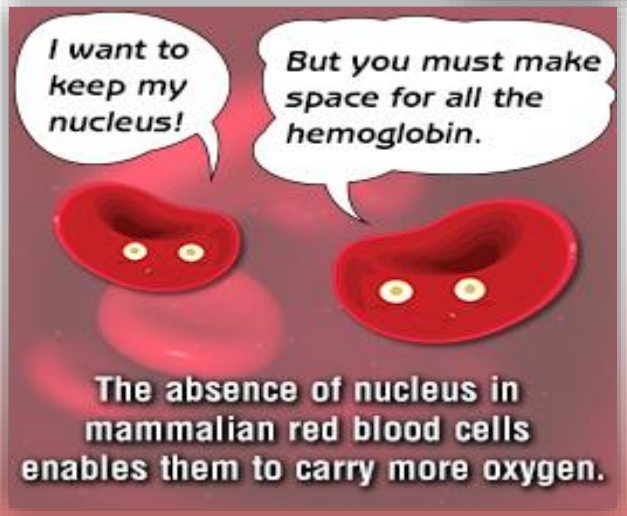
RBC

SLO

- At the end of the class students should be able to
- Define erythropoiesis
- Describe the steps of erythropoiesis
- Describe the regulation of it
- Discuss the various blood indices

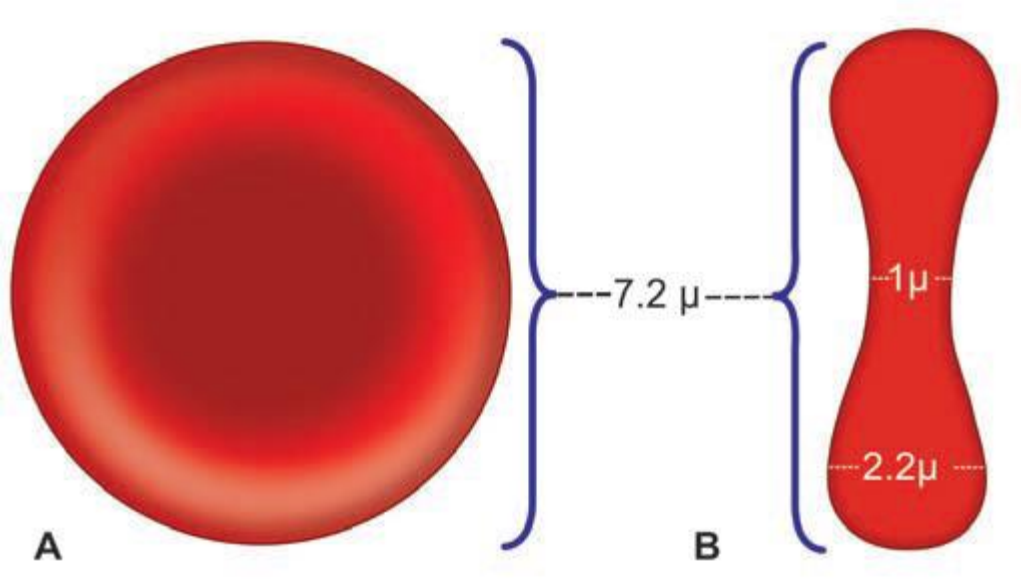
GENERAL FEATURES

- Dimensions
- Normal Count
- Composition
- Functions
- Fragility
- Variations
- Fate & Lifespan

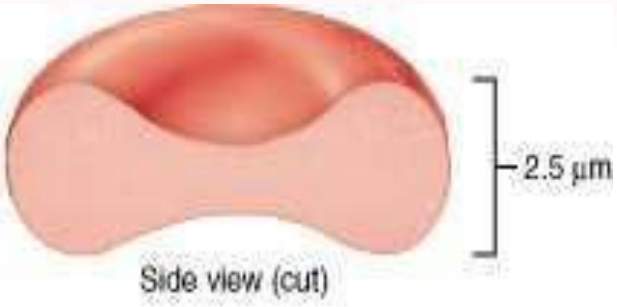


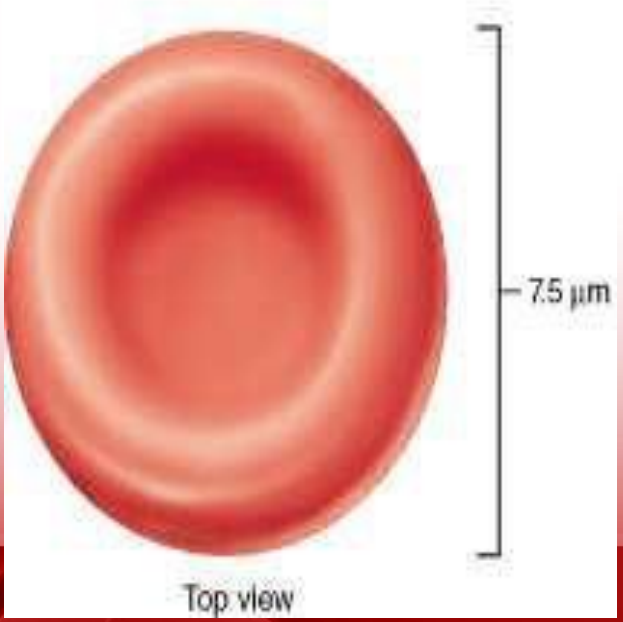
DIMENSIONS

- Shape: Biconcave
- Size: 7.2um in diameter
- Thickness: 2um at the periphery and 1um at the center
- Volume: 87um^3



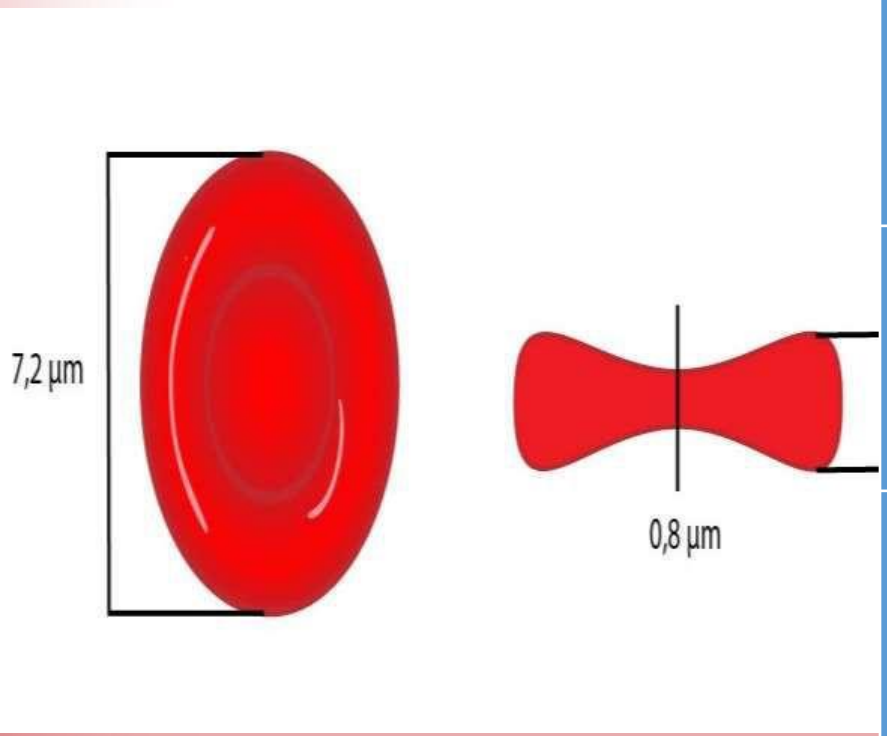
ERYTHROCYTES





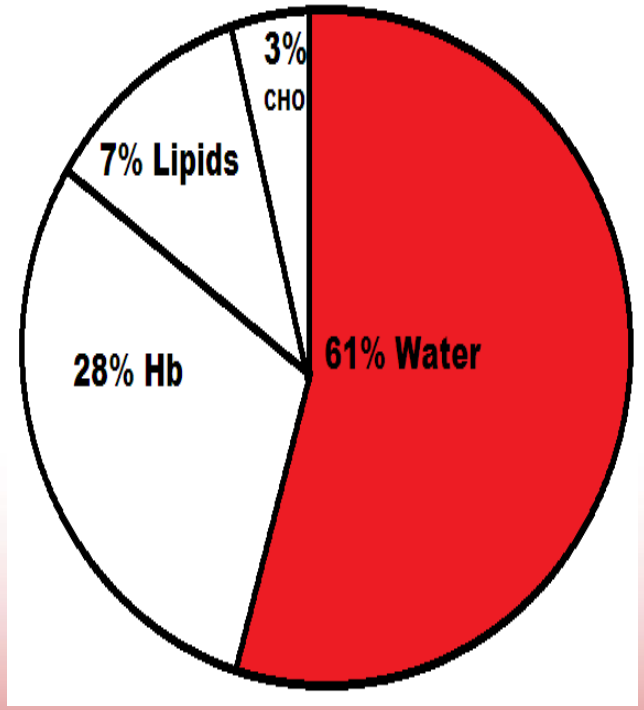
- ✓ Surface area is 120µ2.
- ✓ Normal volume is 80-94 µ3.
- ✓ Normal life span of an RBC is 120 days.

ERYTHROCYTES



Normal Erythrocyte Count	
Males	5-5.5 million cells/mm3
Females	4.5-5 million cells/mm3
Infants	6 – 7 million cells/mm3

STRUCTURE OF RBC



- **Lipids:** Cholesterol, phospholipid, and glycolipids
- **Proteins:** Spectrin, actin, ankyrin.
- **The glycolipids:** constitute the ABO blood group substances (agglutinogens).

Metabolism

It is met by the glucose metabolism through the anaerobic ***Embden- Meyerhof (EMF) pathway*** (90%) and the ***pentose phosphate shunt*** (10%).

Advantages of Biconcave Shape of RBCs:

- Greater surface area for exchange of gases.
- Flexibilty of RBC
- Minimal tension when the volume of cell alters.

How is the shape maintained?

- **Spectrin**

  - a contractile protein

  - maintains shape and flexibility of RBC

  - Antigen on cell membrane – helps in blood group classification

COMPOSITION

- 62.5% water
- 35% Hemoglobin
- 2.5% :

  - Sugar – glucose

  - Lipids – Cephalin, Cholesterol & Lecithin

  - Protein – Glutathion : insoluble protein which acts as a reducing agent and prevents damage of hemoglobin

  - Enzymes – Carbonic anhydrase and catalase

  - Ions – Na^{+}, K^{+}, Ca^{2+}, PO_4^{3-}

FUNCTIONS

- Respiratory
- Acid Base balance
- Maintain viscosity
- Pigment: various pigments are derived from hemoglobin after disintegration of RBC.

FRAGILITY AND HEMOLYSIS

- *Hemolysis*- Breakdown of RBC and liberation of hemoglobin.
- *Fragility*- Susceptibility of RBC to hemolysis or tendency to break easily.
- There are 2 types:

  1. Osmotic fragility- due to exposure to hypotonic saline.

  2. Mechanical fragility- due to mechanical trauma

VARIATIONS

Physiologic causes of increase count:

- Age
- Gender
- High altitude
- Exercise
- Temperature
- Meal

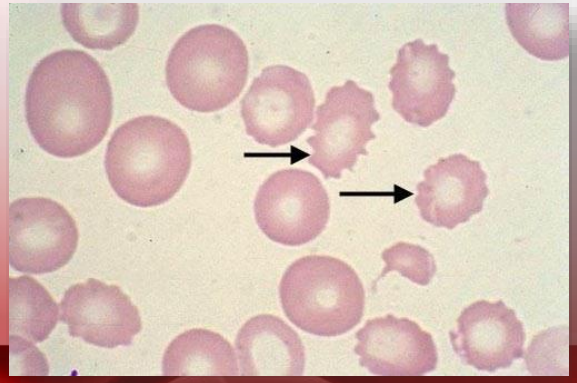
Decrease in count: High barometric pressure, Pregnancy, sleep

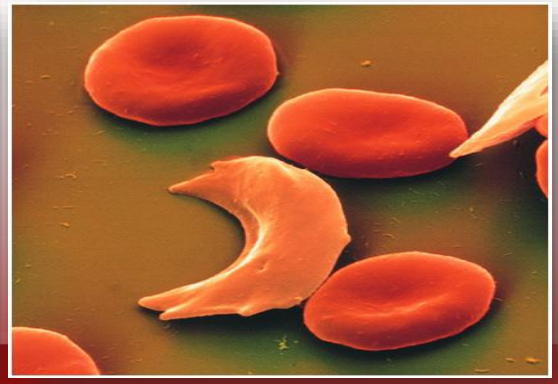
Pathological variations:

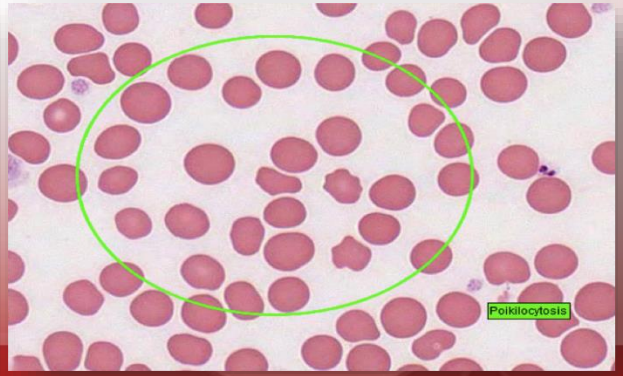
- Increase: Polycythemia
- Decrease: Anaemia

VARIATIONS IN SHAPE

- Crenation: Shrinkage as in hypertonic solutions.
- Spherocytosis: Globular form as in hypotonic conditions.
- Elliptocytosis: elliptical shape
- Sickle cell: Crescent shape
- Poikilocytosis: Flask, hammer or any other unusual shape.







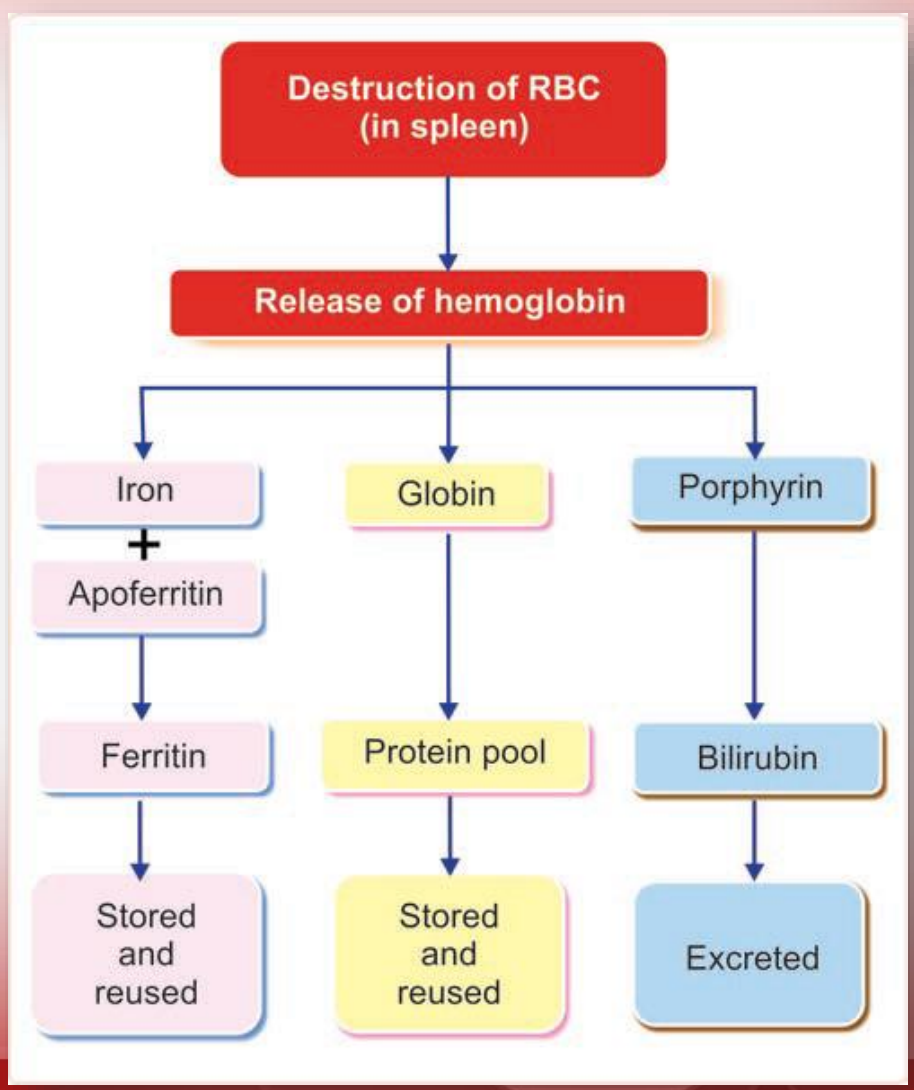
VARIATIONS IN SIZE

- Physiological conditions: RBC in venous blood slightly larger than those in arterial blood.
- Pathological conditions:
  1. Microcytes – smaller cells
  2. Macrocytes – larger cells
  3. Anisocytes – cells of different sizes

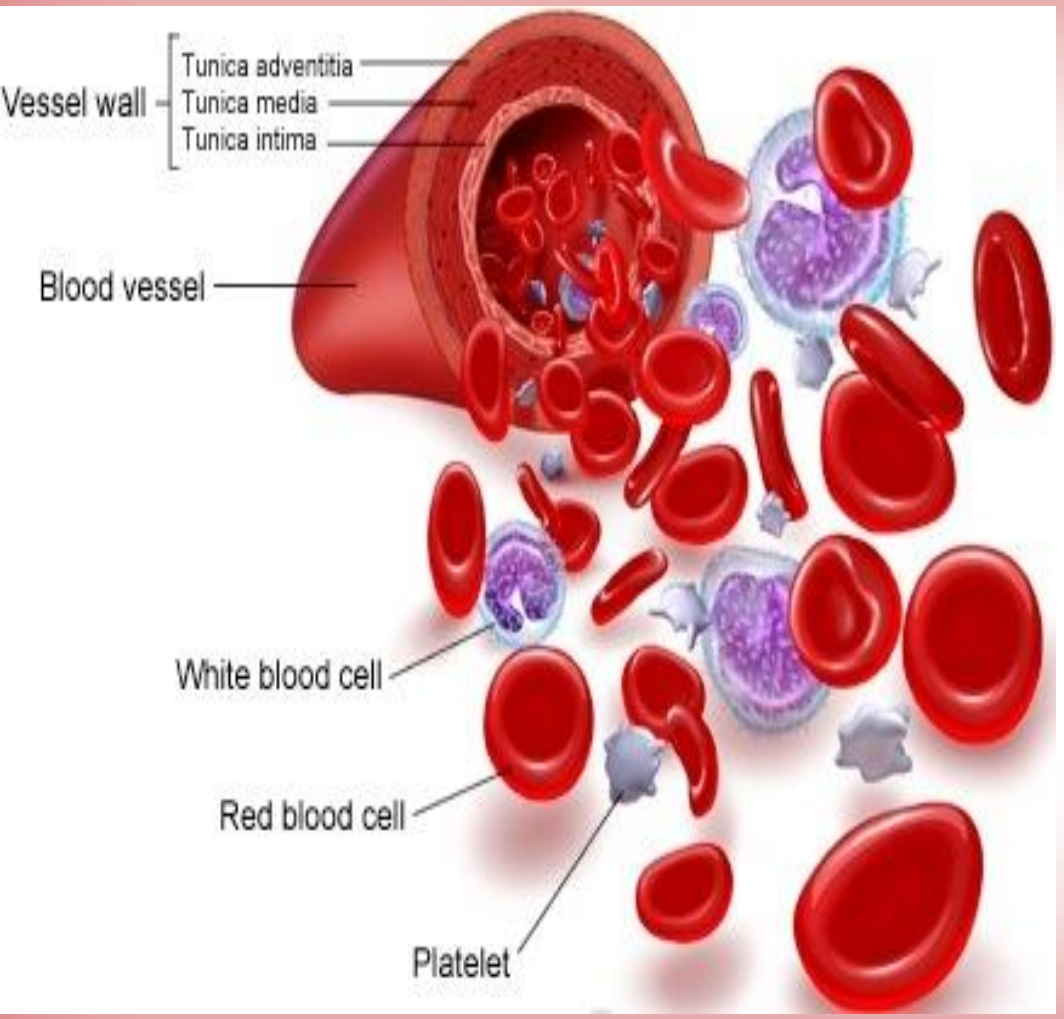
LIFESPAN AND FATE OF RBC

- Lifespan – 120 days
- Site of destruction:

  Reticuloendothelial system

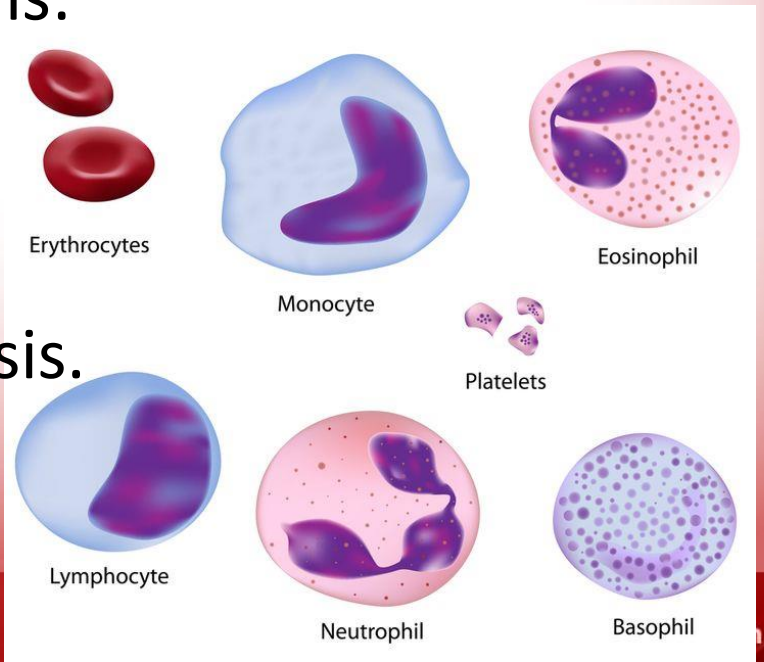


ERYTHROPOIESIS



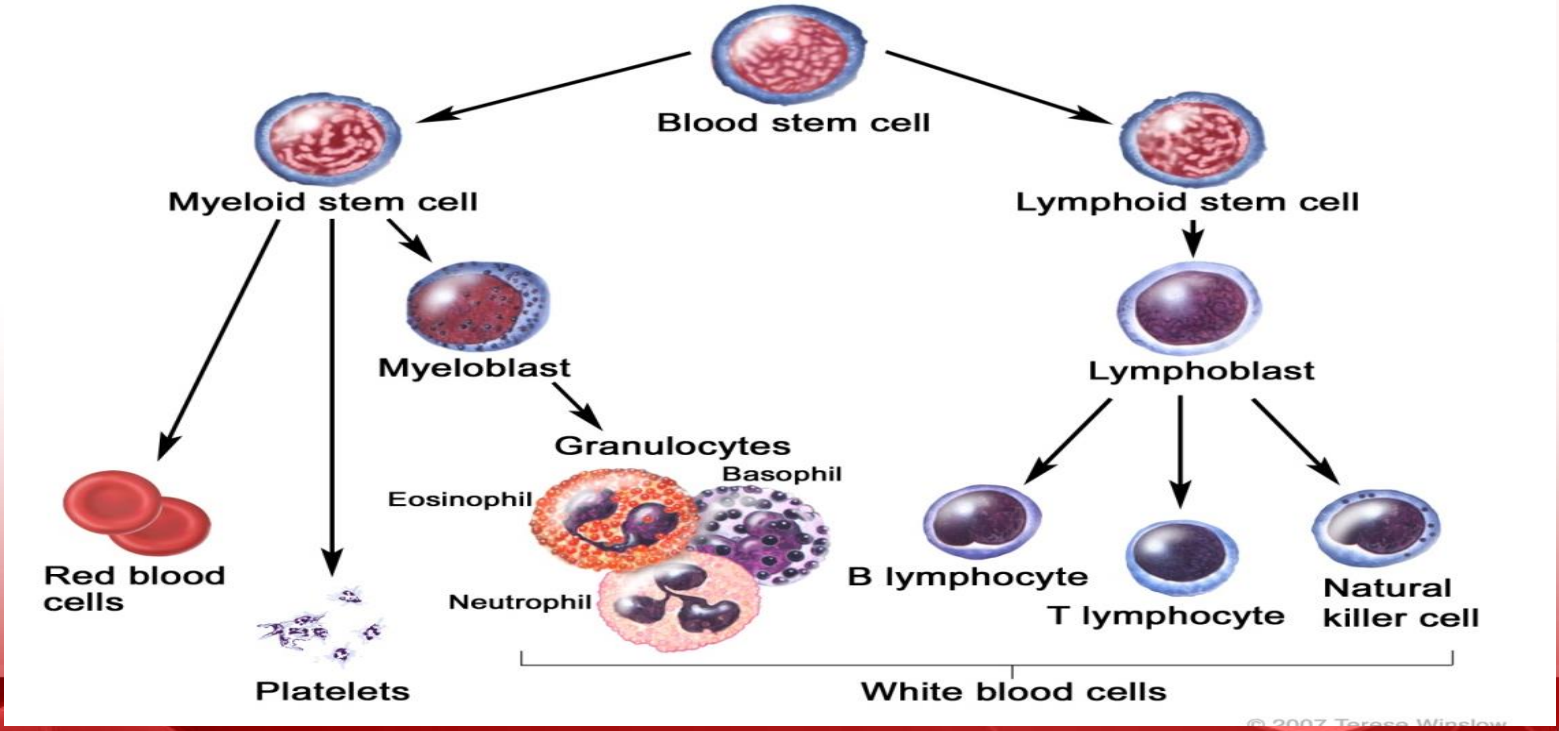
Hemopoiesis

- Hemo: Referring to blood cells
- Poiesis: “The development or production of”
- The word Hemopoiesis refers to the production & development of all the blood cells:
  - Erythrocytes: **Erythropoiesis**
  - Leucocytes: Leucopoiesis
  - Thrombocytes: Thrombopoiesis.



DEFINITION

It is the process of **development**, **differentiation** and **maturation** of RBCs from primitive stem cells



Theories of erythropoiesis

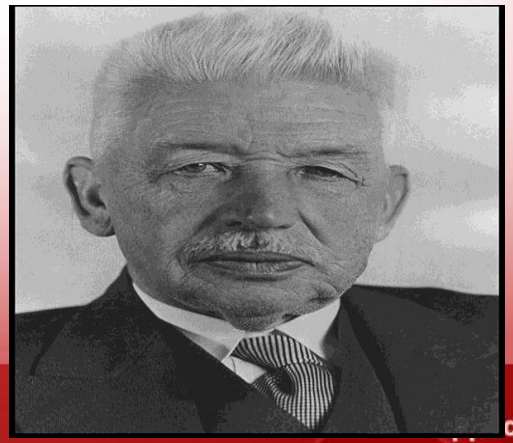
- **Monophyletic theory**

- Also known as **unitary theory.**
- There is a common parent cell of all formed elements of blood.

Alexander A. Maximow

- **Polyphyletic theory**

- Also known as trialistic theory
- Suggests different group of stem cells gives rise to different blood cells.



L. Aschoff

Intravascular erythropoiesis

Site of Erythropoiesis

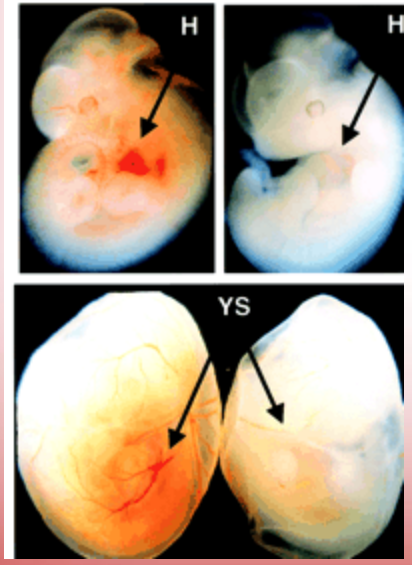
Extravascular erythropoiesis

- **During intrauterine life**

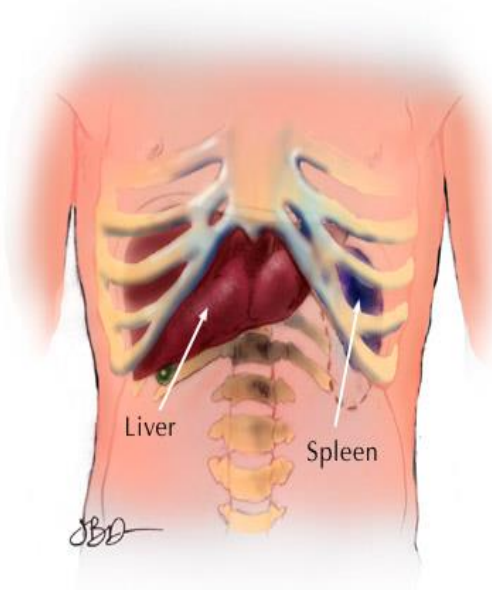
  Mesoblastic stage (3rd week to 3 months)

  Hepatic stage (after 3 months)

  Myeloid stage (3rd trimester)

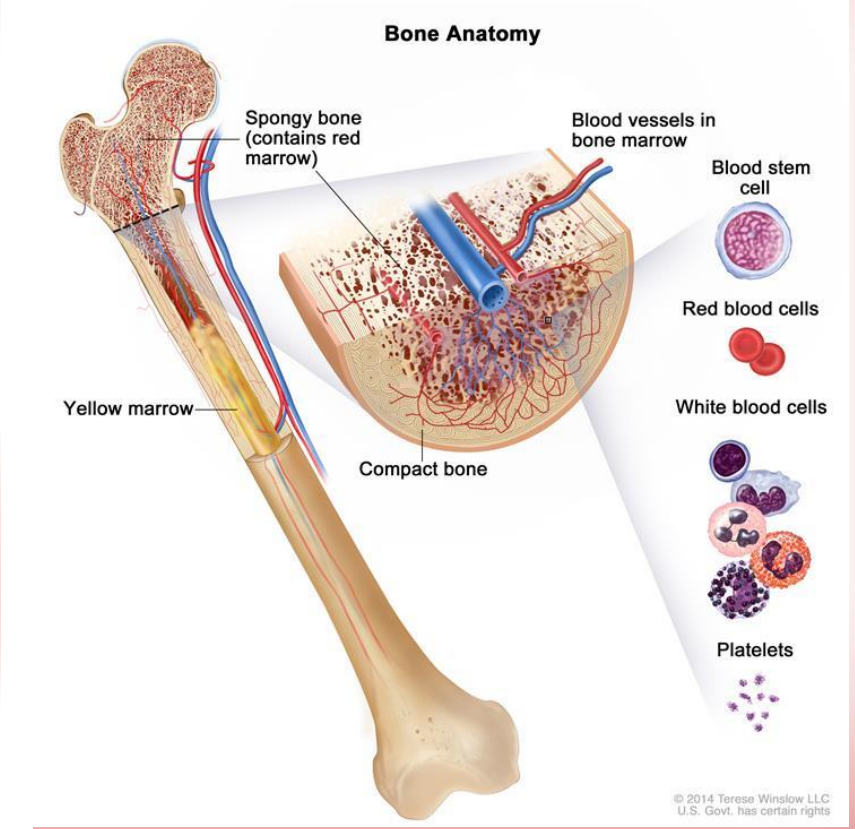


Yolk sac



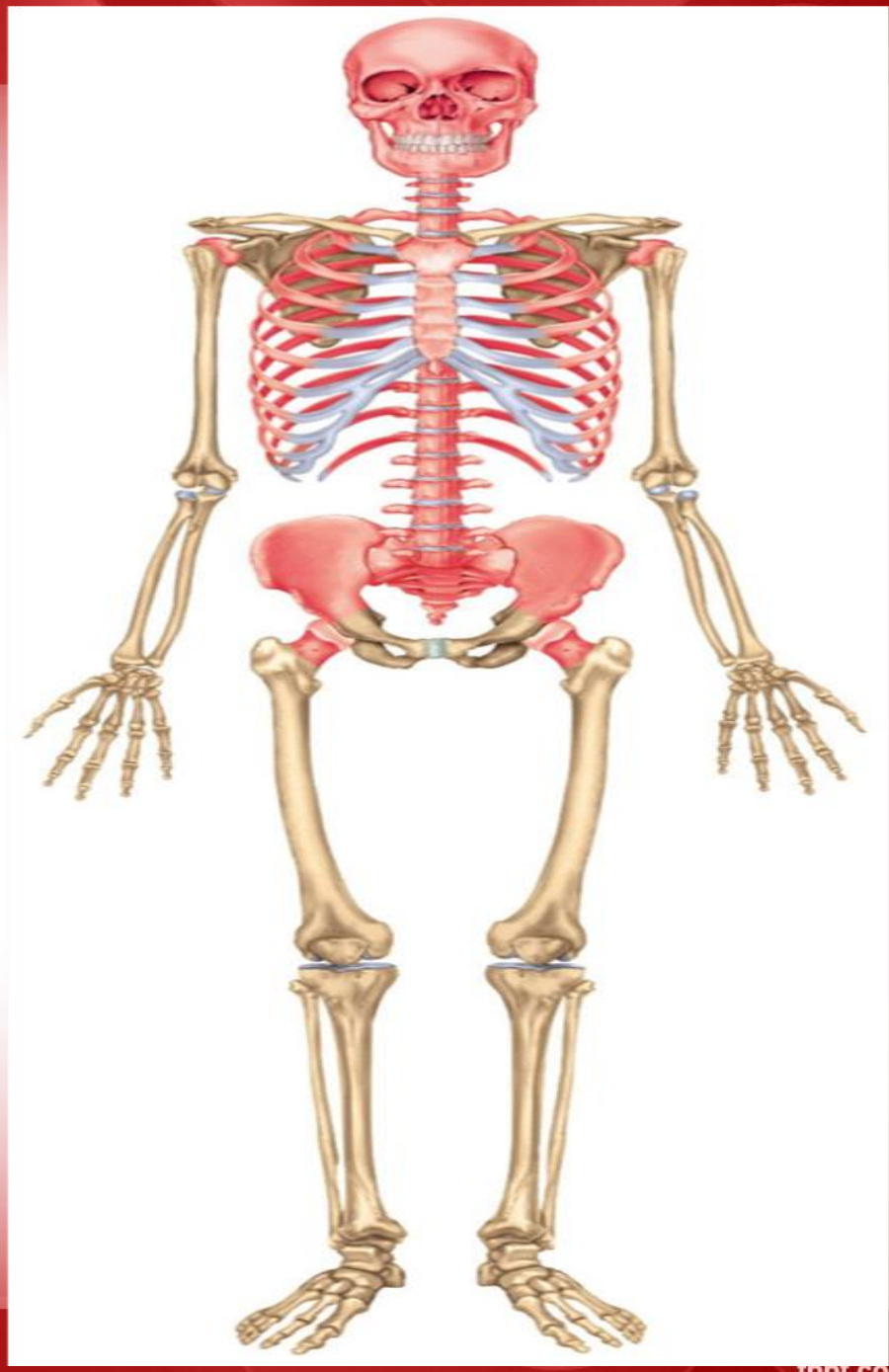
Liver & spleen

Nucleated RBCs

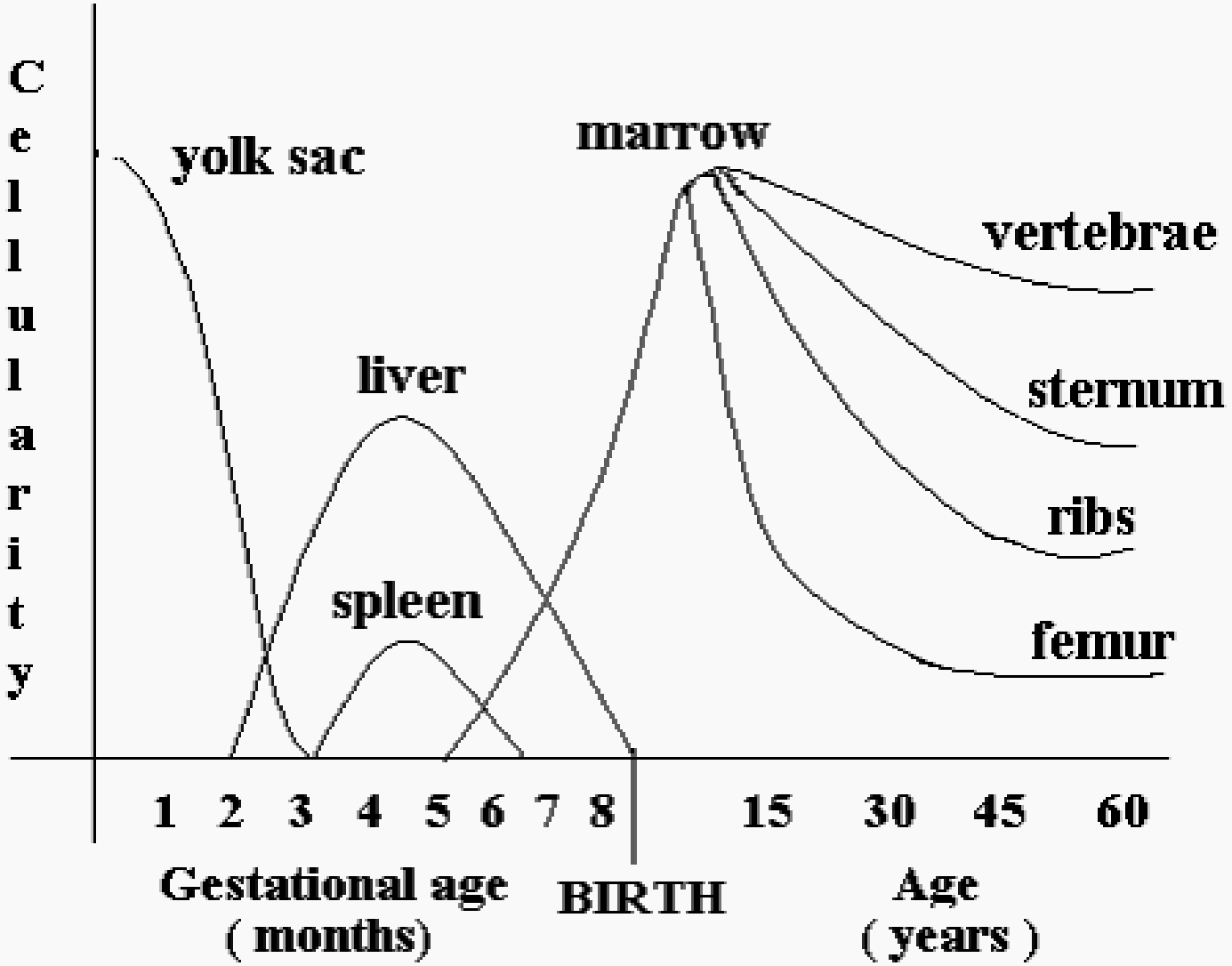


Bone marrow

- **In children**
- All bones with red bone marrow
- Liver & spleen
- **In adults (after 20yrs)**
- Ends of long bones like femur, humerus
- Skull
- Vertbrae
- Ribs
- Sternum
- pelvis



SITES OF ERYTHROPOIESIS



ERYTHROPOIESIS

PHSC Pluripotent Hemopoietic stem cell

IL-1,IL-6,IL-3 → ← GM CSF erythro

BFU-E (Burst Forming Unit Erythrocyte)

↓

CFU-E (Colony Forming Unit Erythrocyte)

↓ ← GM CSF erythro

PROERYTHROBLAST

↓

BASOPHILIC ERYTHROBLAST

↓

POLYCHROMATOPHILIC ERYTHROBLAST

↓

ORTHOCHROMATIC ERYTHROBLAST

↓

RETICULOCYTE

↓

ERYTHROCYTE

ERYTHROPOIESIS

PHSC Pluripotent Hemopoietic stem cell

IL-1,IL-6,IL-3 → ← GM CSF erythro

BFU-E (Burst Forming Unit Erythrocyte)

↓

CFU-E (Colony Forming Unit Erythrocyte)

↓ ← GM CSF erythro

PROERYTHROBLAST

↓

EARLY NORMOBLAST

↓

INTERMEDIATE NORMOBLAST

↓

LATE NORMOBLAST

↓

RETICULOCYTE

↓

ERYTHROCYTE

Hematopoietic stem cells (HSCs) are bone marrow cells that are capable of producing all types of blood cells.

They differentiate into one or another type of committed stem cells (progenitor cells). These in turn form the various differentiated types of blood cells.

There are separate pools of progenitor cells for megakaryocytes, lymphocytes, erythrocytes, eosinophils, and basophils; neutrophils and monocytes arise from a common precursor.

1. STEM CELLS

- These cells have extensive proliferative capacity and also the:
  - Ability to give rise to new stem cells (**Self Renewal)**
  - Ability to differentiate into any blood cells lines **(Pluripotency)**
- Hematopoietic stem cells (HSCs) are bone marrow cells that are capable of producing all types of blood cells.
- They differentiate into one or another type of committed stem cells (progenitor cells).

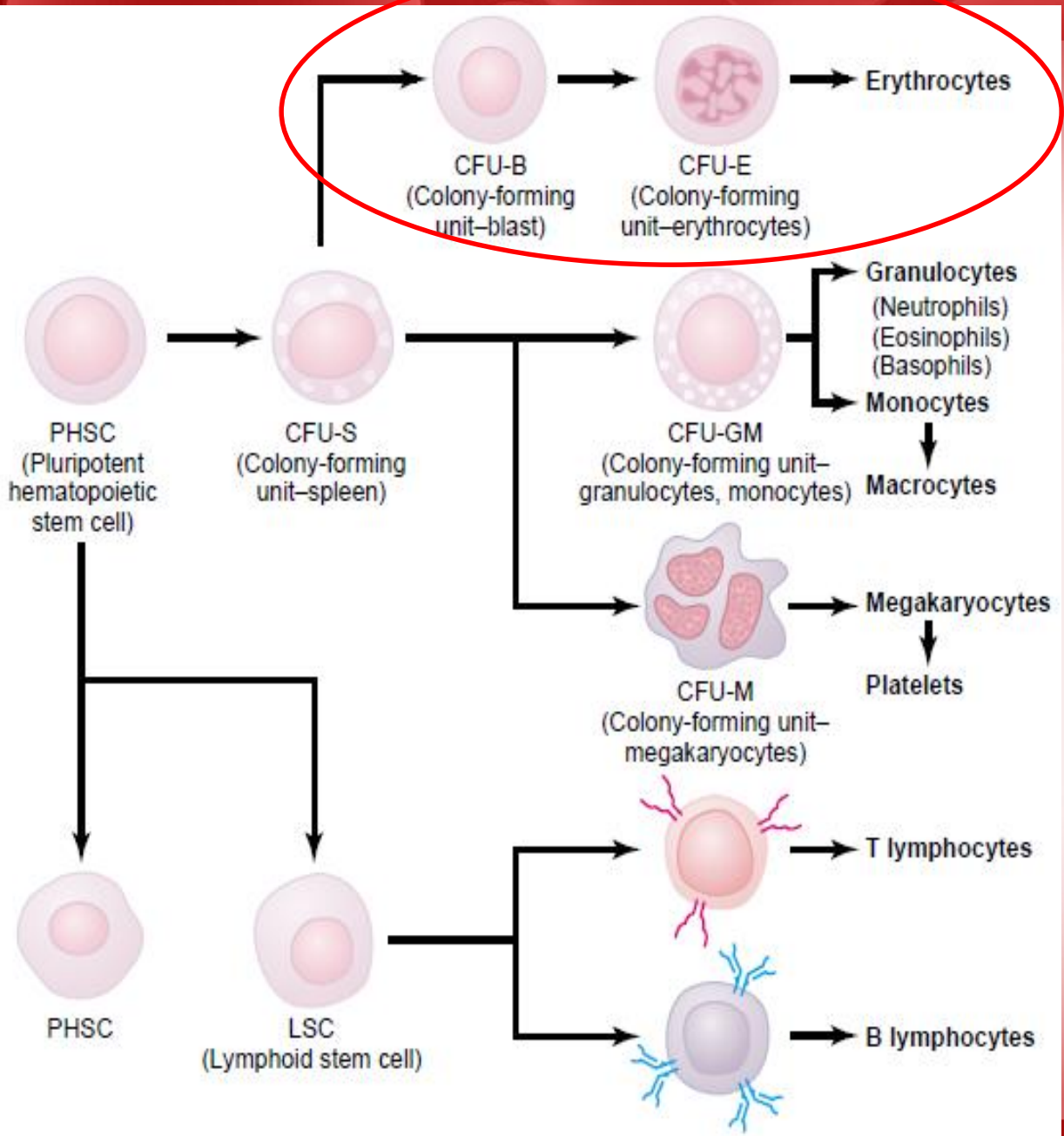


Figure 32–2

Formation of the multiple different blood cells from the original *pluripotent hematopoietic stem cell* (PHSC) in the bone marrow.

2. Progenitor cells BFU-E & CFU-E

- Committed stem cells lose their capacity for self-renewal.
- They become irreversibly committed.

– BFU-E Give rise each to thousands of nucleated erythroid precursor cells.
– Undergo some changes to become the Colony Forming Units-Erythrocyte (CFU-E)
– Regulator: Burst Promoting Activity (BPA)

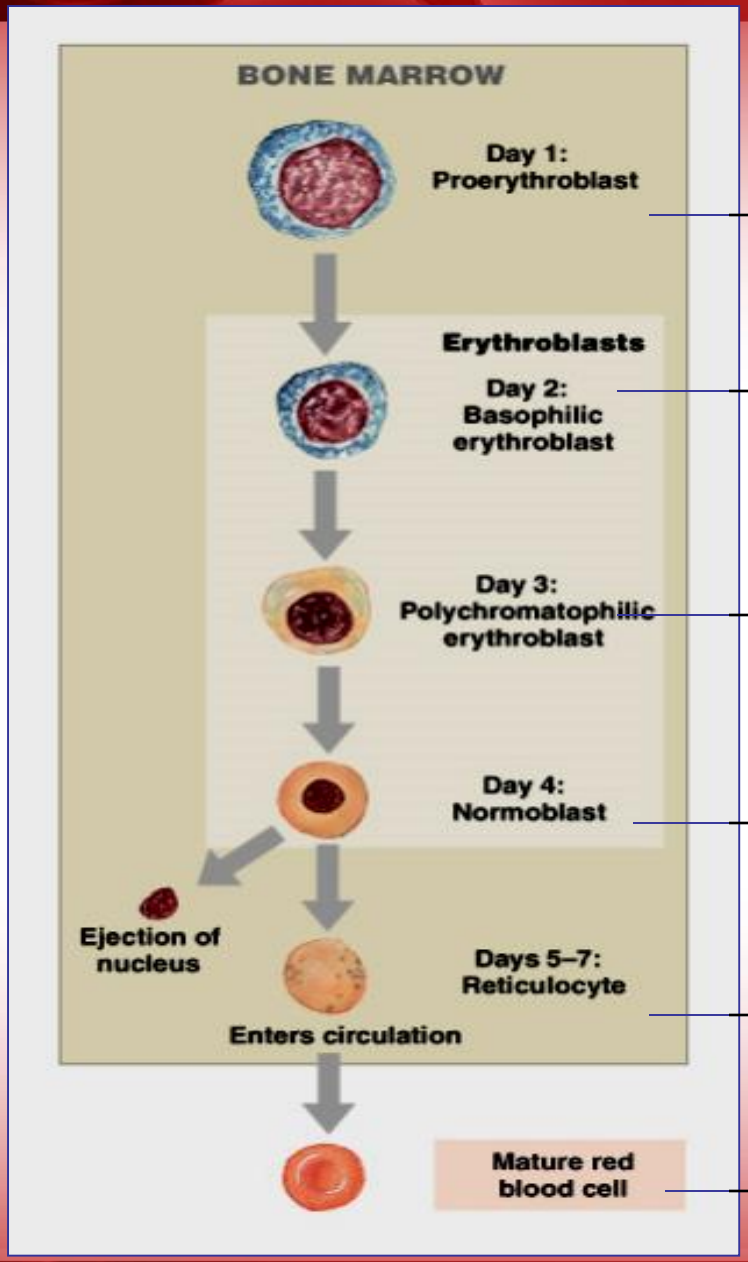
Burst forming unit BFU(E)

- Unipotent progenitor cell
- Less sensitive to erythropoietin
- Responds to other stimulus

forms

Colony forming unit CFU (e)

- Highly sensitive and dependent on erythropoietin



ERYTHROPOIESIS

15-20µm- basophilic cytoplasm, nucleus with nucleoli.

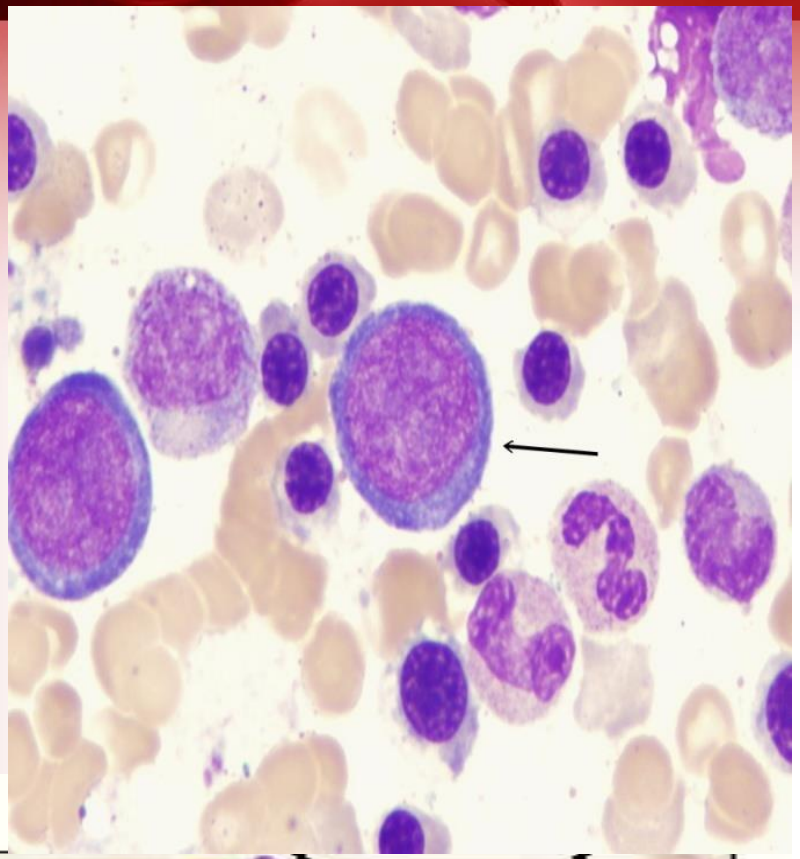
14-17µm-mitosis, basophilic cytoplasm, nucleoli disappears.

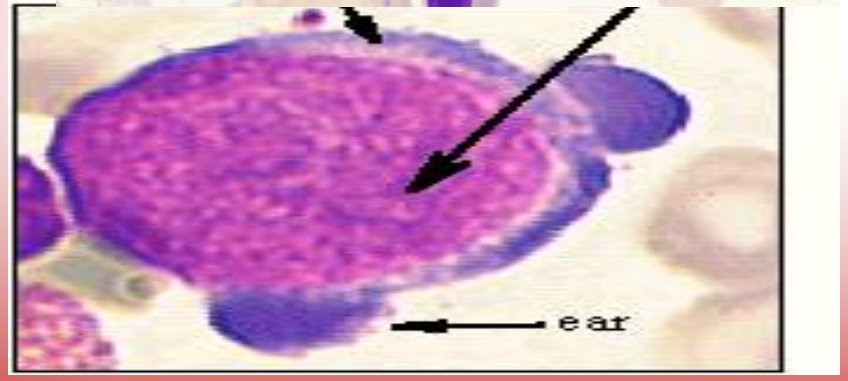
10-15µm- ’POLYCHROMASIA’
Hb appears, nucleus condenses.

7-10µm- PYKNOTIC Nucleus.
Extrusion, Hb is maximum.

7.3µm- Reticulum of basophilic material in the cytoplasm.

7.2µm- Mature red cell with Hb.





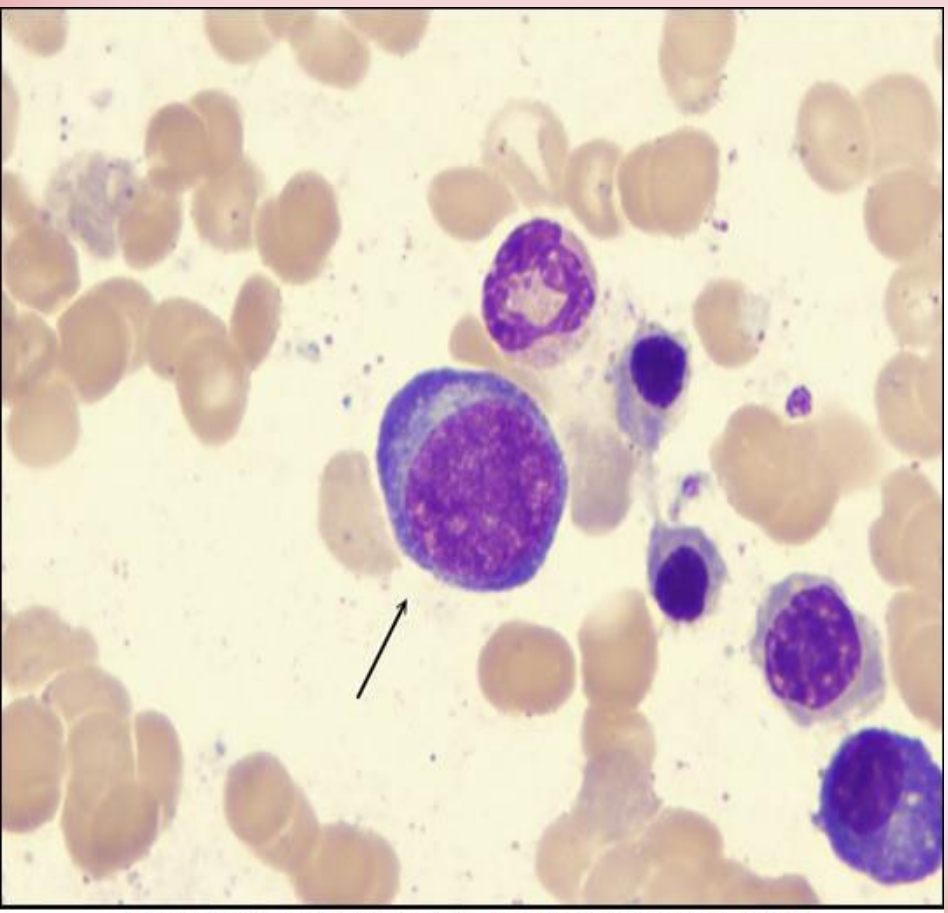
3. Proerythroblast

- 15-20 microns

- Nucleus with multiple nucleoli
- Basophilic cytoplasm with perinuclear halo

- No hemoglobin
- Mitosis present

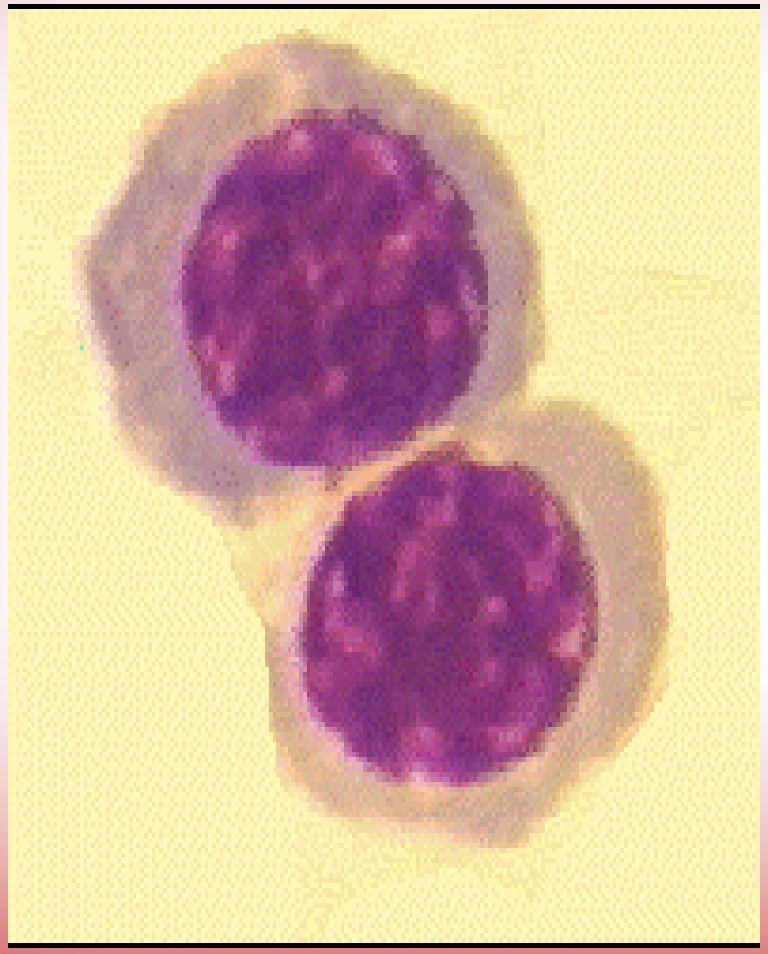
4. Basophilic/ early normoblast



Bone marrow aspirate smear, Wright-Giemsa stain, 1000x

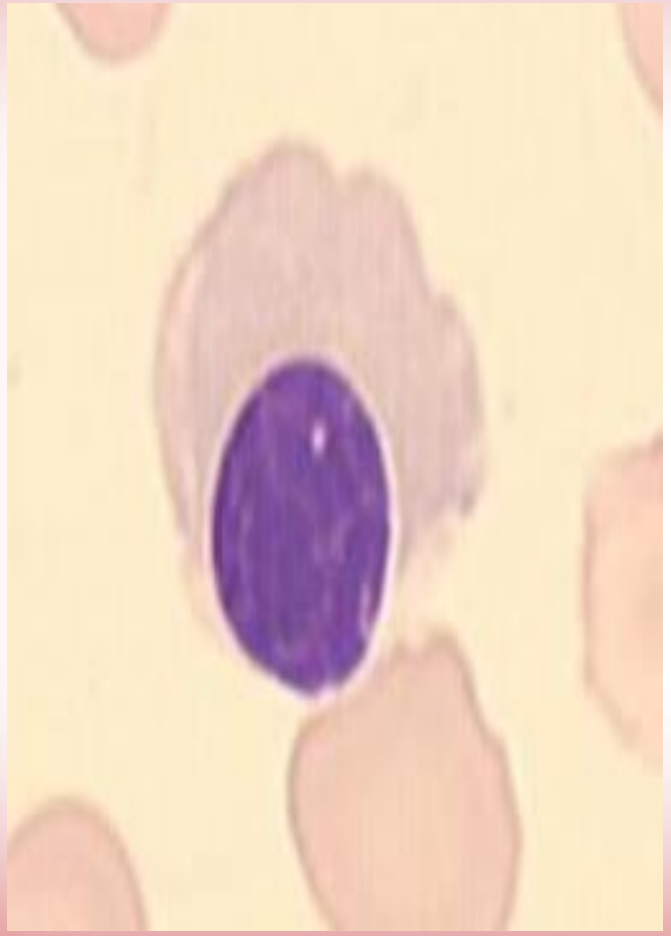
- Slight reduction in size 14-17µm
- Large nucleus, **nucleoli reduce in number**
- Basophilic cytoplasm
- Active mitosis

5. Polychromatophilic/ intermediate normoblast



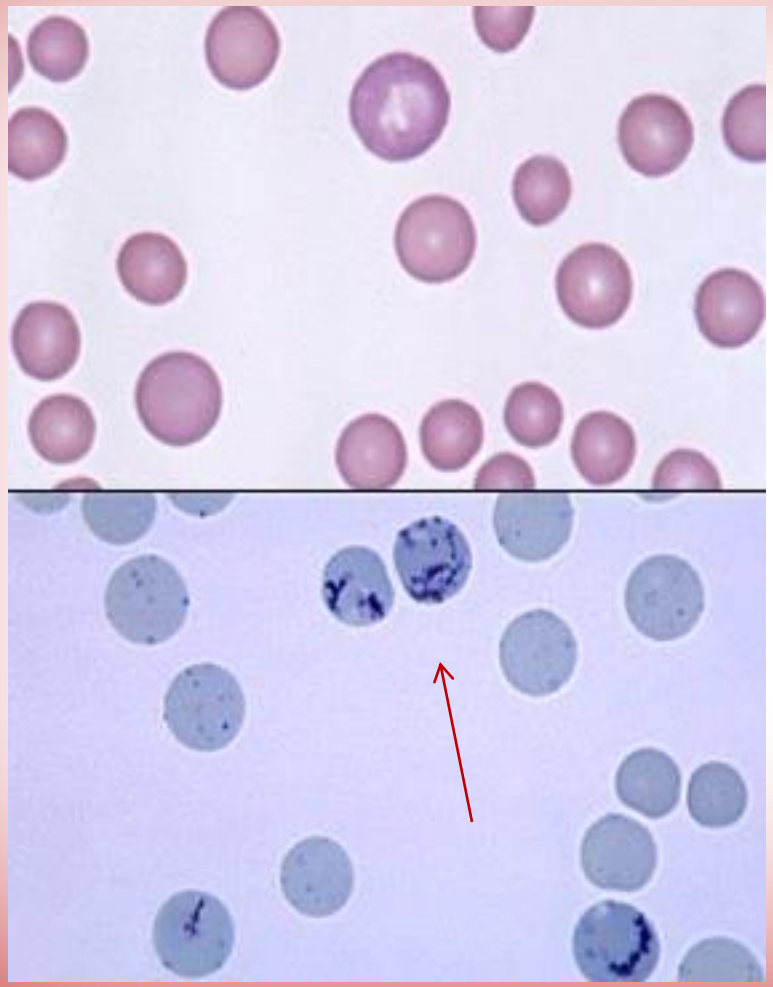
- 10-15µm size
- 'POLYCHROMASIA'
- nucleus condenses Chromatin lumps
- Hb starts appearing
- Reduced mitoses

6. Orthochromatic normoblast

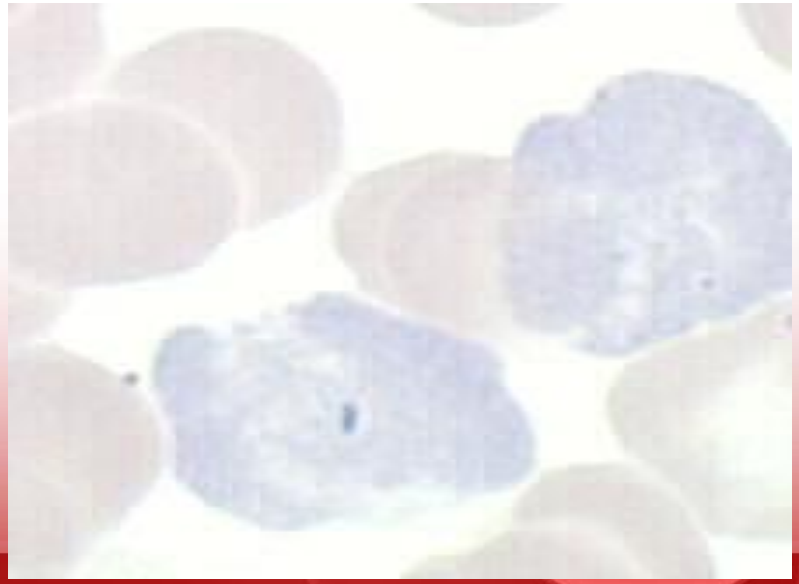


- 7-10µm
- Acidophilic erythroblast which is the last precursor with a nucleus.
- Nucleus is compact & situated near the membrane pyknotic nucleus is extruded
- Cytoplasm is like mature red cell, reflecting a high Hb content.
- Mitosis absent

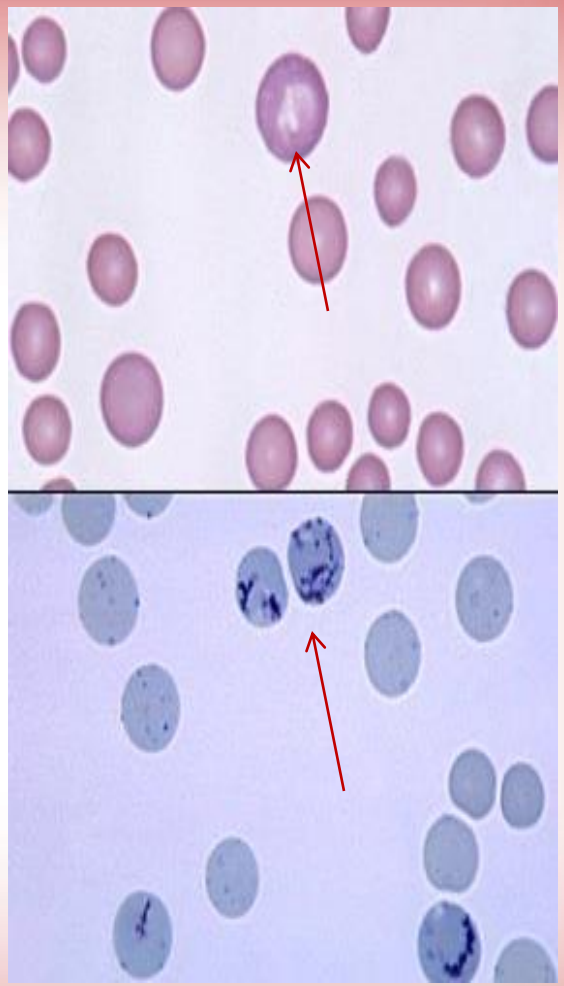
7. Reticulocyte



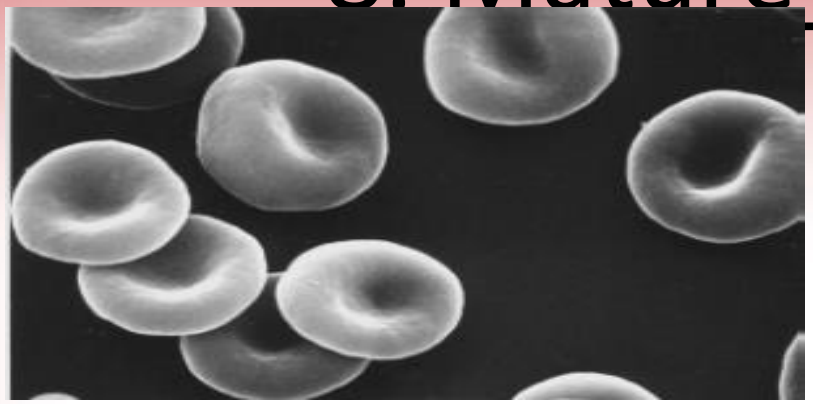
- Reticular nuclear fragments
- Nucleus extruded
- Slightly larger than RBCs

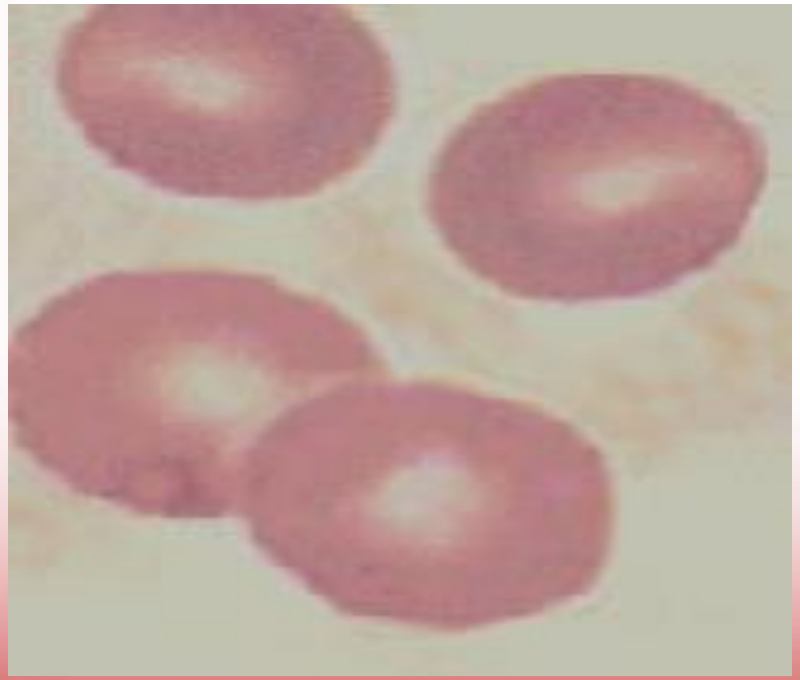


- The Reticulocyte
  - Has no nucleus
  - Has no organelles
  - Is larger than the mature RBC
  - Is not concave
  - Has many polyribosomes
  - In severe anemia, many of these are released into the blood prematurely→ Reticulocyte response.
  - Normally 1% of circulating blood, are reticulocytes.

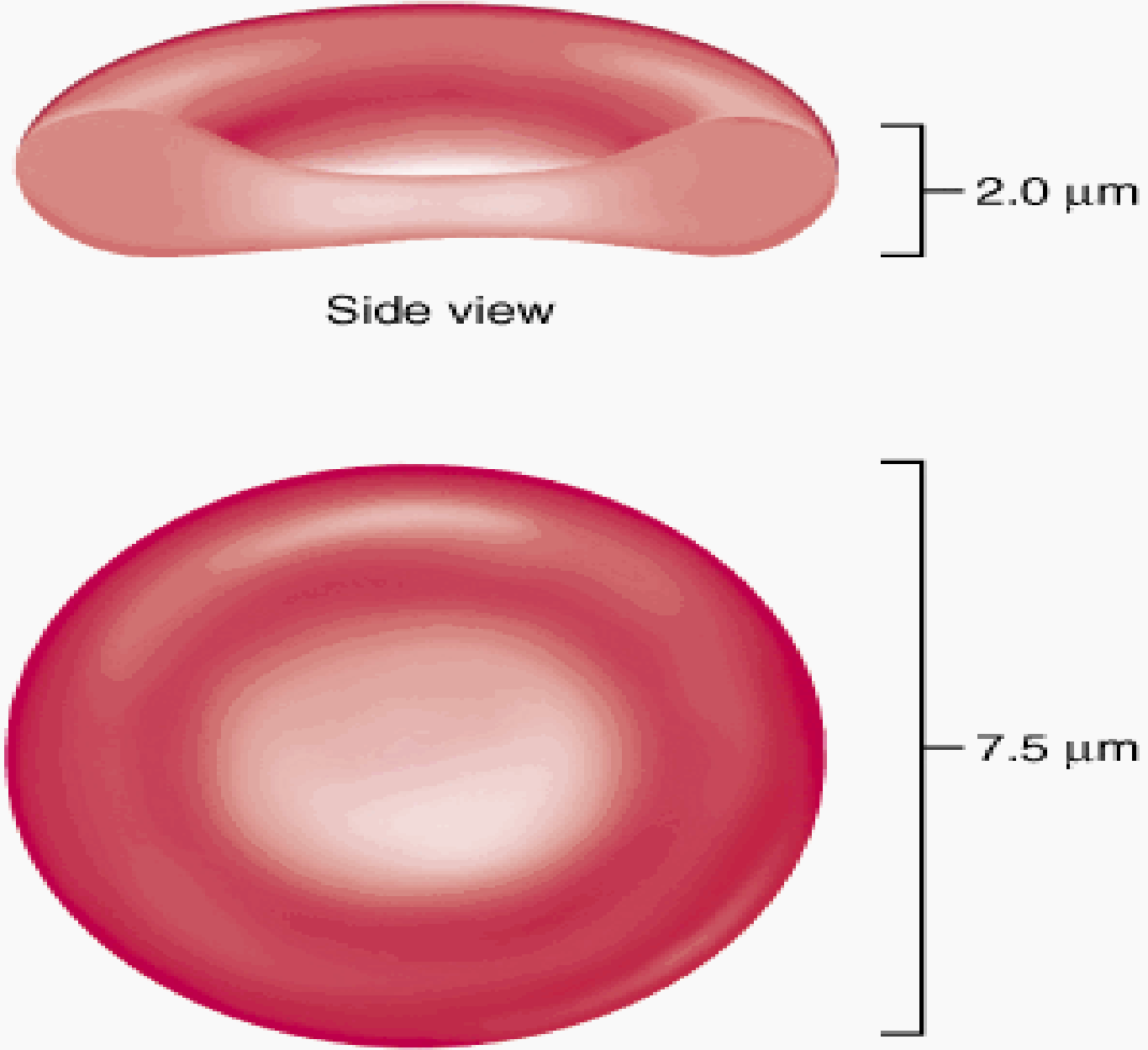


8. Mature erythrocyte





- Reddish, circular, biconcave cells
- 7-8 µ
- No visible internal structure
- High Hb content
- Bright at centre due to biconcave shape

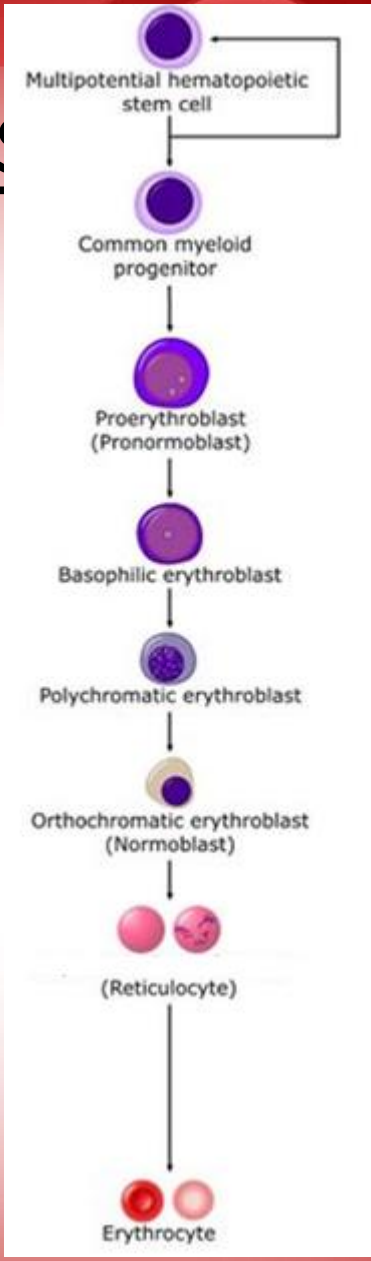


Duration of erythropoiesis

HSC to RBC- 21 days

Differentiation phase: from pronormoblast to reticulocyte phase- 5 days

Maturation phase: from reticulocyte to RBC- 2 days



Changes during erythropoiesis

- Decrease in size
- Loss of mitotic activity (later part of intermediate.normo)
- Hemoglobinization (intermediate normoblast)
- Change of cell shape (from globular to biconcave)
- Disappearance of nucleus, mitochondria, RNA, etc
- Change of staining (basophilic – eosinophilic)

Regulation of erythropoiesis

- **General factors**
- Hypoxia → erythropoietin
- Growth inducers
- Vitamins
- **Maturation factors**
- Vitamin B 12
- Folic acid
- **Factors necessary for hemoglobin production**
  - Vitamin C →Helps in iron absorption (Fe+++ → Fe++)
  - Proteins → Amino Acids for globin synthesis
  - Iron & copper → Heme synthesis
  - calcium, bile salts, cobalt & nickel.

General

Hypoxia → erythropoietin

ERYTHROPOIETIN

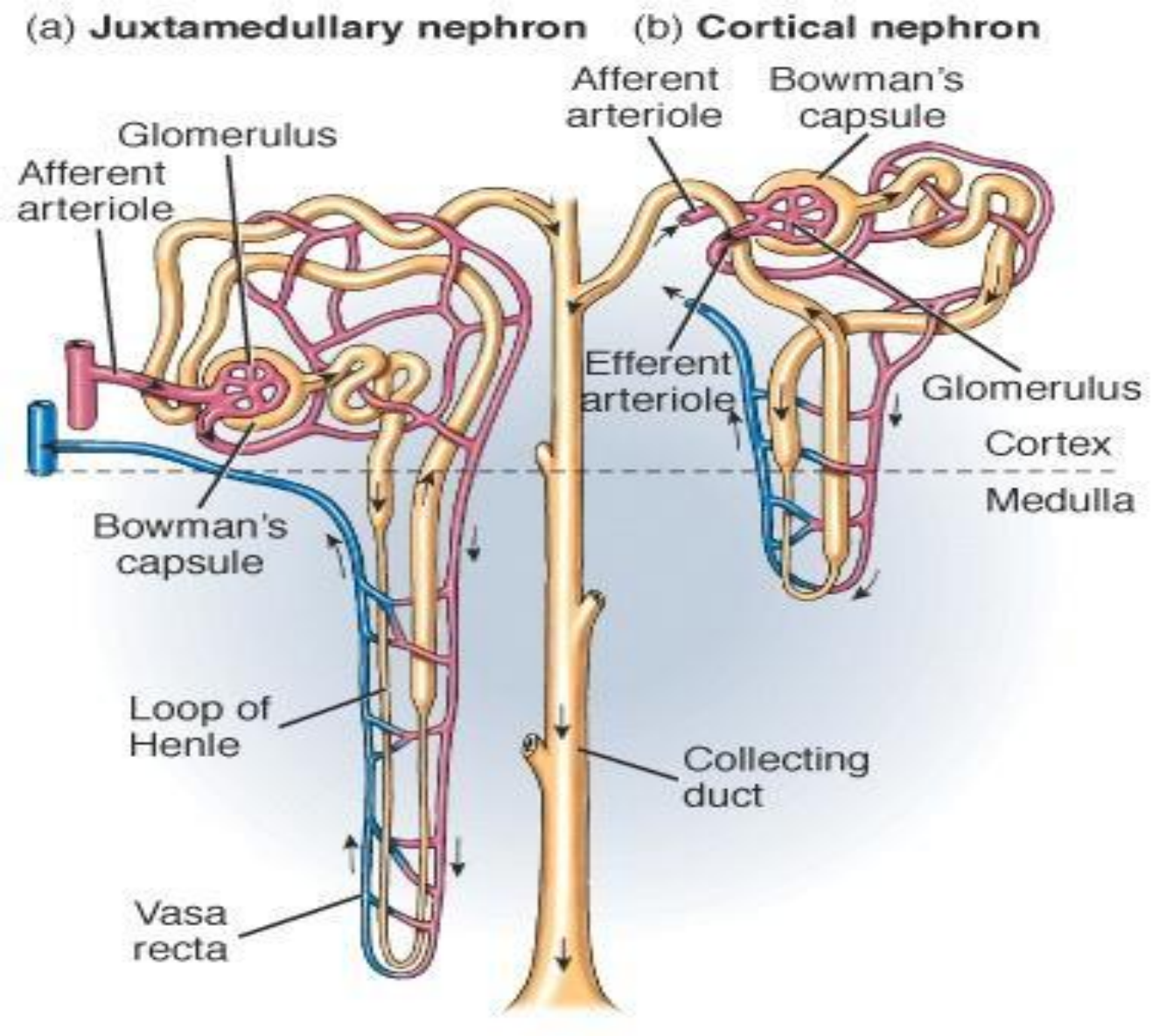
- Glycoprotein MW-34000 (165 AA residues)

Formation

- 85% formed in endothelial cells of the peritubular capillaries of the renal tubules.
- 15% formed in liver, hepatic cells & Kupffer cells.

Breakdown

- In liver. Half life is 5hours


(a) Juxtamedullary nephron
(b) Cortical nephron
Afferent arteriole
Bowman's capsule
Glomerulus
Afferent arteriole
Efferent arteriole
Glomerulus
Cortex
Medulla
Bowman's capsule
Loop of Henle
Collecting duct
Vasa recta

ERYTHROPOEITIN

Stimuli for production

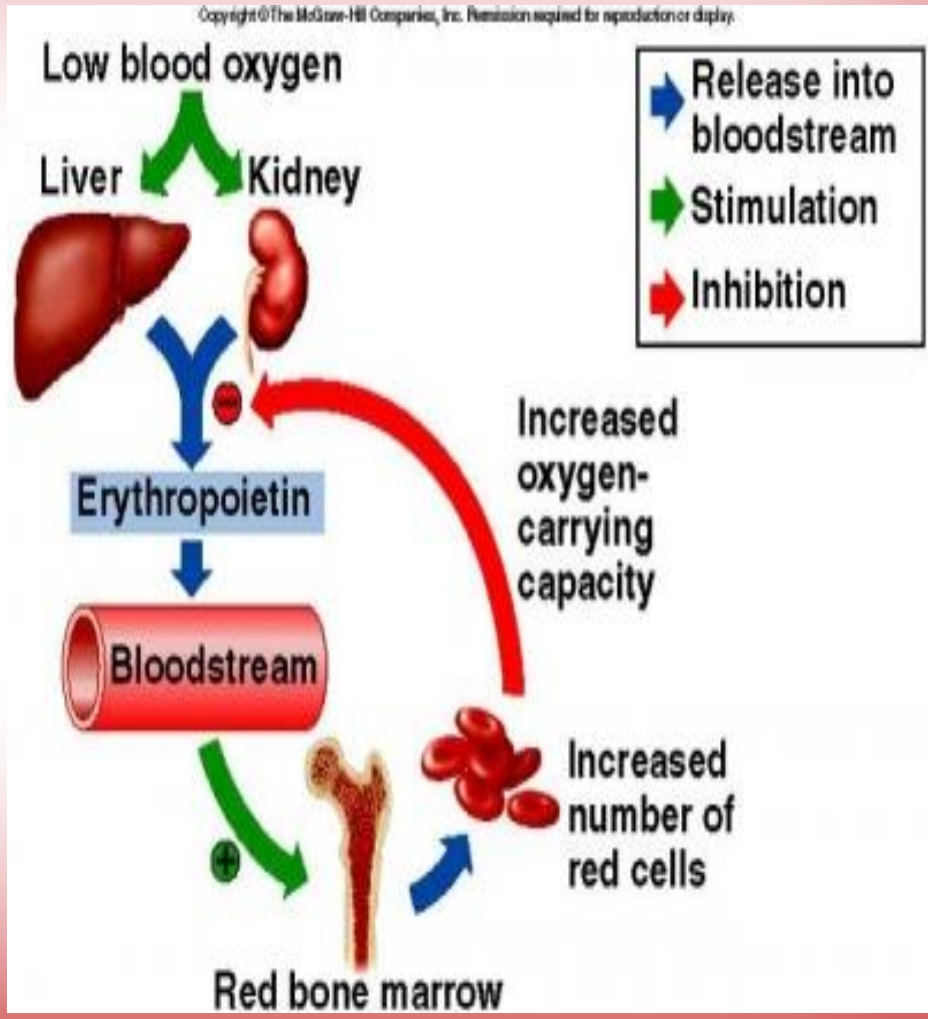
- **Hypoxia**
- Products of RBC destruction
- High altitude
- Anemia
- Chronic lung diseases
- Catecholamines
- Prostaglandins
- **Androgens**

Inhibition

- Blood transfusion

Functions of Erythropoietin

- Erythropoietin increases RBC production in 3 ways:
  - Promotes pronormoblast production
  - Shortens the transition time through the normoblast stage
  - Promotes the early release of reticulocytes.

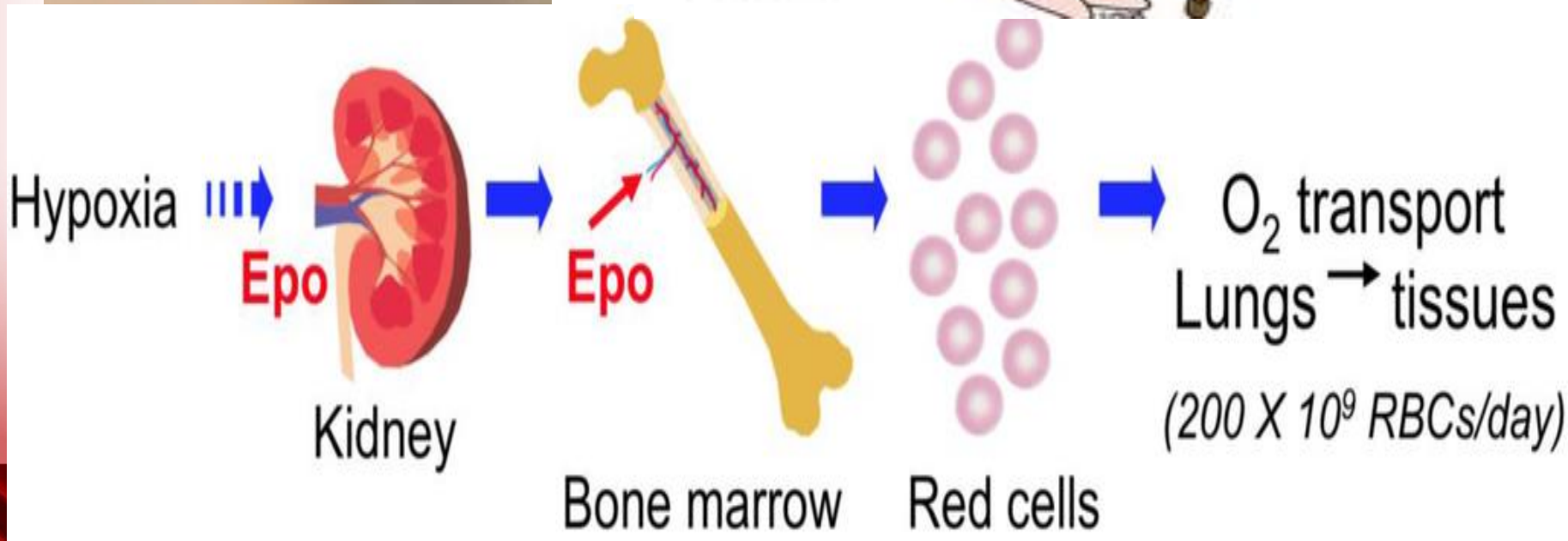


Re

CHRONIC RENAL FAILURE (CRF)

- RENAL INSUFFICIENCY -

- Headaches
- ↓ Ability to Concentrate Urine
- Polyuria → Oliguria
- ↑ BUN & Serum Creatinine
- Edema
- GFR - progressively decreases from 90 to 30 ml/min
- Mild Anemia
- ↑ BP
- Weakness & Fatigue



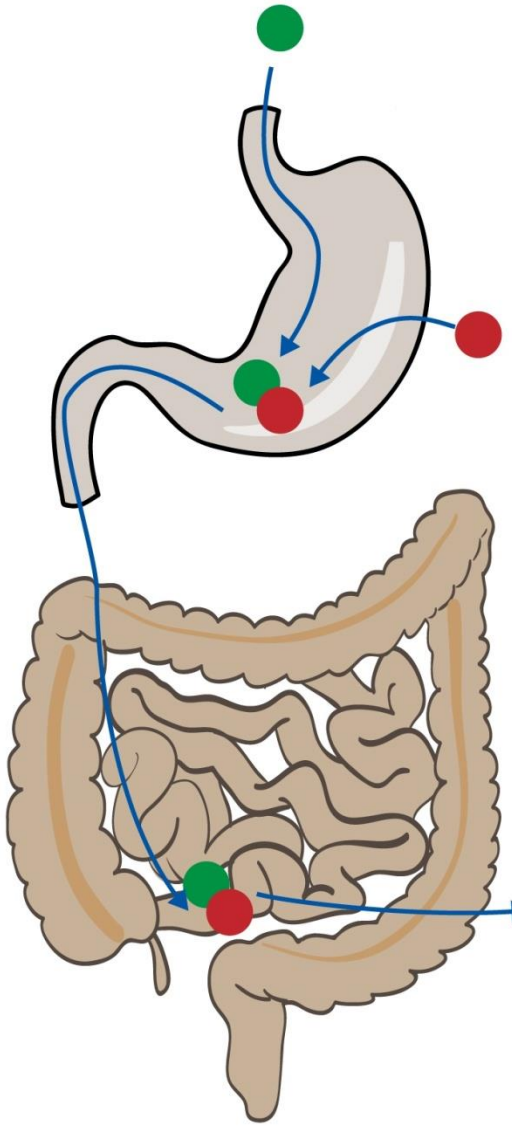
Growth inducers/ Differentiation inducers

- Interleukin 1, 3, 6 (IL-3 is a growth inducer for all cell lines)
- CSF- E (colony stimulating factor – erythro)

Vit B-12

- Source : only animal tissues
- Absorption from ileum
- Functions
- Promotes maturation of RBCs (plays an important role in folic acid synthesis of nucleic acid-DNA)

Sources of vitamin B12



stomach

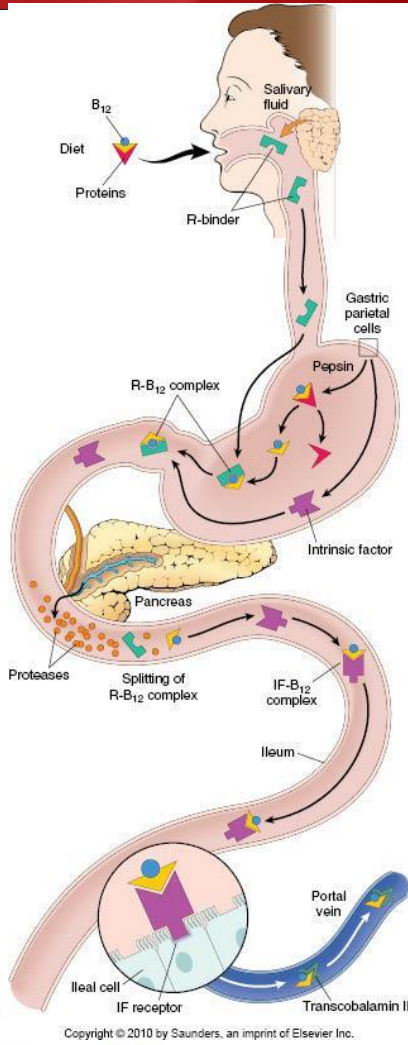
intrinsic factor

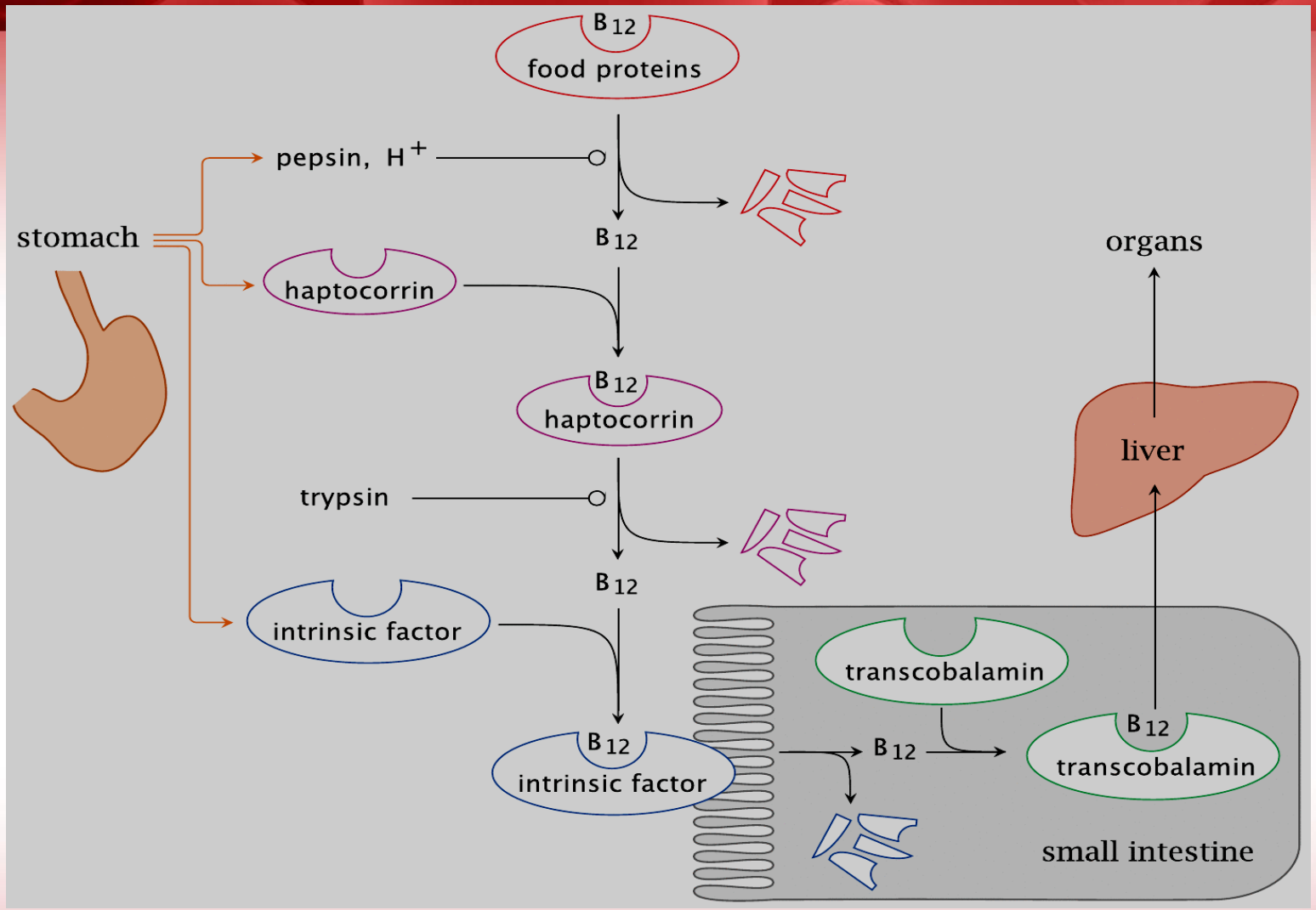
terminal ileum

transcobalamin II

blood transport

cell

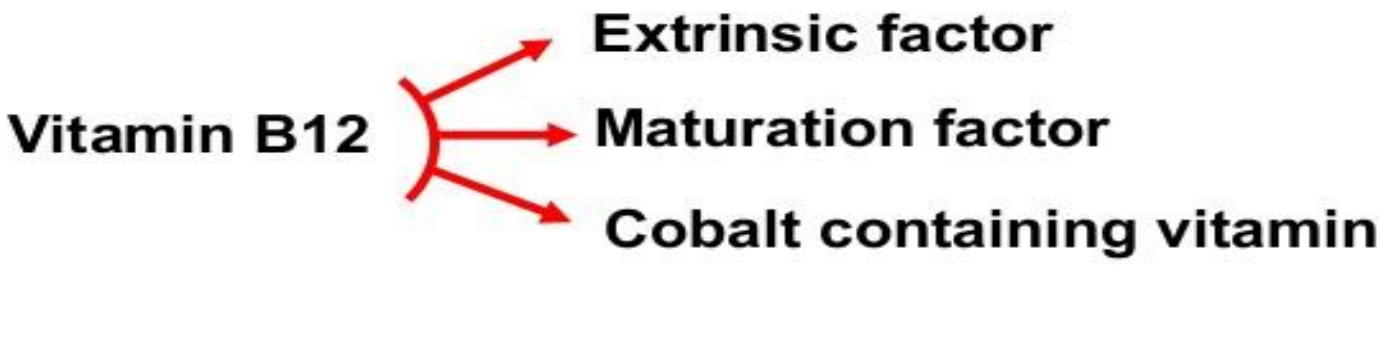


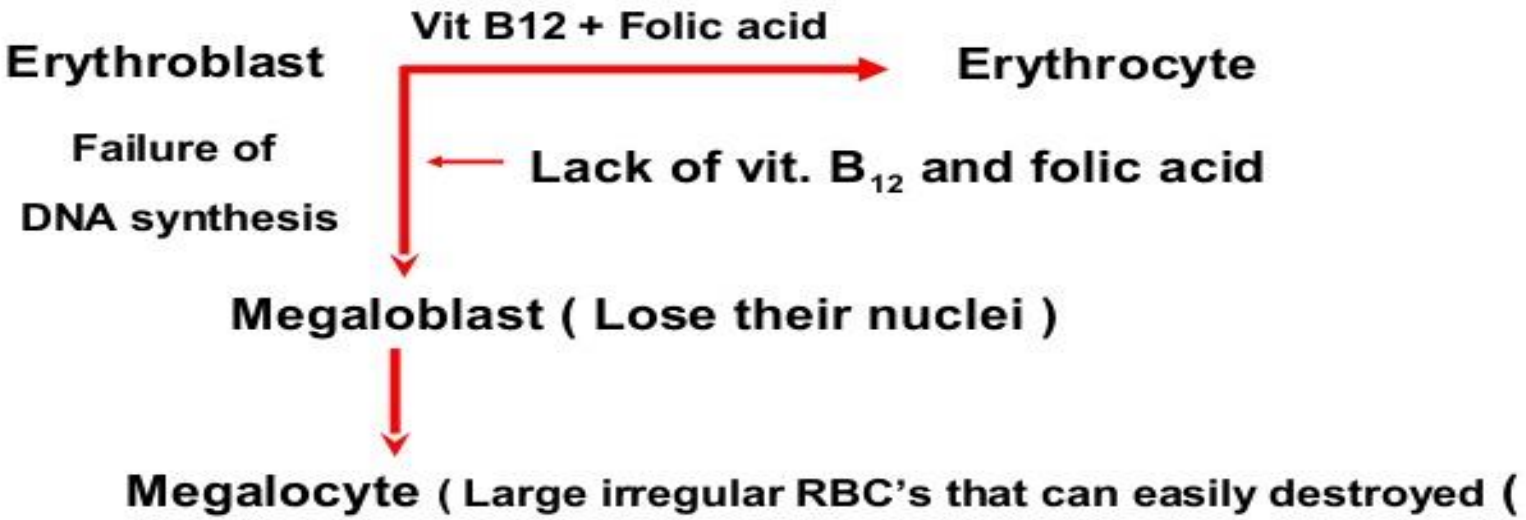


Absorption of Vitamin B12 and the role of Intrinsic factor

Folic acid

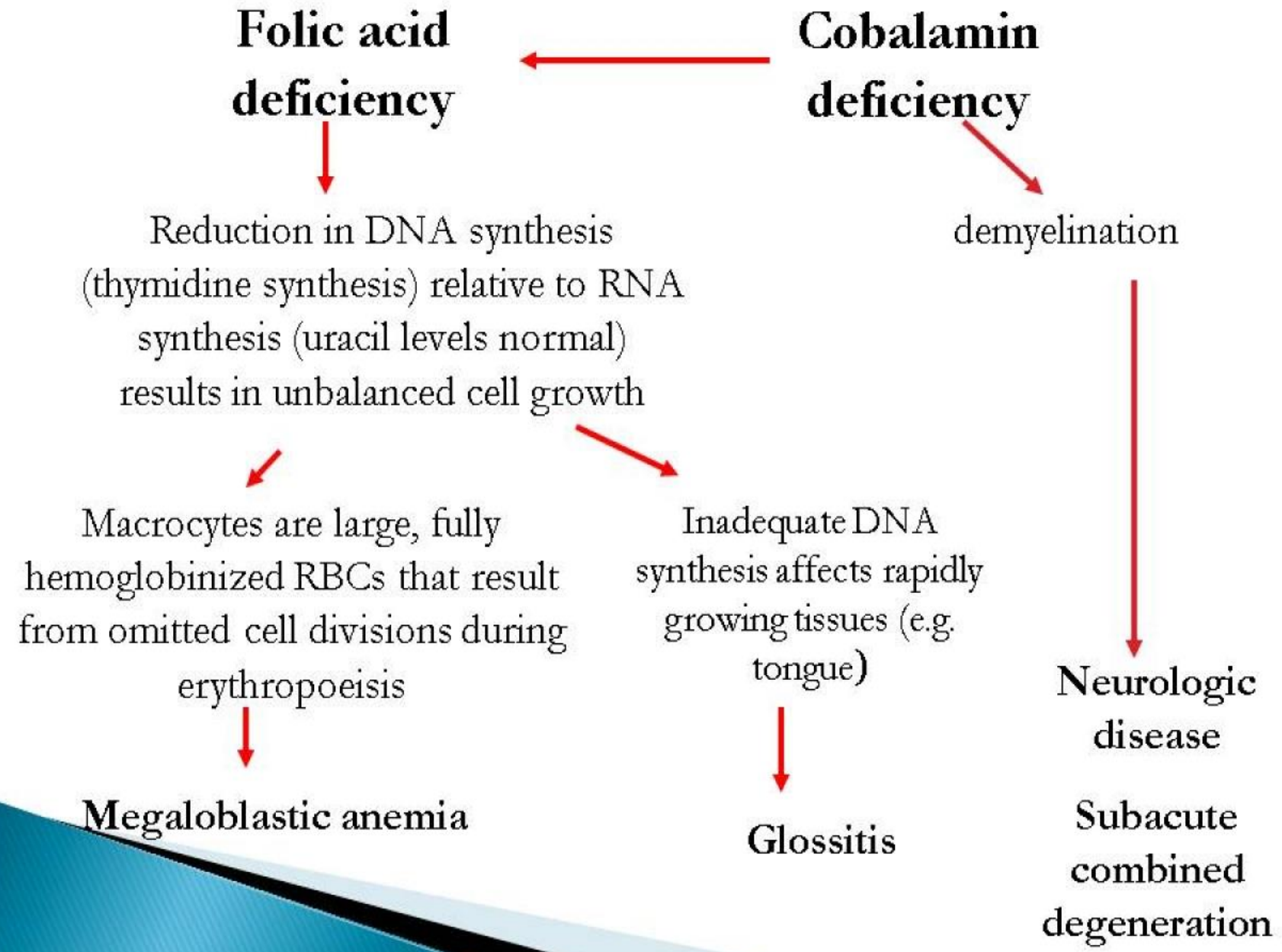
- Green leafy vegetables , yeast, liver
- Function : maturation of RBC

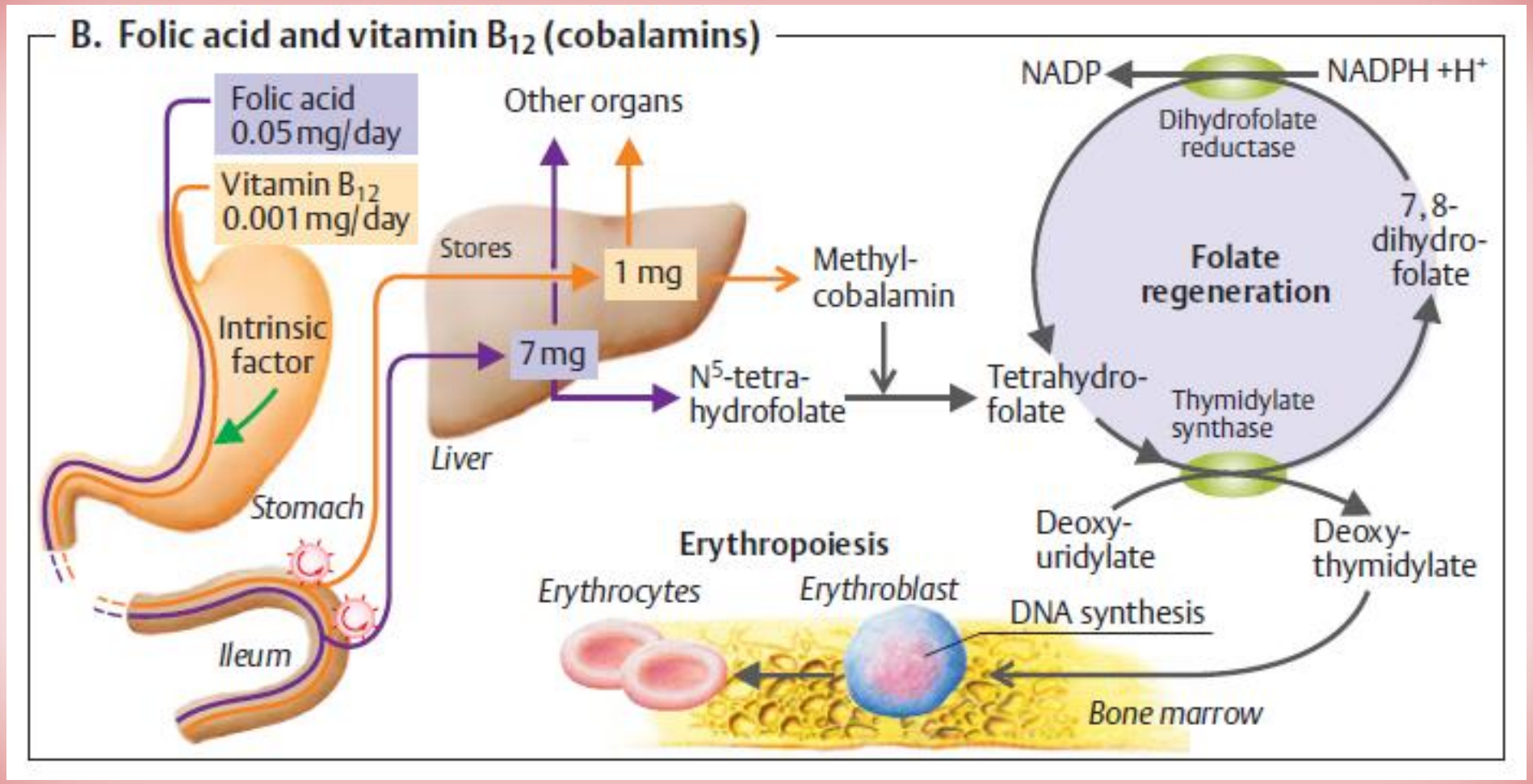




LIFE SPAN OF MEGALOBLAST IS 40 DAYS

Relationship between cobalamin and folate deficiency




B. Folic acid and vitamin B_{12} (cobalamins)
Folic acid 0.05 mg/day
Vitamin B_{12} 0.001 mg/day
Intrinsic factor
Stomach
Ileum
Other organs
Stores
1 mg
7 mg
Liver
Methyl-cobalamin
N^5-tetra-hydrofolate
Tetrahydro-folate
NADP
NADPH +H^+
Dihydrofolate reductase
7,8-dihydro-folate
Folate regeneration
Thymidylate synthase
Deoxy-uridylate
Deoxy-thymidylate
Erythropoiesis
Erythrocytes
Erythroblast
DNA synthesis
Bone marrow

Pernicious Anemia

Intrinsic factor of Castle- secreted by parital cells of gastric mucosa

Essential for absorption of Vitamin B12 by enteric route

Other Factors Regulating erythropoiesis

NUTRITIONAL FACTOR

- Proteins

MINERALS

- Iron – for Hb
- Cu, Zn, Co– Hb synthesis

HORMONES

- Testosterone
- Thyroxine, Adrenal hormones
- Pituitary hormones – stimulate Erythropoietin

VITAMINS

- B12 & folic acid – for synthesis of DNA
- Riboflavin – Normal BM division
- Pyridoxine – Heme synthesis
- Vitamin C – absorption of Fe from gut

NEURAL

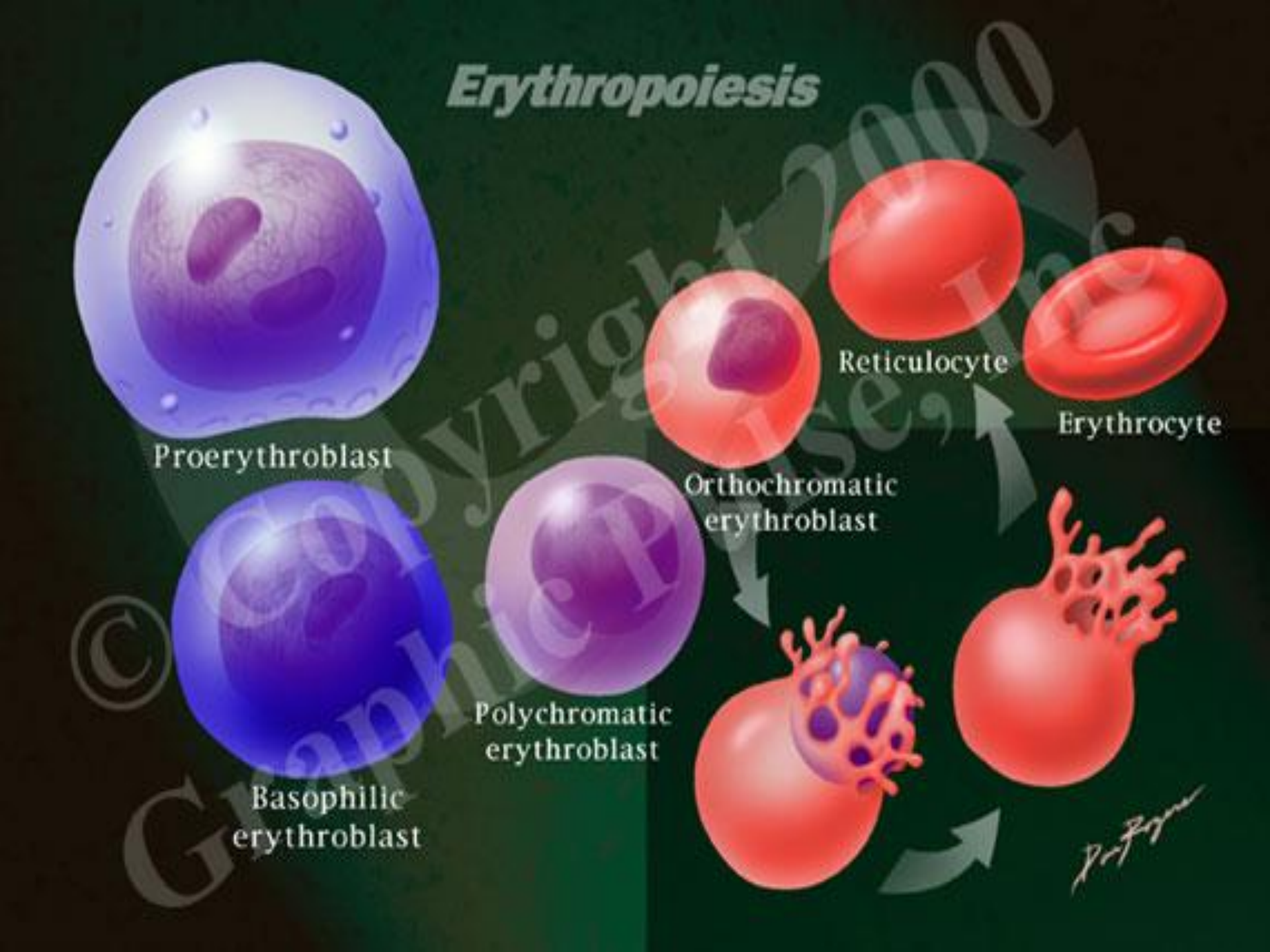
Stimulation of Hypothalamus
↑ RBC production

Clinical Aspects

Anemias: Reduced RBC count / reduced Hb concentration

Polycythemia: Increased RBC count

- Polycythemia vera
- Secondary polycythemia- due to hypoxia



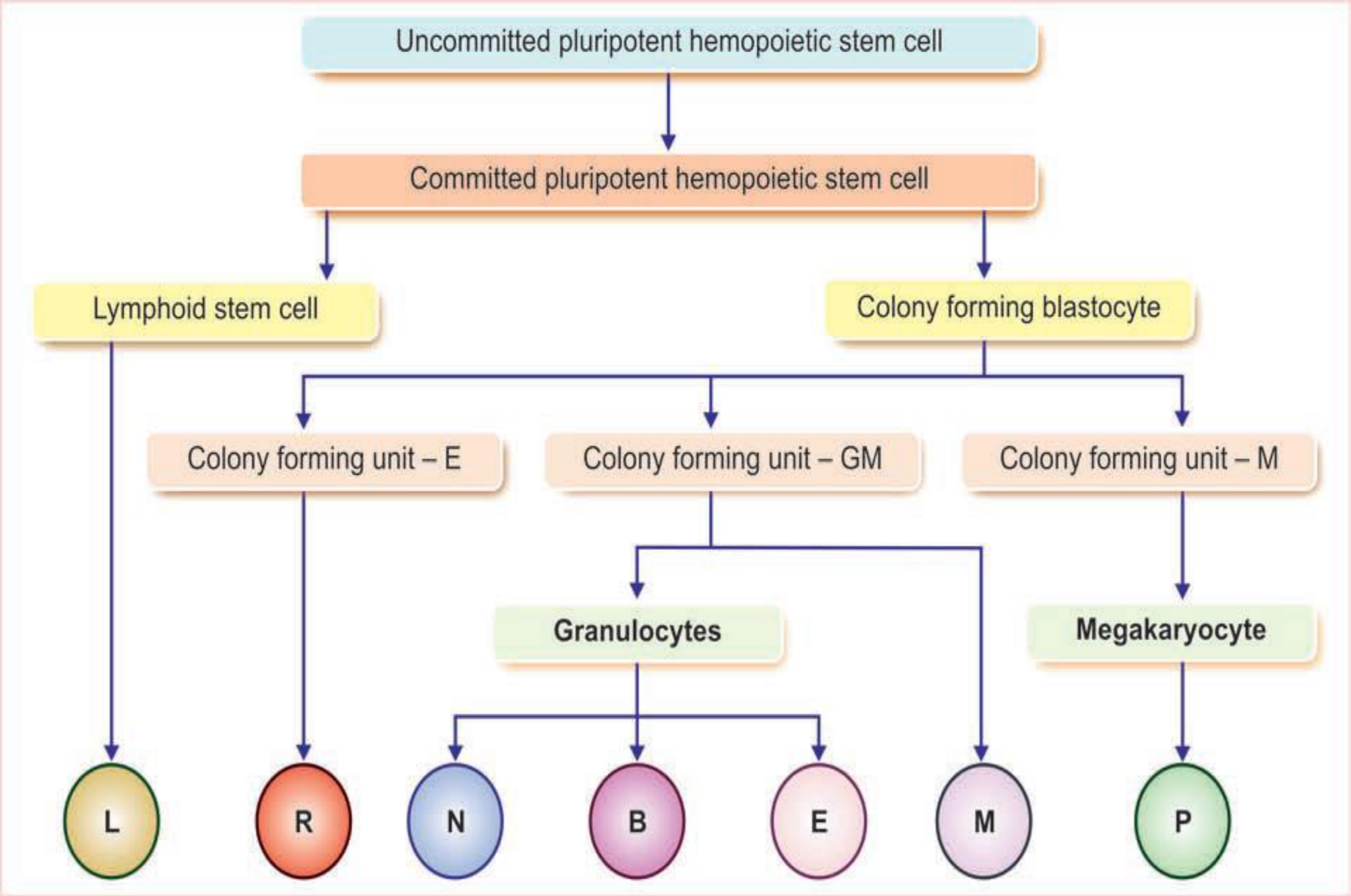


FIGURE 10.1: Stem cells. L = Lymphocyte, R = Red blood cell, N = Neutrophil, B = Basophil, E = Eosinophil, M = Monocyte, P = Platelet.

- Factors necessary for erythropoiesis:

1. General factors:

-Erythropoietin

-Thyroxine

-Vitamins

2. Maturation factors:

-Vitamin B_{12} (Cyanocobalamin)

-Intrinsic factor of Castle

-Folic acid

HEMOGLOBIN

- Hb is the iron containing coloring pigment of RBC.
- 95% dry weight of RBC; 30 – 34% wet weight.
- Molecular weight of Hb is 68,000
- Normal value:

  -At birth: 25g/dl

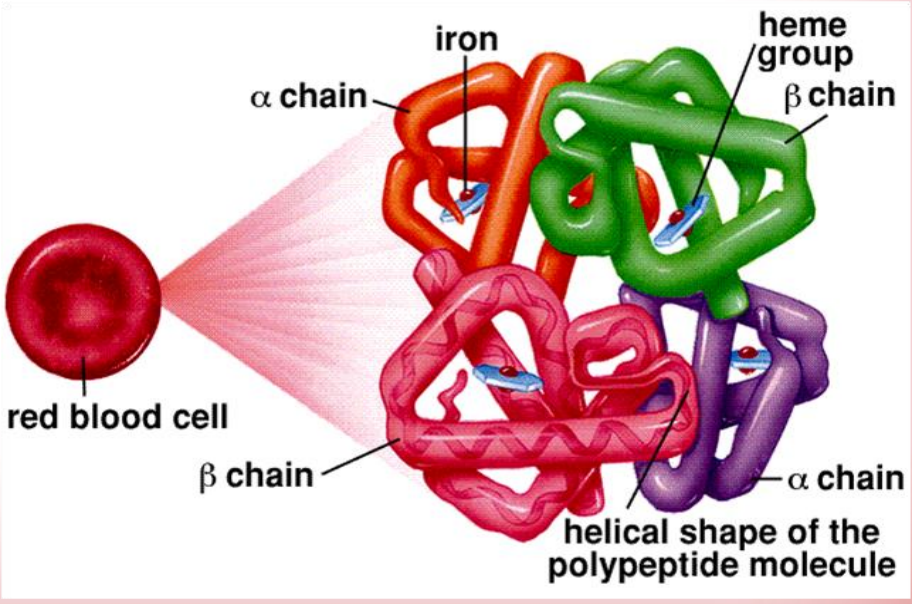
  -From puberty: 14-16 g/dl

  -Adult males: 15g/dl

  -Adult females: 14.5g/dl

STRUCTURE OF HEMOGLOBIN

- Conjugated protein
- Protein part called Globin and iron containing pigment called heme.
- Heme part is called porphyrin and is formed by 4 pyrole rings
- Globin is made up of 4 polypeptide chains – 2 alpha and 2 beta chains.



Types of Hemoglobin:

- Normal: - Adult Hb

  - Fetal Hb

- Abnormal derivatives:

  -Carboxyhemoglobin

  -Methemoglobin

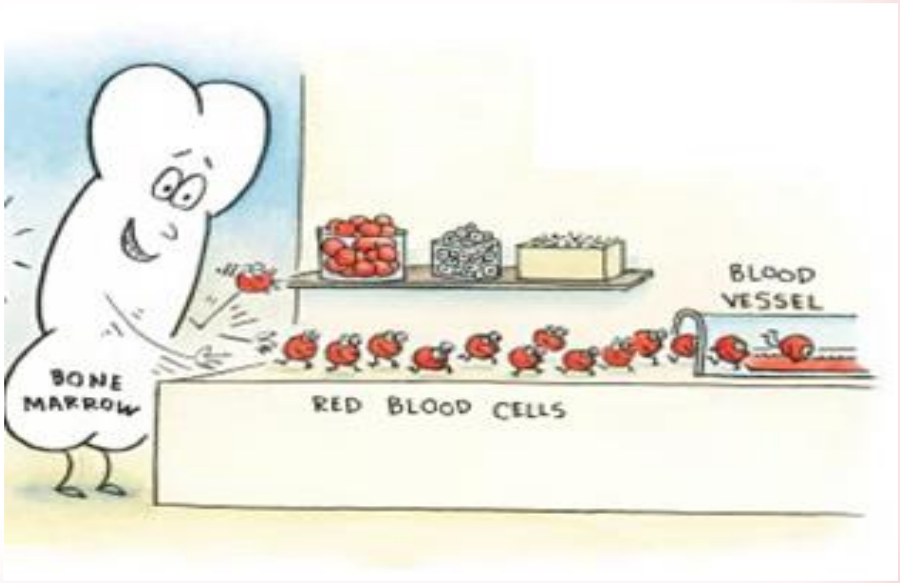
  -Sulfhemoglobin

ERYTHROPOIESIS

- The process of origin, development and maturation of erythrocytes.

➢ Site of erythropoiesis:

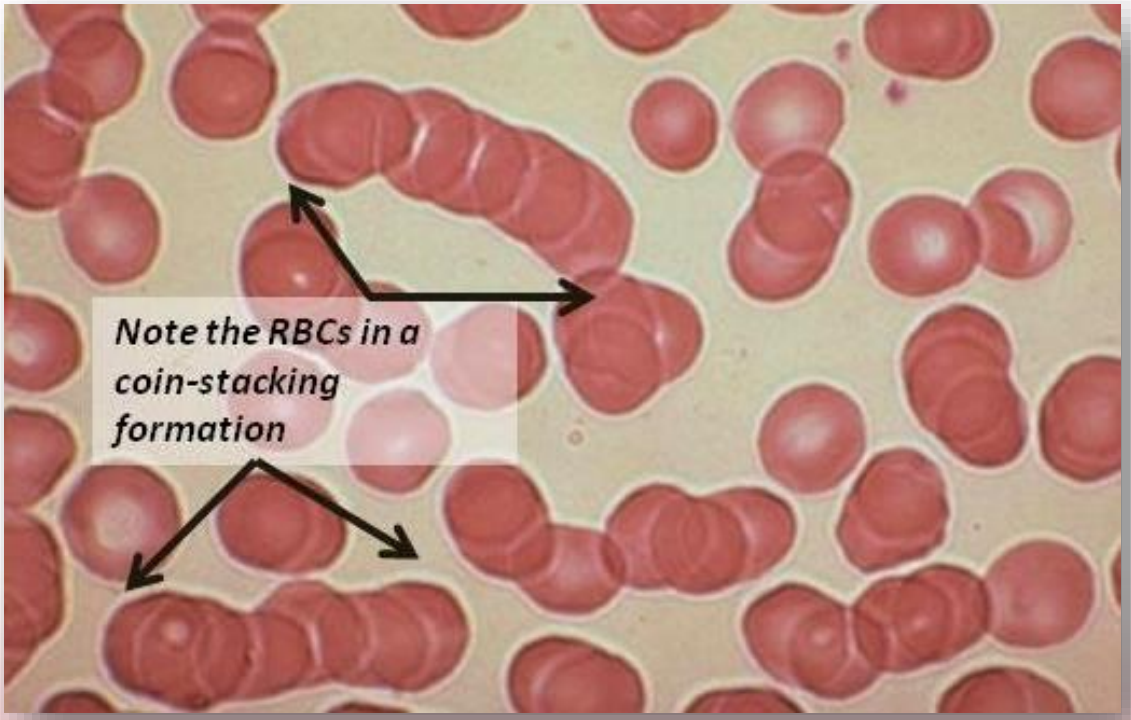
- In fetal life: - Mesoblastic stage
  - Hepatic stage
  - Myeloid stage
- In newborns, children and adults



ERYTHROCYTE SEDIMENTATION RATE

- Red cells have the property of Rouleaux (piling one on the other) formation.
- Piled red cells are heavier than the individual ones.
- The rate at which the red cells fall is known as ESR.
- Normal values:

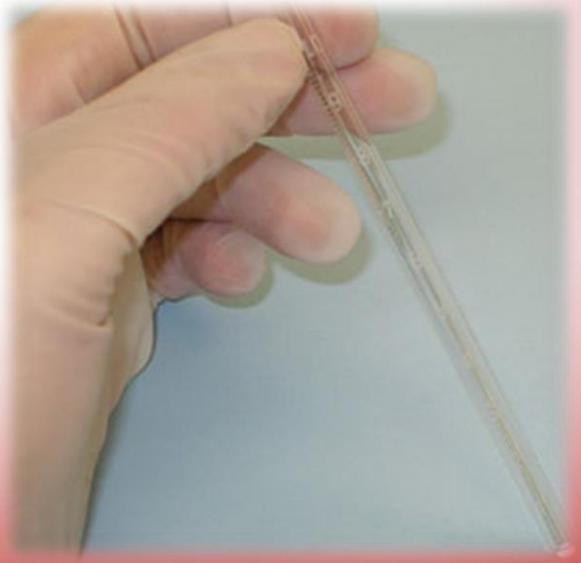
Wintrobe's method:	Males: 0 – 9mm/hr
	Females: 0 – 20mm/hr
Westergren's method:	Males: 3 – 7mm/hr
	Females: 5 – 9 mm/hr



Westergren's method:

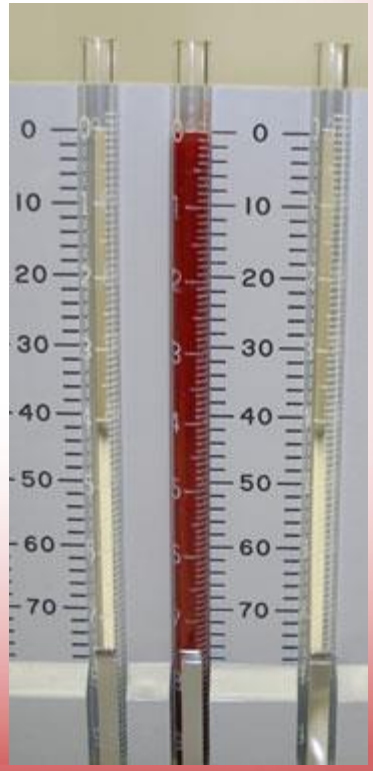
- Westergren's tube is used which is 300 mm long & opened at both the ends.
- It requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature

or 6 hours at 4 °C. The blood is drawn into the tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature,



Wintrobe's method:

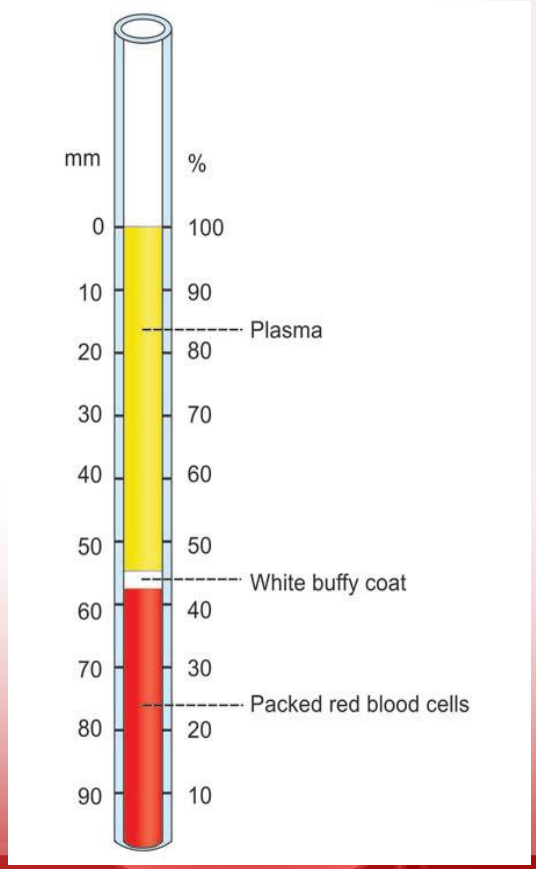
- The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long.
- EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour.



PACKED CELL VOLUME

- Hematocrit is the fractional volume of blood that the erythrocytes occupy
- It is a reliable index of red cell population.
- Normal values : Males – 46%

  Females – 42%



RBC INDICES

- **MCV (MEAN CORPUSCULAR VOLUME)**
  - The average volume of single RBC

$$MCV = \frac{\text{PCV per 100ml blood}}{\text{RBC count (million/cu mm)}} \times 10 um^3$$

Normal range : 78 – 94 um^3

- RBC with normal volume are called Normocytes
- RBC with less then normal volume, Microcytes
- RBC with more than normal volume, Macrocytes

- **MCH (mean corpuscular hemoglobin)**
  - The average content of Hb in average RBC.

$$\text{MCH} = \frac{\text{Hb in gm\%}}{\text{RBC count (million/cu mm)}} \times 10\text{pg}$$

- Normal range: 28 – 32pg

- **MCHC (mean corpuscular hemoglobin concentration)**
  - Express the average concentration of hemoglobin per unit volume of RBC.
  - It defined as the ratio of the weight of hemoglobin to volume of RBC.

$$\text{MCHC} = \frac{\text{Hb in gm\%}}{\text{PCV per 100ml blood}} \times 100$$

Normal range: 33 – 38 gm/100ml of cells

DISORDERS OF RBC

- Anemia

  Morphologic classification

  Etiologic classification

- Polycythemia

  Polycythemia Vera

  Relative polycythemia

  Secondary polycythemia

ANEMIA

- Anemia is defined as Hb concentration in blood below the lower limit of the normal range for the age and sex of the individual.
- In adults, the lower extreme of normal Hb is taken as 13g/dl for males and 11.5g/dl for females.

POLYCYTHEMIA

- Abnormal increase in the number of RBCs in the peripheral blood, usually with increase in Hb level.
- Types:
  - Polycythemia Vera
  - Relative Polycythemia
  - Secondary Polycythemia

POLYCYTHEMIA VERA

- Polycythemia rubra vera / Osler's disease / Erythremia / Vaquez's disease
- Uncontrolled proliferation of erythroid stem cells leading to excess of erythroid cell mass in the body.

Clinical Features:

- Male predilection – Middle age
- Skin appears flushed, reddened
- Spleen is palpable

Case study

- A 30 yrs old female came to medicine opd with history of generalized weakness, pounding of heart on exertion
- Physician examined for pallor which is present
- Investigation was done:
- Hb-9g/dl
- RBC count:3.5 millions/cubic mm
- What is your provisional diagnosis?