RBC Structure and Function and Erythrocyte Destruction

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Today’s Discussion

What is an RBC?

RBC Maturation Sequence

RBC Structure

RBC Metabolic Pathway

Erythrokinetics

RBC Function

Red Blood Cell (erythrocyte)

- Approximately 4-5 million RBC per L of blood
- Main Function
  - Transport oxygen from the lungs to the tissues through hemoglobin
  - Return CO_2 to the lungs
  - Buffer the pH of the blood
- Lifespan is 120 days

Normoblastic Maturation

Burst forming unit-erythroid (BFU-E)

↓ 1 week

Colony-forming unit-erythroid (CFU-E)

↓ 1 week

Pronormoblast

↓ 6-7 days

Mature RBC in circulation

Identification of Erythroid Precursor

- Stage of maturation is determined by examination of nucleus and the cytoplasm
- As the RBC matures:
  - Overall diameter of cell decreases
  - Diameter of the nucleus decreases
  - N:C ratio decreases
  - Nuclear chromatin pattern becomes coarser, clumped, and condensed
  - Nucleoli disappear
  - Cytoplasm changes from blue → gray blue → pink

Maturation sequence


Pronormoblast


Basophilic Normoblast


Polychromatic Normoblast


Orthochromic Normoblast

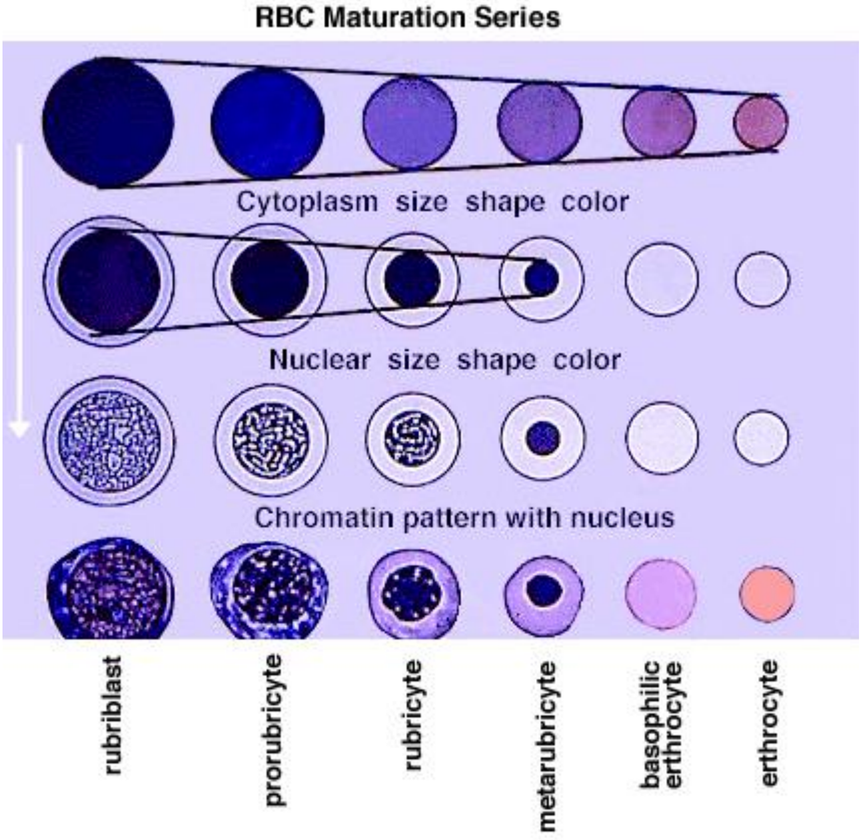

Polychromatic Erythrocyte (Reticulocyte)


Erythrocyte

https://www.researchgate.net/publication/357031646_Red_Blood_Cell_Classification_Based_on_Attention_Residual_Feature_Pyramid_Network

Maturation sequence

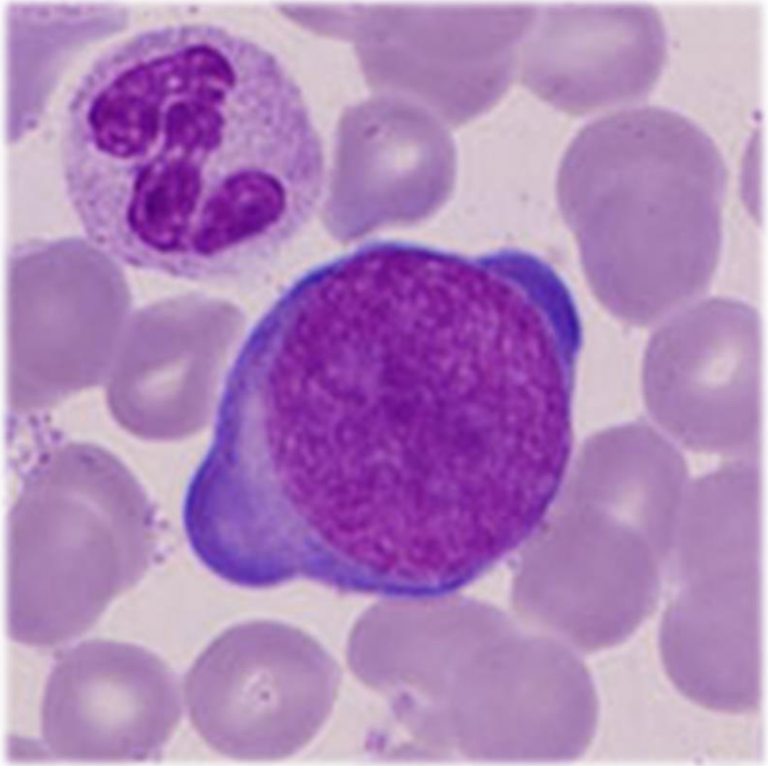
- Defining each stage based on:
  - Nucleus
  - Cytoplasm
  - Division
  - Location
  - Cellular activity
  - Length of time at this stage



https://www.medical-labs.net/normoblastic-erythropoiesis-3381/

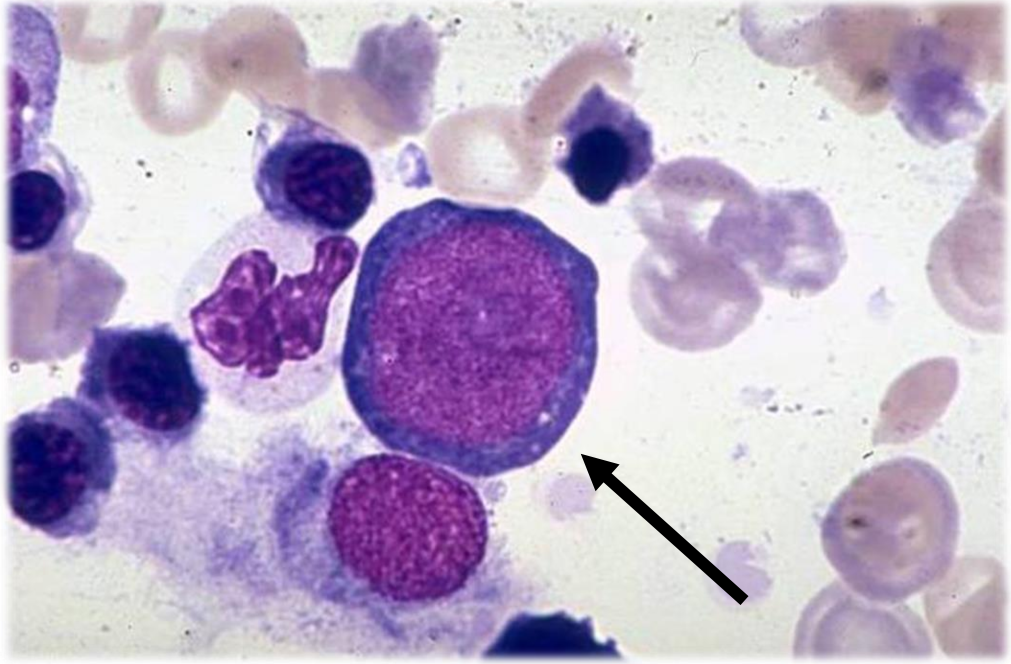
Pronormoblast

- Nucleus
  - Increased N:C ratio(8:1) , nucleoli present, purple/red chromatin
- Cytoplasm
  - Dark blue (increase in RNA)
- Division
  - Mitosis
- Location
  - Bone marrow in healthy states
- Cellular activity
  - Begin to accumulate components necessary for hemoglobin production
    - Proteins and enzymes necessary for iron uptake and protoporphyrins synthesis are produced
    - Globin production begins
- Length of time at this stage
  - Slighter longer than 24 hours

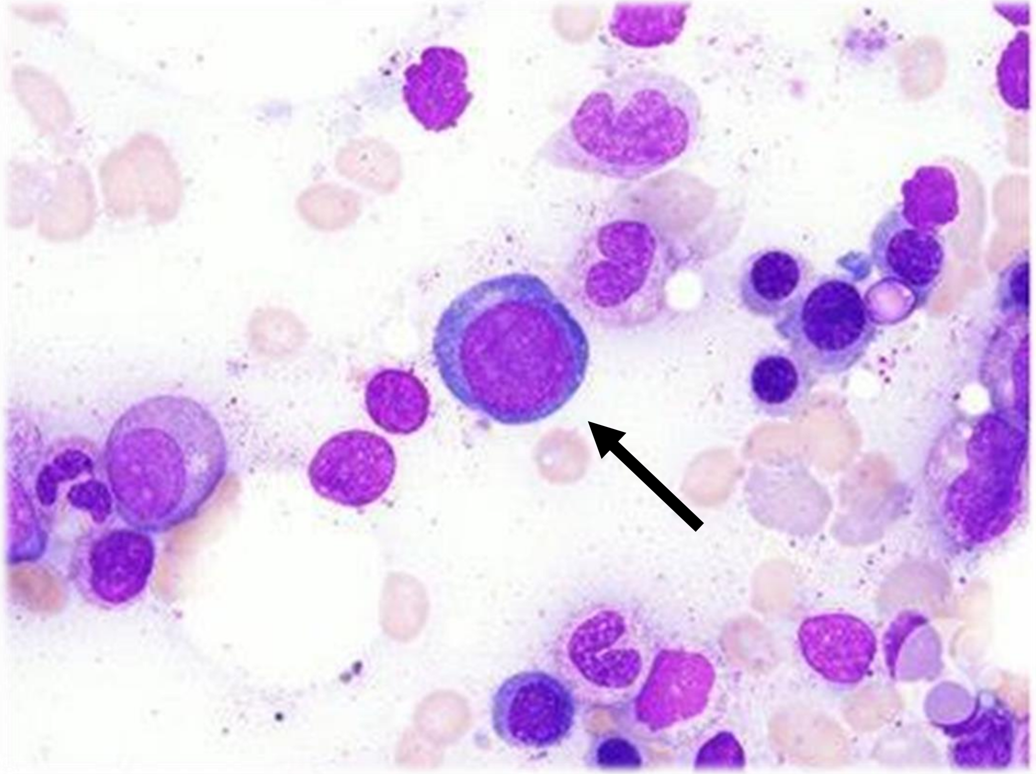


http://www.hematologyatlas.com/seq36.htm

Pronormoblast



https://www.wikidoc.org/index.php/Proerythroblast



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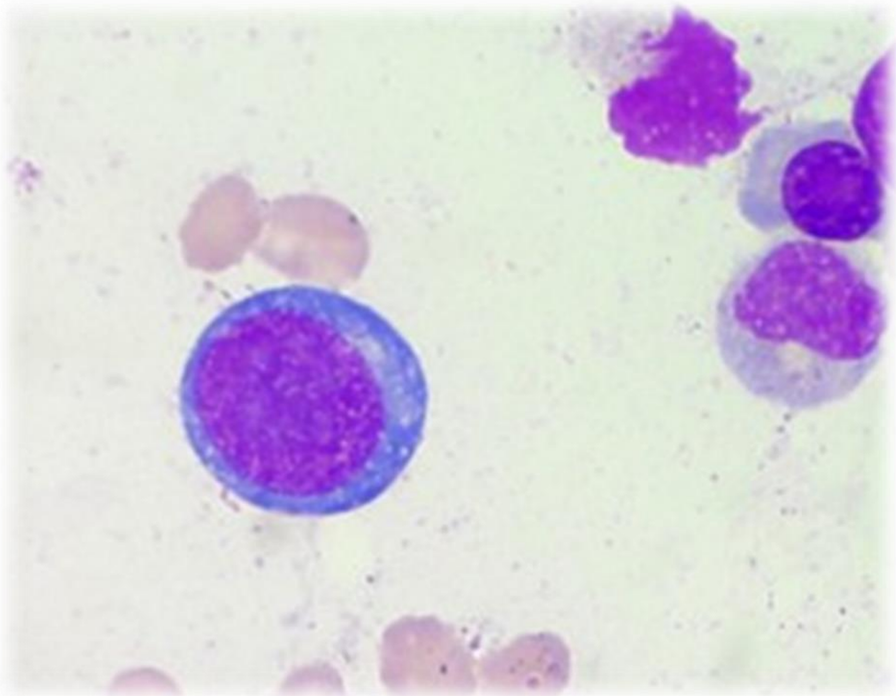
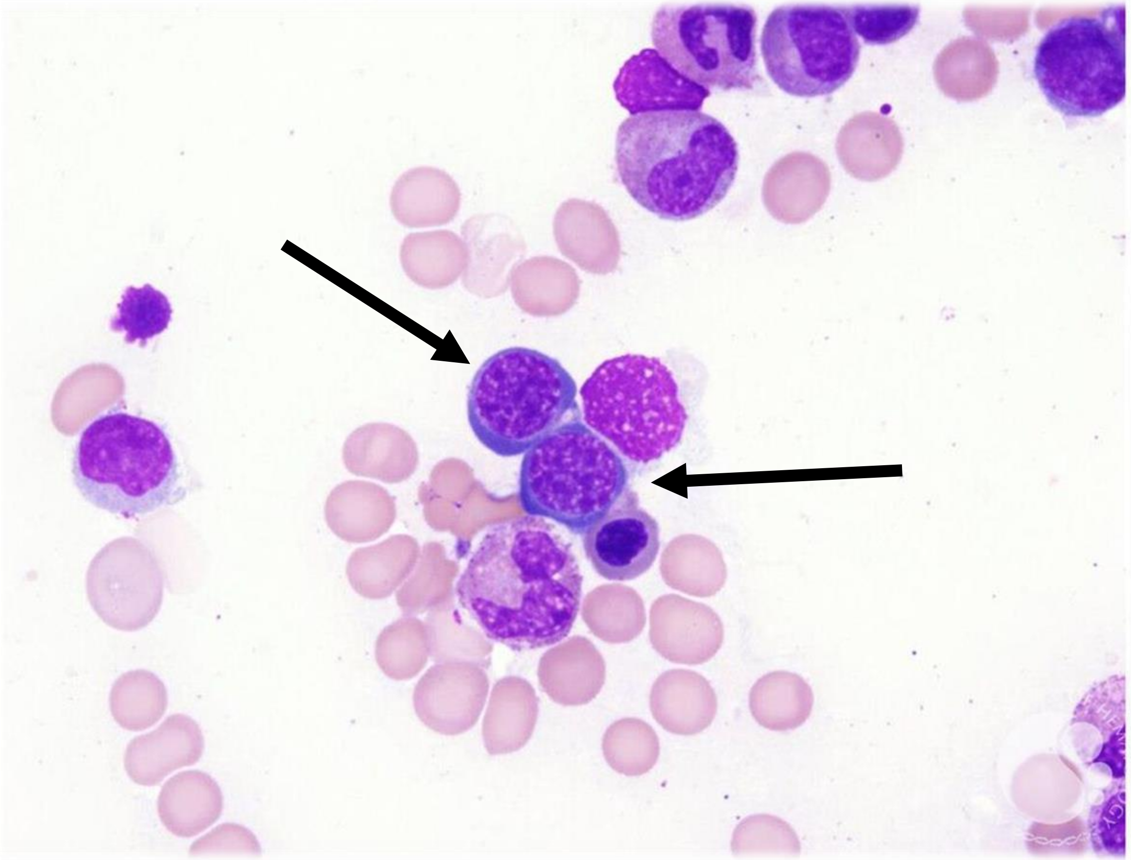
Basophilic Normoblast

- Nucleus
  - N:C ratio decreases (6:1), chromatin condenses (clumping), nucleoli may/may not be present
- Cytoplasm
  - Deeper/richer blue than pronormoblast
- Division
  - Mitosis
- Location
  - Present in the bone marrow in healthy states
- Cellular activity
  - Hemoglobin synthesis occurs
- Length of time at this stage
  - Slightly longer than 24 hours



https://www.shutterstock.com/image-photo/basophilic-normoblast-667781029

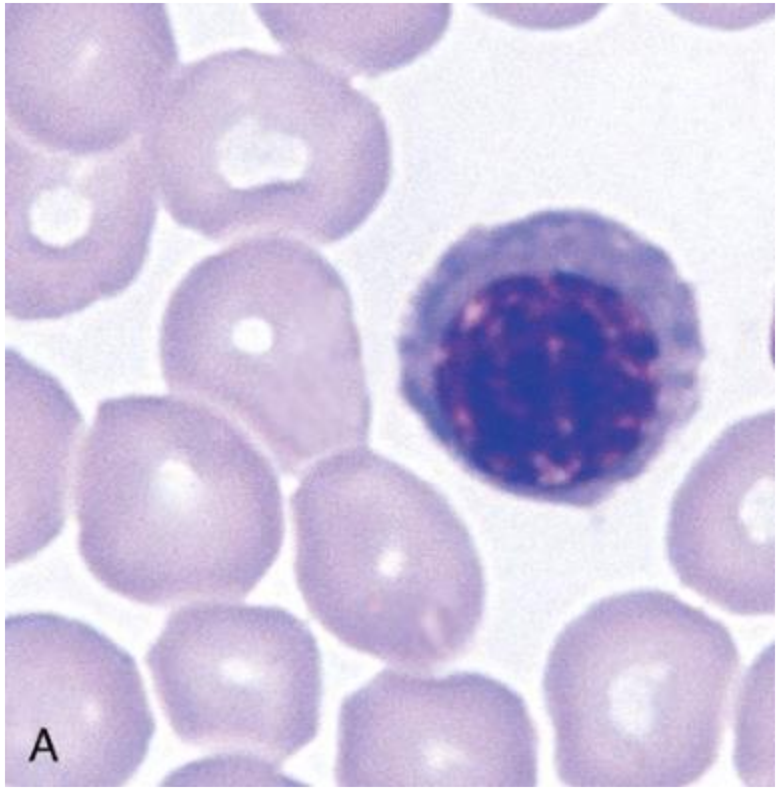
Basophilic Normoblast



https://imagebank.hematology.org/image/60298/basophilic-normoblasts?type=upload

Polychromatic Normoblast

- Nucleus
  - N:C ratio drops from 4:1 (beginning of stage) to 1:1
  - no nucleoli present
- Cytoplasm
  - "murky gray blue" cytoplasm, pink color begins to be seen
- Division
  - Last stage cell is capable of undergoing mitosis
- Location
  - Present in the bone marrow in healthy states
- Cellular activity
  - Increase in hemoglobin synthesis
  - Decrease in nucleus (decrease in DNA transcription)
- Length of time in this stage
  - Lasts approximately 30 hours



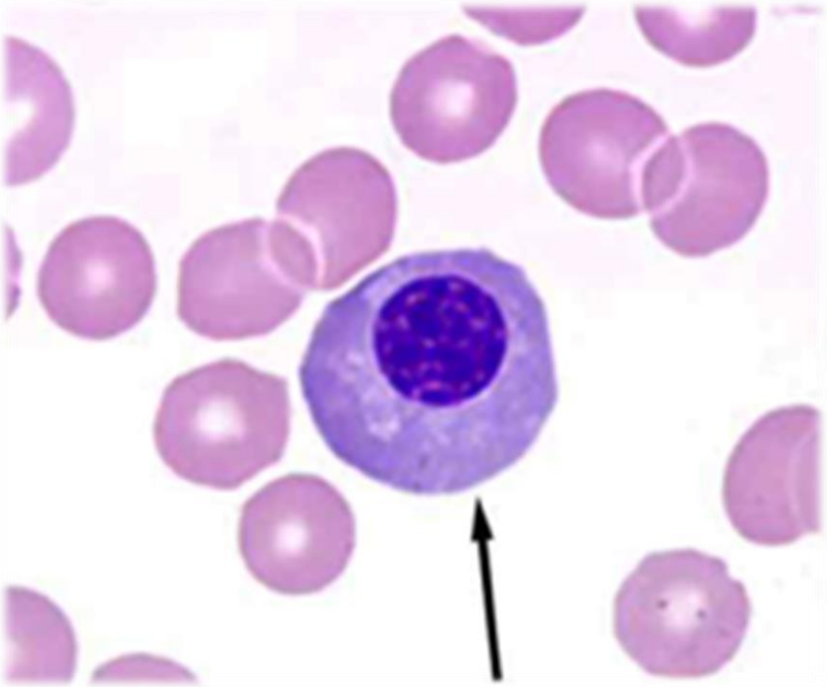
Rodak's Hematology, Clinical Principles and Applications 6th Edition

Orthochromic Normoblast

- Nucleus
  - Pyknotic nucleus, low N:C ratio (1:2)
- Cytoplasm
  - Increase in pink salmon color with a slight bluish hue
- Division
  - No division occurs
- Location
  - Present in the bone marrow of healthy individuals
- Cellular activity
  - Hemoglobin production continues
  - Later in this stage the nucleus is ejected from the cell
- Length of time in this stage
  - Approximately 48 hours

https://imagebank.hematology.org/atlas/60294

Orthochromic Normoblast



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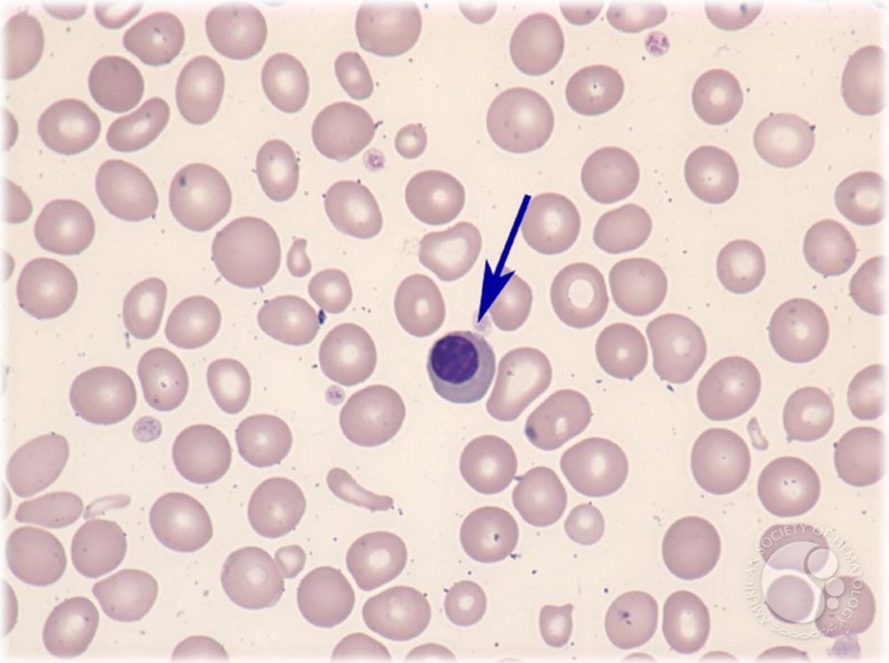


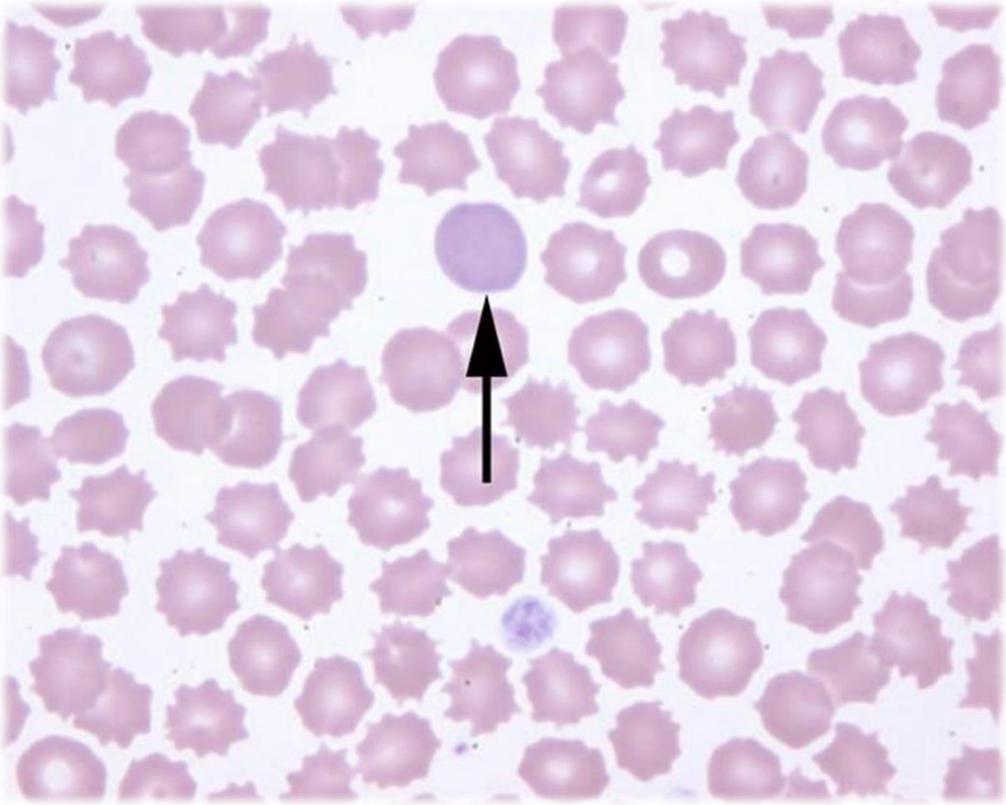
https://imagebank.hematology.org/image/4103/nucleated-red-blood-cell--1?type=upload

Orthochromic Normoblast to Reticulocyte

- As the cell matures, it loses vimentin
- The nucleus moves towards the cell membrane into a pseudo-pod like projection
  - The projection is pinched off from the cell, through the help of myosin from the cell membrane
- The enveloped extruded nucleus (pyrenocyte) is eaten by a macrophage in the bone marrow
  - Macrophage recognizes the “eat me” Phosphatidylserine flag on the pyrenocyte surface
- Small nucleus fragments left behind inside the RBC is called a Howell-Jolly bodies
  - Removed from the RBCs by pitting in the spleen
- Final result gives rise to the polychromatic erythrocyte (reticulocyte)

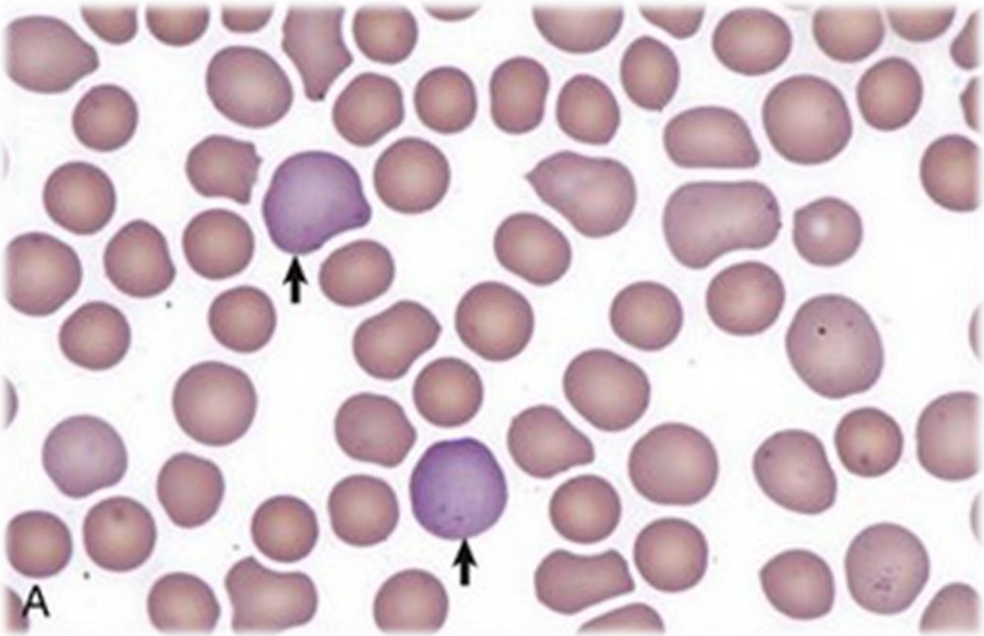
Polychromatic erythrocyte (reticulocyte)

- First stage with no nucleus
- Cytoplasm
  - Predominant pink color with bluish tinge due to residual ribosomes and RNA
  - At the end of the stage, cell is the same color as a mature RBC
- Location
  - Reside in the bone marrow for 1 to 2 days then moves to the peripheral blood for about 1 day before reaching maturity
  - Can be retained in the spleen for pitting inclusions and membrane polishing by splenic macrophages
- Cellular activity
  - Completes production of hemoglobin from small amount of mRNA
  - Cytoplasmic protein production is dismantled
    - Endoribonuclease digests ribosomes



http://criticati.com/showarticle.php?artid=5851

Polychromatic erythrocyte (reticulocyte)



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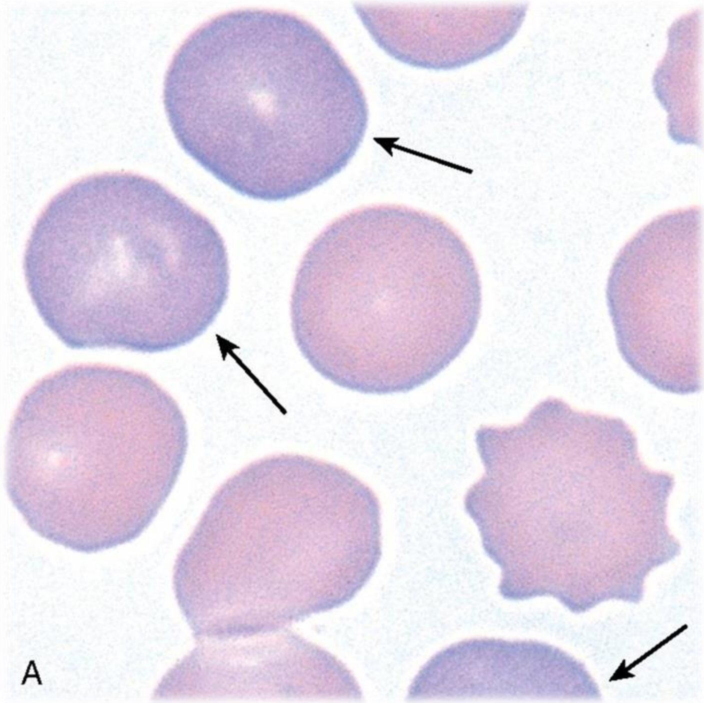
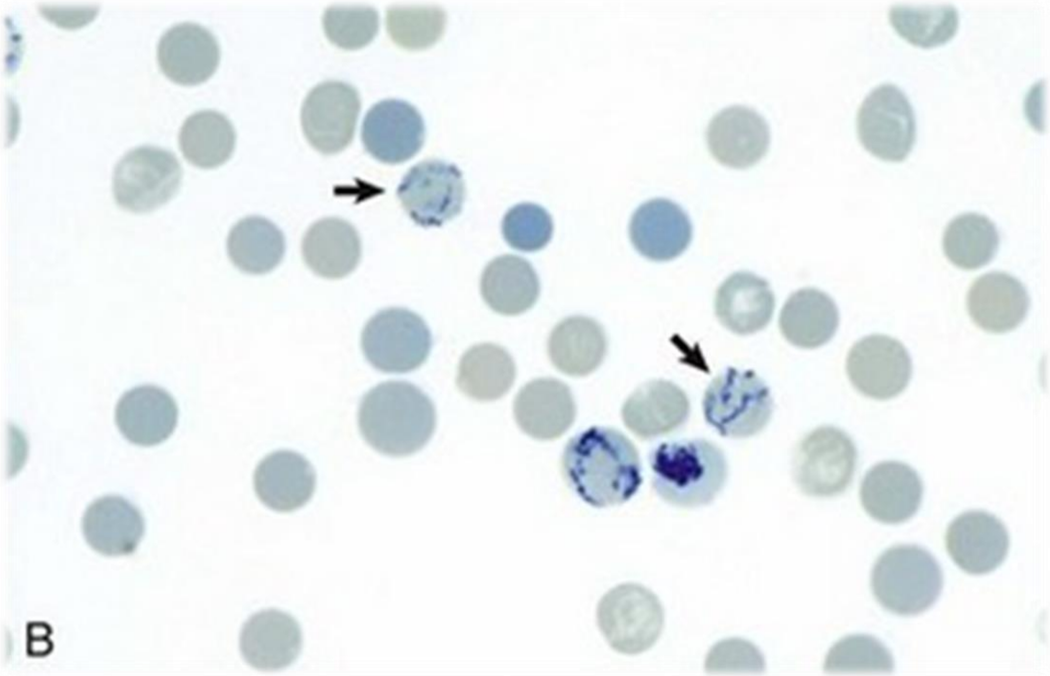


https://doctorlib.info/hematology/rodak-hematology-clinical-principles-applications/9.html

Polychromatic erythrocyte (reticulocyte)

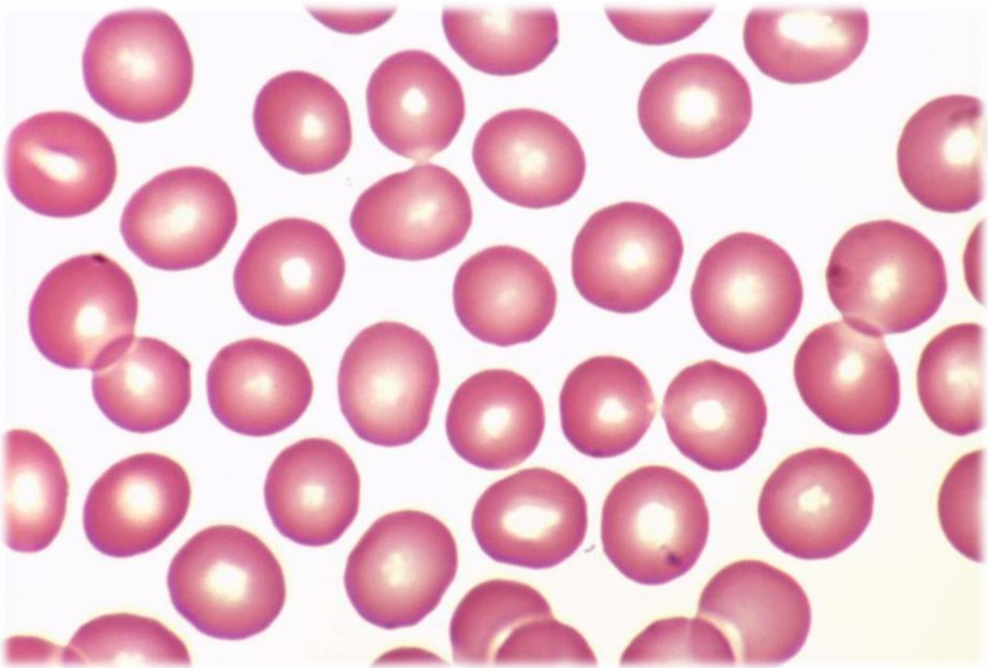
- Vital stain (new methylene blue)
  - Used to visualize the small amount of residual ribosomal RNA that is still present in the reticulocyte
  - Residual ribosomal RNA seen as blue strands (reticulum) or blue dots when more fully digested



https://veteriankey.com/bone-marrow-blood-cells-and-the-lymphatic-system/

Erythrocyte

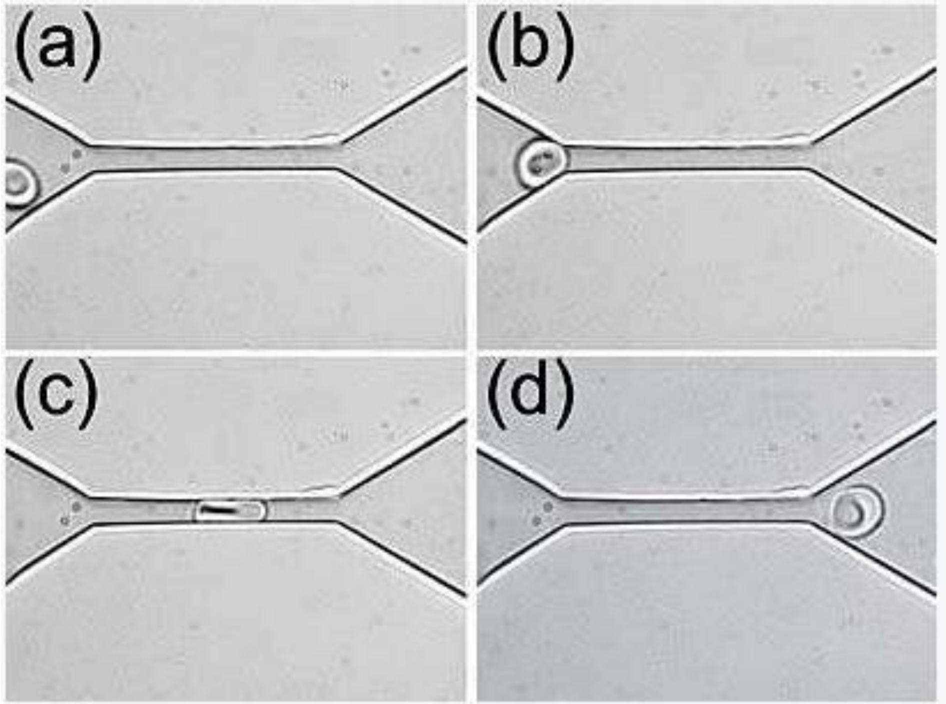
- No nucleus
- Salmon pink with central pale pallor area
- Active circulation occurs for 120 days in the peripheral blood
- 1% of RBCs die each day



http://studymedicalphotos.blogspot.com/2016/09/studying-erythrocyte-rbc-morphology-on.html

RBC Structure

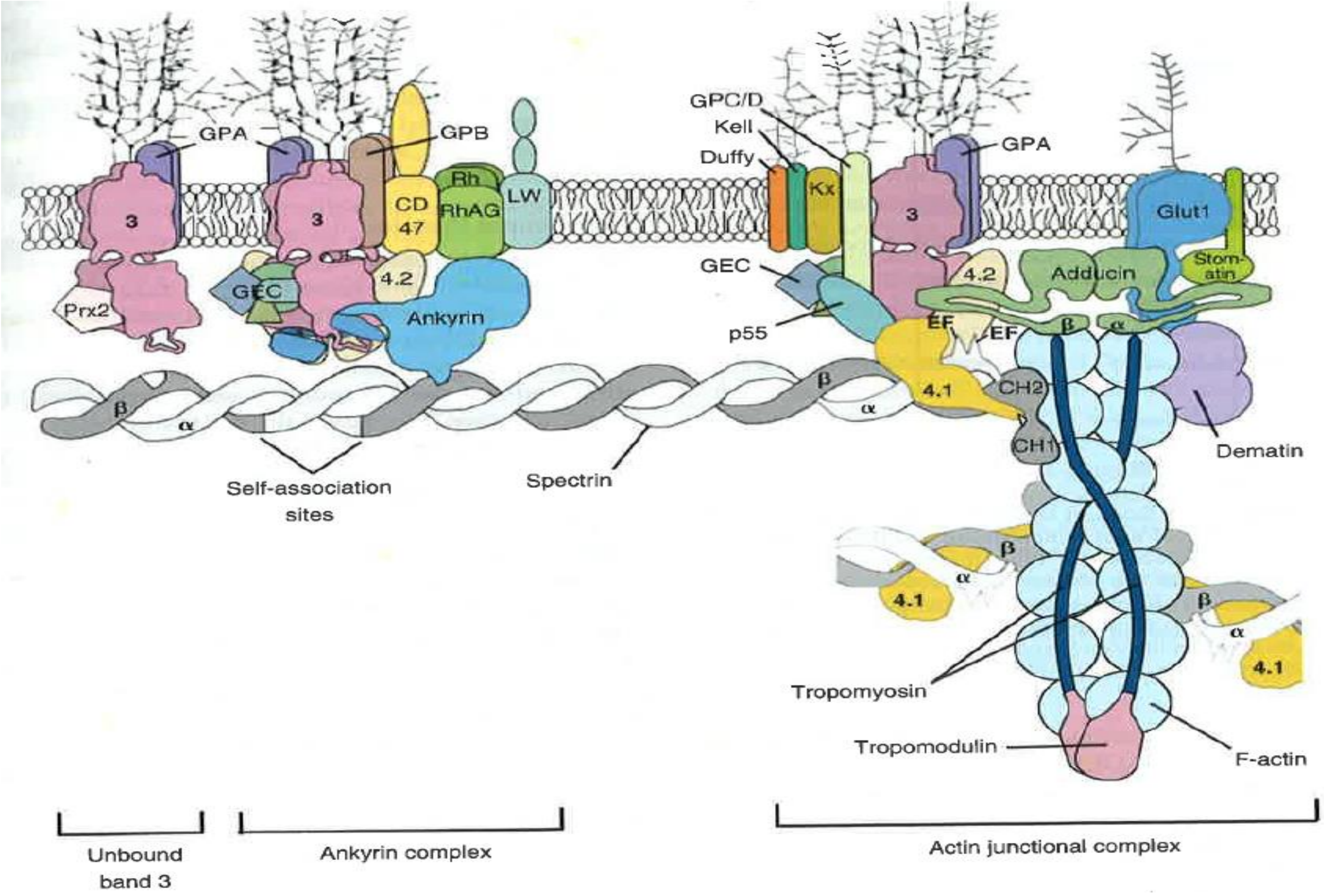
- Structure
  - Biconcave disc with no nucleus
  - Central pallor is 1/3 the diameter of the cell
  - 7-8 µm in diameter
  - Volume of 80-100 fL
- RBC deformability
  - Can stretch 2.5 times their resting diameter without damage
    - Ability to squeeze through basement membrane of BM and red pulp of spleen
  - Decreased flexibility leads to hemolysis
- RBC Membrane
  - 52% protein, 40% lipid, and 8% carbohydrate



https://news.mit.edu/2007/blood

RBC Structure

- Erythrocyte membrane
  - Lipids
    - Equal parts cholesterol and phospholipids
      - Cholesterol provides tensile strength
      - Phospholipids responsible for the impenetrable “bilayer” of the RBC membrane
        - Inner layer- predominantly phosphatidylcholine and sphingomyelin
        - Outer layer- predominantly phosphatidylserine (PS) and phosphatidylethanolamine
  - Proteins
    - Over 300 different membrane proteins and around 50 have been characterized and named
      - Many different functions including transport, adhesion, and signaling receptors
    - Example: blood group antigens



Rodak's Hematology 6th Edition, Figure 6.3

RBC Metabolic Pathway

With a lack of a nucleus and mitochondria, the RBC relies on anaerobic glycolysis for its energy

ATP is produced within the cytoplasm via Embden-Meyerhof pathway (EMP)

Embden-Meyerhof pathway (EMP)

- Anaerobic glycolysis
- Occurs for the lifetime of the cell
- Generates 90% of the ATP
- Passive function that requires glucose to generate ATP
- Glycolysis is organized into 3 phases
- Results in a net gain of 2 ATP molecules

RBC Metabolic Pathways

Glucose diversion pathways (shunts)

- 3 alternative pathways that branch from the glycolytic pathway

- Hexose monophosphate pathway (HMP)
- Methemoglobin reductase pathway
- Rapoport-Luebering pathway

RBC Metabolic Pathways

Hexose Monophosphate Pathway (HMP)

- Also known as the pentose phosphate shunt
- Extends the functional life span of the RBC by maintaining membrane proteins, lipids, enzymes, and hemoglobin iron in the functional, reduced ferrous state (Fe^{2+})
- Converts glucose → pentose and generates the reduced form- NADPH
- NADPH reduces GSSG →GSH
  - Reduces peroxides
  - Protects proteins, lipids, and heme iron from oxidation
- Extends the life of the RBC molecule by protecting RBC from degradation

RBC Metabolic Pathways

Methemoglobin reductase

- Converts ferric heme (Fe^{3+}, methemoglobin) → ferrous form (Fe^{2+})
  - Able to bind O_2
- Maintains iron in the Fe^{2+} state for effective oxygen delivery

Rapoport-Leubering Pathway

- Generates 2,3-biphosphoglycerate (2,3-BPG or 2,3-DPG)
  - Will bind between globin chains to stabilize it in the deoxygenated state
    - Enhances oxygen delivery to the tissues

Erythrokinetics

- Erythrokinetics: dynamics of RBC production and destruction
- Red blood cells can be quantified in two ways:
  - Erythron: collection of all stages of erythrocytes in the body
  - RBC mass: erythroid cells in circulation

Production

- Primary oxygen sensing system of the body is located in the peritubular fibroblasts in the kidneys
- Hypoxia- too little oxygen in the tissue
  - Detected by fibroblasts
- Fibroblasts will produce erythropoietin (EPO), major stimulatory cytokine for RBCs
  - Normally does not fluctuate
  - Increase in EPO produced during hemorrhage (RBC destruction) or other factors that decrease oxygen carrying capacity of blood
- Increase in EPO production, caused by hypoxia, is regulated by transcription factor proteins called hypoxia inducible factors (HIFs)
  - Respond to hypoxia
  - Bind to kidney hypoxia response element located on EPO gene

Erythropoietin (EPO)

- True hormone
- Produced in the kidney and acts on the bone marrow
- EPO binds to it’s receptor (EPOR) on the surface of the EPO-responsive immature erythroid cells
  - Will begin a cascade of events
    - ↑ cell division and maturation
    - ↑ intestinal iron absorption
    - ↑ hemoglobin synthesis
    - ↑ RBCs entering circulation
- EPO will increase productions of RBCs by:
  - Allowing early release of reticulocytes from the bone marrow
  - Preventing apoptosis
  - Reduce time needed for cells to mature in the bone marrow

EPO: Early release of Reticulocytes

- Normally, RBCs held in bone marrow due to expression of surface membrane receptors for adhesive molecules on the bone marrow stroma
- EPO allows the early release through 2 mechanisms
  - Increase width of advential cell layer or the bone marrow/ sinus barrier for RBC to egress into the sinus
  - Down regulate surface membrane receptors on the reticulocyte
    - Unable to attached to the adhesive molecules on the bone marrow stroma
      - Example: Fibronectin

EPO: Prevention of apoptotic cell death

- EPO increases the number of cells that will be able to mature into circulating erythrocytes
- Apoptosis: programed cell death
  - Used to get rid of RBC progenitors (CFU-Es)
- Normal RBC apoptosis
  - Fas: death receptor expressed by young erythroid precursors
  - FasL: death ligand expressed older erythroid precursors
  - These cross link together to induce apoptosis
- EPO causes indirect avoidance of apoptosis by removing an apoptosis induction signal
- EPO causes direct avoidance of apoptosis by binding to CFU-E to reduce the production of Fas ligand and stimulate production of anti-apoptotic molecules

EPO: Reduce marrow maturation time

- Increase the rate at which surviving precursors can enter circulation by
  - Increasing rate of cellular process
  - Decreasing cell cycle times
- Stimulate RNA synthesis in erythroid precursors
- Increase hemoglobin production
- Stimulate erythroid precursors to produce erythroferrone
  - Acts on hepatocytes to decrease hepcidin production
    - Allows more iron to be absorbed from the intestines for an increase in hemoglobin synthesis
- Loss of adhesive receptors (fibronectin receptor) and acquisition of egress-promoting surface molecules

Erythrocyte Destruction

- Average RBC life span is 120 days
- RBC lacks a nucleus and relies on glycolysis for ATP
  - Loss of glycolytic enzymes leads to senescence (cellular aging)
    - Leads to phagocytosis by macrophages
- <u>Extravascular Hemolysis</u>
  - Macrophage-mediated hemolysis
- <u>Intravascular (Fragmentation) Hemolysis</u>
  - Mechanical hemolysis

Extravascular Hemolysis

- Accounts for 90% of RBC death
- Lysis of the RBCs by macrophages in the spleen (and sometimes in the liver or bone marrow)
- Spleen
  - Movement of RBCs through red pulp is sluggish
    - Glucose depleted, glycolysis slows
    - pH is low, which promotes iron oxidation
    - RBC expending more energy to survive
- Decreased ATP leads to oxidation of membrane lipids and proteins
  - Phosphatidylserine (PS) exposed to macrophage receptors
- Intracellular Na^+ increases and K^+ decreases
  - Water enters the cell due to selective permeability being lost
  - Discoid shape is lost

Extravascular hemolysis

- Inflexible RBC is unable to leave the spleen
- Macrophages recognize signals on the senescent RBCs and they are targeted for ingestion and lysis
  - Iron removed from heme and stored in macrophages as ferritin
  - Globin of hemoglobin is broken down into amino acids
  - Protoporphyrin is degraded to bilirubin and released into the blood
    - Excreted as bile in the liver

Intravascular Hemolysis

- Represents 10% of normal erythrocyte destruction
- RBCs rupture intravascularly (within the lumen of blood vessels)

- Process
  - Complement is activated on the RBC membrane and lyses the erythrocytes
  - RBCs experience physical or mechanical trauma that causes destruction
    - Turbulent vasculature
    - Clots or vessel breakage
  - Toxic substances which result in lysis
    - Bacteria

- Results in fragmentation and release of cell contents into the blood

RBC Function

- Main Function
  - Transport oxygen from lungs to the tissue
- Secondary functions
  - Return carbon dioxide to the lungs
  - Buffer the pH of the blood
- Things to remember
  - Structure of the mature erythrocyte is key to being able to perform these functions effectively

References

- Rodak's Hematology, Clinical Principles and Applications 6th Edition