HAEMATOPOIESIS

HAEMATOPOIESIS

OBJECTIVES

- Embryonal ,fetal, new born & adult haematopoiesis
- Stem cell
- Bone marrow microenvironment

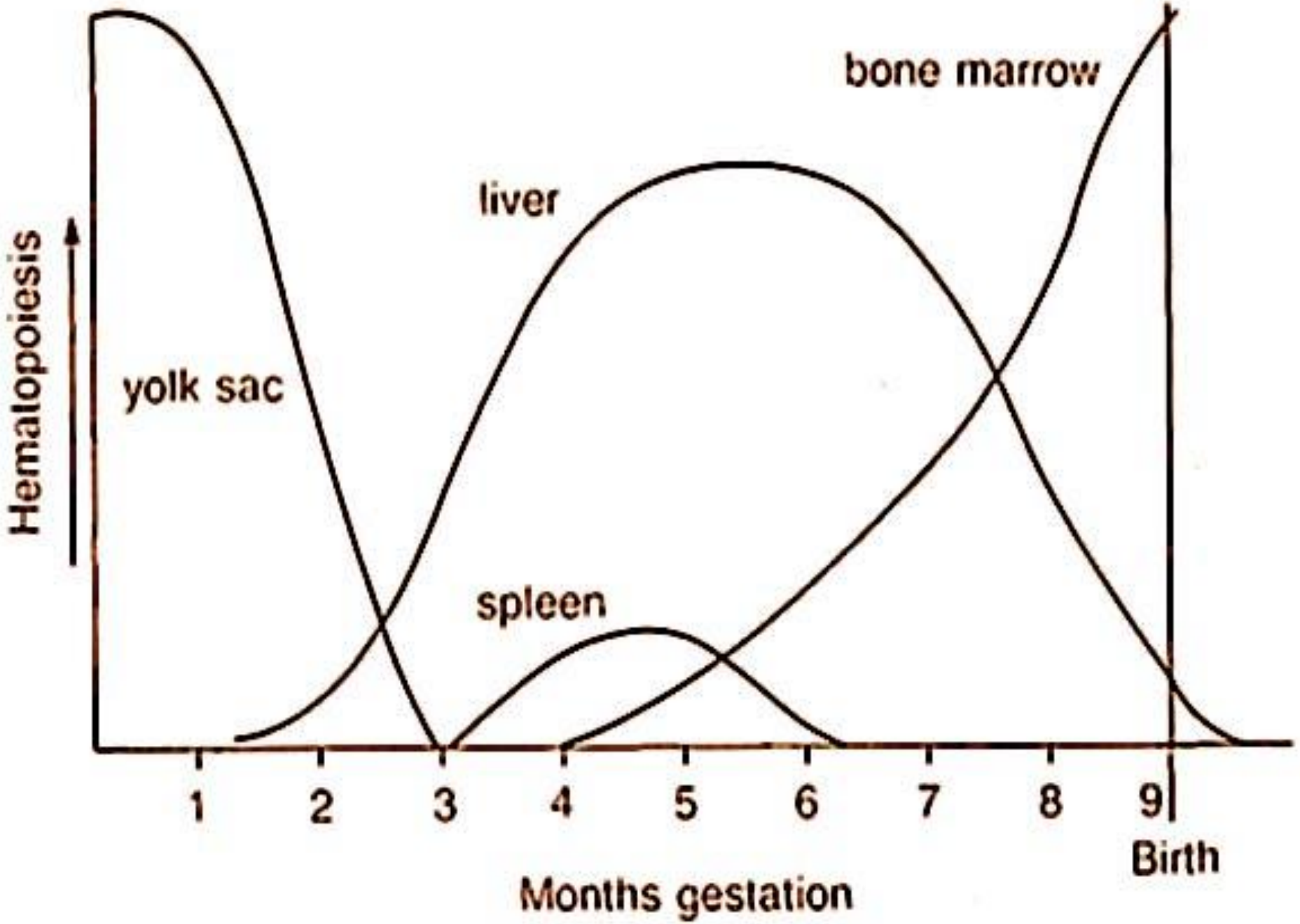
DEVELOPMENT OF HEMATOPOEITIC SYSTEM (EMBRYONIC PHASE)

- Clusters of mesenchyme, mesodermal cells proliferate and expand (2 week)
- Vascular channels develop and primitive embryonic circulatory system is formed.
- Proliferation of early hematopoietic cells
- Differentiation of hematopoietic precursors

DEVELOPMENT OF HEMATOPOEITIC SYSTEM

FETAL HAEMATOPOIESIS - from 10^{TH} week of gestation till the entire 2^{nd} trimester, the major sites are liver and spleen

- Proliferation of early hematopoietic cells
- Differentiation of hematopoietic precursors
- Third trimester the sites shift to medullary cavities of bones.


bone marrow
liver
yolk sac
spleen
Hemalopoiesis
1
2
3
4
5
6
7
8
9
Birth
Months gestation

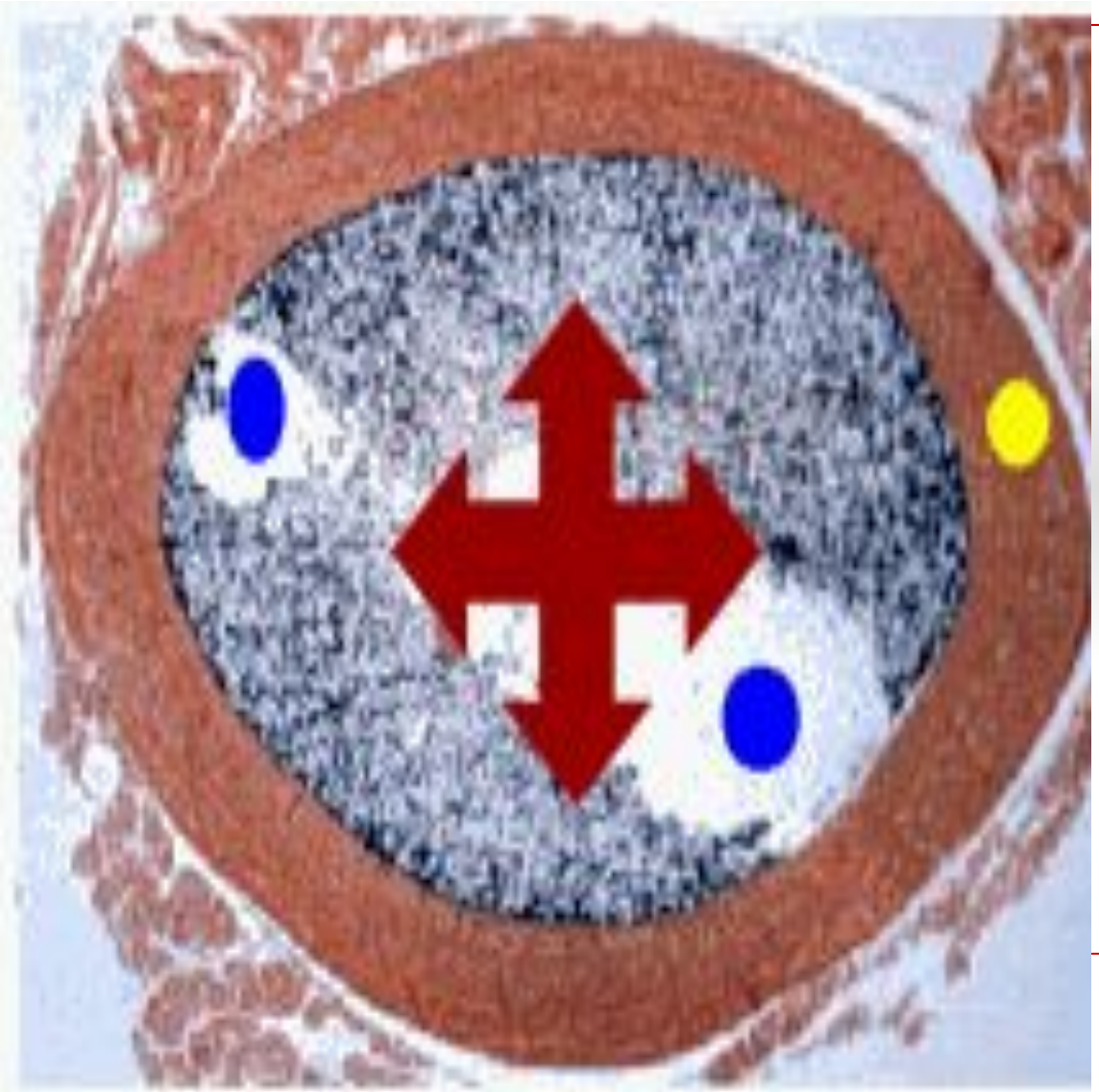
DEVELOPMENT OF HEMATOPOEITIC SYSTEM

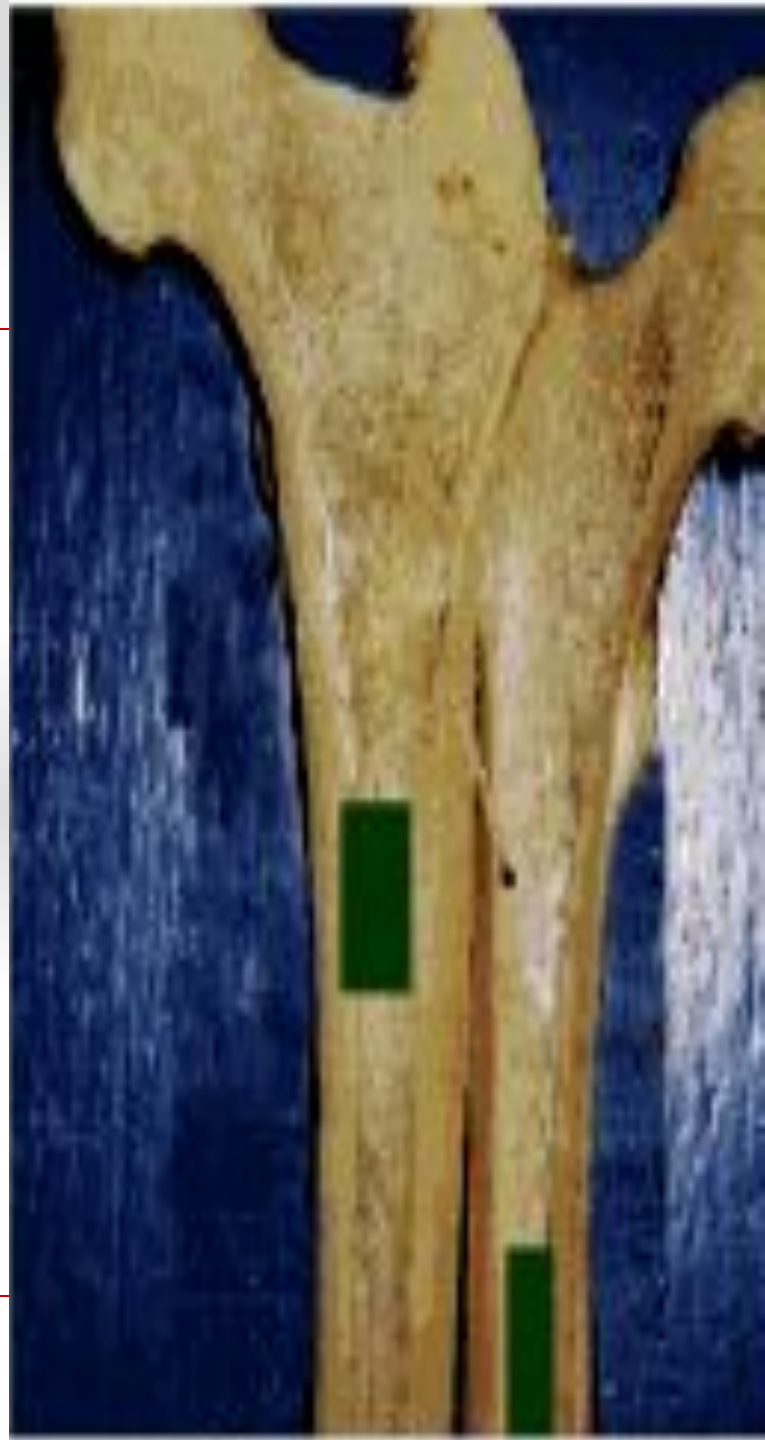
- By birth, medullary cavities of almost **every bone** contributes to provide mature functional hematopoietic cells.
- **Pluripotent cells** remain as rest cells in the bone marrow and other organs of reticuloendothelial cell system.

AGE CHANGES

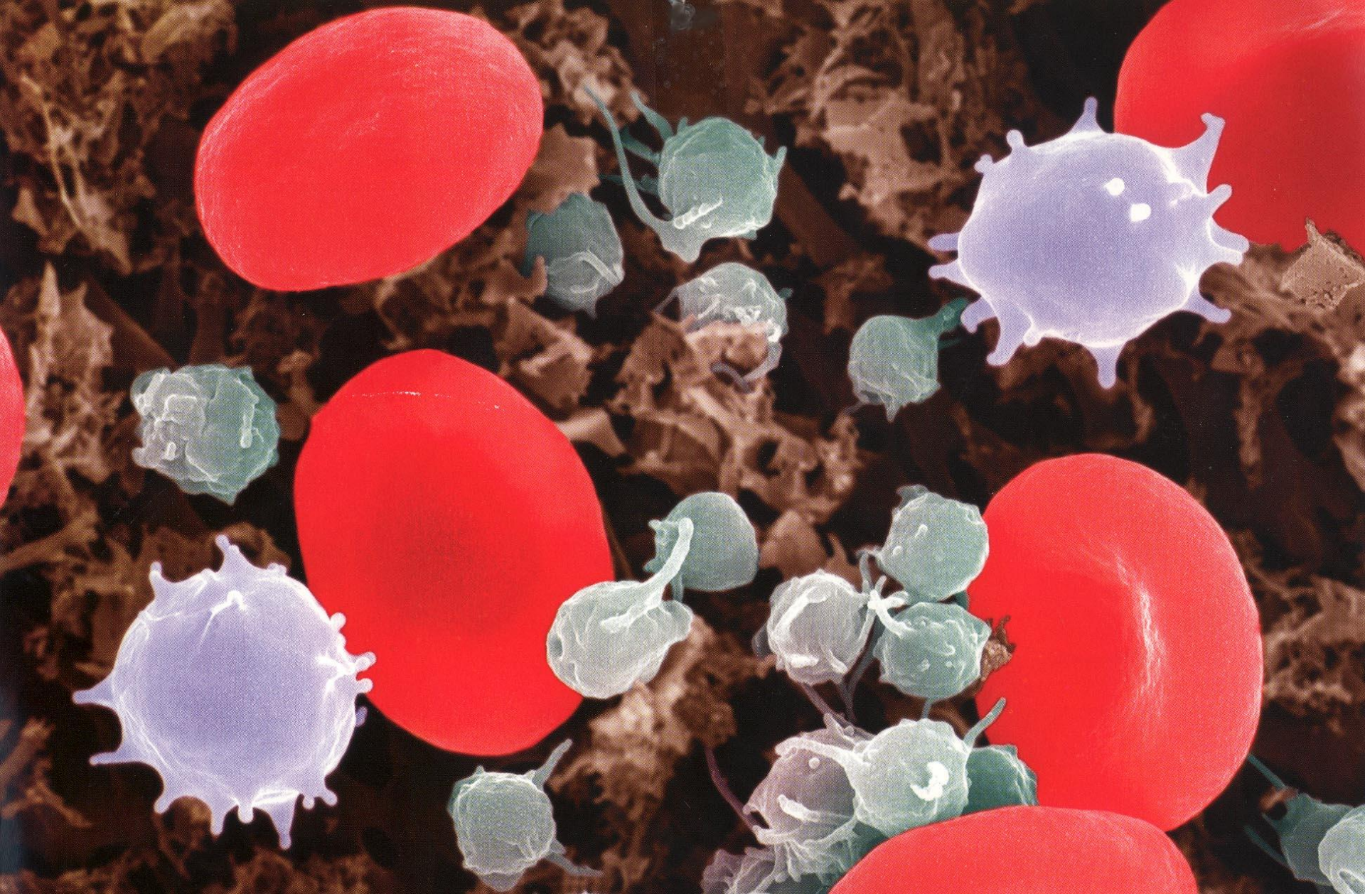
A: NEWBORN
B: ADULT
BONE
MARROW

Bone Marrow

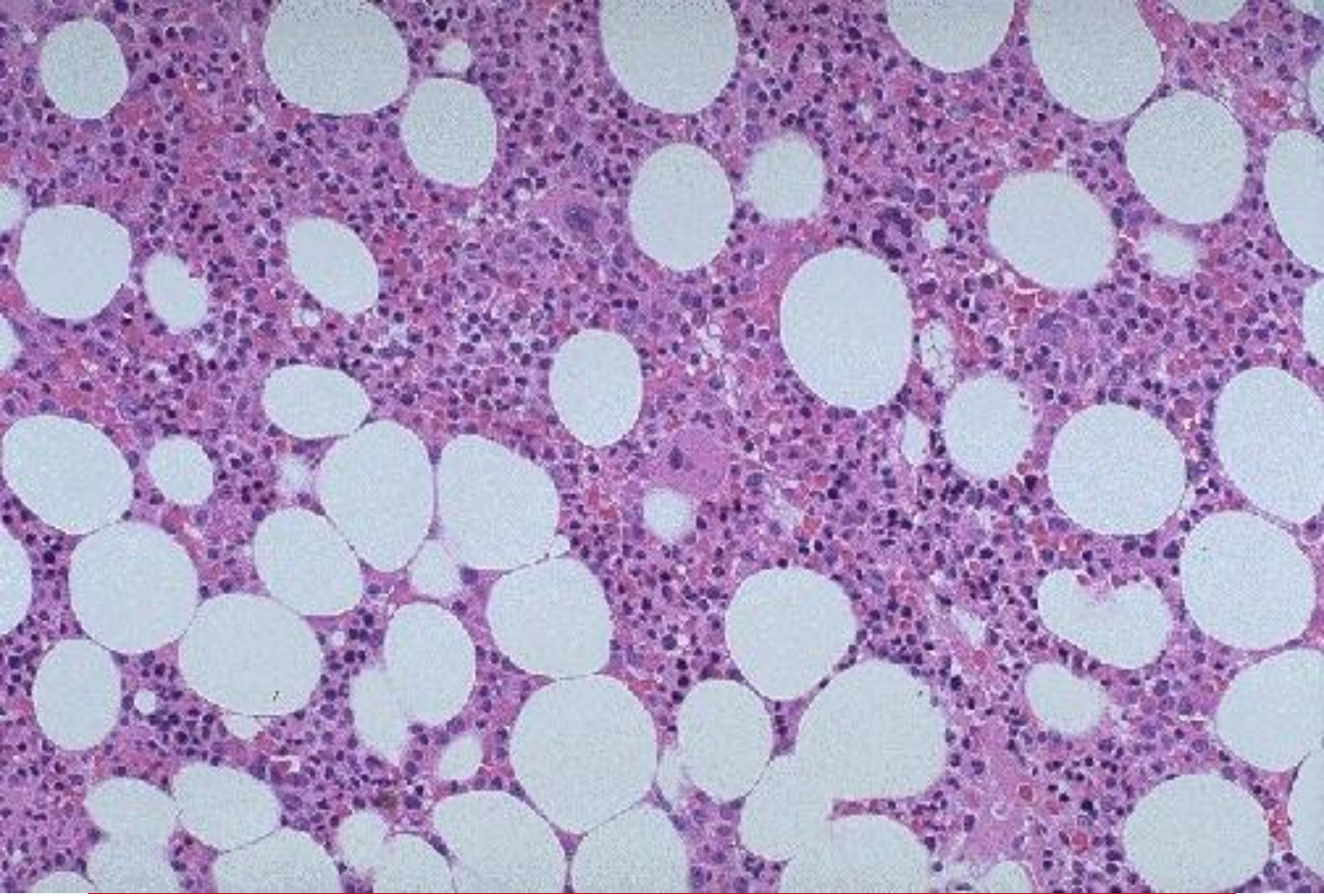




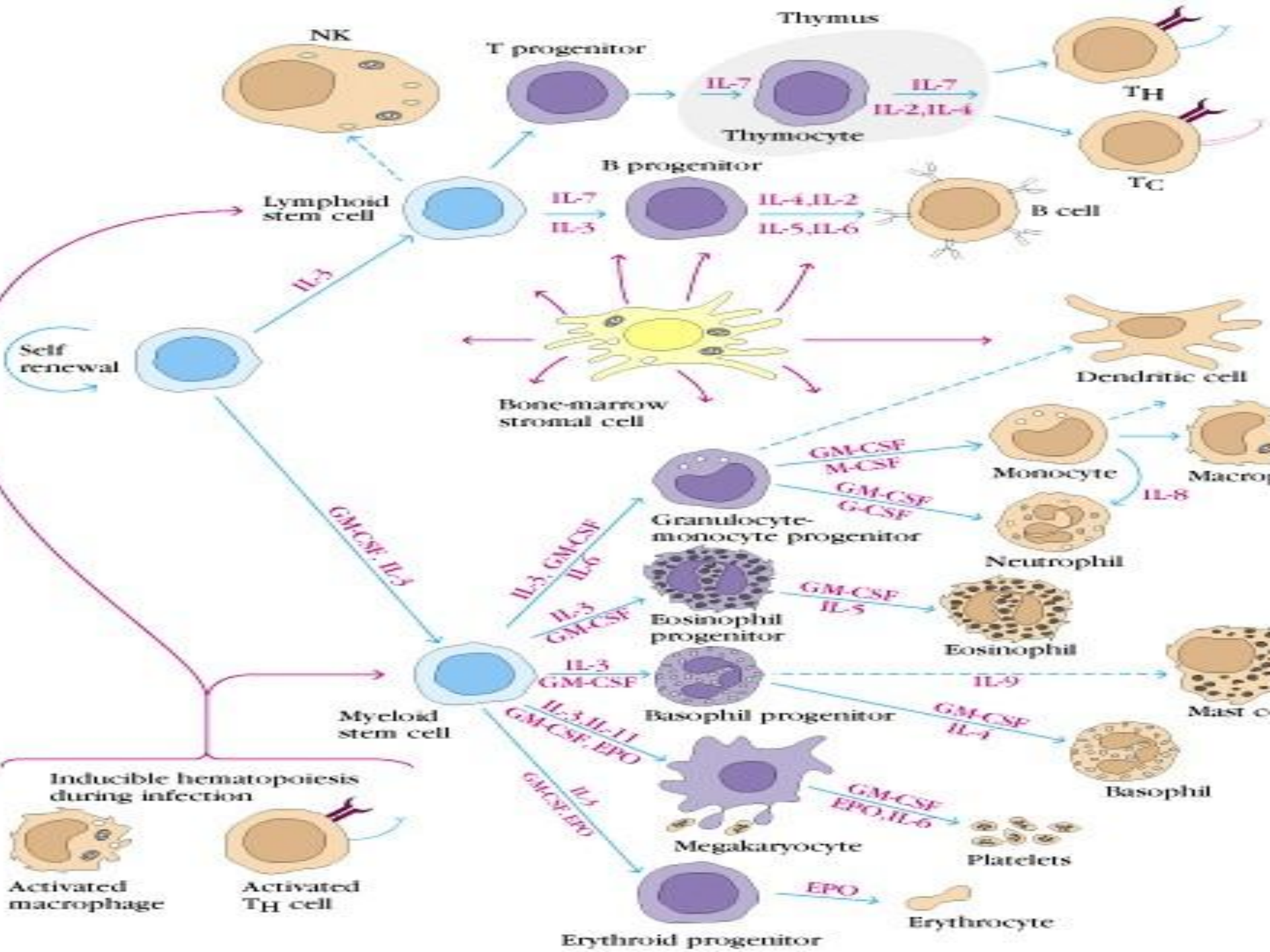
Inside of a blood vessel
SEM x 2,500



Erythrocytes, leukocytes and thrombocytes SEM x1,825

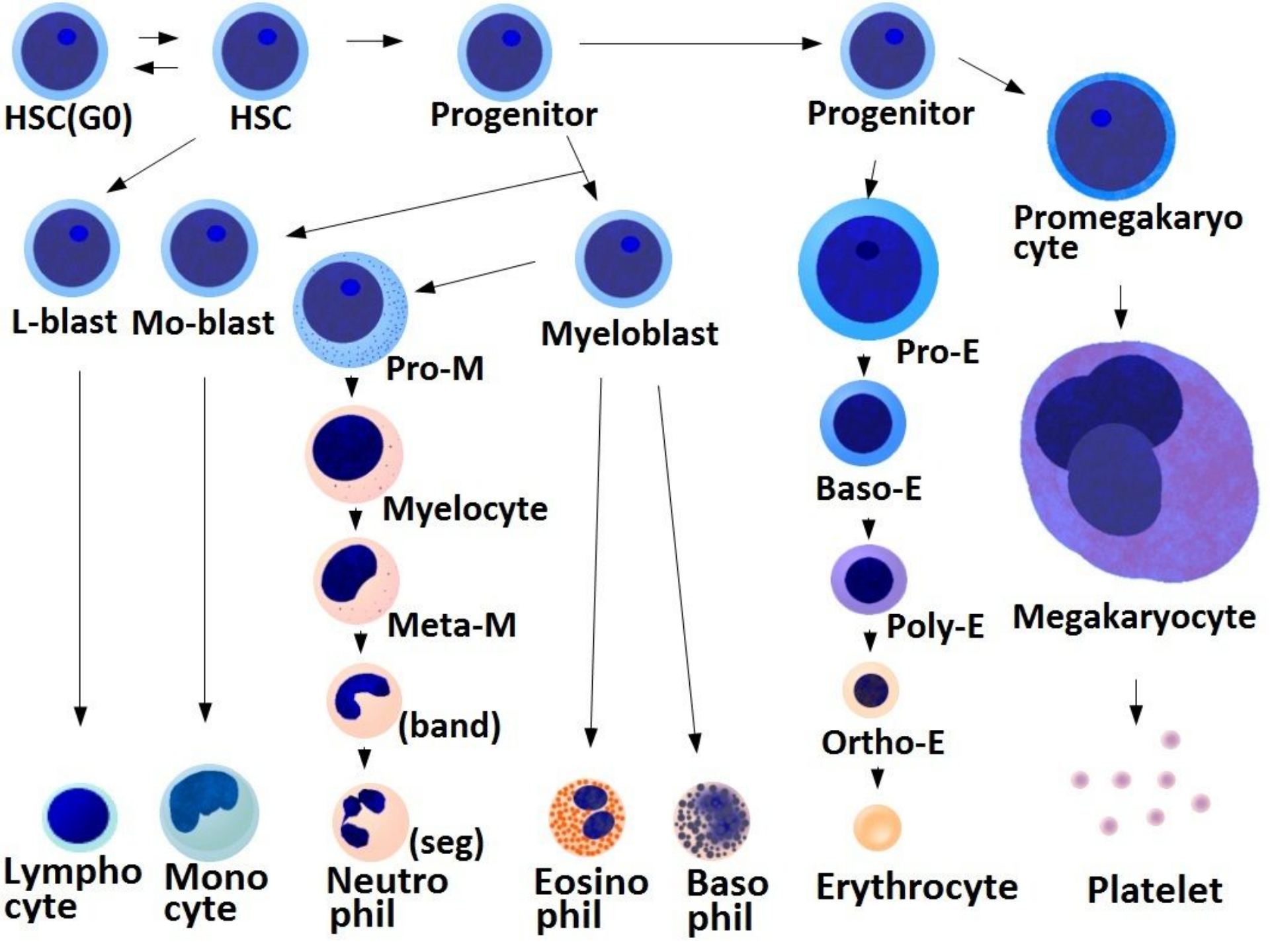


BONE MARROW

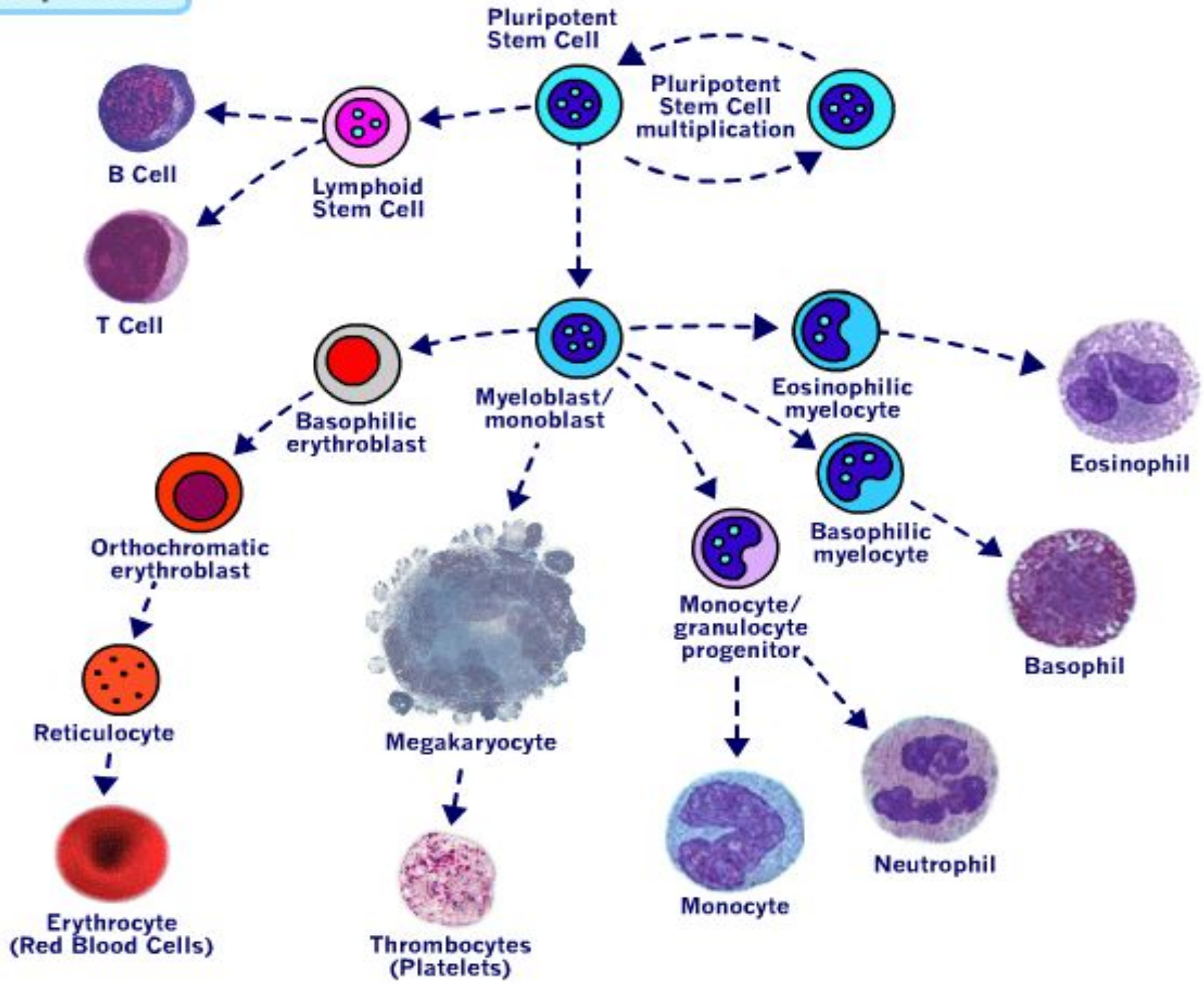

NK
T progenitor
Thymus
IL-7
Thymocyte
IL-7
IL-2,IL-4
TH
TC
B progenitor
IL-7
IL-3
IL-4,IL-2
IL-5,IL-6
B cell
Lymphoid stem cell
IL-3
Self renewal
Bone-marrow stromal cell
Dendritic cell
GM-CSF, IL-3
IL-3, GM-CSF IL-6
IL-3 GM-CSF
Granulocyte-monocyte progenitor
GM-CSF M-CSF
GM-CSF G-CSF
Monocyte
Macrop
IL-8
Neutrophil
Eosinophil progenitor
GM-CSF IL-5
Eosinophil
Myeloid stem cell
IL-3 GM-CSF
Basophil progenitor
IL-9
GM-CSF IL-4
Mast c
Basophil
IL-3,IL-11 GM-CSF, EPO
IL-3 GM-CSF, EPO
Megakaryocyte
GM-CSF EPO,IL-6
Platelets
Inducible hematopoiesis during infection
Activated macrophage
Activated TH cell
EPO
Erythrocyte
Erythroid progenitor

HEMATOPOIETIC STEM CELLS

- Is a cell **that can divide**, through mitosis and **differentiate** into specialized cell types and
- That can **self-renew** to produce more stem cells


HSC(G0)
HSC
Progenitor
Progenitor
Promegakaryo cyte
L-blast
Mo-blast
Pro-M
Myeloblast
Pro-E
Baso-E
Poly-E
Ortho-E
Erythrocyte
Megakaryocyte
Platelet
Myelocyte
Meta-M
(band)
(seg)
Lympho cyte
Mono cyte
Neutro phil
Eosino phil
Baso phil

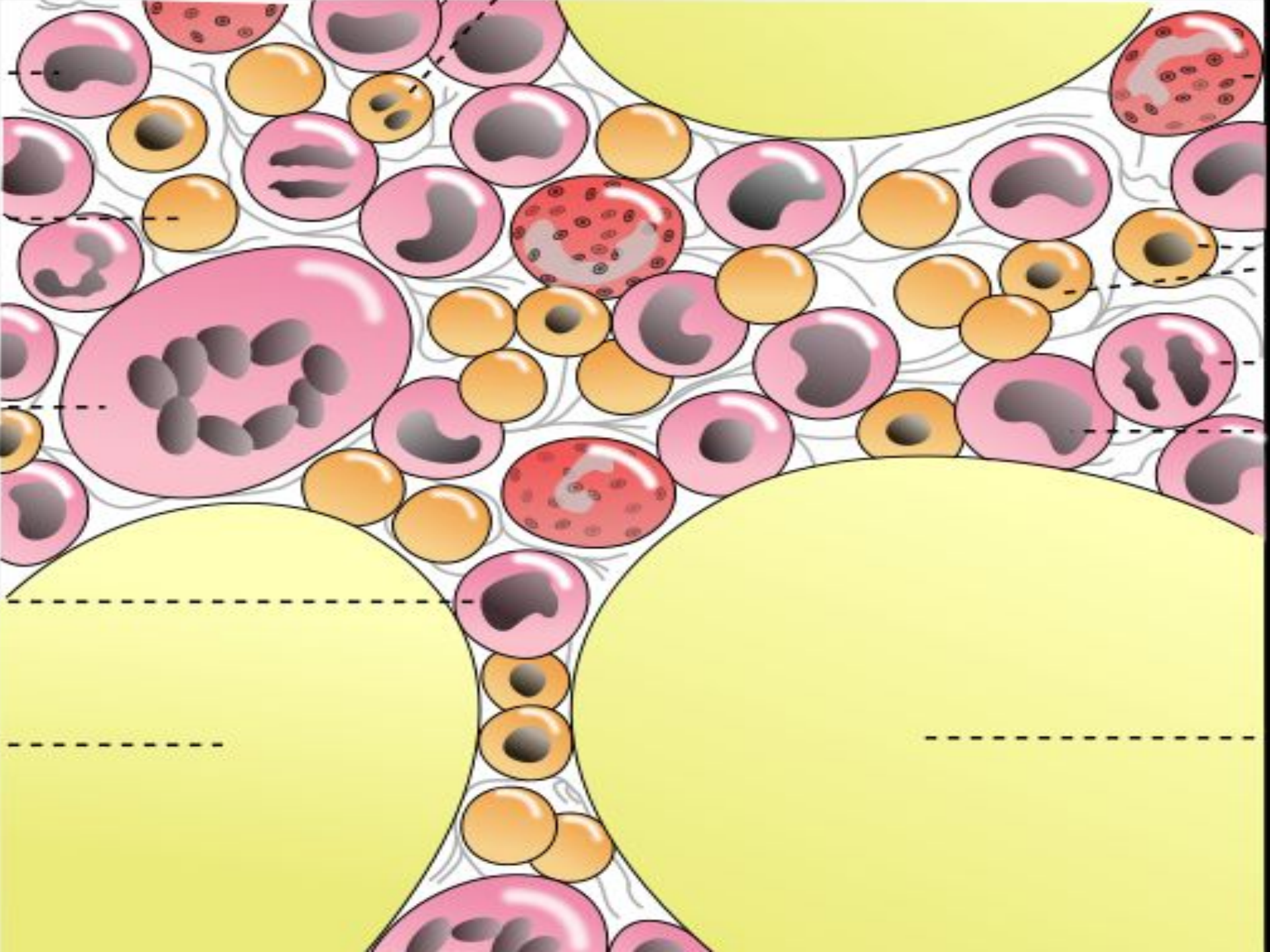
Hematopoiesis



HEMATOPOIETIC STEM CELLS

- Differentiate into **multiple cell lines**.
- Proliferation is under influence of hematopoietic **growth factors** present in reticuloendothelial system.
- Morphologically they resemble **large immature lymphocytes**
- **Cell membrane phenotyping** with monoclonal antibodies has identified them by presence of surface markers.


© Frantisek Staud
www.phototravels.net



ERYTHROPOIESIS

- In normal state, the balance of production and destruction is maintained at remarkably **constant rate**
- Both endocrine and exocrine hormones make important contributions to this dynamic well balanced mechanism
- The earliest recognizable erythroid precursor seen in the bone marrow is large basophilic staining cell,15-20 um
- Contains a single large well defined, rounded nucleus, ribosomes, mitochondria and Golgi apparatus

ERYTHROPOIESIS

- **As the early precursor cell matures,** its nucleus increases in size. As maturation goes on cell becomes smaller and more eosinophilic indicating hemoglobin.
- During **intermediate stages of maturation,** cytoplasm becomes polychromatic indicating mixture of basophilic proteins and eosinophilic hemoglobin.

ERYTHROPOIESIS

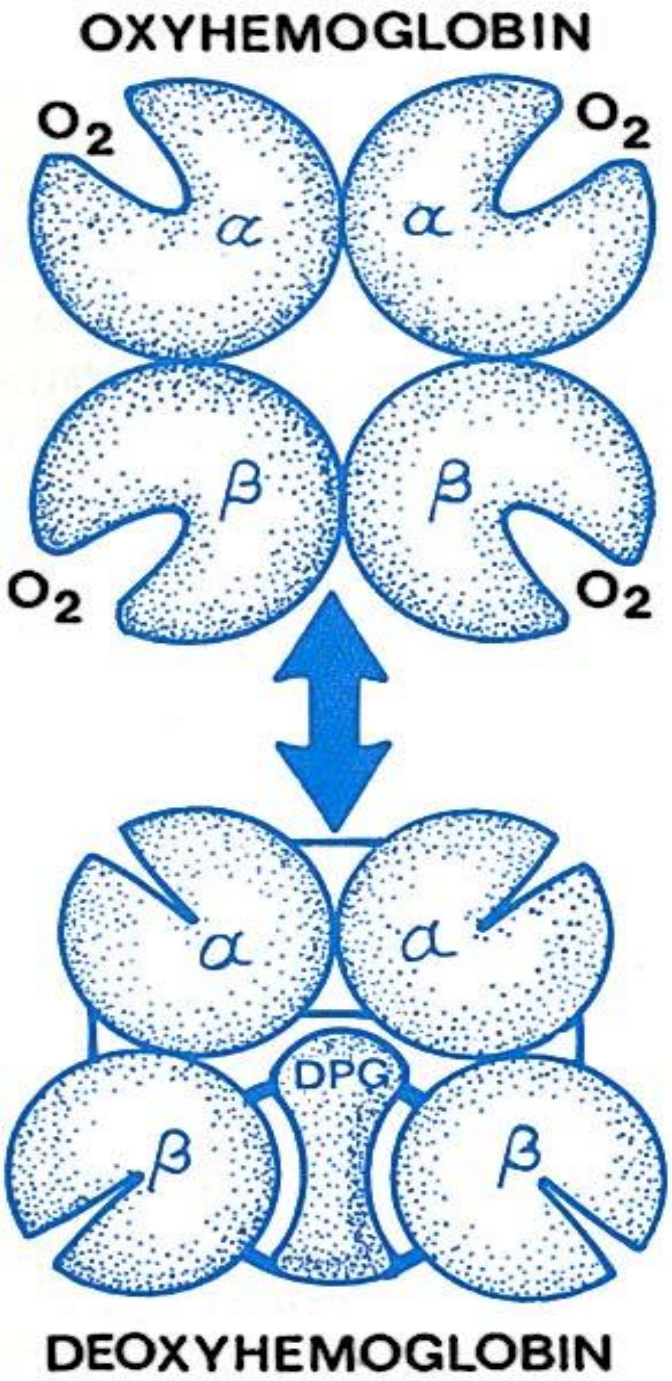
- **Further maturation,** hemoglobin synthesis continue and cytoplasm becomes entirely eosinophillic.
- **Late stages of maturation,** hemoglobin is abundant, few mitochondria and ribosomes are present, nucleus is small dense and well circumscribed.

ERYTHROKINETICS

- Number is constant normally as their life span is 120 days approximately.
- Differentiation and maturation from a basophillic erythroblast occurs in 5 to 7 days.
- 10-15% of erythroid precursors never mature and are destroyed.

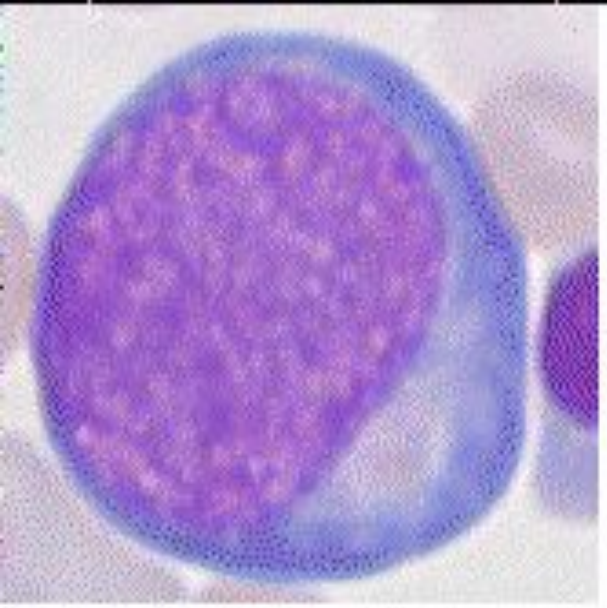
Hemoglobin

- Normal adult Hb A
  - 2 α globin chains
  - 2 β globin chains
  - Heme: porphyrin plus Fe^{++}
- Minor Hb's
  - $\alpha 2\delta 2$: Hb A_2
  - $\alpha 2\gamma 2$: Hb F

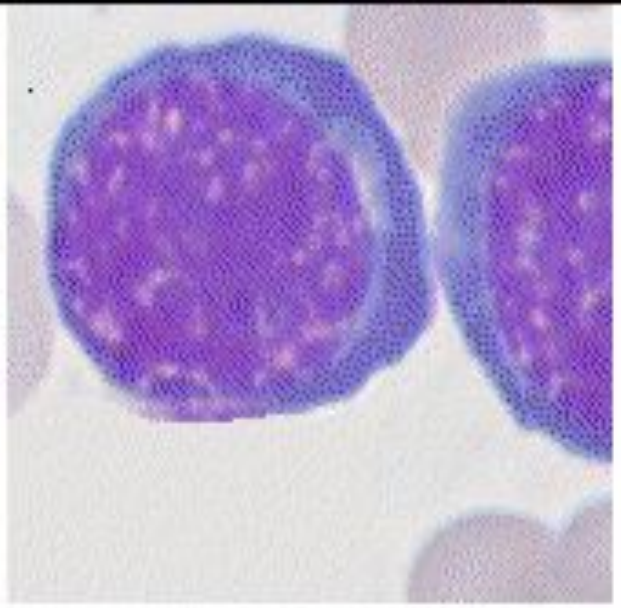


Kinds of Hemoglobin

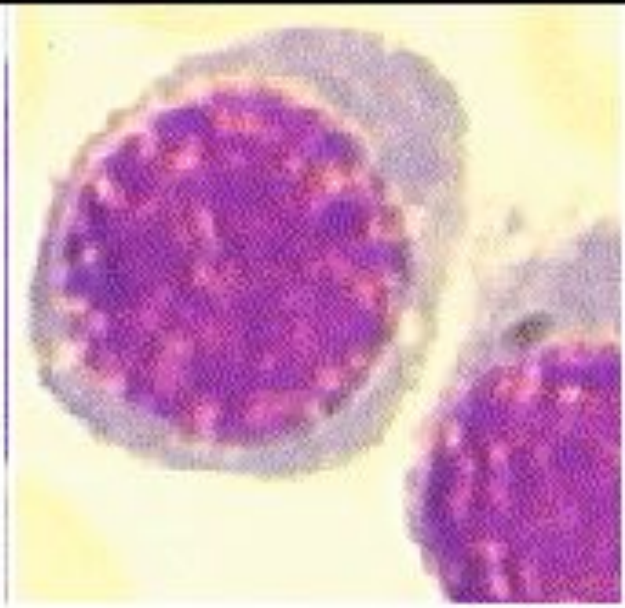
- Oxyhemoglobin: O_2
- Reduced (deoxy-) hemoglobin: no O_2
- Methemoglobin: oxidized
- Carboxyhemoglobin: 218 times affinity to CO than O_2
- Sulfhemoglobin: sulfa drugs



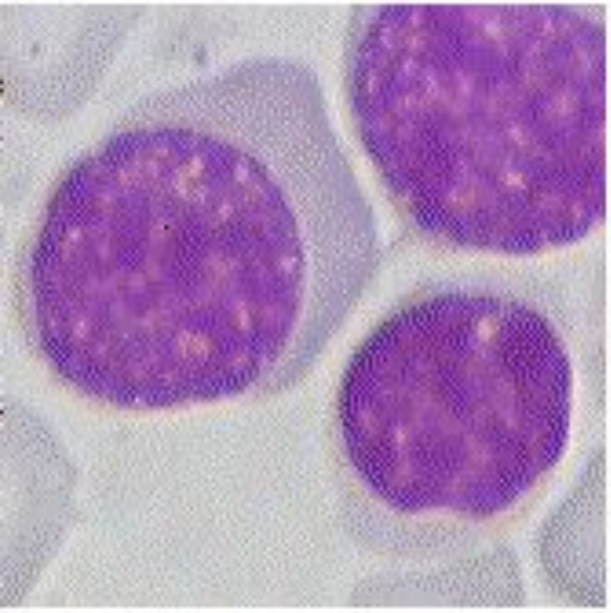
Proerythroblast



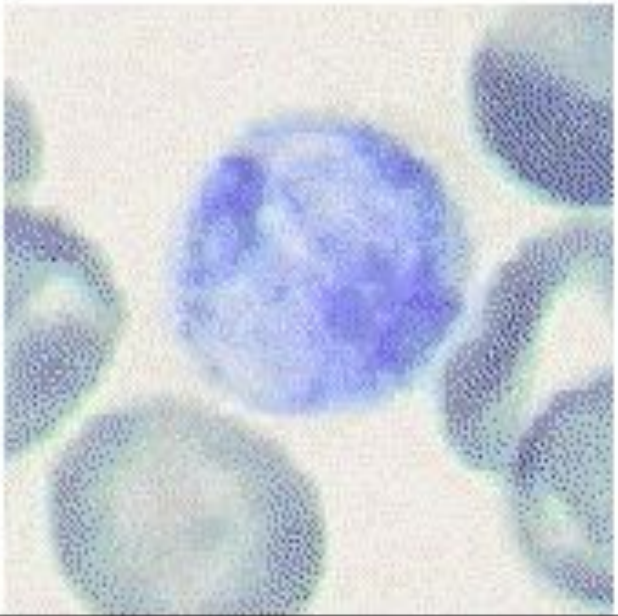
Basophilic Normoblast



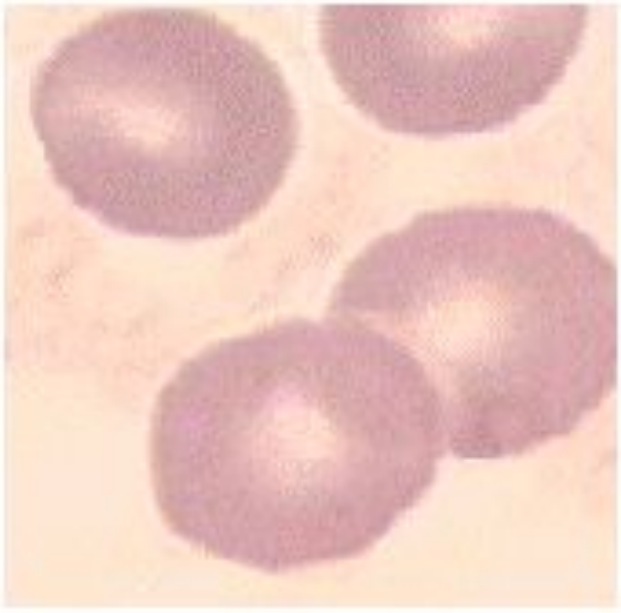
Polychromatic Normoblast



Orthochromatic Normoblast

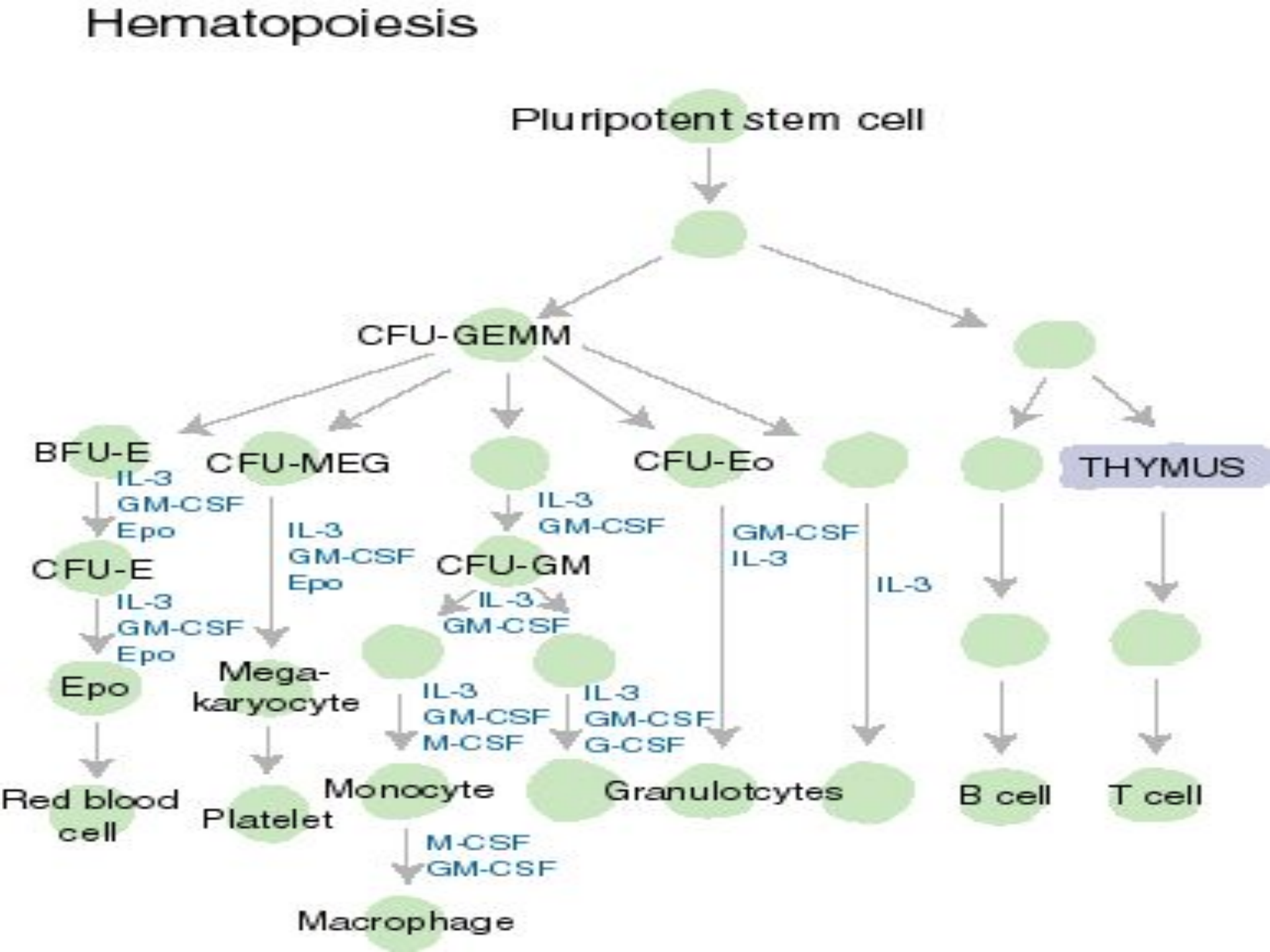


Reticulocyte



Erythrocyte

Hematopoiesis



GRANULOPOIESIS

- Committed myeloid stem cells differentiate into three types of cells, Neutrophils, Basophils and Eosinophils
- **FORMATION OF NEUTROPHILLS**
- **Myeloblast,** an early precursor cell, diameter 15-20um, lower nuclear cytoplasmic ratio, no cytoplasmic granules.

GRANULOPOIESIS

- **2.Promyeloctes,** is the next stage of maturation, similar in size and appearance to Myeloblast but has numerous azurophillic primary granules in cytoplasm, that contain variety of enzymes. (myeloperoxidase, acid phosphates, beta galactosidase, 5-nucleotidase)

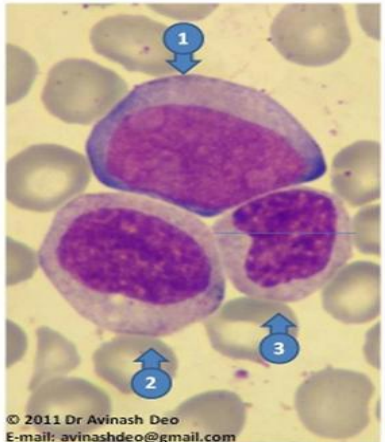
GRANULOPOIESIS

- 3.**Myelocyte**
- Secondary granules become apparent.
- Increased size and and smaller primary granules.
- Secondary granules have several bactericidal enzymes
- Nucleus becomes indented,

GRANULOPOIESIS

- **4.Metamyelocytes:** Next stage in myelopoiesis is a cell having more indented and smaller nucleus and having more granule
- **5.Mature neutrophils:** arise from stem cells in approx 10 days. Remain viable in systemic circulation for 8-12 hrs.

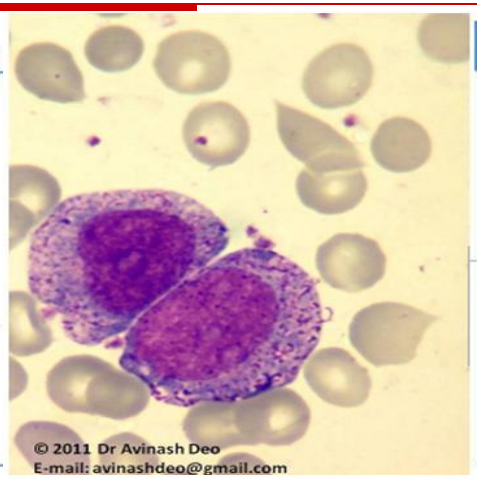
GRANULOPOESIS



Neutrophilic Myeloid Precursors

Morphological changes in the nucleus and cytoplasm accompanying maturation of myeloid cells are evident in this picture (details discussed in the text). The three cells are

1. **Myeloblast**: (~20μ) The nucleus has fine chromatin and the cytoplasm is blue without granules
2. **Myelocyte**: (~15μ) The nucleus is oval and eccentrically placed lacks nucleoli the chromatin is coarser than the myeloblast and the cytoplasm shows a pink secondary granules which first appear at this stage. A few primary granules can also be seen.
3. **Metamyelocyte**: (~12μ) The nucleus is indented and the chromatin coarser than the myelocyte.

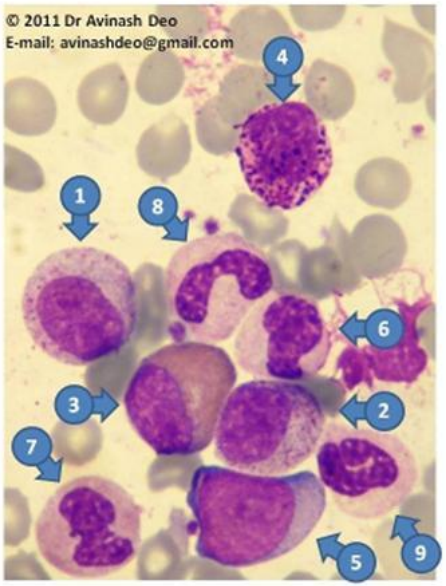


Promyelocytes

The cells are about 20μ with three features characterizing the promyelocyte stage

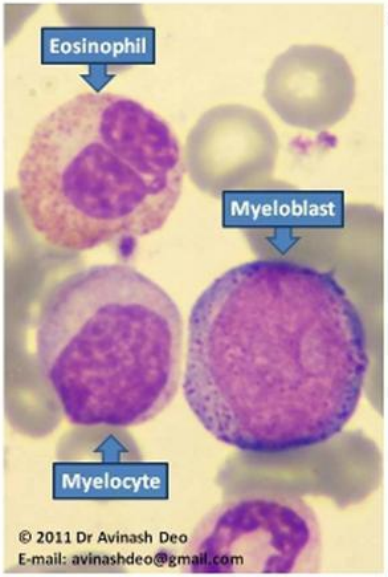
1. Nucleoli (pale staining areas in the nucleus)
2. Basophilic (blue) cytoplasm
3. Prominent Primary (reddish purple) granules.

The nucleoli are more prominent in the cell on the right which also has a finer chromatin pattern. The chromatin of the cell on the left is coarser and more clumped but unlike a myelocyte the nucleoli are still visible



The Myelocytes

Myelocytes are cells with a round to oval nucleus that may be flattened on one side. The nucleus lacks nucleoli. The cytoplasm shows secondary granules that are pink in neutrophilic myelocytes (cells 1 and 2), brick red in eosinophilic myelocyte (cell 3) and dark blue in basophilic myelocyte (cell 4). Morphological distinction between the three garnulocytic lines is first evident at the myelocyte stage. Electron microscopy can differentiate promyelocytes from the three series. The nucleus and cytoplasm of the myelocytes contrasts with that of the myeloblast (cell 5). Cells 6-9 are neutrophilic band forms band forms.



Myeloblast, Myelocyte and Eosinophil

The myeloblast has a large nucleus with fine chromatin and nucleoli. Features that differentiate the lymphoblasts and proerythroblast on include

1. **Lymphoblast** have a coarser chromatin, fewer nucleoli and the chromatin shows condensation along the nuclear membrane.
2. **Proerythroblasts** have a coarser chromatin a perinuclear halo.

It may be impossible to tell the lineage of very immature blasts. The fine chromatin of the myeloblast is a contrast from the clumped chromatin of the myelocyte. The blast has azurophilic primary granules. Secondary granules are seen in the myelocyte (pink) and eosinophil (brick red).

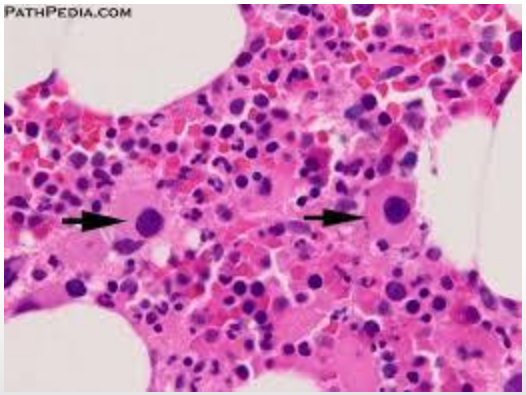
THROMBOPOIESIS

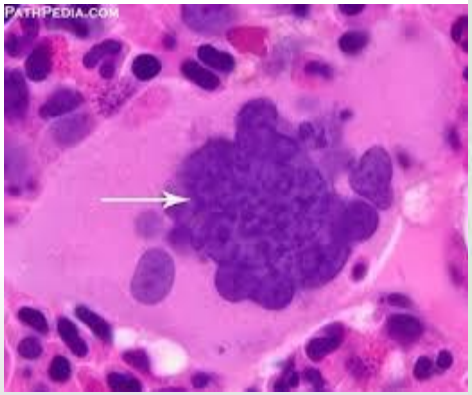
- Megakaryocytes differentiate from myeloid stem cell and are responsible for production of platelets.
- THREE STAGES OF MATURATION OF MEGAKARYOCYTES
- **Basophilic stage**, megakaryocyte is small, has diploid nucleus and abundant basophilic cytoplasm.

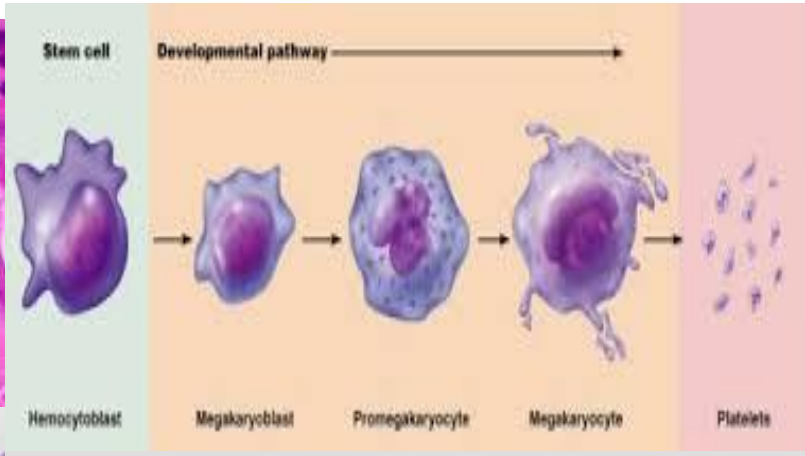
THROMBOPOIESIS

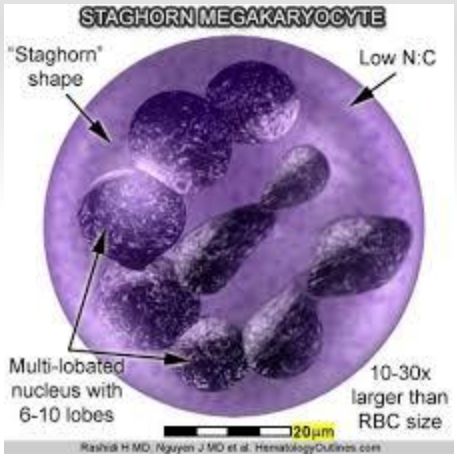
- **2.Granular stage,** here the nucleus is more polypoid, cytoplasm is more eosinophilic and granular
- **3.Mature stage**, megakaryocyte is very large, with approx 16-32 nuclei, abundance of granular cytoplasm. It undergoes shedding to form platelets.

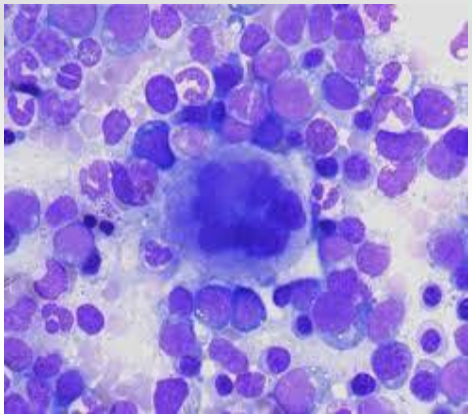
MEGACARYOCYTES











LYMPHOPOIESIS

- Lymphocytes are derived from committed stem cells that originate from pluripotent stem cell.
- Early lymphoid cells further differentiates into B & T lymphocytes.
- **B-LYMPHOCYTES** – main component of humoral immunity
- As they mature in specialized organ in birds called ***bursa of Fabricus***. They proliferate and mature into antibody forming cells.

LYMPHOPOIESIS - B

- Bone marrow or fetal liver are the organs in humans for development of B-lymphocytes from uncommitted lymphocytes.
- Maturation culminates in migration of B lymphocytes to other lymphoid organs and tissues throughout the body (e.g. spleen, gut, liver , tonsils, lymph nodes) where they meet antigens

LYMPHOPOIESIS

Plasma cells

- Normally found in Bone marrow, lymphoid organs few circulating in blood and lymph.
- Little capacity to undergo mitosis.
- Ultimate stage for synthesis and secretion of antibodies or immunoglobulins.
- Clones of plasma cells and B. cells can expand and contract under influence of many regulating factors.

LYMPHOPOIESIS

T.LYMPHOCYTES.

- Depends on **thymus** for their maturation and specialized functions.
- 60-70% of circulating lymphocytes are able to cycle from blood, through lymphoid tissue and then back to blood via lymphatics.

LYMPHOPOIESIS

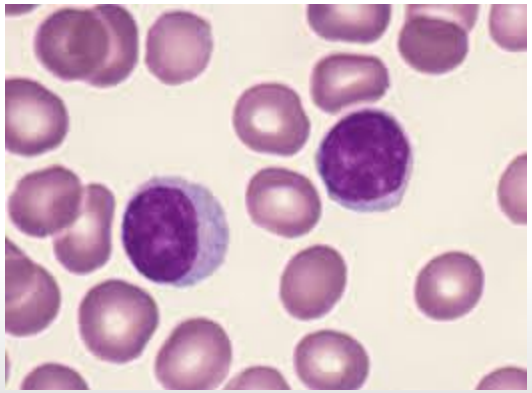
T.LYMPHOCYTES

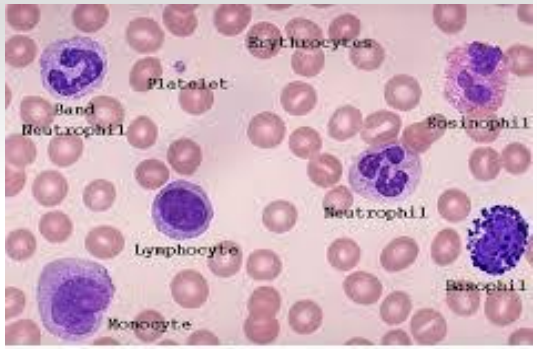
- Secrete cytokines (LYMPHOKINES).
- Regulate proliferation and differentiation of other T.cells, B.cells and macrophages.
- Main component of cell mediated immunity.

LYMPHOPOIESIS - T

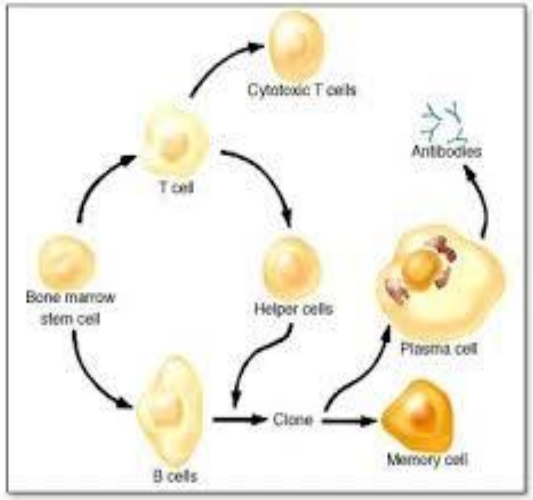
- Differentiation and maturation of uncommitted lymhocytes take place in thymus, these Thymocytes loose their antigenic surface molecules and finally mature into **helper/ effector T lymphocytes** and **suppressor T lymphocytes.**
- The helper and suppressor cells can be differentiated by presence of specific cell membrane molecules and receptors – CD4, CD8

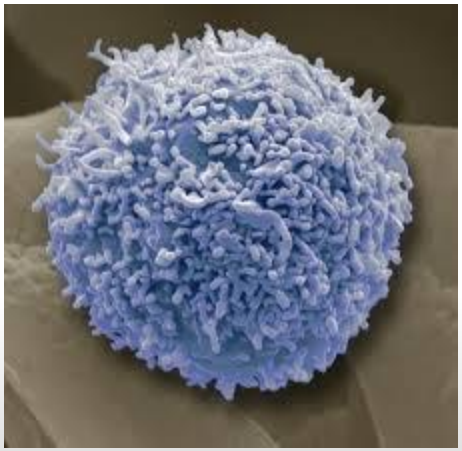
LYMPHOCYTES

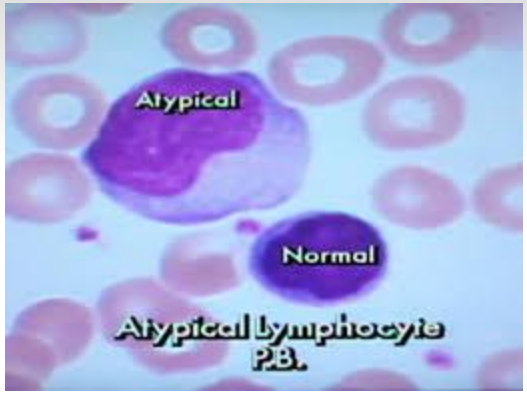




Wright-stained smear of normal blood (x1000) The RBC's are biconcave discs stained buff-pink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.







HEMATOPOIETIC GROWTH FACTORS

- Heterogeneous group of cytokines that stimulate the progenitor cells and induce proliferation, differentiation and maturation
- Glycoproteins synthesized by variety of cells in marrow – stroma, endothel, macrophages
- They bind to specific receptors on the surface of various cells of the hematopoietic system

HEMATOPOIETIC GROWTH FACTORS

- Naturally occurring hormones.
- Low molecular weight glycoprotiens.
- Variable degrees of species specificity.
- Available in purified form by recombinant DNA technology.
- Responsible for stimulation and release of other growth factors and cytokines.

Hematopoietic Growth Factors

ERYHTROPOIETIN

- Synthesized by peritubular cells of kidney in response to hypoxemia
- Present in minute amounts in urine
- Liver secretes 10% of endogenous erythropoietin.
- Half life of 6-9 hrs

Hematopoietic Growth Factors

Thrombopoietin

- A glycoprotein hormone produced mainly by liver and kidney that regulates the production of platelets in bone marrow.
- Stimulates the production and differentiation of Megakaryocytes

Hematopoietic Growth Factors

- **3. GM-CSF:**
- Produced by fibroblasts, stromal cells, T.lymphocytes and endothelial cells.
- Stimulate progenitors for granulocytes, monocytes and erythrocytes
- **4. G-CSF:**
- LMW glycoprotein
- Stimulates proliferation and maturation of granulocyte precursors.
- Produced by stromal cells, monocytes, macrophages, and endothelial cells.

Hematopoietic Growth Factors

❖5. M-CSF

- ❖ Secreted by stromal cells, macrophages and fibroblasts.
- ❖ Heavily glycosylated glycoprotein
- ❖ Potent stimulator of macrophage function and activation as it increases the expression of MHC class II antigens on macrophages.

Thank you