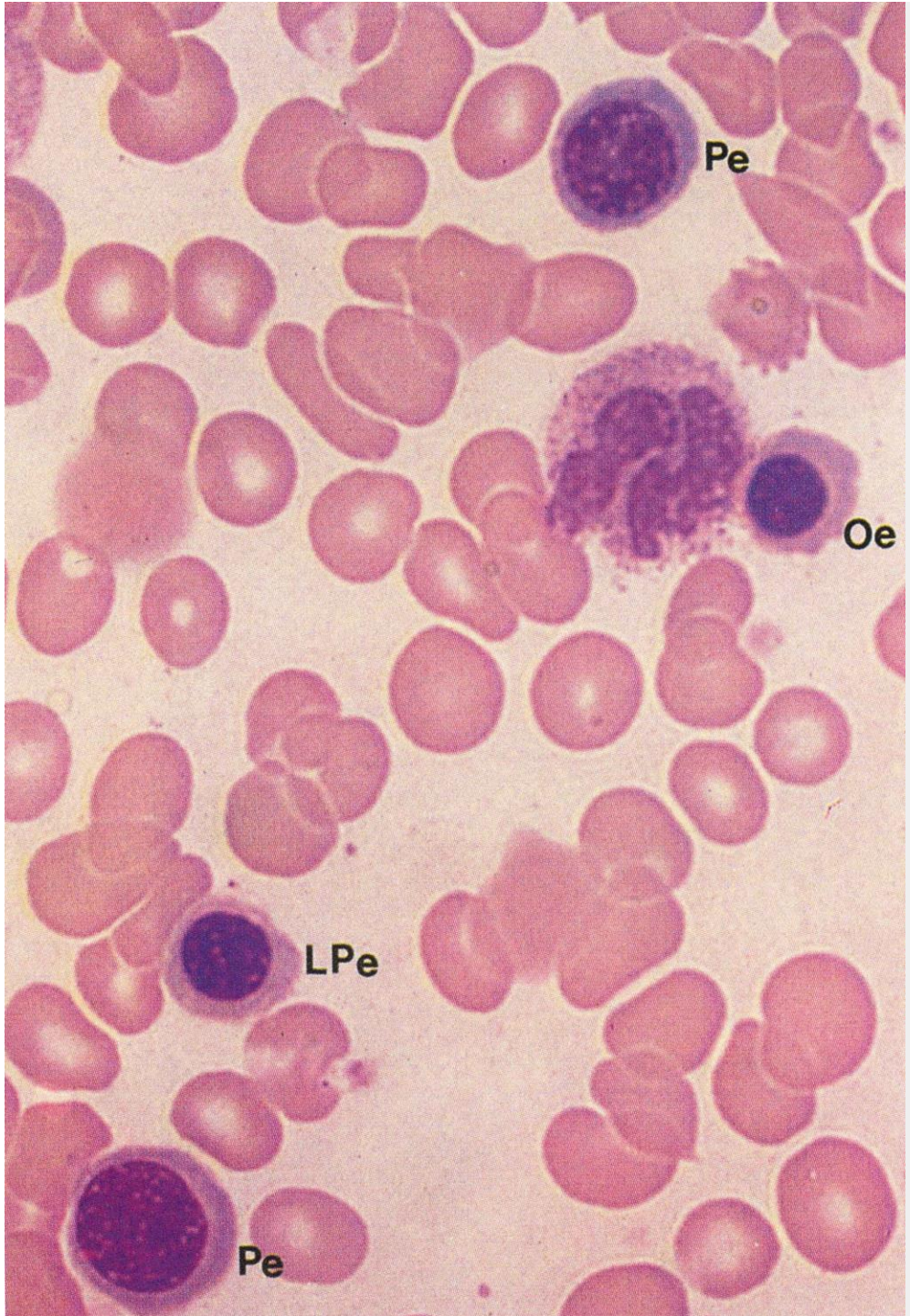


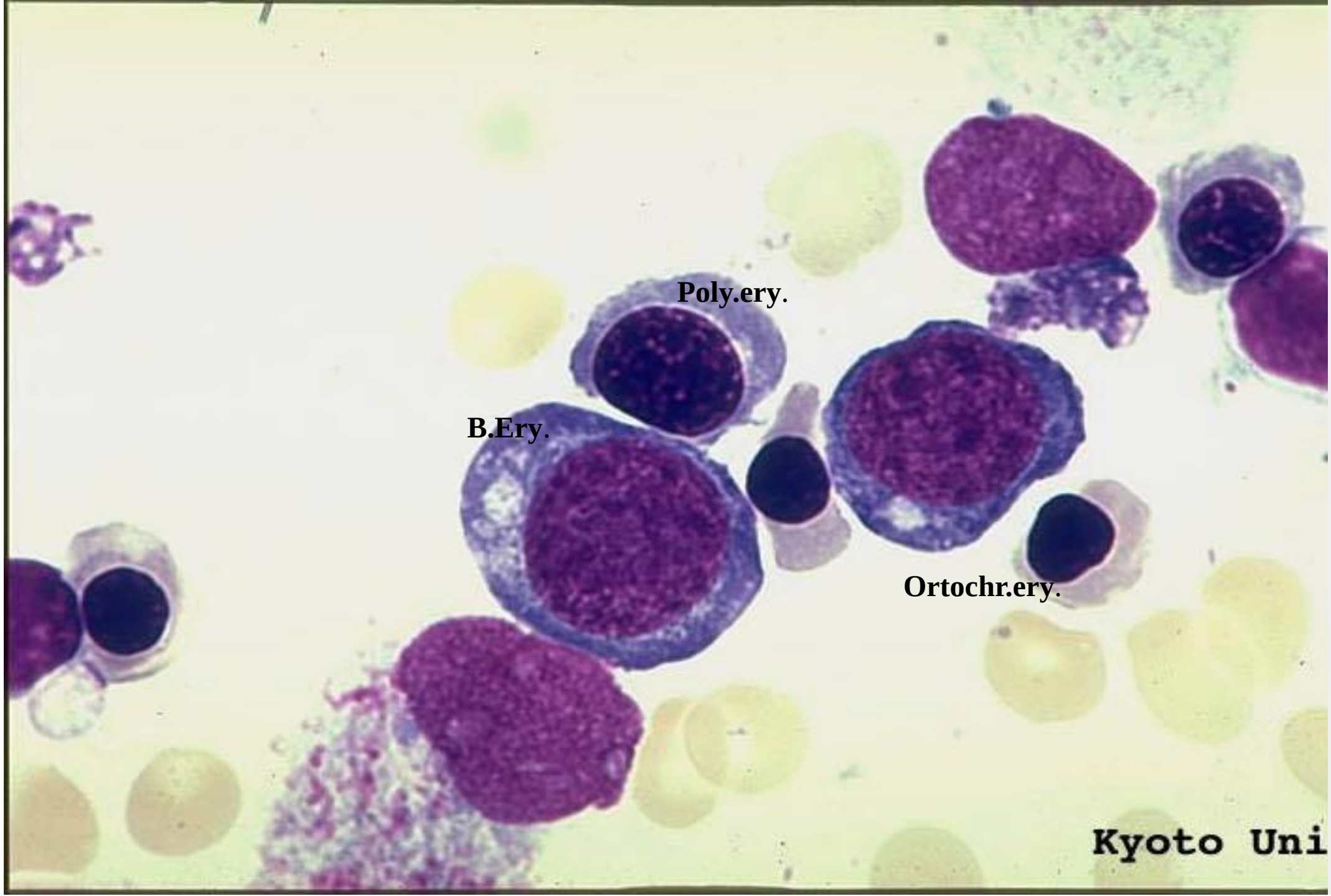
Oe = early ortochromatophilic erythroblast **8 – 12 µm**

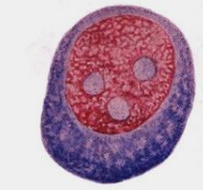




Pe = polychromatophilic erythroblast
LPe = late polychromatophilic erythroblast
Oe = late ortochromatophilic erythroblast






Proerythroblast

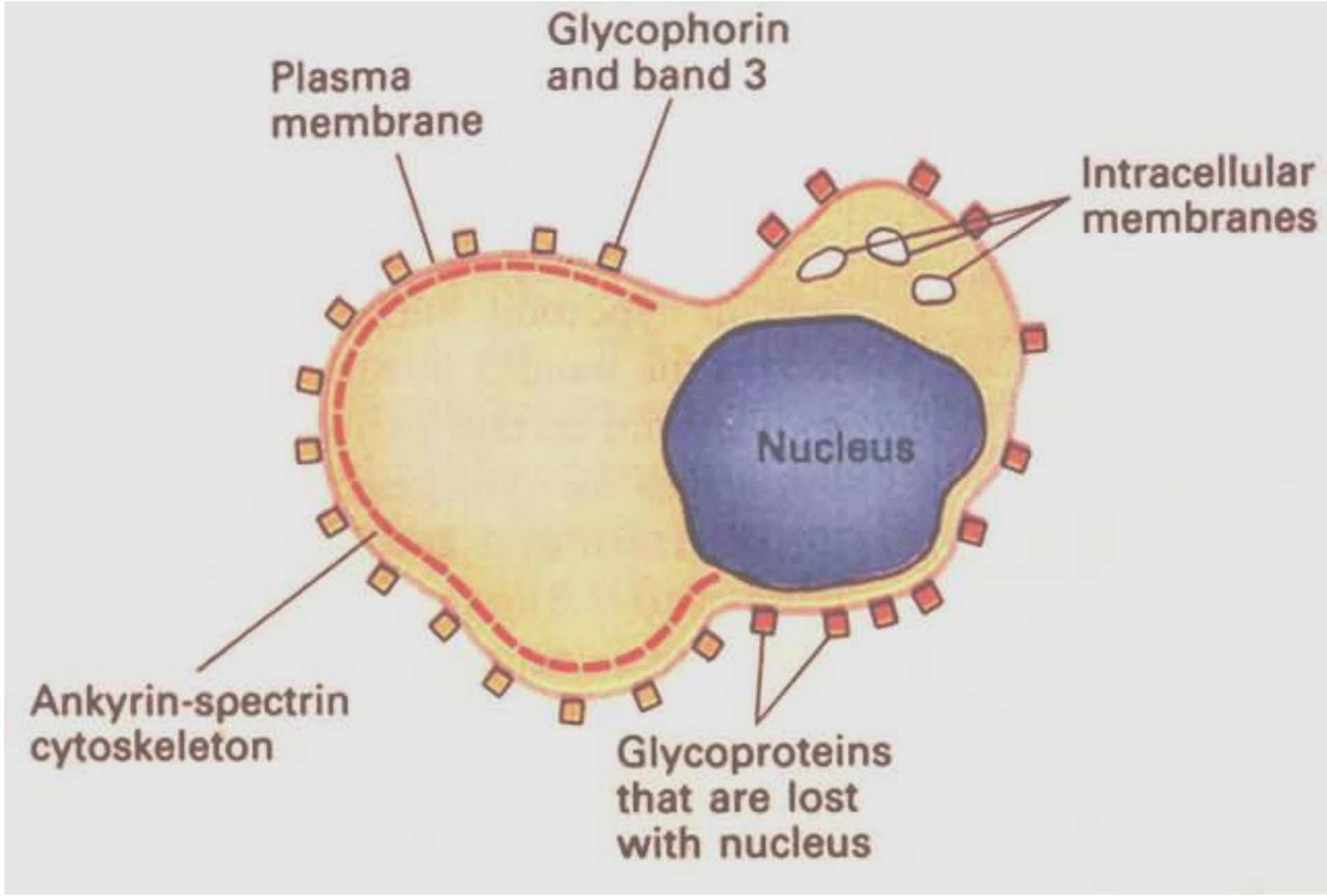

Basophilic erythroblast


Polychromatophilic erythroblast


Orthochromatophilic erythroblast

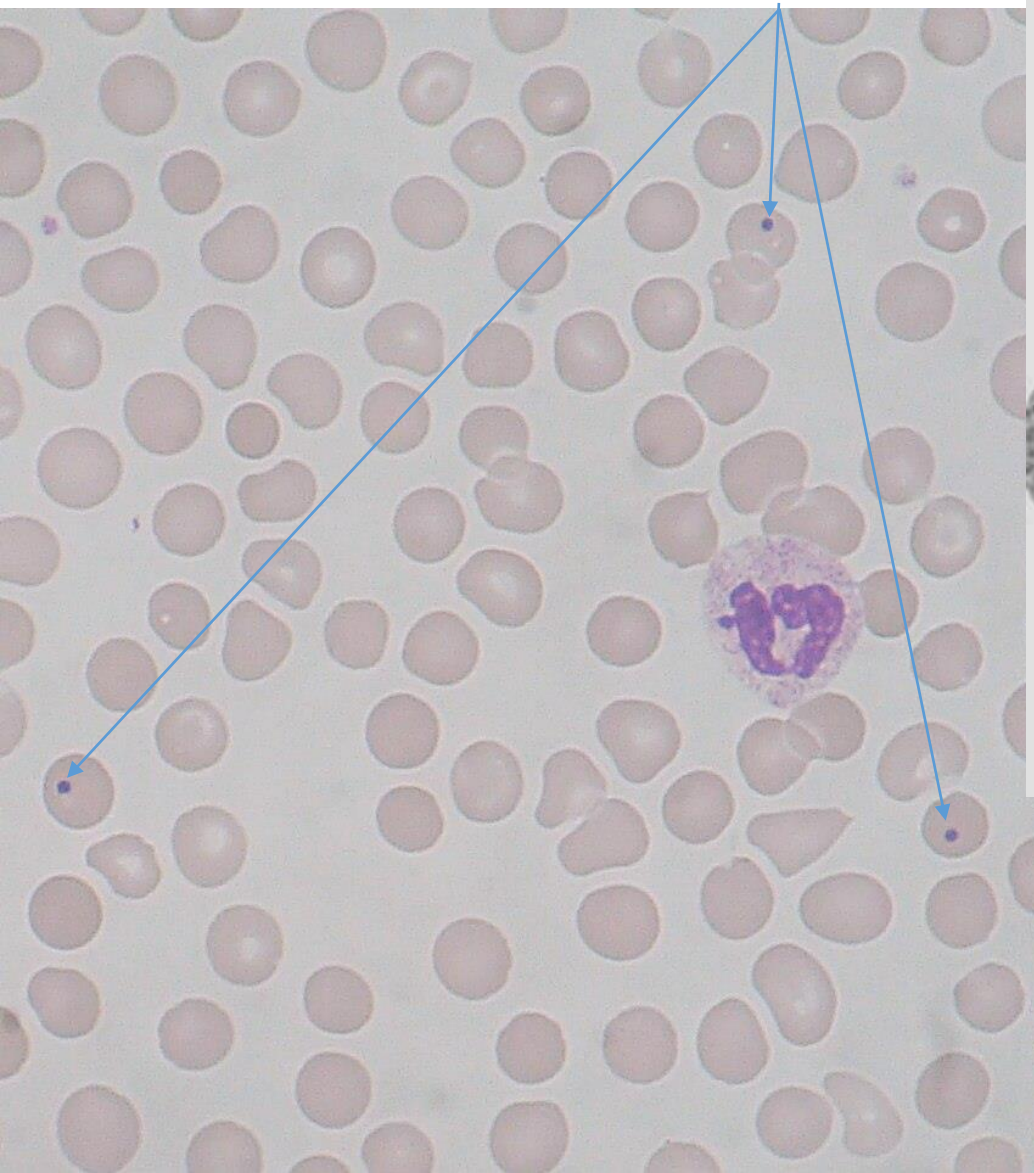

Reticulocyte

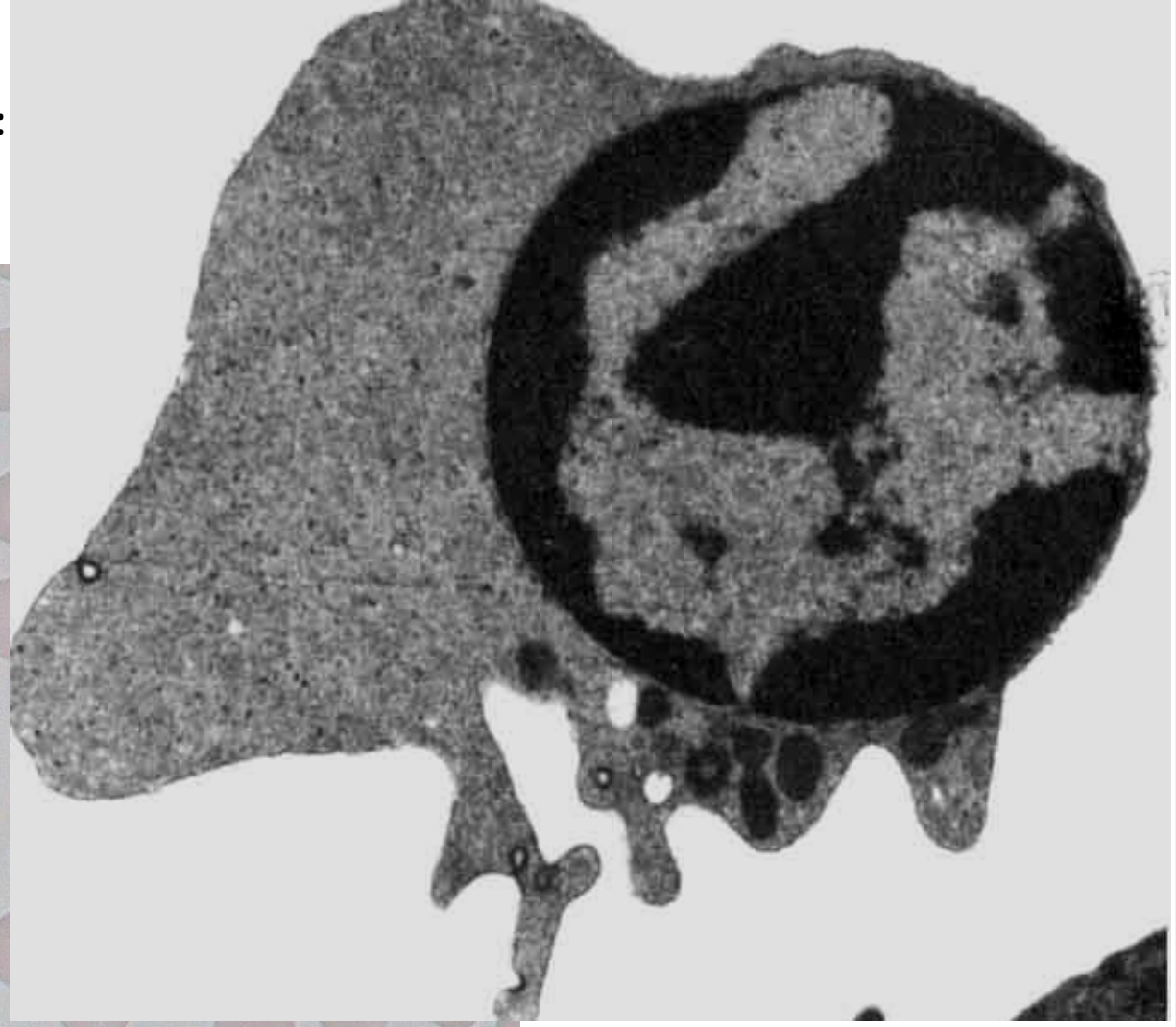

Erythrocyte


Plasma membrane
Glycophorin and band 3
Intracellular membranes
Nucleus
Ankyrin-spectrin cytoskeleton
Glycoproteins that are lost with nucleus


Proerythroblast
Basophilic erythroblast
Polychromatophilic erythroblast
Orthochromatophilic erythroblast
Reticulocyte
Erythrocyte

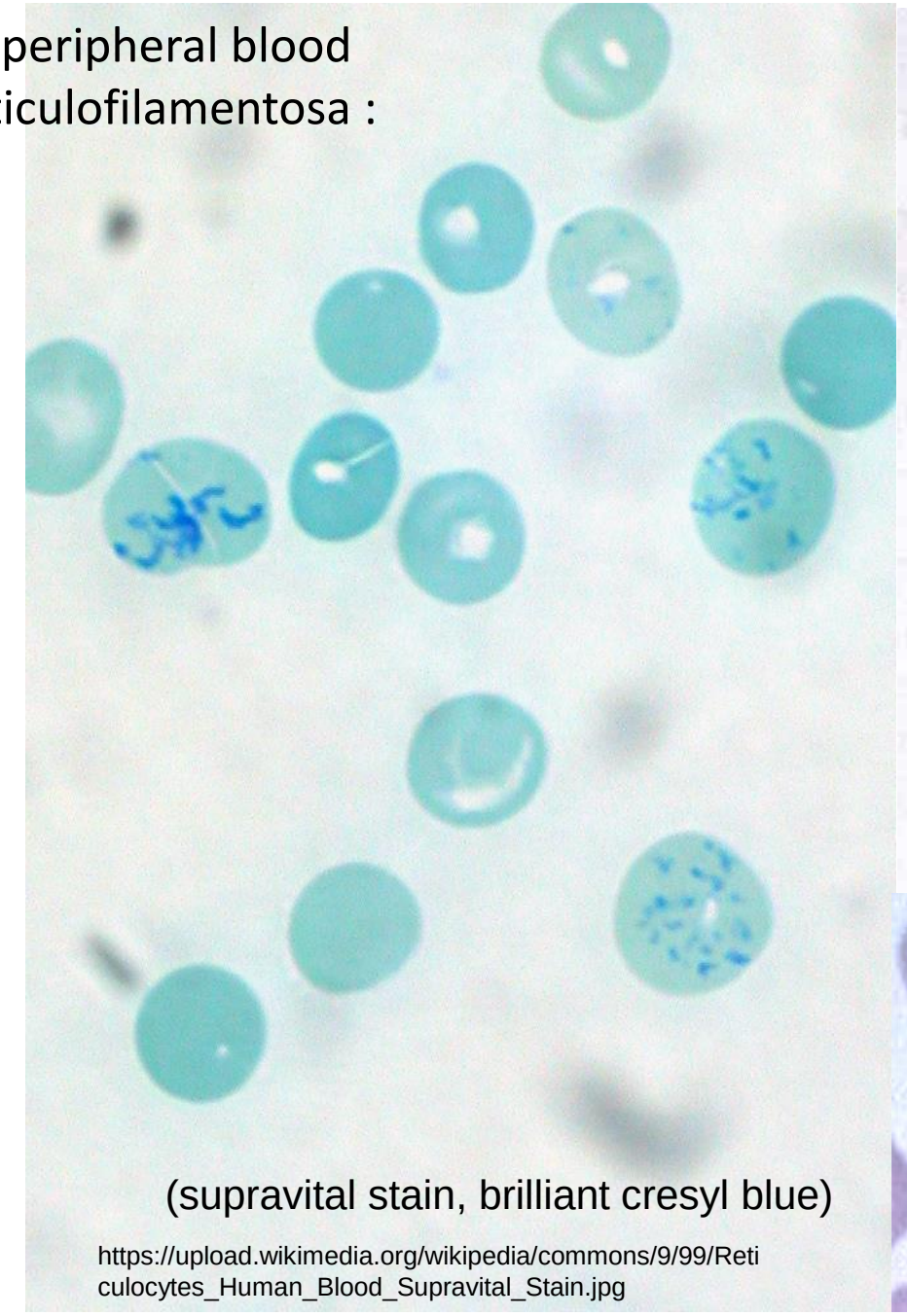
Fragments of nuclei: Howell – Jolly bodies





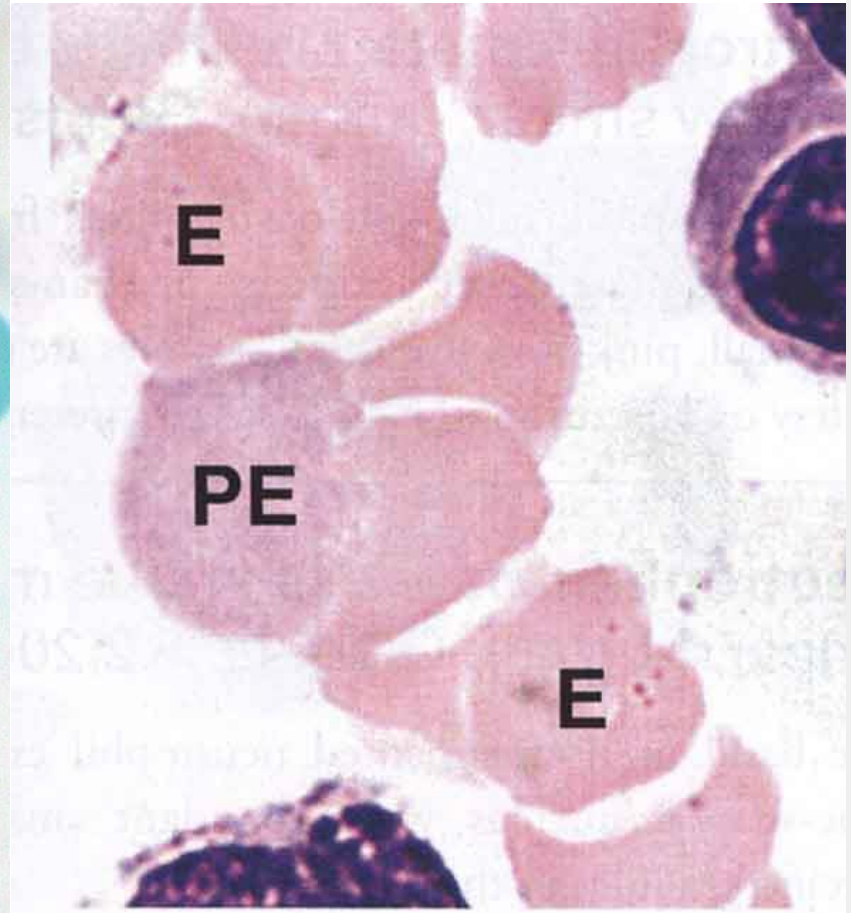
Reticulocytes

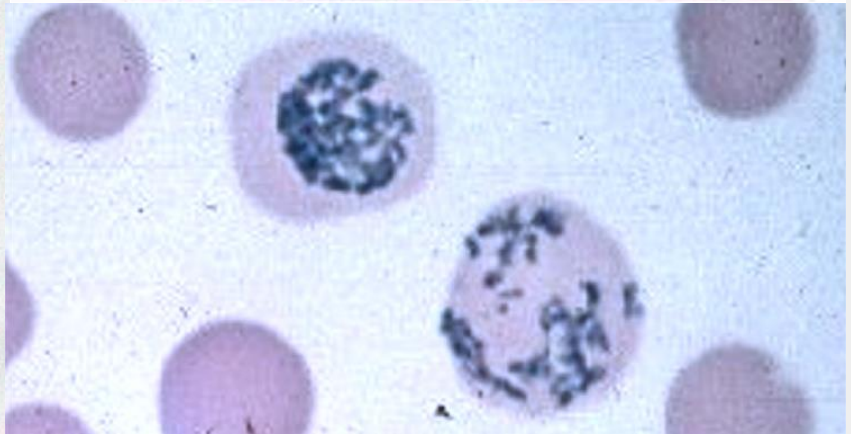
0,5 – 2,5 % in peripheral blood
Substantia reticulofilamentosa :
residual RNA

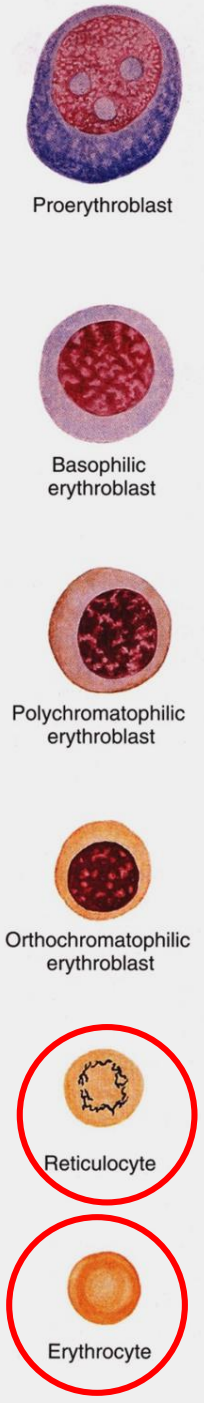


(supravital stain, brilliant cresyl blue)

https://upload.wikimedia.org/wikipedia/commons/9/99/Reticulocytes_Human_Blood_Supravital_Stain.jpg





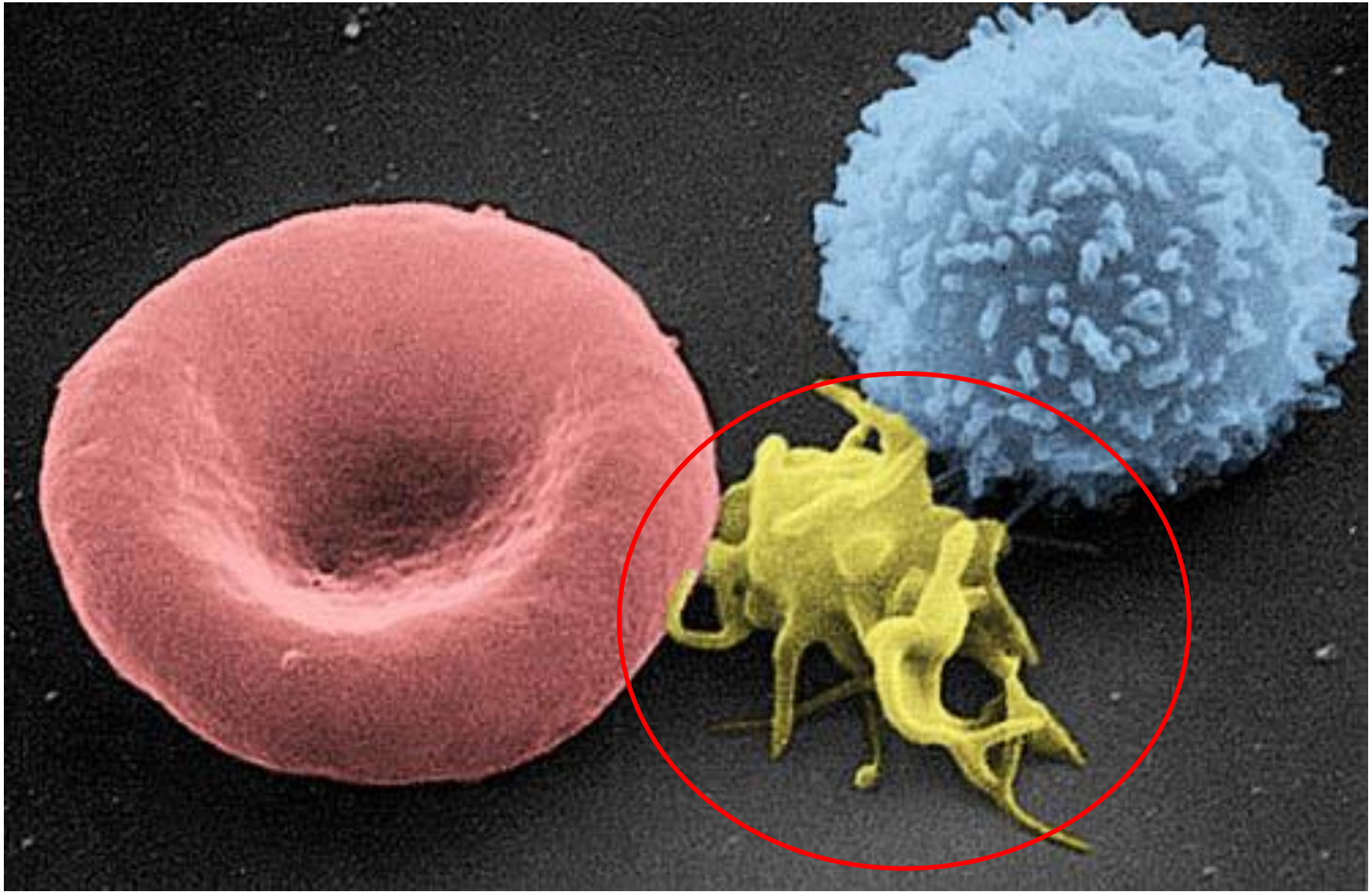


Erythropoiesis

- Erythroblasts develop into erythrocytes, the process requires a lot of iron
- Stimulated by erythropoietin produced in the kidneys (e.g. during hypoxia)
- Erythroblasts have several main tasks
  - Synthesis of hemoglobin, at first basophilic cytoplasm (ribosomes), then eosinophilic (hemoglobin)
  - Shrinkage, condensation, extrusion of the nucleus (the cell is filled with hemoglobin and does not need a nucleus), remnants of the nucleus sometimes persist as Howell - Jolly bodies
  - Reduction in cell size from about 20 mm to 7.8 mm
  - Many erythrocytes are formed from one proerythroblast (multiple divisions during development)

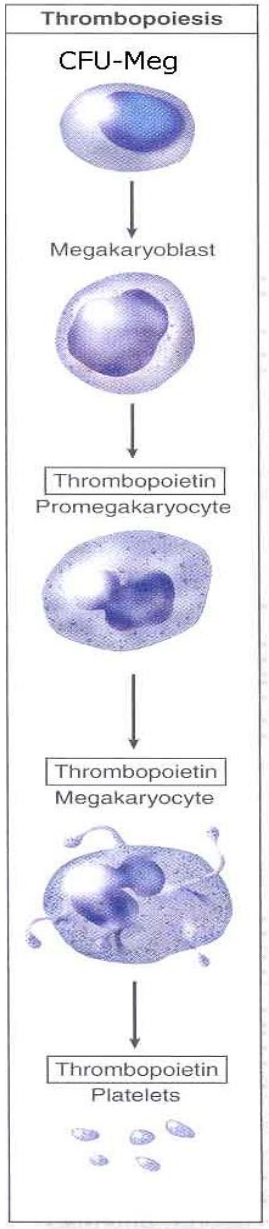
Brutus does not shy away from doping to secure victory (EPO). (Asterix at the Olympic Games)

Trombopoiesis



Trombopoiesis

- Cell size
- Nucleus size
- ploidy
- Lobulation of the nucleus



30 μm

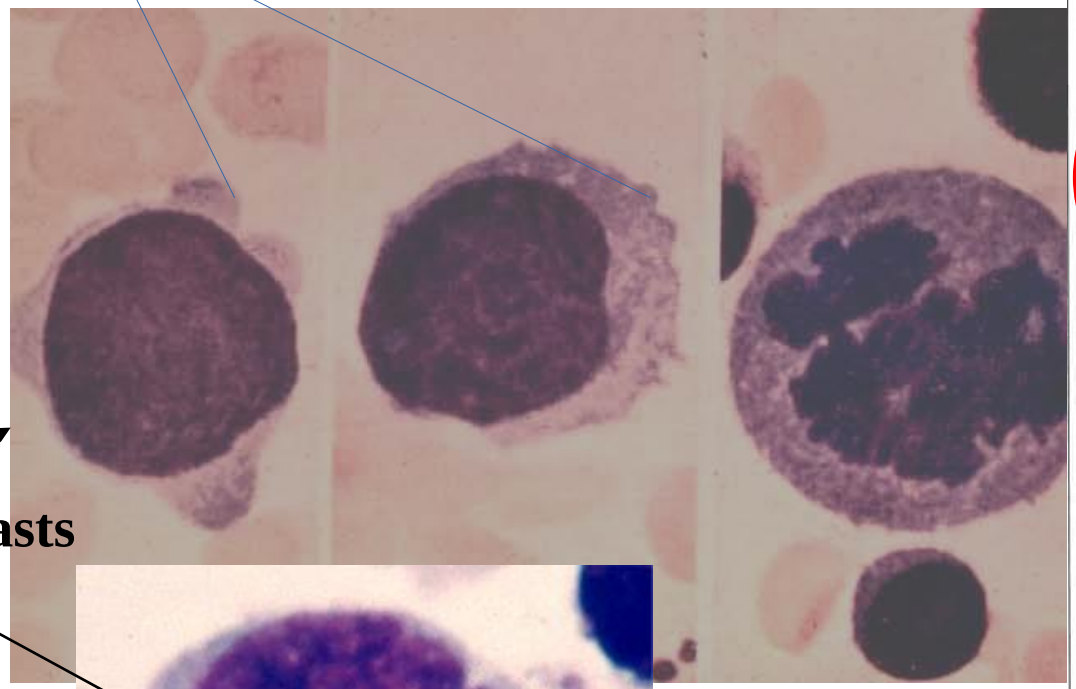
50 - 70 μm

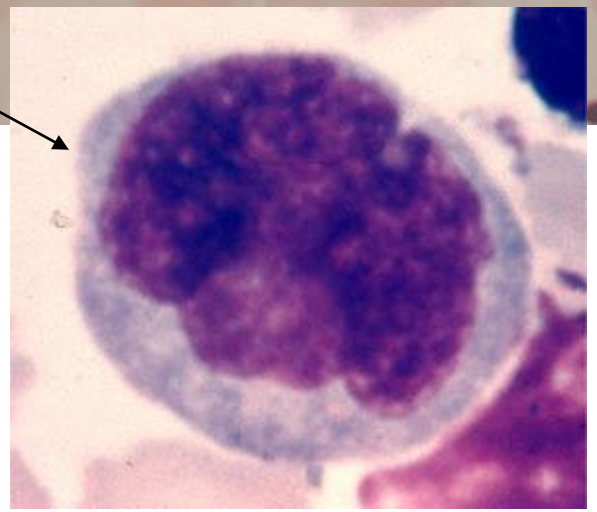
Thrombocytes
Thrombopoiesis

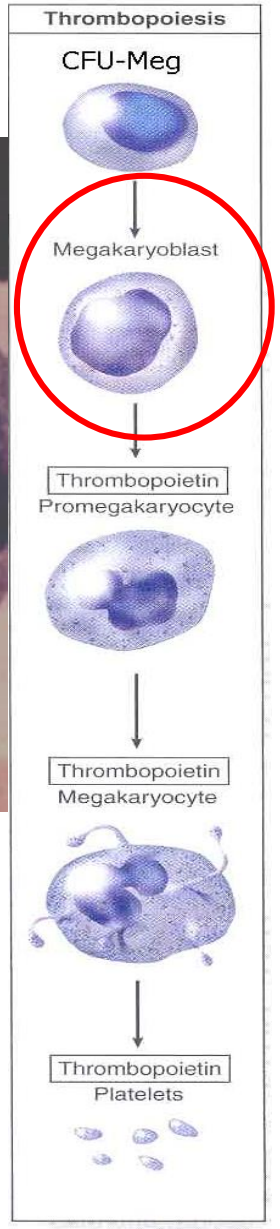
endomitosis

Megakaryoblasts
15 – 35 µm

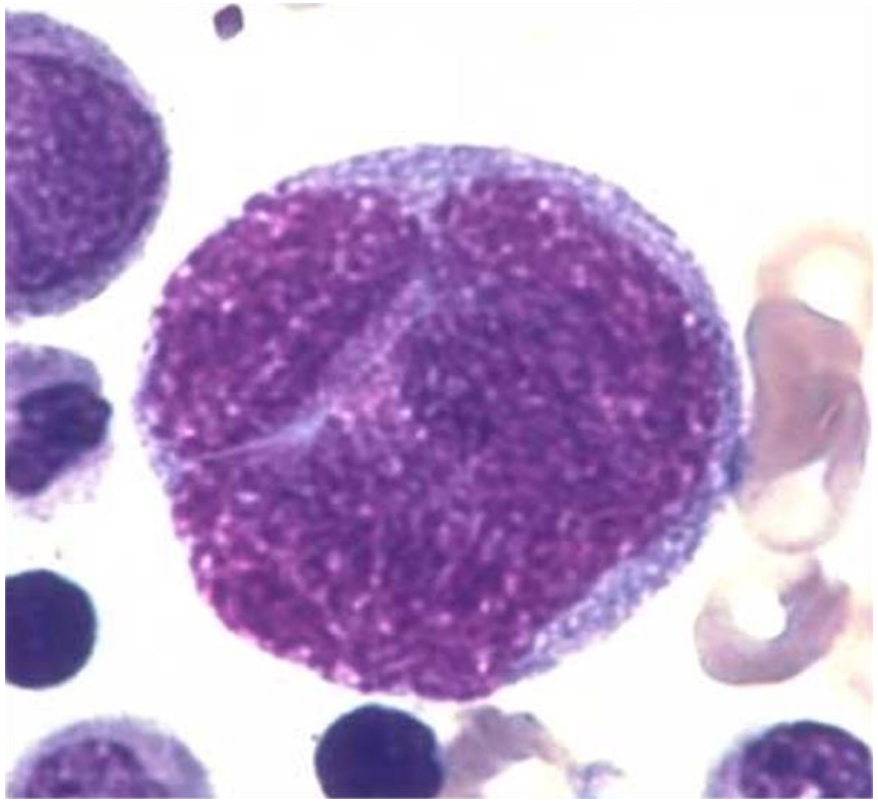
Polyploidy up to 64n
Basophilic cytoplasm
No granules

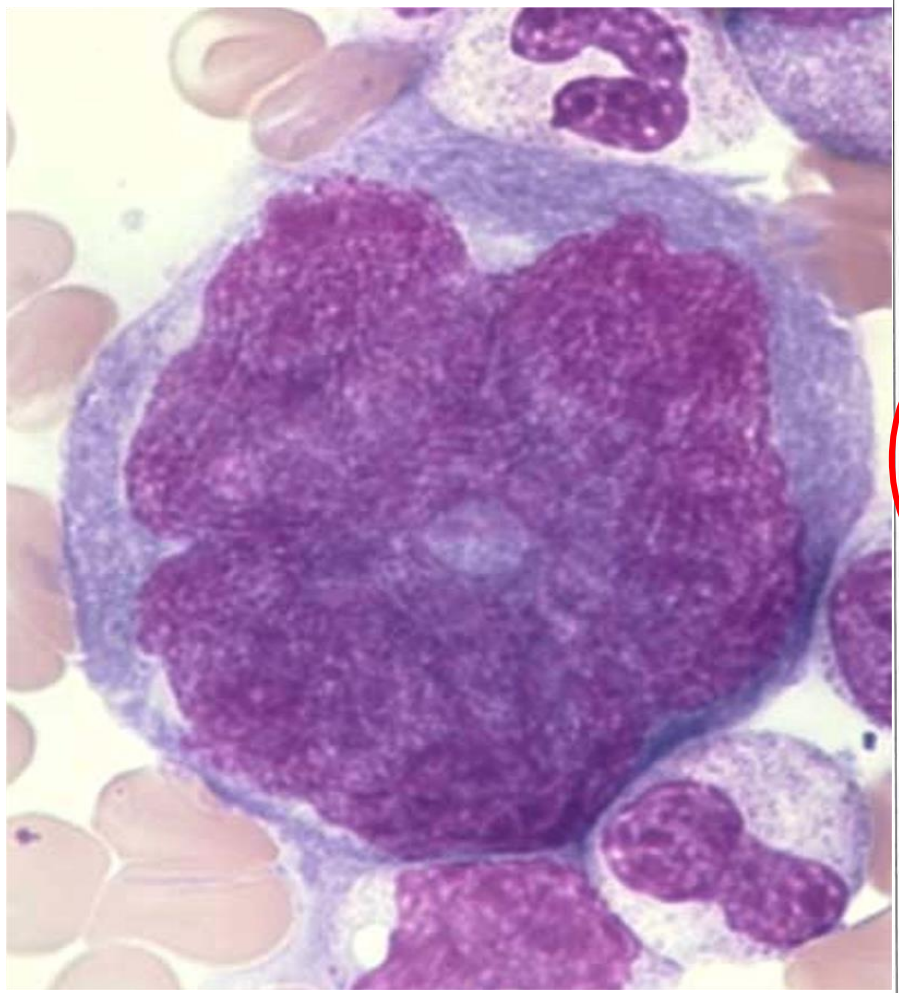




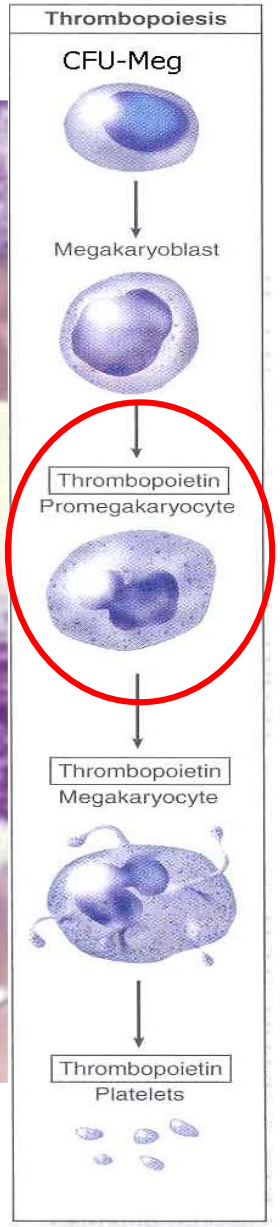


Promegakaryocytes
30 – 60 µm

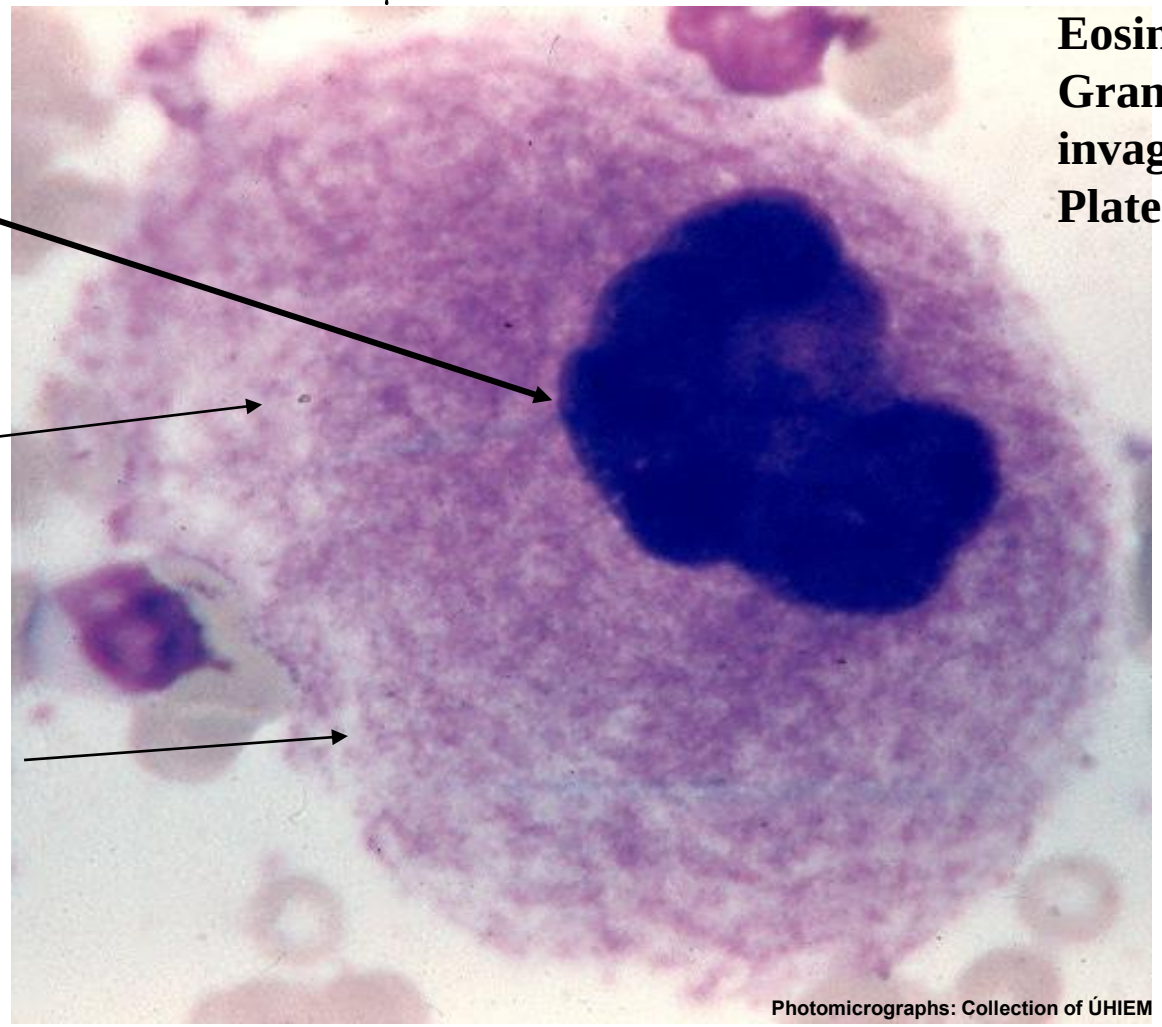




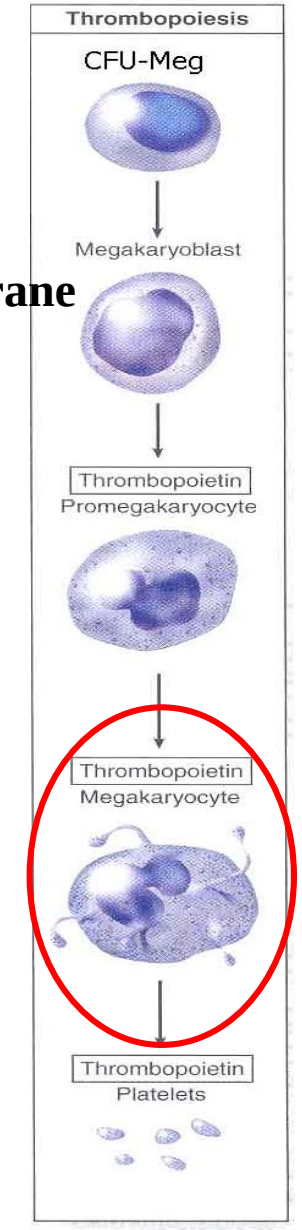
Basophilic cytoplasm, granules
No pinching off of platelets



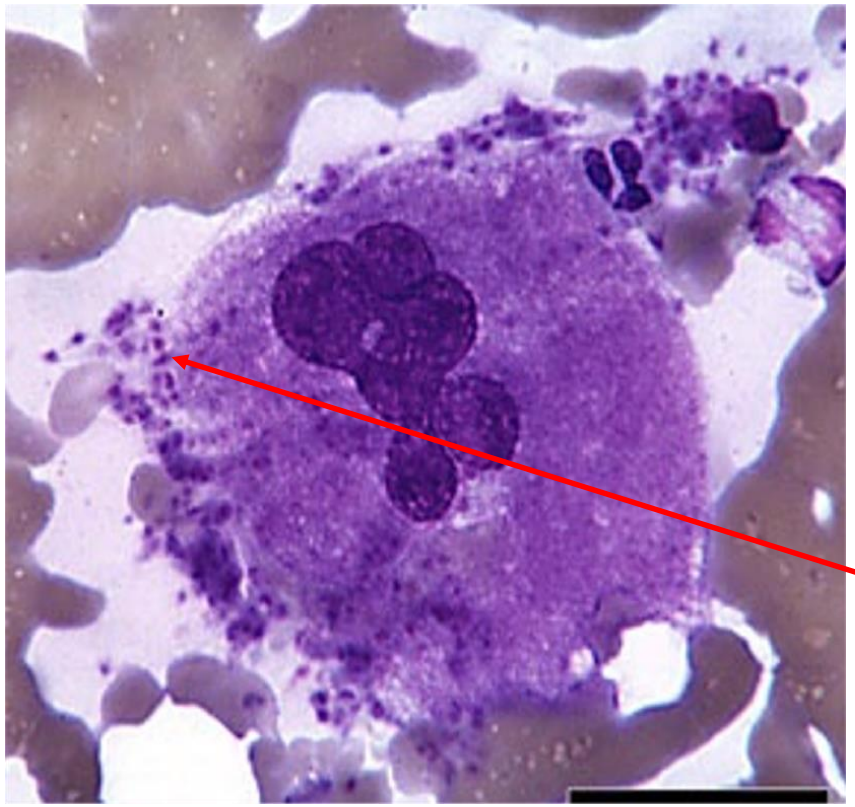
MEGAKARYOCYTE - size : 50 - 100 μm

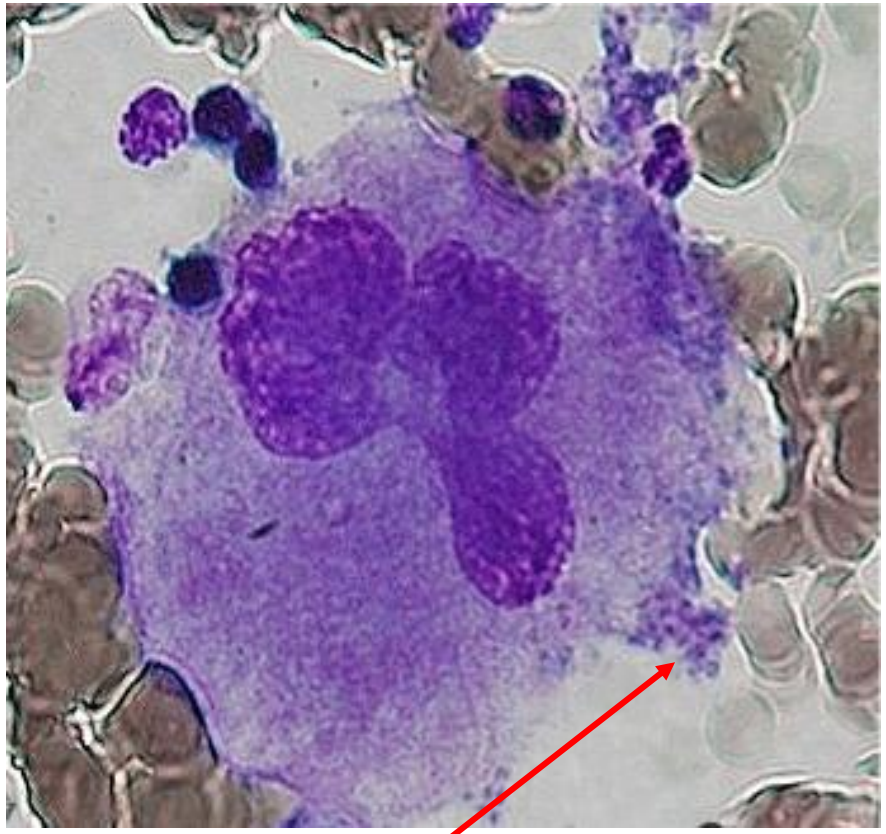


Lobulated, dark nucleso
Eosinophilic cytoplasm
Granula
invaginations of the cell membrane
Platelets pinch off

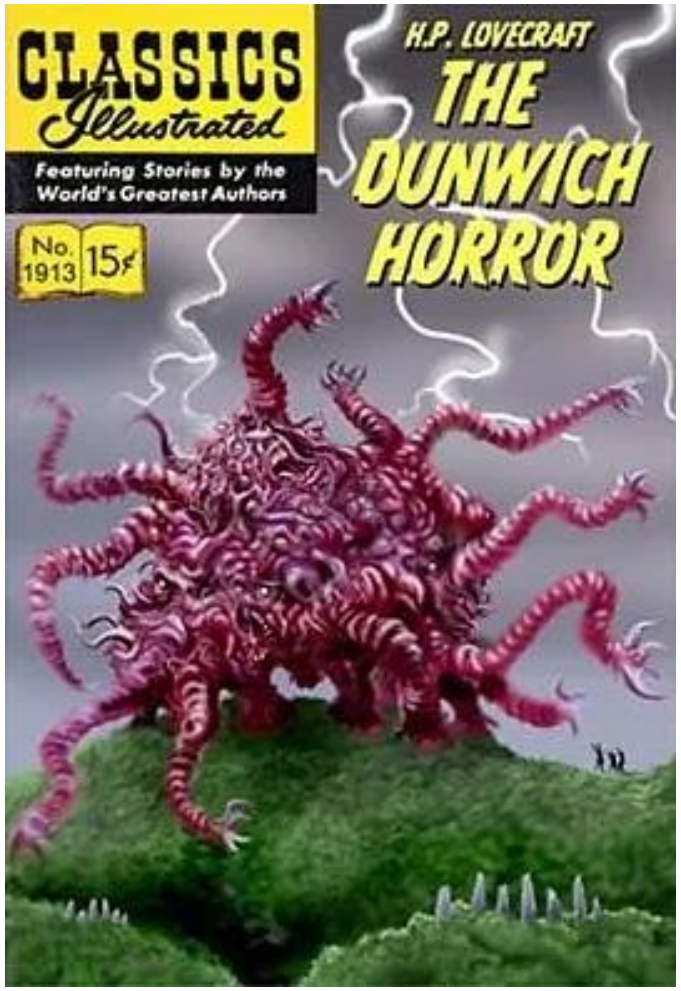


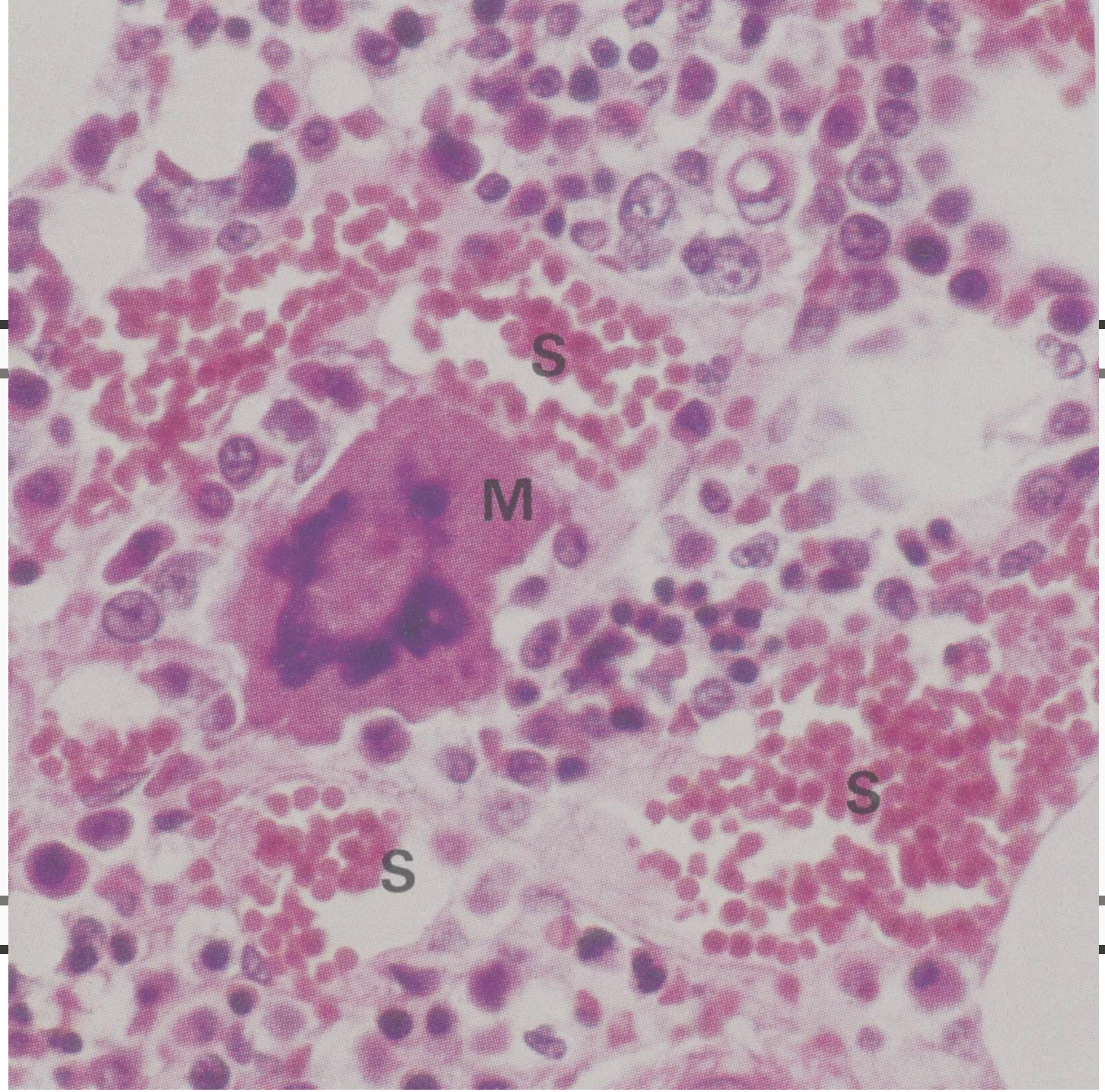
Megakaryocytes





Fragmentation of thrombocytes from the cytoplasm 2000 - 4000 thrombocytes directly into the sinusoid

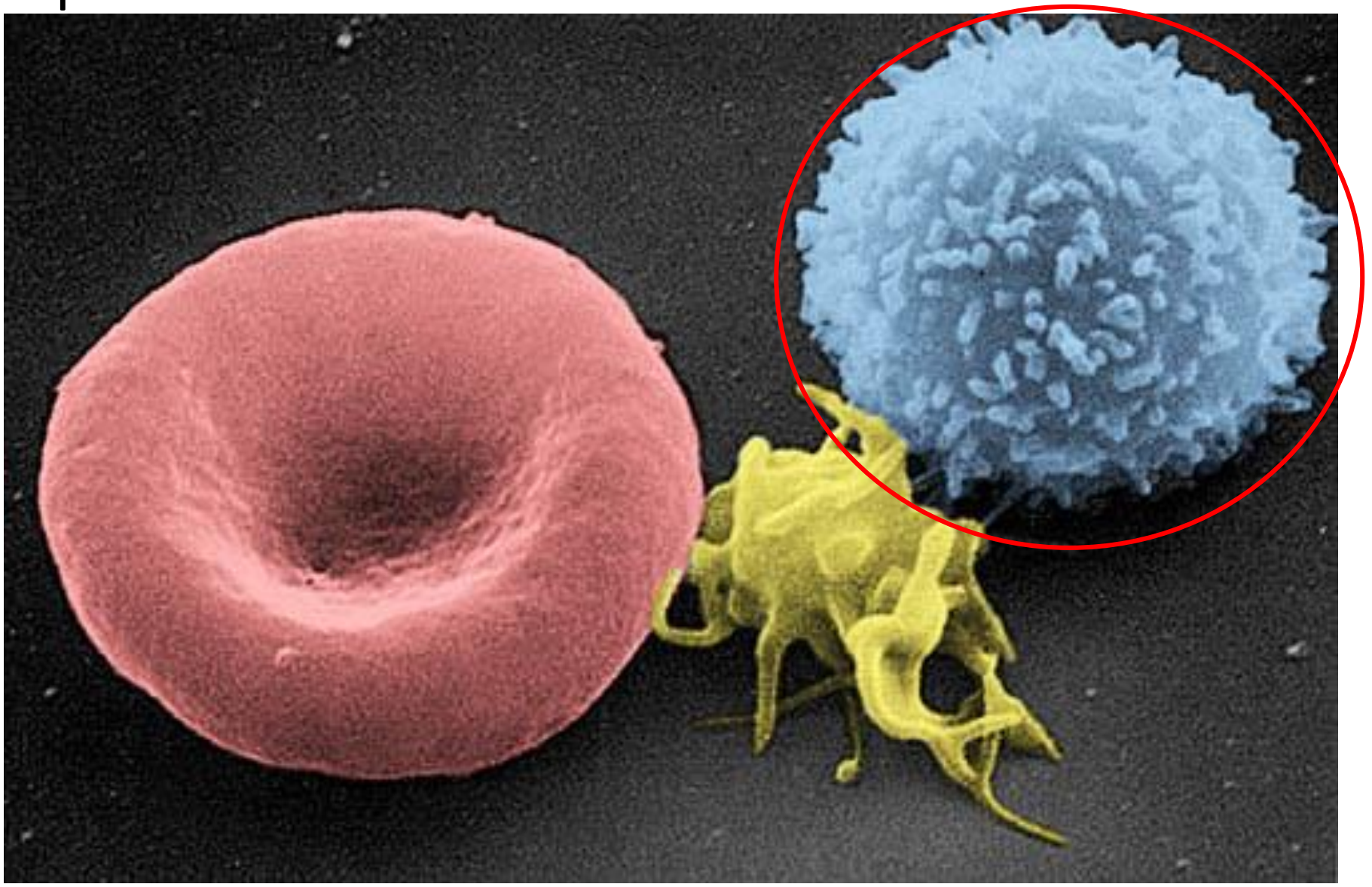



S
M
S
S

Trombopoiesis

- The precursor is common with erythroblasts (MEP)
- Megakaryoblast undergoes endomitosis and transforms into a promegakaryocyte and then into a megakaryocyte
- Megakaryocyte is a polyploid giant cell with eosinophilic cytoplasm, progressively sloughing off individual platelets
    - We can observe granules and demarcation lines
- A single megakaryocyte releases thousands of platelets into the blood sinusoids

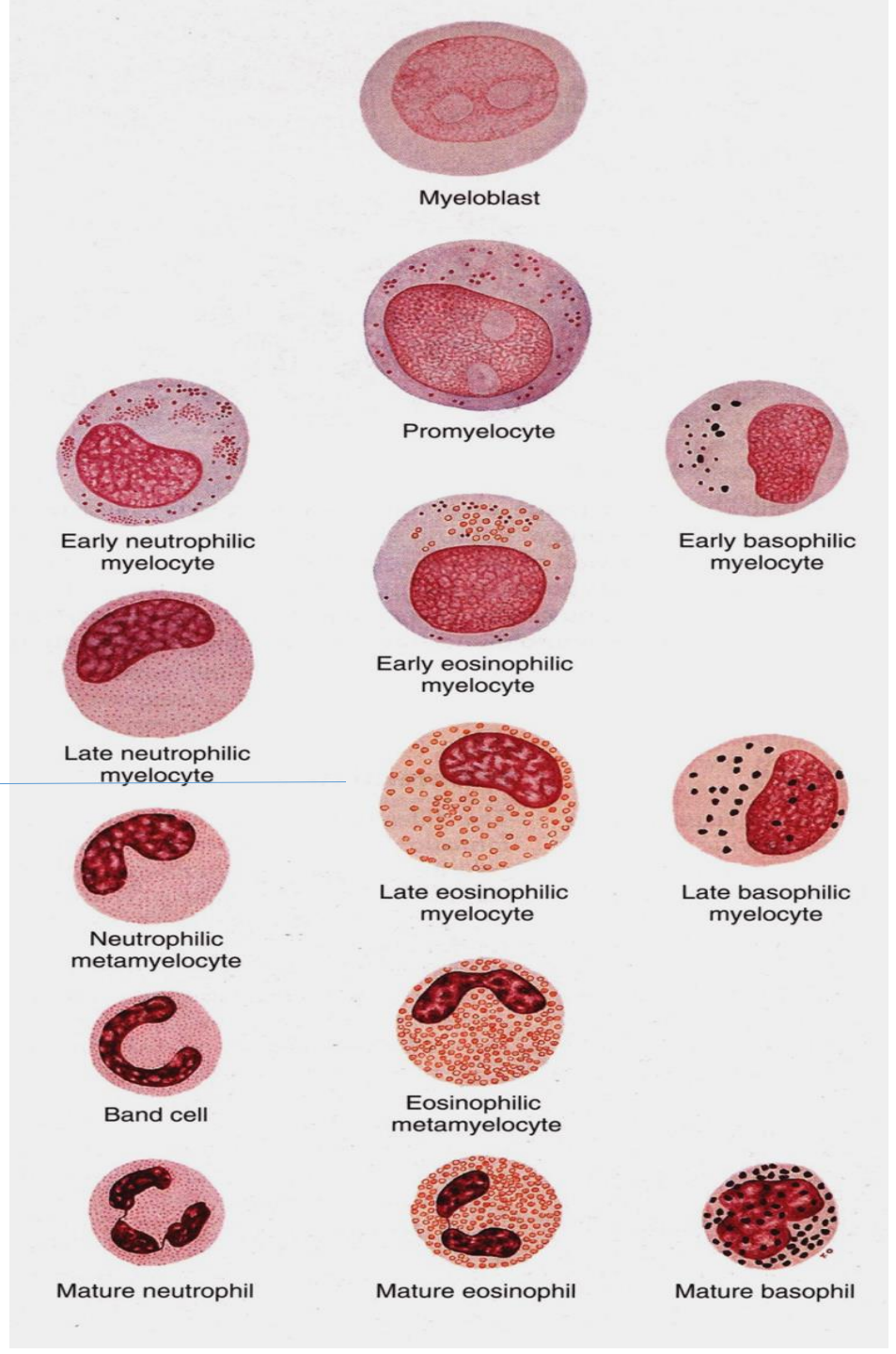
Leukopoiesis



Granulocyte development, granulopoiesis, myelopoiesis

- Cell size
- Nucleus size
- Condensation and segmentation of the nucleus
- Azurophilic granules
- Specific granules

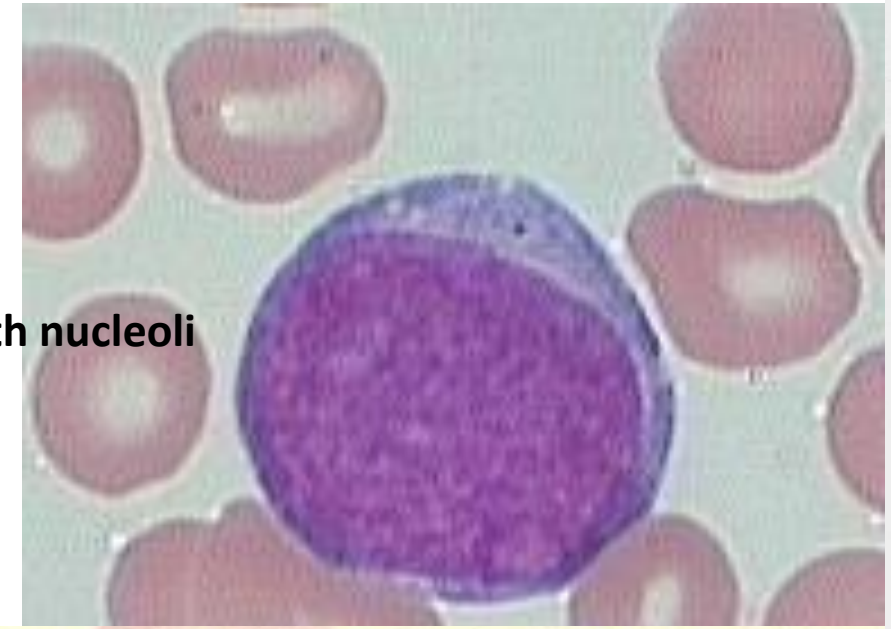
mitosis

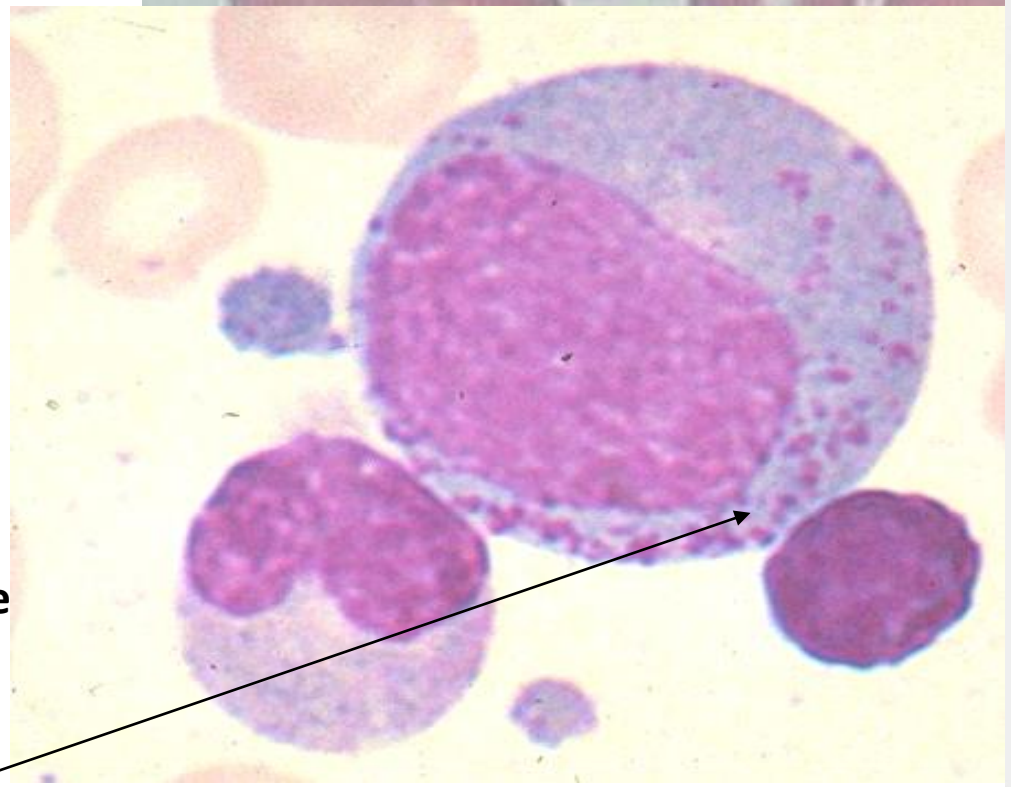


Granulopoiesis

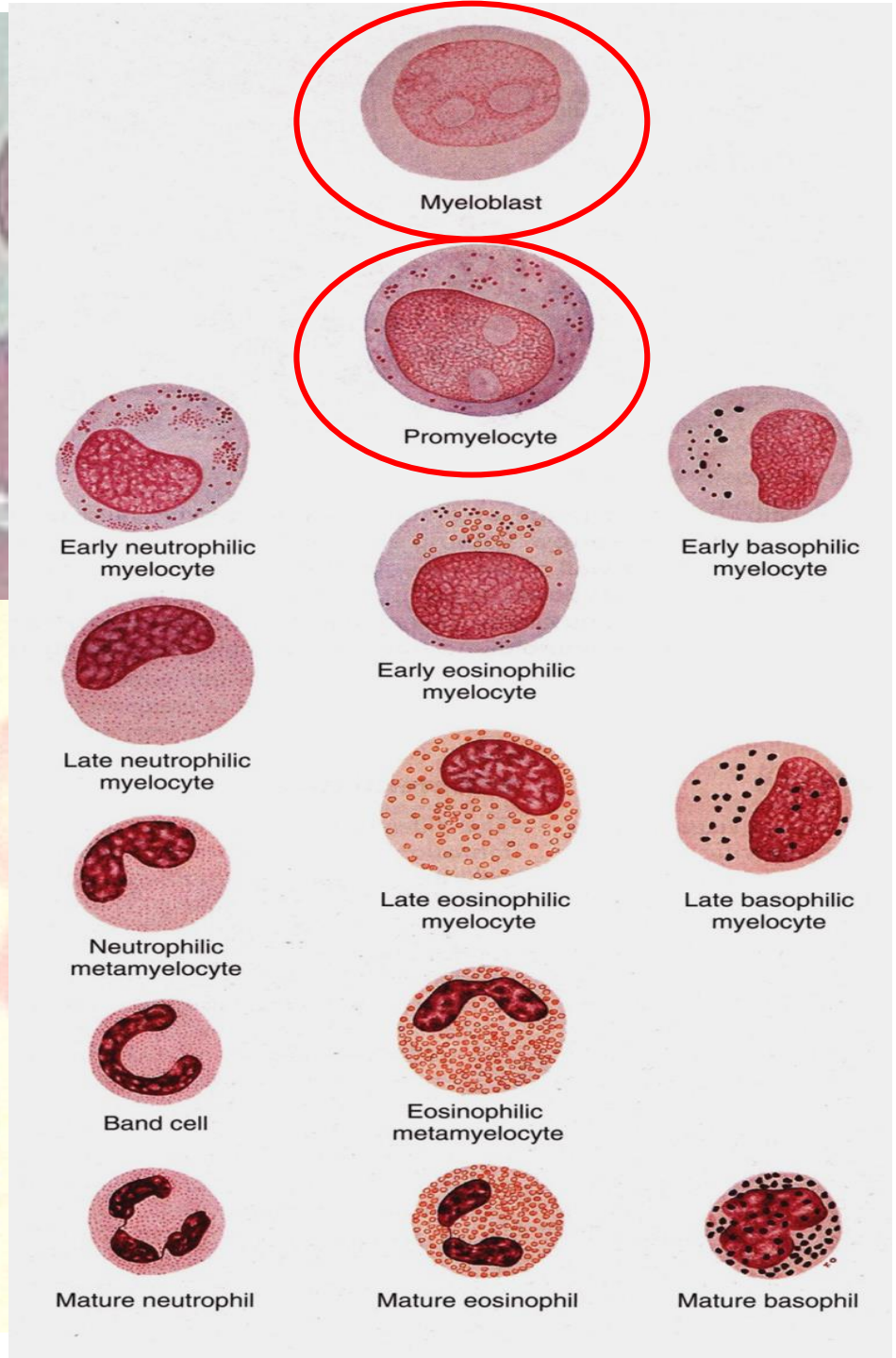
Myeloblast
10 – 20 µm

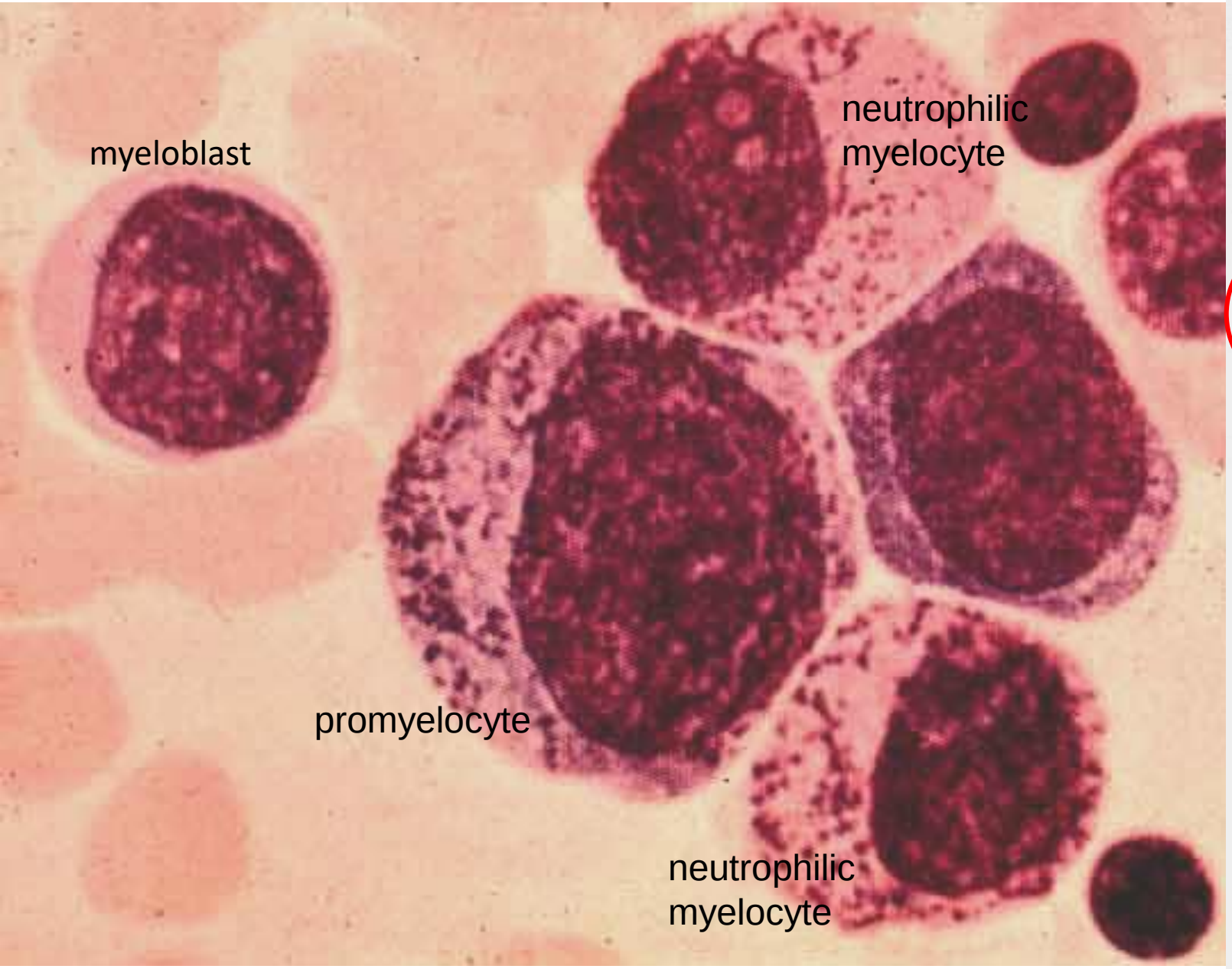
Large spherical nukleus with nucleoli
Basophilic cytoplasm

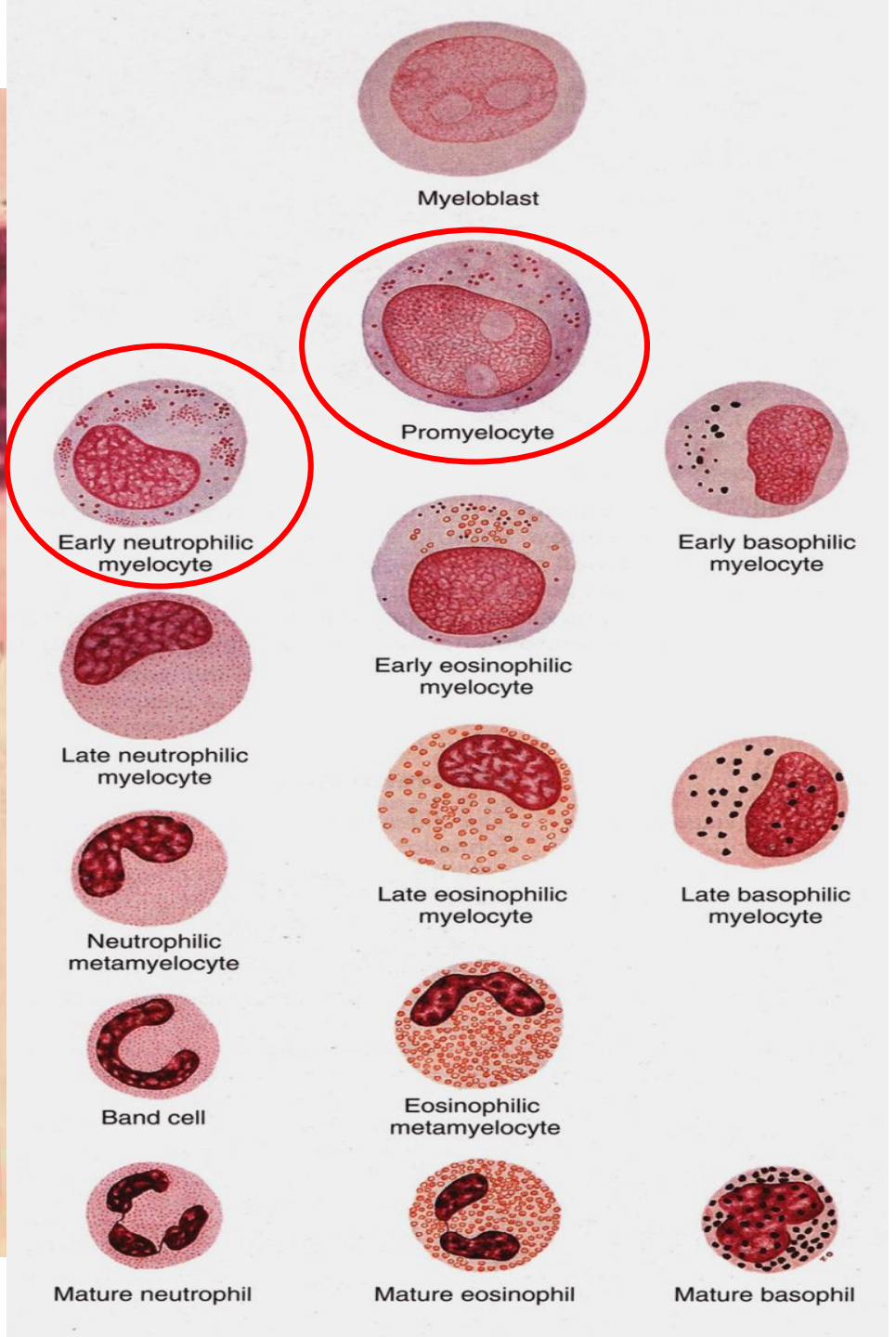




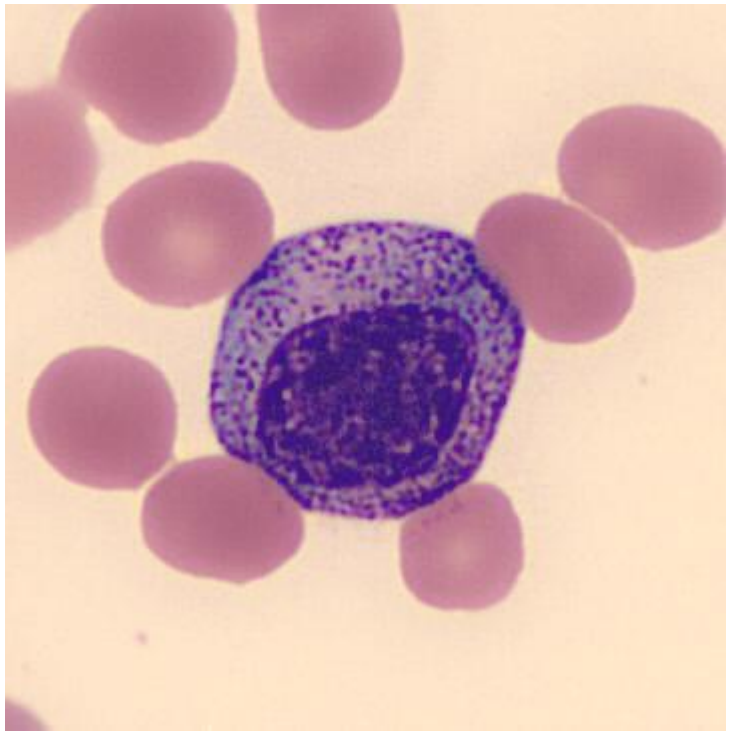
Promyelocyte
15 – 24 µm
Azurophilic granules

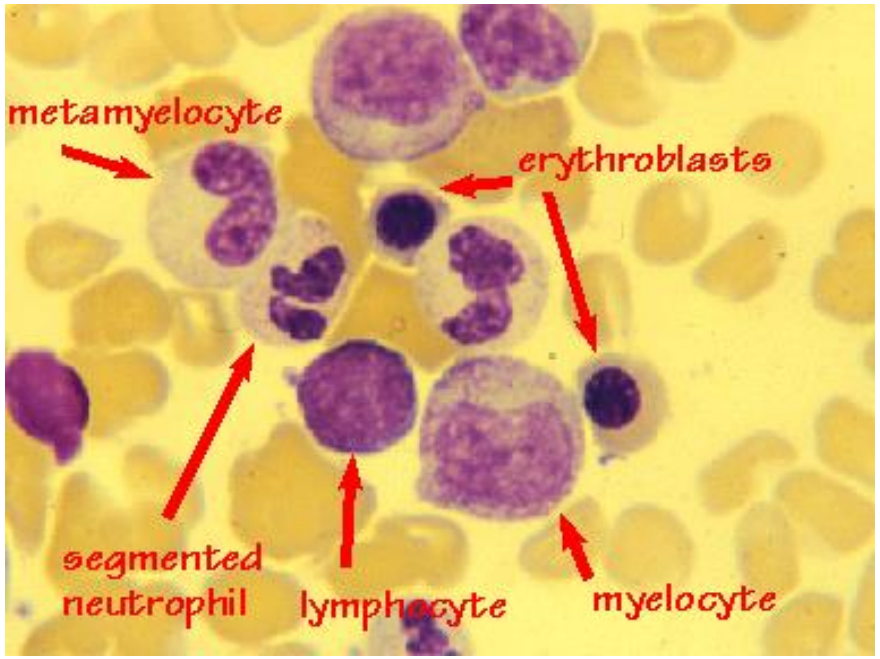



myeloblast
neutrophilic
myelocyte
promyelocyte
neutrophilic
myelocyte


Myeloblast
Promyelocyte
Early neutrophilic myelocyte
Early basophilic myelocyte
Early eosinophilic myelocyte
Late neutrophilic myelocyte
Late eosinophilic myelocyte
Late basophilic myelocyte
Neutrophilic metamyelocyte
Band cell
Eosinophilic metamyelocyte
Mature neutrophil
Mature eosinophil
Mature basophil

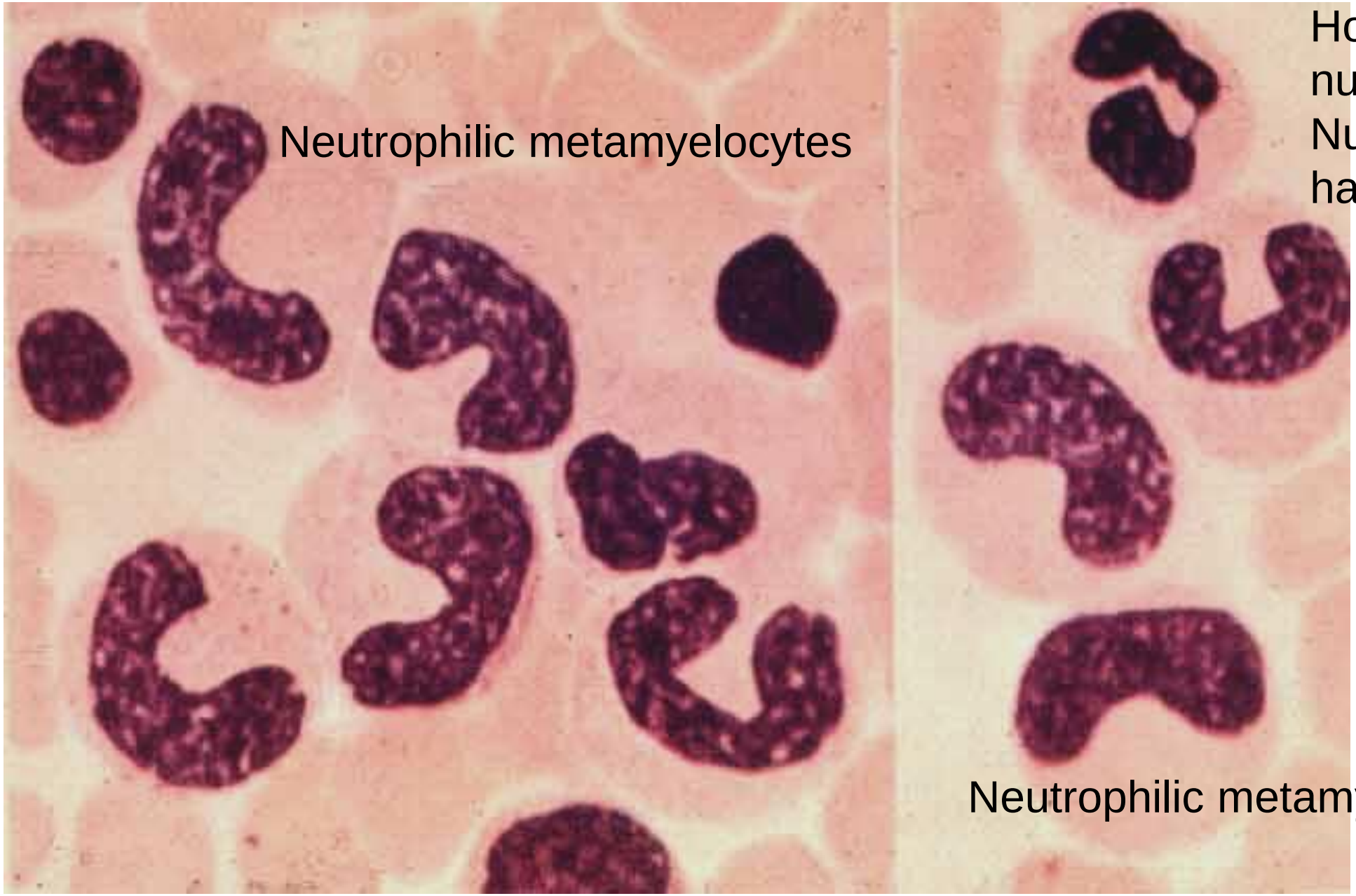
myelocyte





Gradual dondensation and deformation of the nucleus
Specific granules
can undergo mitosis

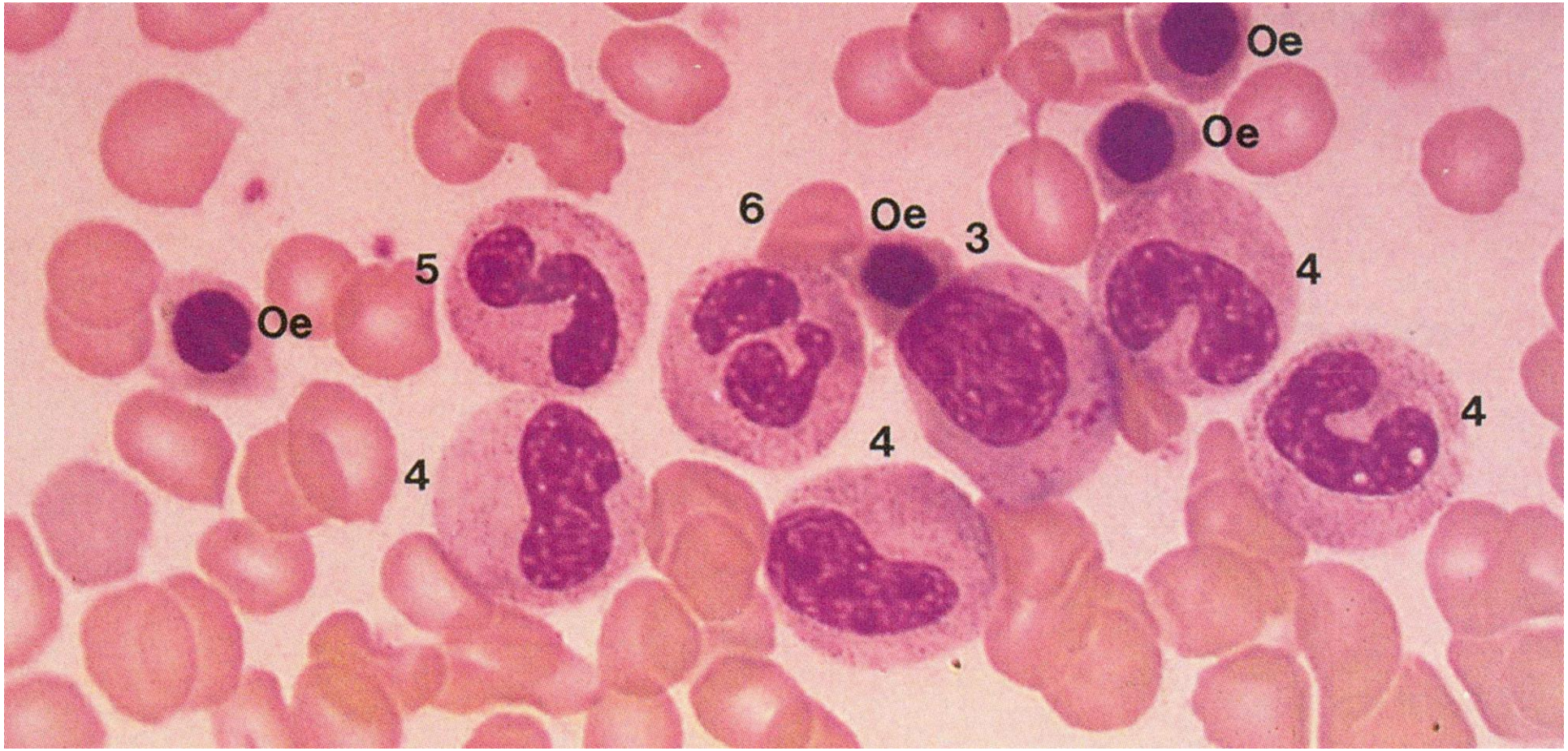
https://web.archive.org/web/20070827124407/http://meds.queensu.ca/medicine/deptmed/hemonc/anemia/myelo.htm

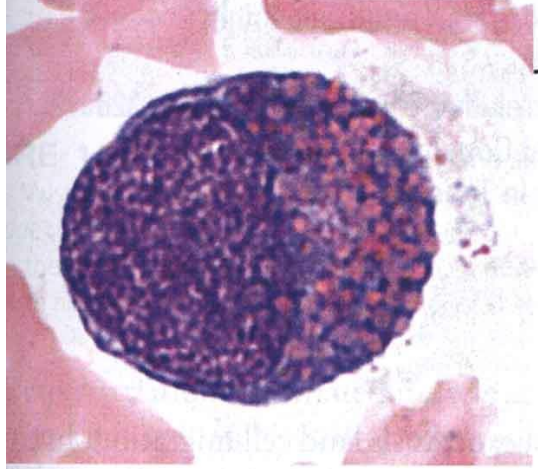


Horseshoe shaped nucelus
Nuclear segmentation hallmarks maturation

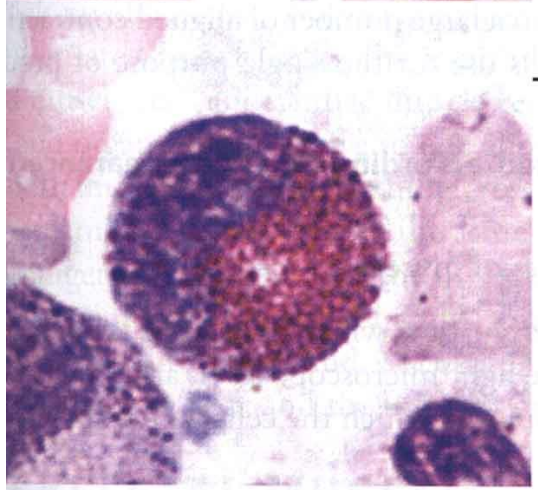
Neutrophilic metamyelocytes

3 = neutrophilic myelocyte
4 = neutrophilic metamyelocyte
5 = neutrophilic band
6 = neutrophilic granulocyte

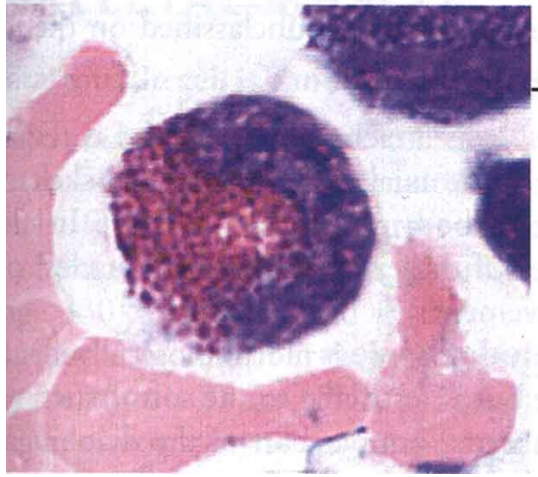




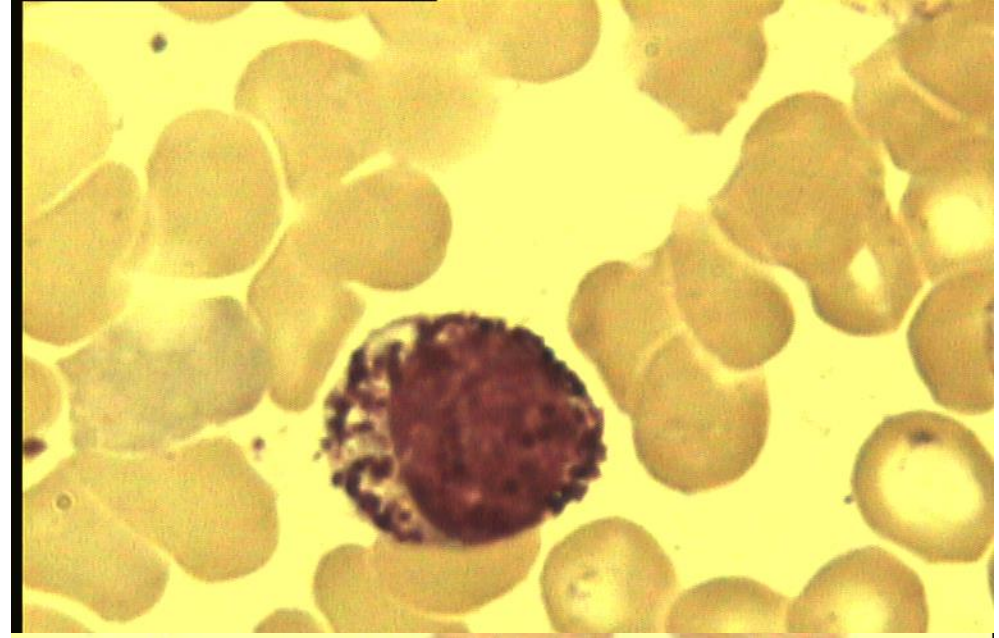
—eosinophilic myelocyte

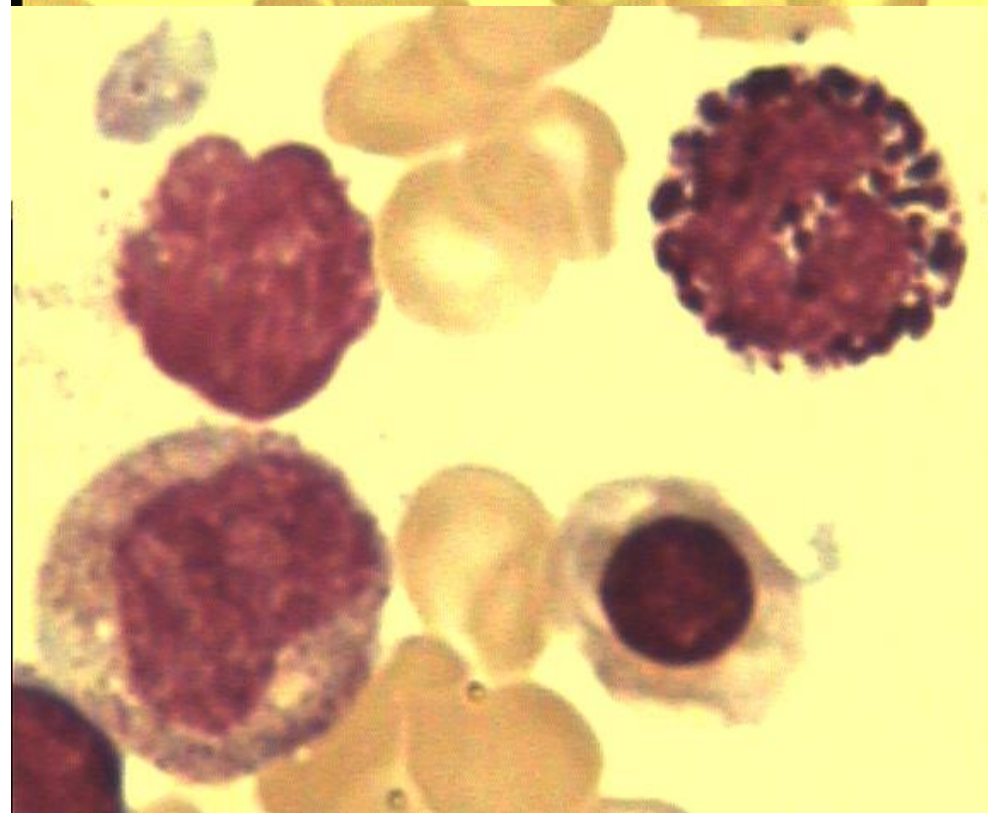


—eosinophilic metamyelocyte



—eosinophilic band cell



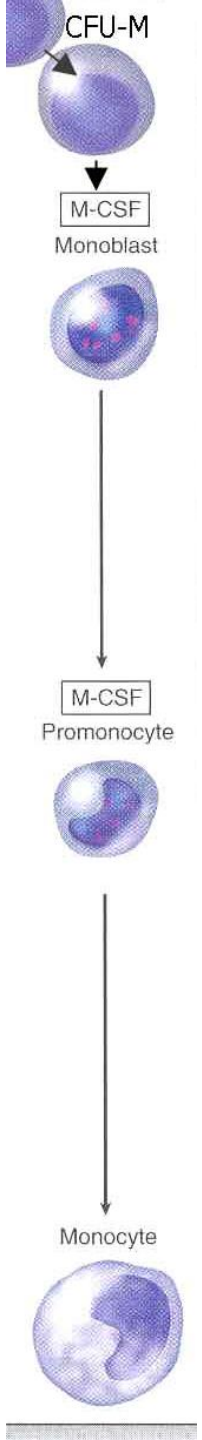


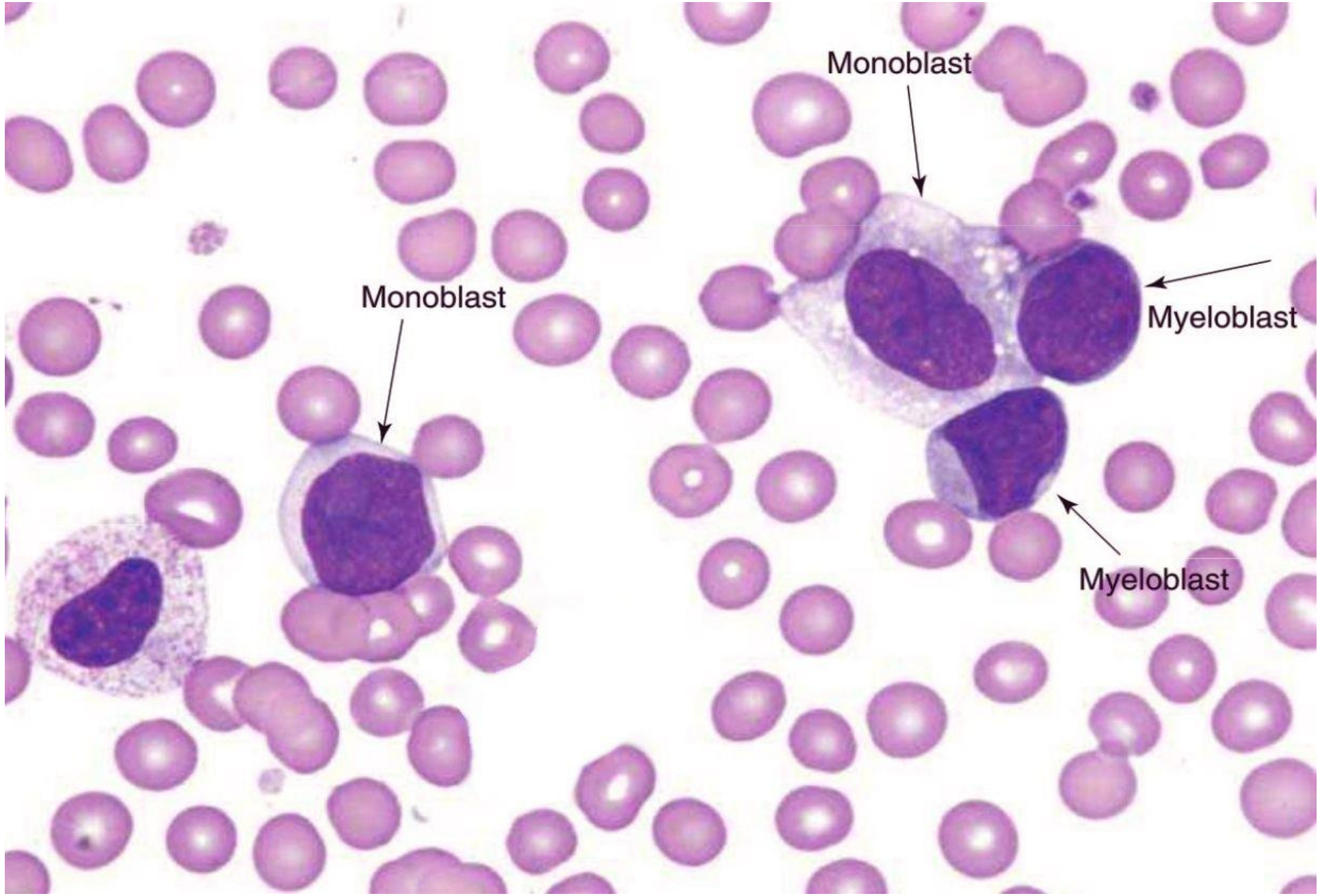
Myelopoiesis

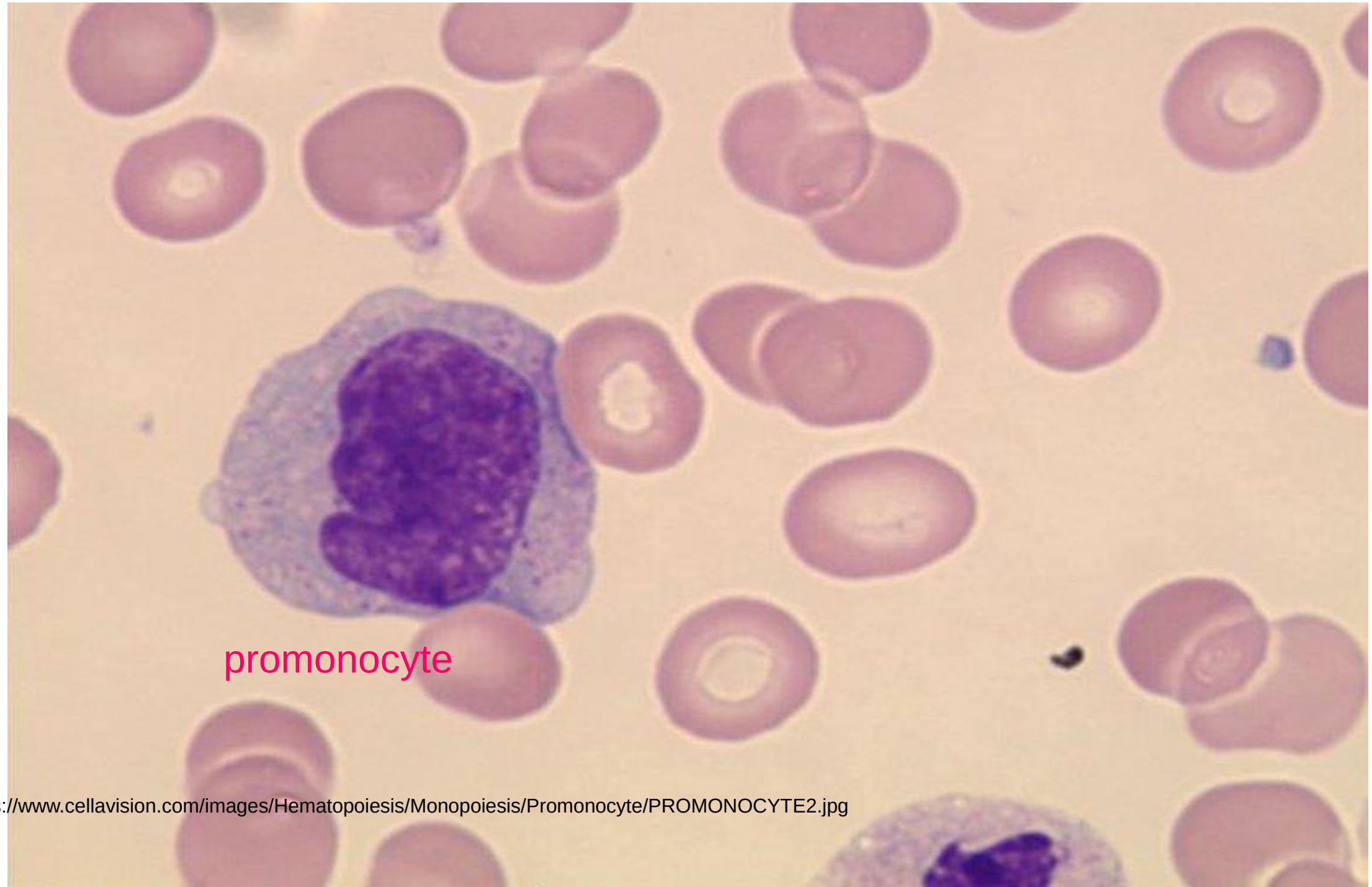
- Myeloblast with basophilic cytoplasm is a common precursor for all granulocytes
- The largest cell of granulopoiesis is the promyelocyte, azurophilic granules are formed only at this stage, at subsequent divisions their density decreases
- Myelocyte is the last dividing stage, it forms specific granules (granulocyte types can be distinguished), D-shaped nucleus
- Metamyelocyte has a cytoplasm corresponding to a mature granulocyte, but the horseshoe-shaped nucleus is still not segmented
- A young granulocyte with an unsegmented nucleus is called a band cell

Monocyte development, monopoiesis

- A monoblast is virtually indistinguishable from a myeloblast
- Promonocytes are larger with an indented nucleus



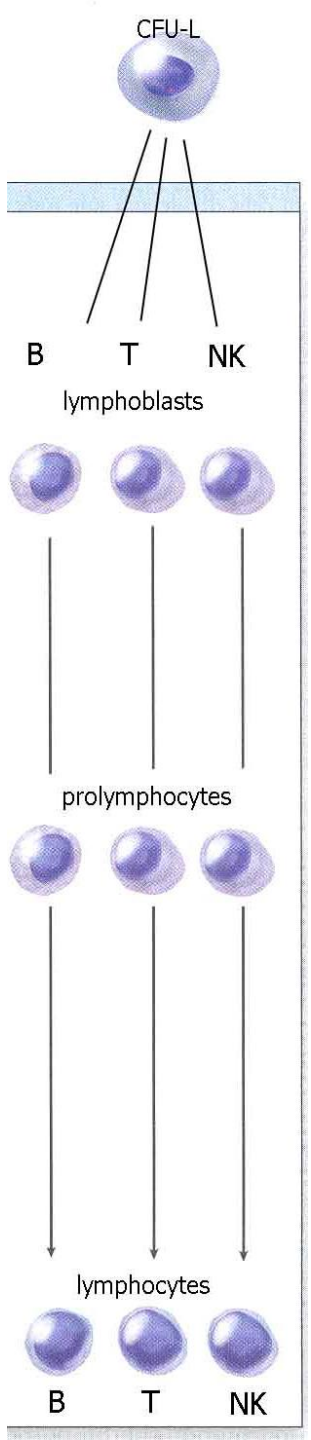

Monoblast
Monoblast
Myeloblast
Myeloblast



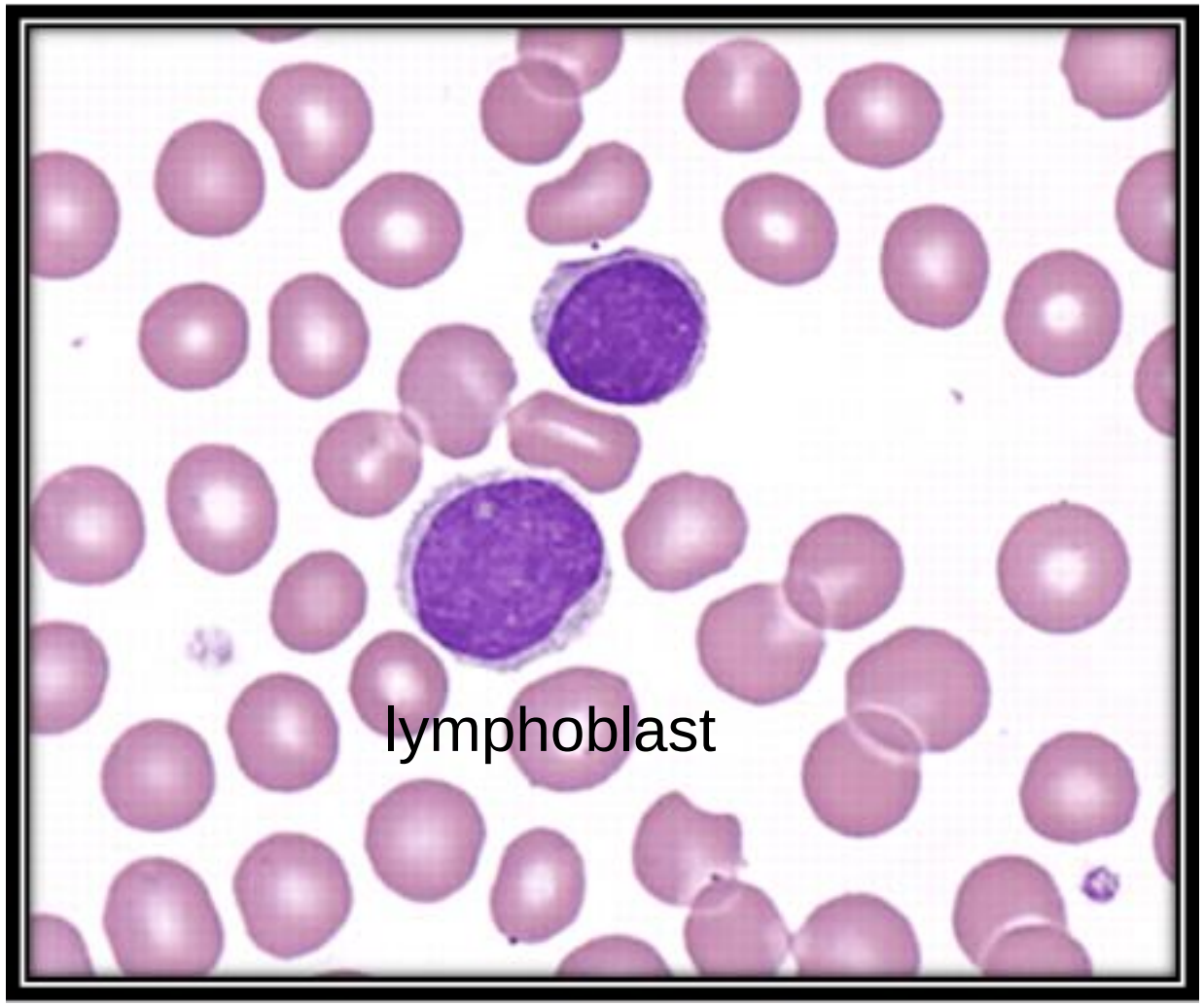
https://www.cellavision.com/images/Hematopoiesis/Monopoiesis/Promonocyte/PROMONOCYTE2.jpg

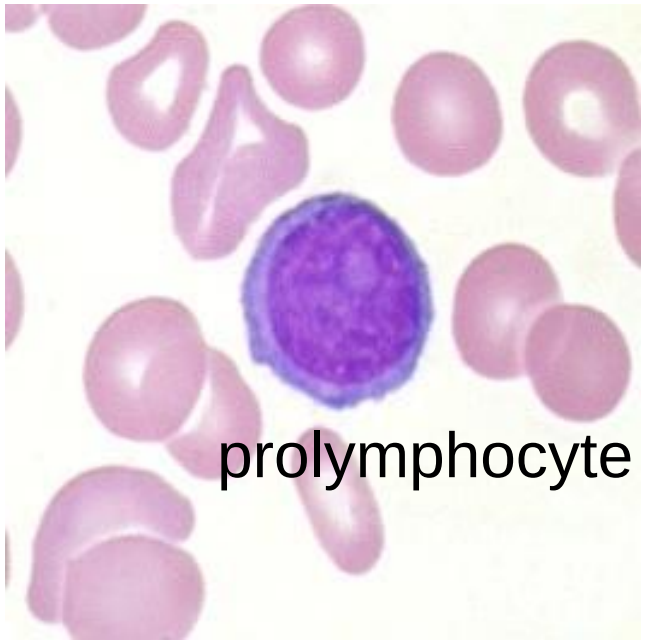
Vývoj lymfocytů, lymfopoéza

- Cell size ↓
- Nucleus size ↓
- Nuclear condensation ↑

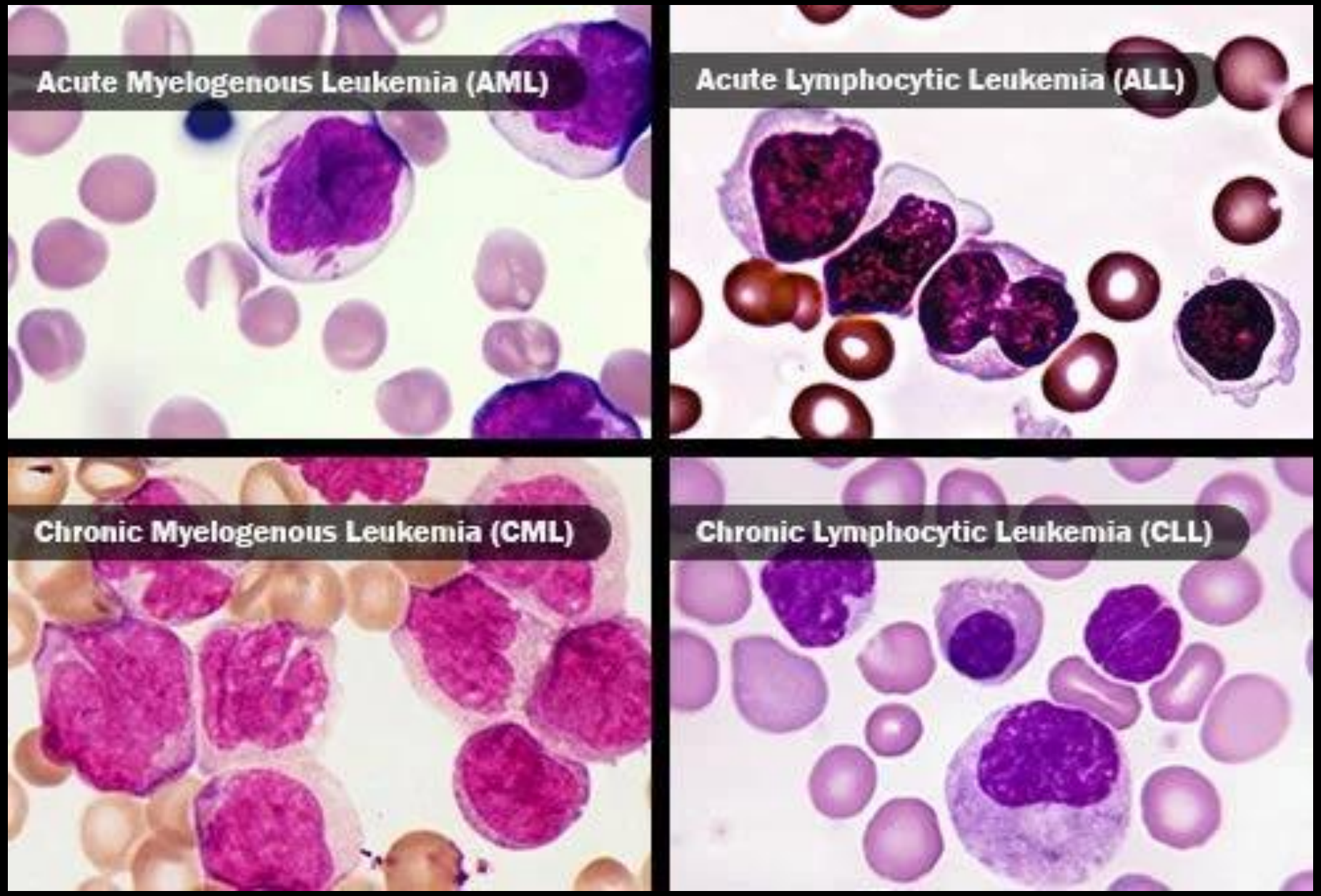


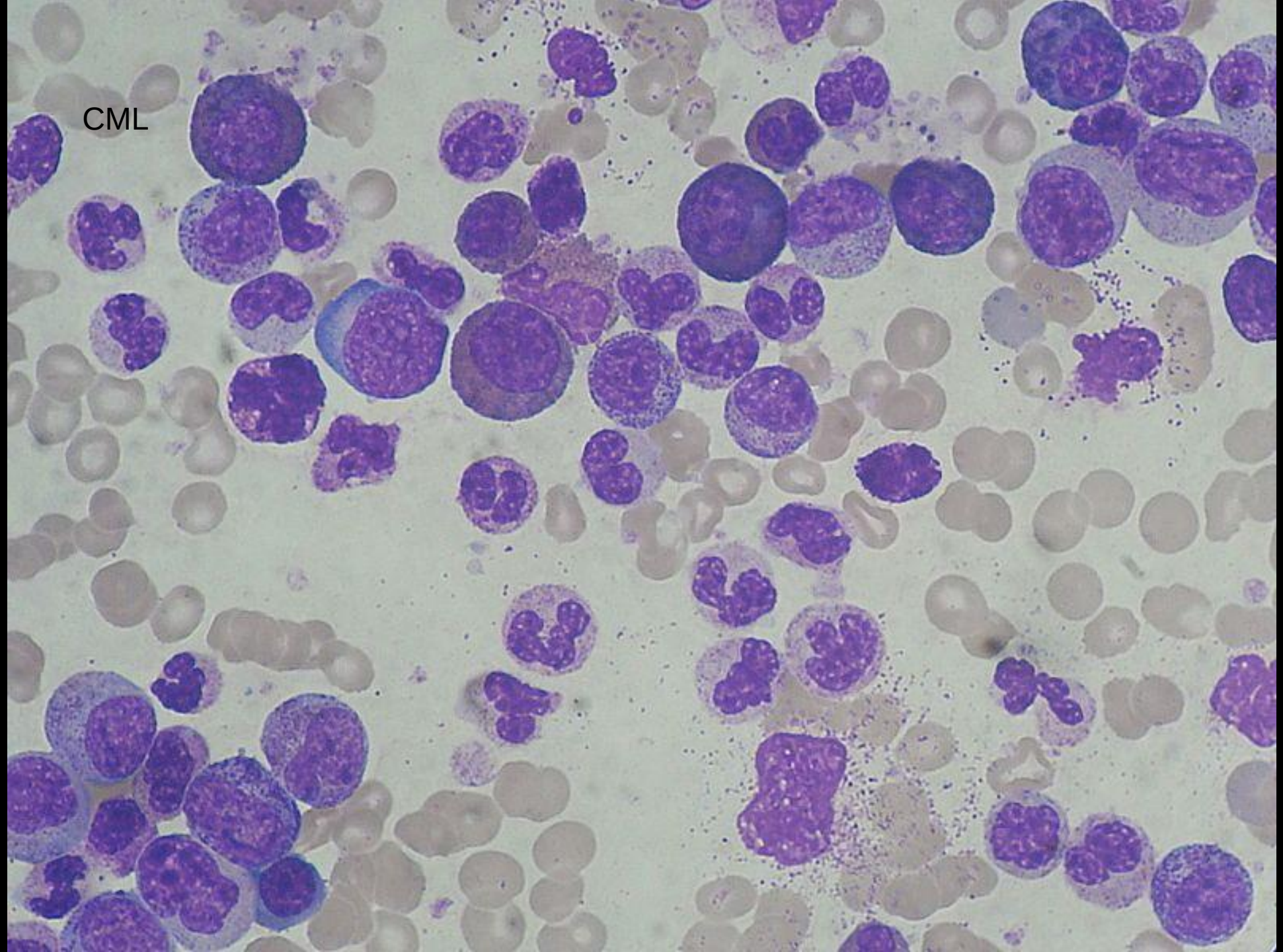
Morphologically, the individual stages are very similar, differentiated by molecular markers





https://classconnection.s3.amazonaws.com/421/flashcards/940421/png/lymphoblast1321553343200.png


Acute Myelogenous Leukemia (AML)
Acute Lymphocytic Leukemia (ALL)
Chronic Myelogenous Leukemia (CML)
Chronic Lymphocytic Leukemia (CLL)


CML

https://askhematologist.com/bone-marrow-examination/