LIFE CYCLE OF ERYTHROCYTES

Hematopoietic System and Disorders (MED202)

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Lecture outline

- Erythrocyte death and phagocytosis
- Recycling of erythrocyte components
  - Break down of Hb, Globin, and Heme
  - Transport, storage and absorbtion of Iron
- Stimulation of red blood cell production by EPO
- Stages of erythrocyte maturation
- Factors necessary for erythropoiesis
  - Vitamin B12, B6
  - Folic acid
  - Iron

Erythrocyte count

The number of erythrocytes per microliter of blood.

Normal ranges:

Male	4.7-6.1 million/µL
Female	4.2-5.4 million/µL
New born	6-7 million/µL

Morhology of Erythrocytes

- **Shape:** Biconcave disc
- **Diameter:** 7 – 8 um
- **Thickness:** Thickest → 2,5 um
  Center → 1 um
- **Volume:** 78-94 $\mu m^{3:}$ normocyte
  < microcyte
  > macrocyte
- **Surface area:** 136 μm^2
- **Enucleated:** No nucleus

Erythrocytes

- ✓ Durability of erthrocytes is remarkable
- ✓ No nucleus to direct regenerative processes
- ✓ No mitochondria available for efficient oxidative metabolism
- ✓ No ribosomes for regeneration of lost or damaged proteins
- ✓ No devo synthesis of lipids
- ✓ **Life span:** 120 days

Genesis of Red Blood Cells: **Erythropoiesis**

- Erythroprotein (EPO) supports erythropoiesis or red cell development
- EPO is not absolutely required for early commitment of progenitor cells to the erythroid lineage; it is essential for the differentiation of burst-forming unit–erythroid cells (BFU-Es) to colony-forming unit–erythroid cells (CFU-Es) or **proerythroblasts** (also known as pronormoblasts), which still lack hemoglobin.
- The further maturation of cells downstream of proerythroblasts does not require EPO.

Genesis of Red Blood Cells: **Erythropoiesis**

- The frst cell that can be identifed as belonging to the RBC series is the **proerythroblast**
- Once the proerythroblast has been formed, it divides multiple times, eventually forming many mature RBCs.
- The first generation cells are called ***basophil erythroblasts*** because they stain with basic dyes
- The cell at this time has accumulated very little hemoglobin.
- In the succeeding generations, the cells become filled with hemoglobin to a concentration of about 34 %
- The nucleus condenses to a small size, and its final remnant is absorbed or extruded from the cell.
- At the same time, the endoplasmic reticulum is also eliminated. Cell membraine is reorganized. The cell at this stage is called a ***reticulocyte***

Genesis of Red Blood Cells: **Erythropoiesis**

- During this reticulocyte stage, the cells pass from the bone marrow into the blood capillaries by ***diapedesis*** (squeezing through the pores of the capillary membrane).
- The remaining basophilic material in the reticulocyte normally disappears within 1 to 2 days, and the cell is then a ***mature erythrocyte.***

Enucleation and reticulocyte formation: Role of makrophages in erythropoiesis

- Erythropoiesis in the bone marrow occurs in structures termed **macrophage islands**
  - a central macrophage
  - erythroid cells at different maturation stages
  - reticulocytes.
- In order to facilitate the interaction with erythroblasts, macrophage expresses various adhesion molecules such as VCAM1, ICAM4, CD169, CD163.
- In the erythroid niche, central macrophages play a nursing role for developing erythroblasts.

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Enucleation and reticulocyte formation: Role of macrophages in erythropoiesis

Macrophage facilitates the **enucleation** process by phagocytosing and degrading of extruded nuclei

Enucleation of erythroblasts is a complex process which includes

- condensation of chromatin,
- deacetylation of histones,
- formation of CAR (contractile actin ring) and further abscission

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Enucleation and reticulocyte formation: Role of macrophages in erythropoiesis

- ✓ Phagocyted nucleus is degraded by DNAse-II
- ✓ After expelling its nucleus, the reticulocyte maturation continues, losing 20–30% of the cell surface and eliminating any remaining membrane-bound cytosolic organelles through an autophagy/exosome-combined pathway .
- ✓ Some soluble factors secreted by erythroblasts (eg. VEGF-A, PGF) modify endothelial attachment sites to regulate entering of reticulocytes into the circulation.

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Maturation of reticulocytes in the circulation

- ✓ To adopt a bi-concave morphology, stability, and the ability to deform these cells have to undergo a maturation process.
- ✓ Characterized by progressive loss of RNA and membrane proteins (eg. CD71 or CD49d).
- ✓ Membrane-cytoskeleton rearrangement may be an important step allowing the transition from an unstructured reticulocyte to a morphologically biconcave and functional erythrocyte
- ✓ There are three major events occurring in this time window:
  1) **Volume control,**
  2) **Membrane remodeling,**
  3) **Vesicularization**

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Maturation of Red Blood Cells Requires **Vitamin B_{12}** (Cyanocobalamin) and **Folic Acid**

- ✓ Erythrocyte maturation and rate of production are affected greatly by a person's nutritional status.
- ✓ Especially important for final maturation of the RBCs are two vitamins, ***vitamin B_{12}*** and ***folic acid***.
- ✓ Essential for **DNA synthesis**, reguired for **Thymidine triphosphate synthesis**
- ✓ Lack of either vitamin B_{12} or folic acid causes abnormal and diminished DNA and, consequently, **failure of nuclear maturation and cell division**.
- ✓ **Megaloblasts and immature RBCs** are formed

Maturation of Red Blood Cells Requires **Vitamin B_{12}** (Cyanocobalamin) and **Folic Acid**

- ✓ The erythroblastic cells of the bone marrow, produce mainly larger than normal RBCs called *macrocytes*
- ✓ These cells have a flimsy membrane and is often irregular, large, and oval instead of the usual biconcave disk.
- ✓ Are capable of carrying oxygen normally, but their fragility causes them to have a short life: ***Maturation failure***

A common cause of RBC maturation failure is **failure to absorb vitamin B_{12} from the gastrointestinal tract**.

This situation often occurs in the disease *pernicious anemia,* in which the basic abnormality is an *atrophic gastric mucosa* that fails to produce normal gastric secretions.

The parietal cells of the gastric glands secrete a glycoprotein called ***intrinsic factor,*** which combines with vitamin B_{12} in food and makes the B_{12} available for absorption by the gut.

Absorbtion of vitamin B_{12}: **Intrinsic factor**

- Glycoprotein, MW: 4500 Da
- Secreted by the parietal cell of the stomach
- Vitamin B_{12} combine with IF forming a complex that resist digestion by GIT enzymes.
- Still in the bound state, intrinsic factor binds to specifc receptor sites on the brush border membranes of the mucosal cells in the ileum
- This complex is absorbed at terminal ileum by pinocytosis.
- Vitamin B_{12} is transported to the liver where it is stored.

- Recommended Dietary Intake
  - 0-6 Months: 0.4mcg
  - 7-12 Months: 0.5mcg
  - 1-3 Years: 0.9mcg
  - 4-8 Years: 1.2mcg
  - 9-13 Years: 1.8mcg
  - **Adults: 2.4mcg**
  - Pregnancy: 2.6mcg
  - Lactation: 2.8mcg

Death of erythrocytes: **Eryptosis**

- After about 100-120 days, RBCs are removed from circulation through a process called eryptosis.
- Some changes occur in the plasma membrane of the aging erythrocyte.
- This makes the cell more susceptible to selective recognition by macrophages.
- In this way, the aging and wearing cells are removed in spleen, liver and lymph nodes.
- It occurs at the same rate as erythropoiesis: keeping the total number of erythrocytes in circulation in balance.

- Once the RBC membrane becomes fragile, the cell ruptures during passage through some tight spot of the circulation.
- Many of the RBCs self-destruct in the spleen, where they squeeze through the red pulp of the spleen.

- When RBCs burst and release their hemoglobin, the hemoglobin is phagocytized almost immediately by macrophages in many parts of the body,
  - Kupffer cells of the liver
  - Macrophages of the spleen and bone marrow.
- During the next few hours to days, the macrophages **release iron** from the hemoglobin
- **Porphyrin portion** of the hemoglobin molecule is converted by the macrophages, through a series of stages, into the bile pigment ***bilirubin***
- **Globin** part of the Hb is also degraded into amino acids which then used for protein synthesis

The main element required for erythropoiesis is the **Iron**.

- The total quantity of iron in the body averages 4 to 5 grams,
  - 65 % is in the form of hemoglobin
  - 4 % is in the form of myoglobin
  - 1% is in the form of the various heme compounds
  - 0.1% is in the blood plasma combined with transferrin
  - 15-30% is stored in the form of ferritin: reticuloendothelial system and liver parenchymal cells
- The amount of iron lost per day by urine, feces and bleeding:
  - Male: 0.9 mg / day
  - Women: 1.7 mg / day
- Due to low absorption from the intestines, it is necessary to take 5-20 mg of iron daily.
- Fe^{2+} and Fe^{3+}

The main element required for erythropoiesis is the **Iron**.

- When iron is absorbed from the small intestine, it immediately combines in the blood plasma with a beta globulin, *apotransferrin,* to form ***transferrin*** which is then transported in the plasma.
- The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body.
- Excess iron in the blood is deposited especially in the **liver hepatocytes** and less in the **reticuloendothelial cells of the bone marrow**.
- In the cell cytoplasm, iron combines mainly with a protein, *apoferritin,* to form ***ferritin****. STORAGE IRON*
- When the quantity of iron in the plasma falls low, some of the iron in the ferritin storage pool is removed easily and transported in the form of transferrin in the plasma to the areas of the body where it is needed.