Erythrocyte and Hemoglobin

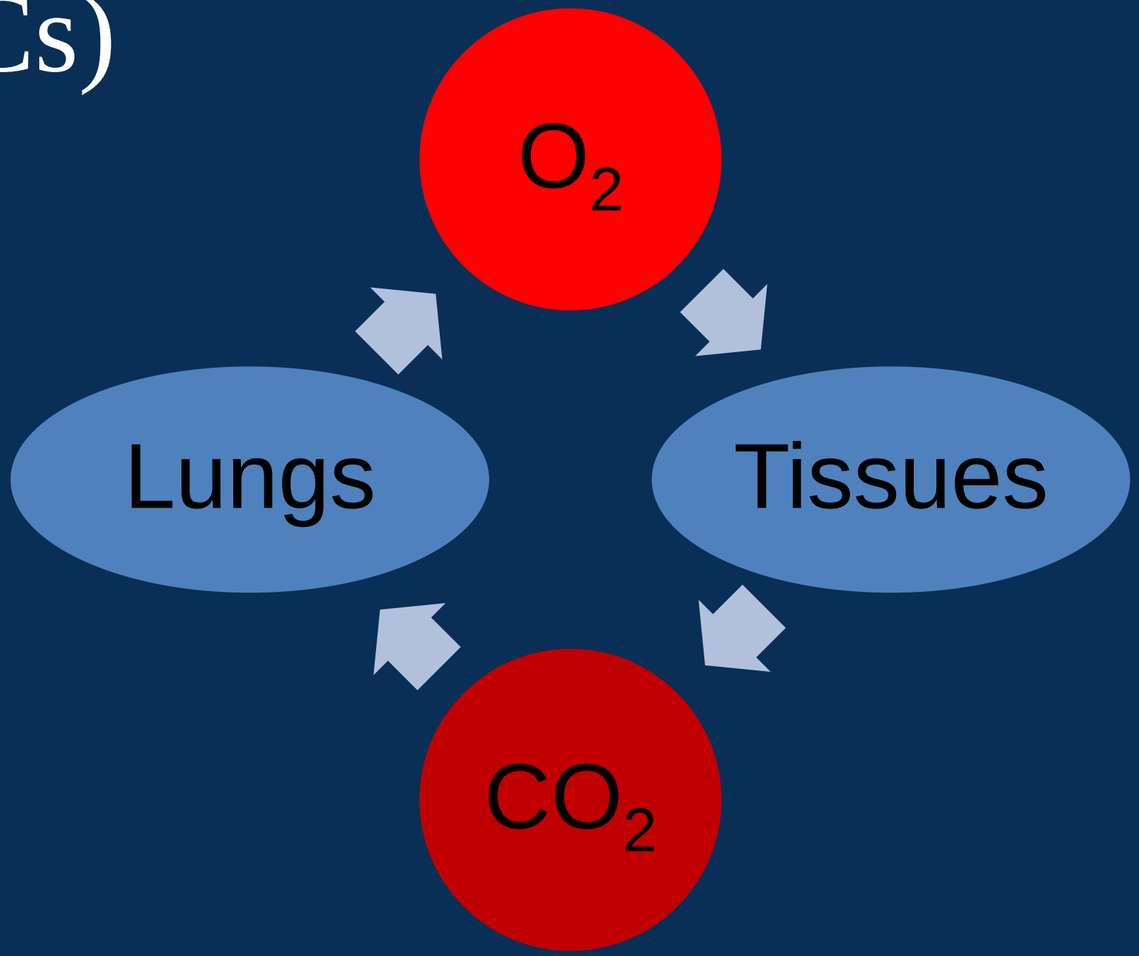
Erythrocyte

Objectives

- Erythrocyte purpose
- Morphology of maturing stages
- Erythrocyte membrane composition and the cytoskeleton support structure
- Membrane composition changes affect erythrocyte shape
- Erythrocyte function - biochemistry and energy production
- End of life processes

Erythrocytes (RBCs)

- Carries O_2 from lungs to tissues
- Carries CO_2 from tissues to lungs
- Anemia – insufficient number of RBCs
  - Tissue hypoxia – inadequate tissue oxygenation
- Erythrocytosis – excess number of RBCs
  - No adverse effect on pulmonary gas exchange

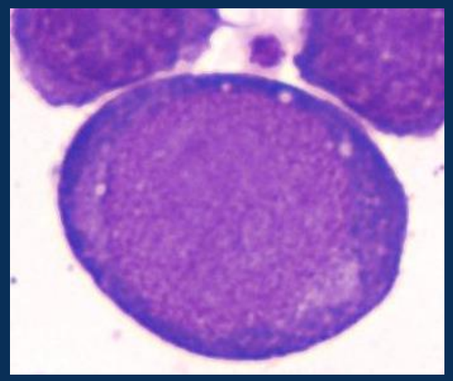


Life Cycle

- Immature
  - EPO is major cytokine regulating erythropoiesis
- Mature
  - Circulating life span of mature RBCs ~ 100–120 days
- Old
  - Senescent cells are destroyed by reticuloendothelial system macrophages
  - Occurs in spleen, liver, bone marrow

RBC Maturation

1. Gradual decrease in cell size with progressive condensation of the nuclear chromatin
2. Cytoplasm in younger cells is deeply basophilic due to the abundance of ribosomes
3. Increase in hemoglobin (acidophilic) as the cell matures, cytoplasm appears pink or salmon color
4. Eventual expulsion of the pyknotic nucleus



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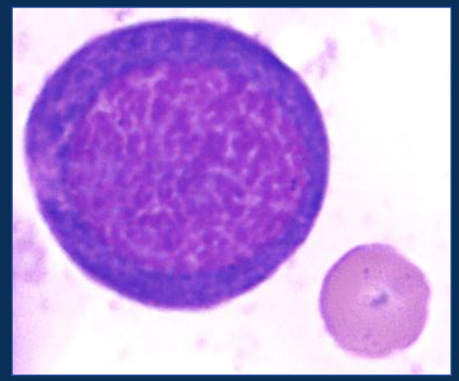

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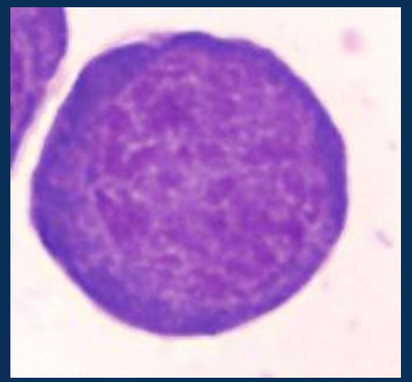

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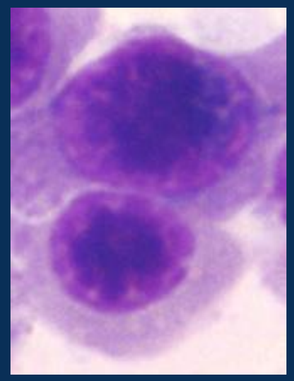

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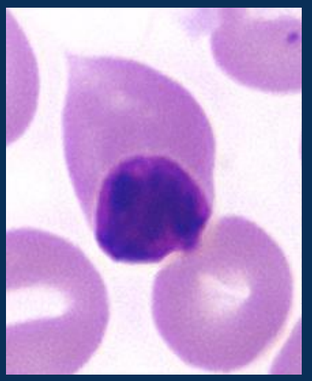

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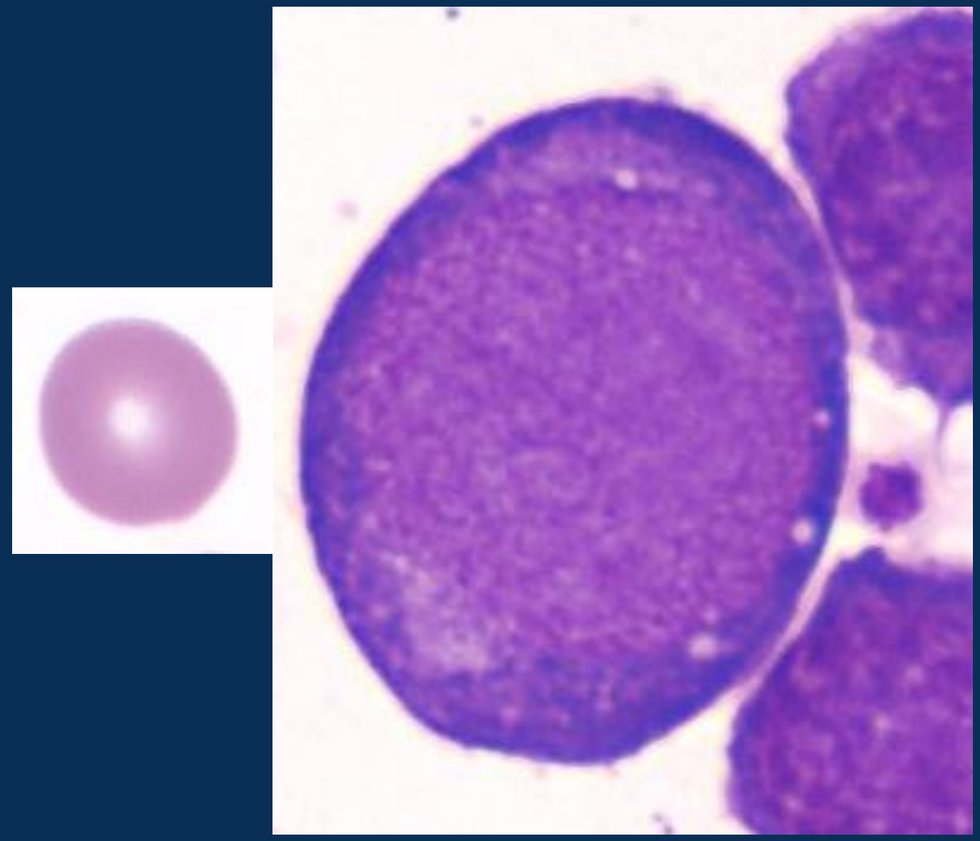
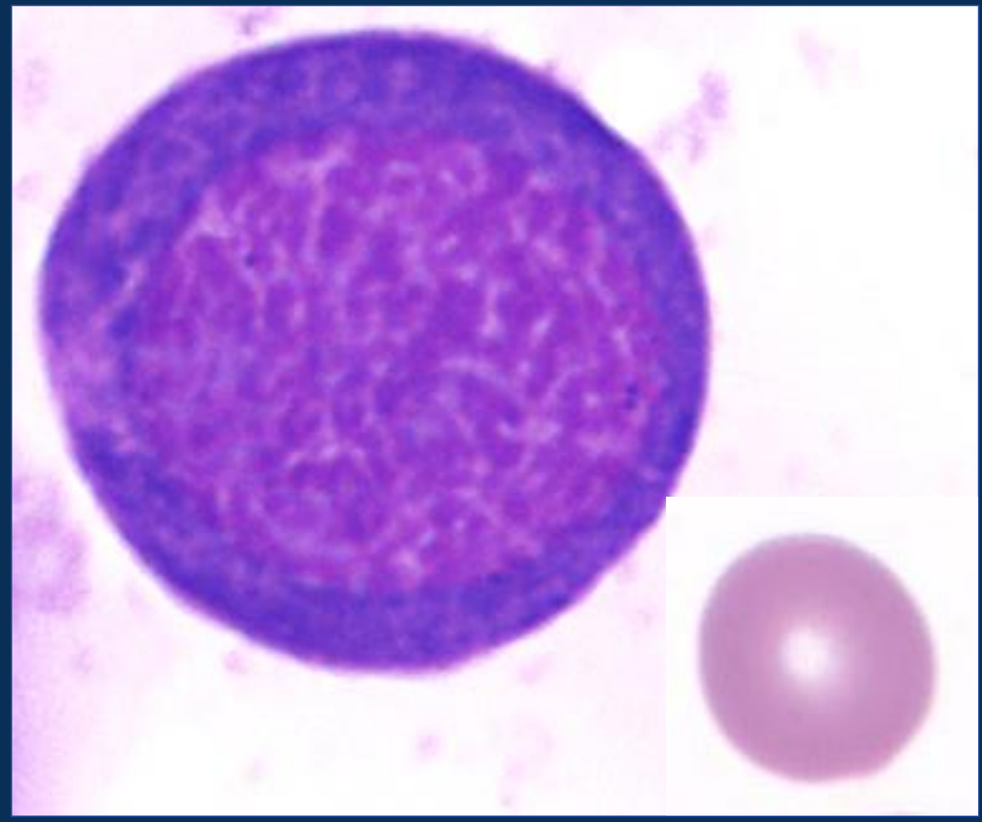
Pronormoblast (Rubriblast)

- ↑↑↑ N:C ratio
- Cytoplasm
  - ↑↑↑ ribosomes (deep basophilia)
  - Gogli apparatus
- Nucleus
  - Fine chromatin (lacy)
  - 1 – 3 nucleoli



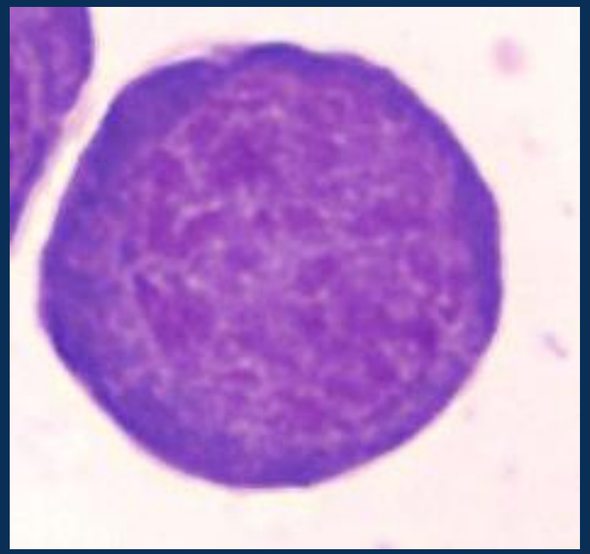
Basophilic normoblast (Prorubricyte)

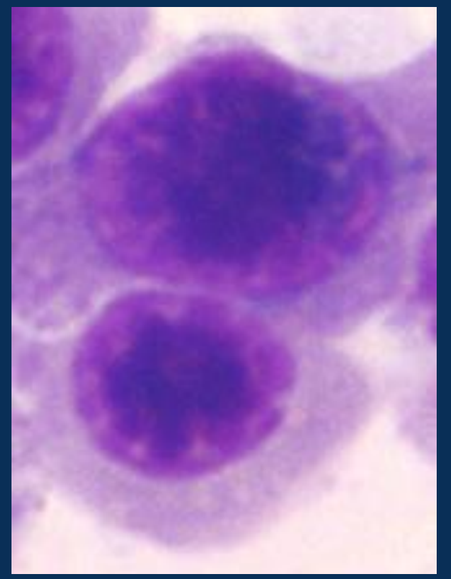
- ↑↑ N:C ratio
- Cytoplasm
  - ↑↑ ribosomes (deep basophilia)
    - Lighter with maturity
  - Perinuclear halo (mitochondria)
- Nucleus
  - Coarse/fine chromatin (wheel spoke)
  - Nucleoli not obvious



Polychromatic normoblast (Rubricyte)

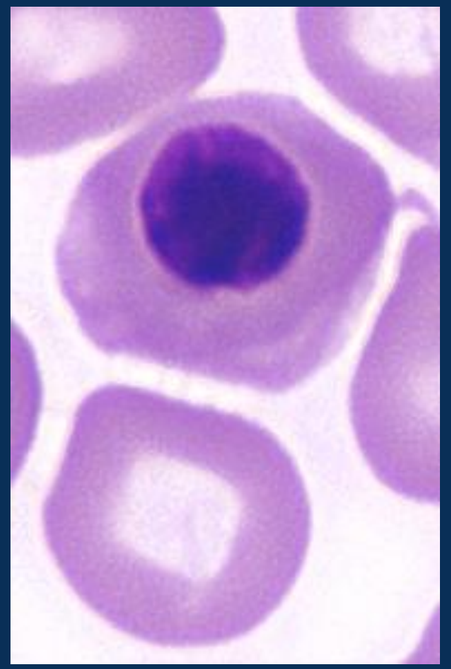
- Decreasing N:C ratio
- Cytoplasm
  - Fewer ribosomes (gray-blue)
    - ↑ hemoglobin
- Nucleus
  - Chromatin condensation
- Final mitotic stage

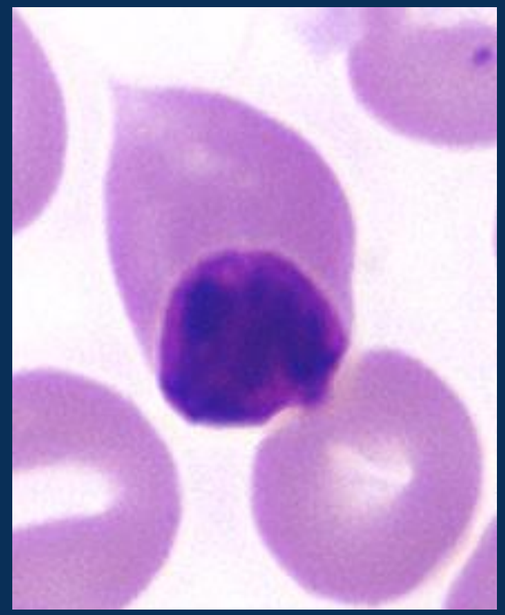




Orthochromic normoblast (Metarubricyte)

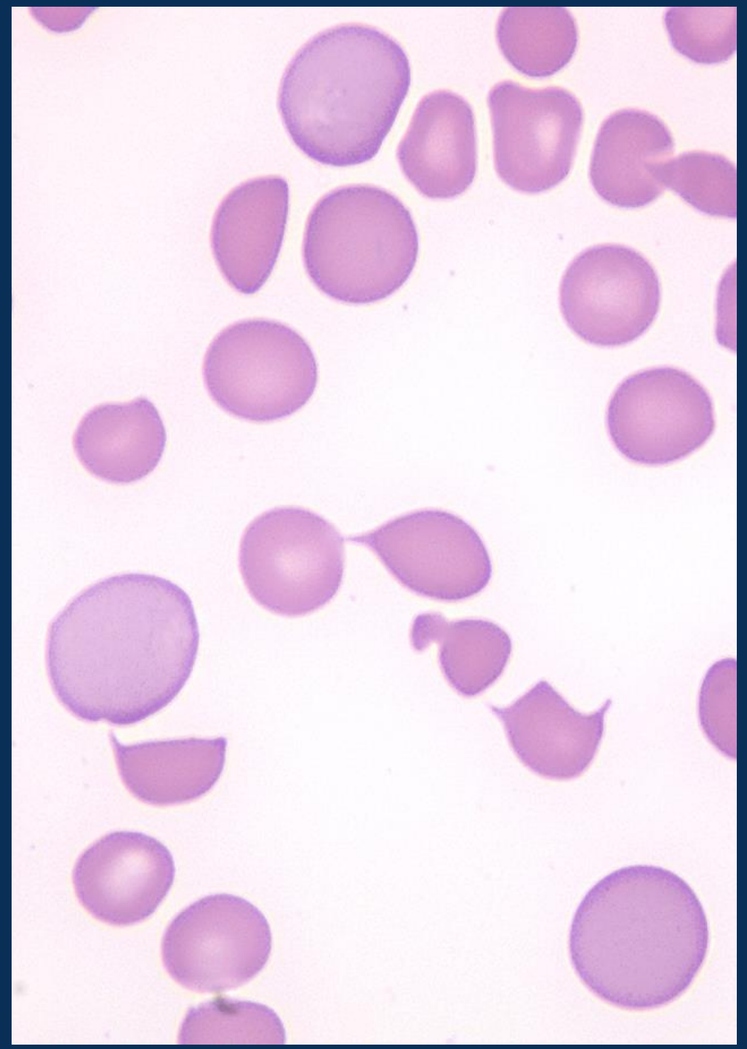
- ↓ N:C ratio
- Cytoplasm
  - ↑↑ hemoglobin (pink/salmon color)
- Nucleus
  - Chromatin heavily condensed
    - Pyknotic
  - Eccentric or partially extruded





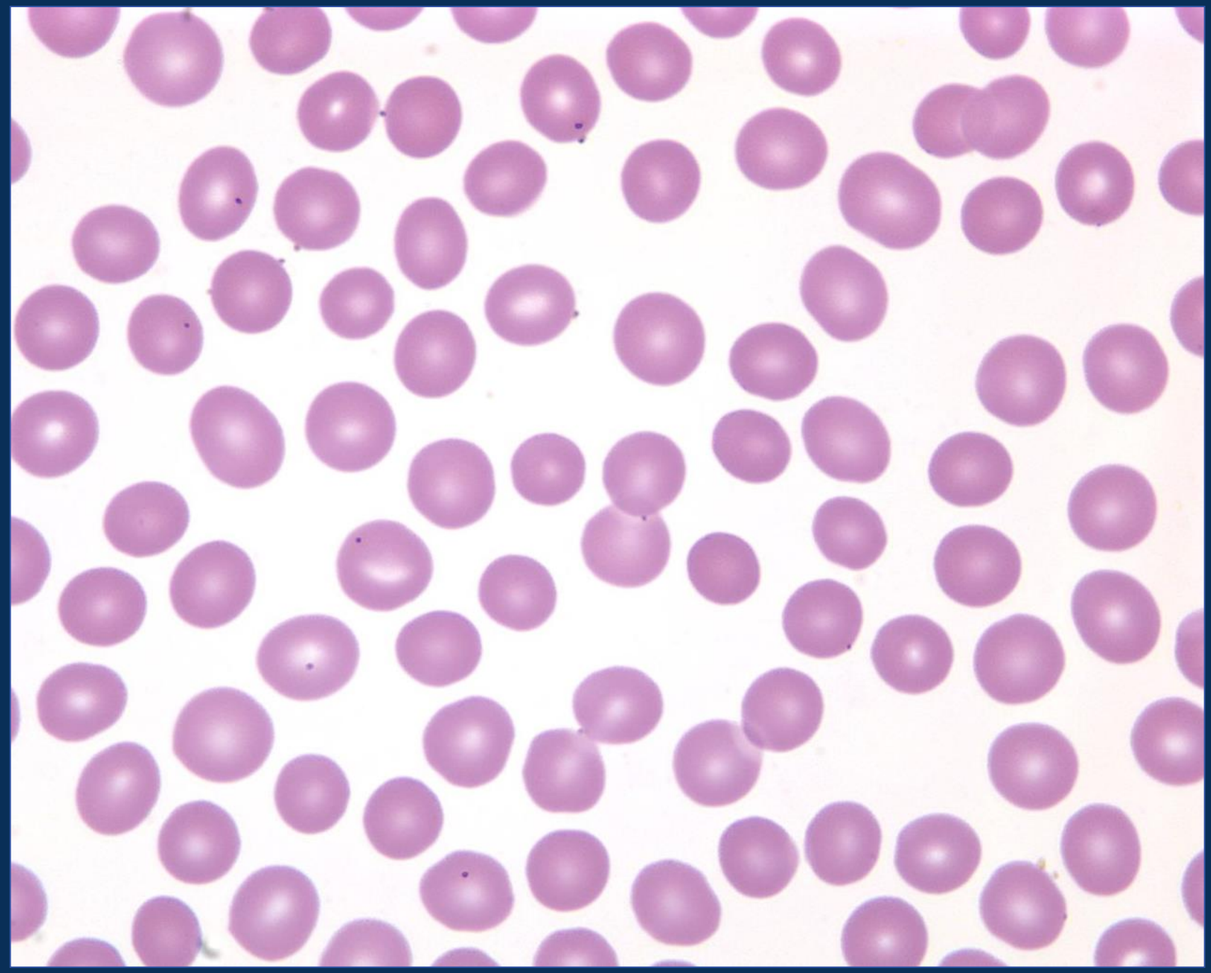
Reticulocyte

- Post extrusion, membrane remodels
- Cytoplasm
  - Bluish tinge
  - Polychromasia evaluation
- Supravital stains
  - Precipitates RNA and mitochondria
    - New methylene blue
    - Brilliant cresyl blue



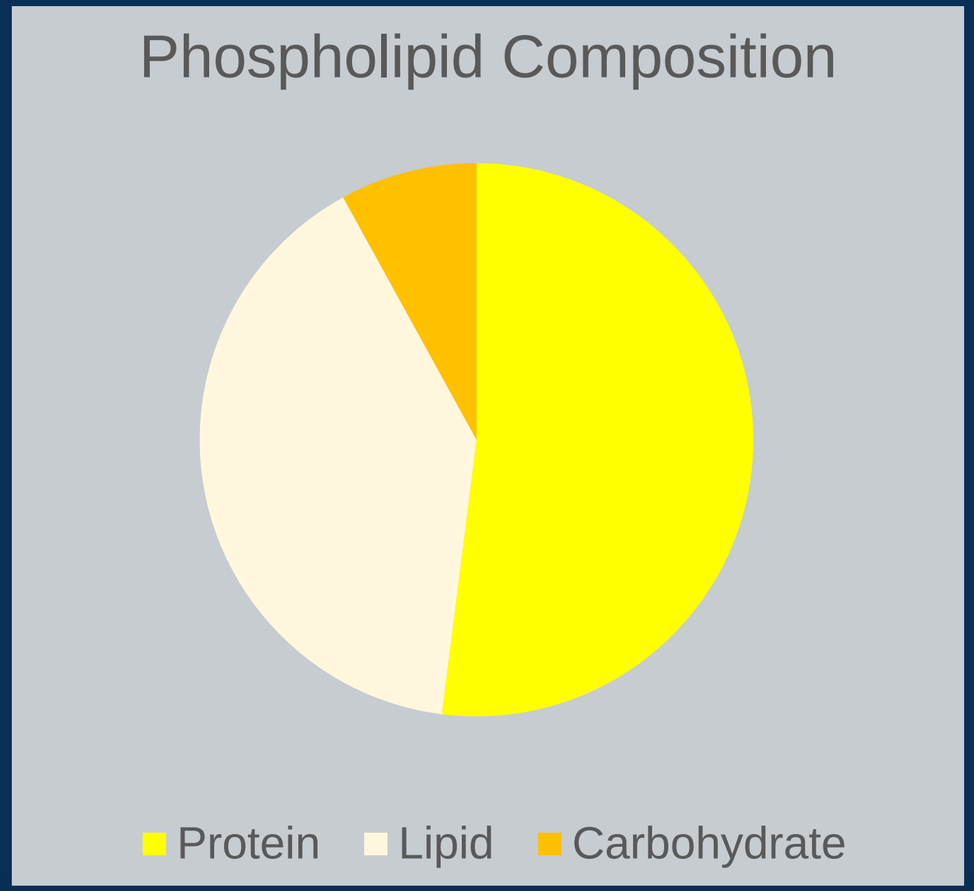
Erythrocyte

- Biconcave disc
- Lack organelles to synthesize new lipids and proteins
  - Membrane damaged RBCs culled or pitted by spleen

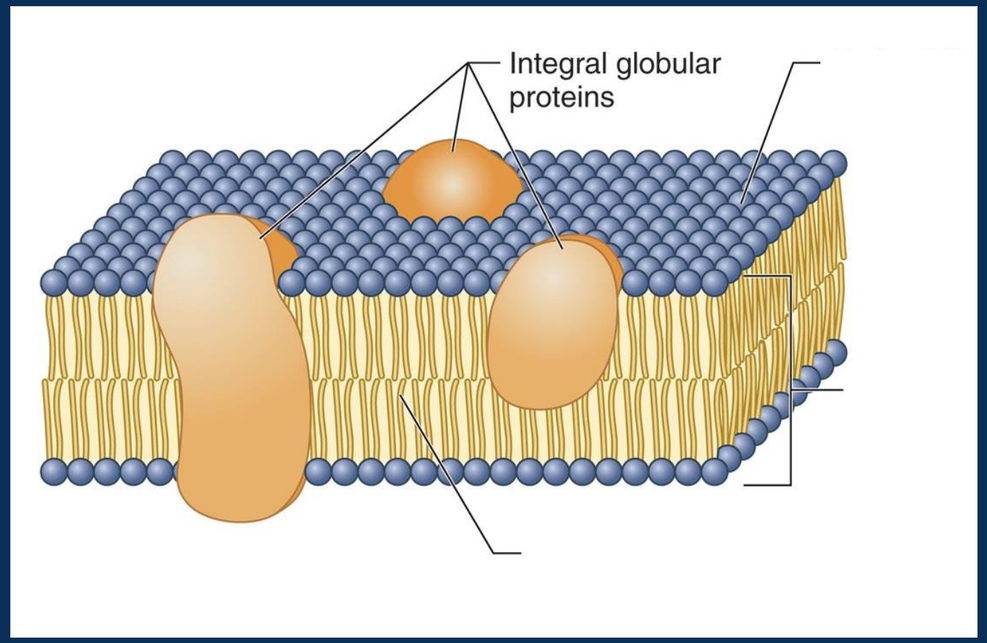


Erythrocyte Membrane

- Balances exchange of bicarbonate and chloride ions
- Controlled permeability maintains osmotic equilibrium
- Cytoskeleton provides strength and flexibility
- Phospholipid bilayer-protein complex
  - ~ 52% protein
  - ~ 40% lipid
  - ~ 8% carbohydrate



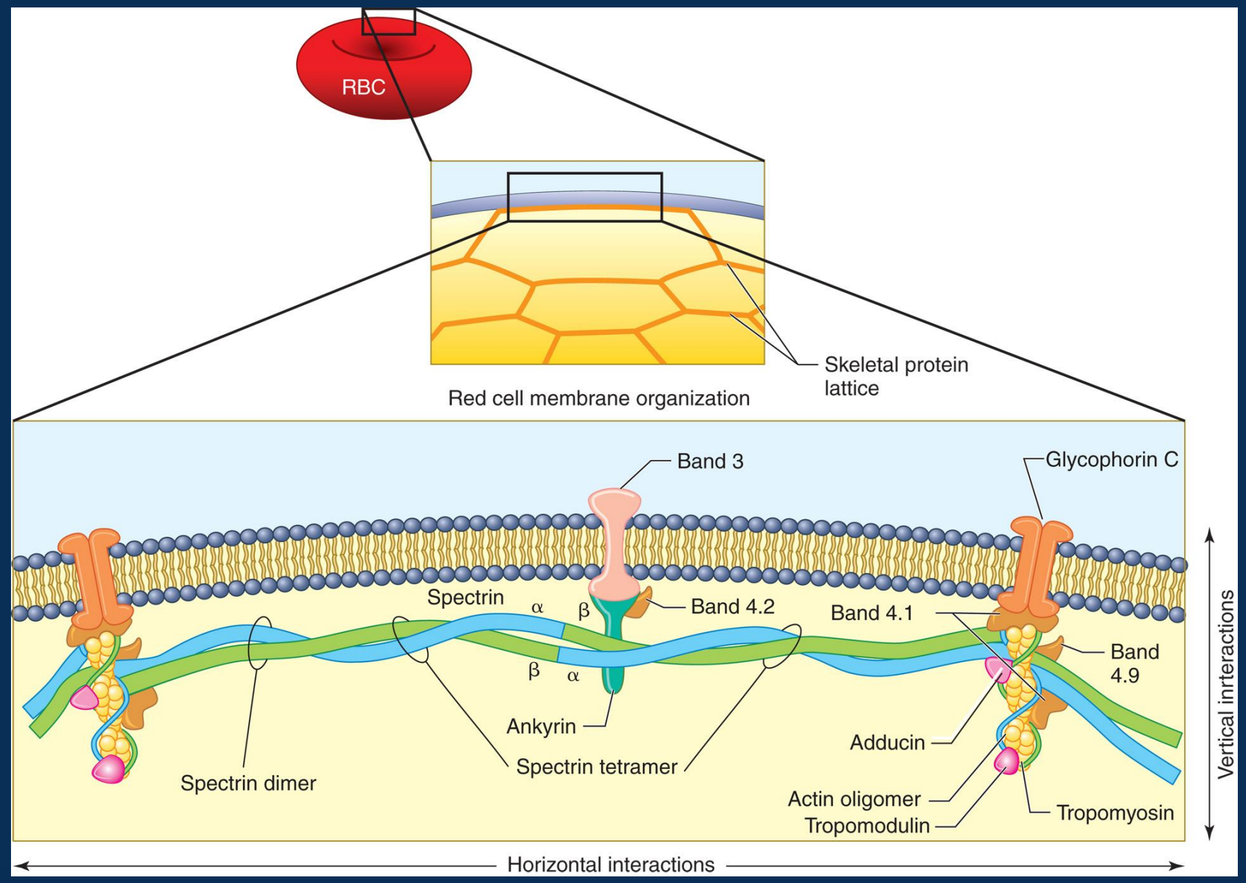
Protein Composition



- Integral proteins
  - Extracellular domain (exterior surface)
    - Responsible for zeta potential (negative surface)
      - Prevent RBCs sticking
  - Band 3
    - Major binding site for cytoplasmic membrane components
    - Transport channel for chloride-bicarbonate exchange

Protein Composition

- Peripheral proteins
  - Includes enzymes and structural proteins (SPs)
  - Spectrin (SP)
    - Predominant skeletal protein
    - Functions like a spring
  - Ankyrin (SP)
    - Binds to Band 3
    - High-affinity binding site of spectrin to inner surface



Lipid Composition

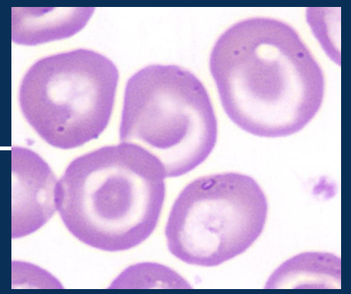
- ~95% of lipid content is equal amounts (1:1 occurrence)
  - Unesterfied cholesterol
  - Phospholipids (PLs)
- New lipids depend on exchange with plasma
- RBC shape change due to disruptions in distribution

Lipid Composition

Cholesterol : Phospholipid

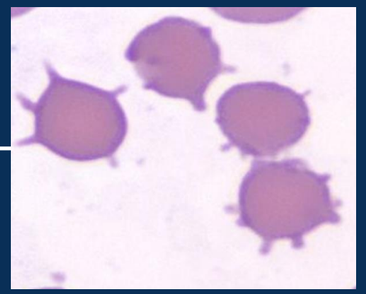
↑ CH : ↑ PL

Macrocodocyte



↑ CH : ↓ PL

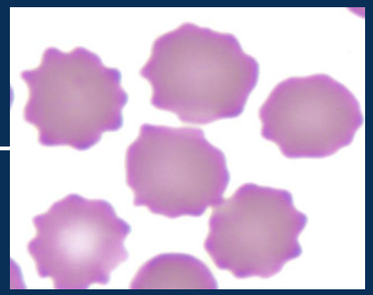
Acanthocyte



Outer Member : Inner Membrane

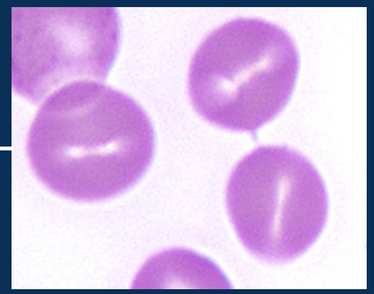
↑ OM : ↓ IM

Echinocyte

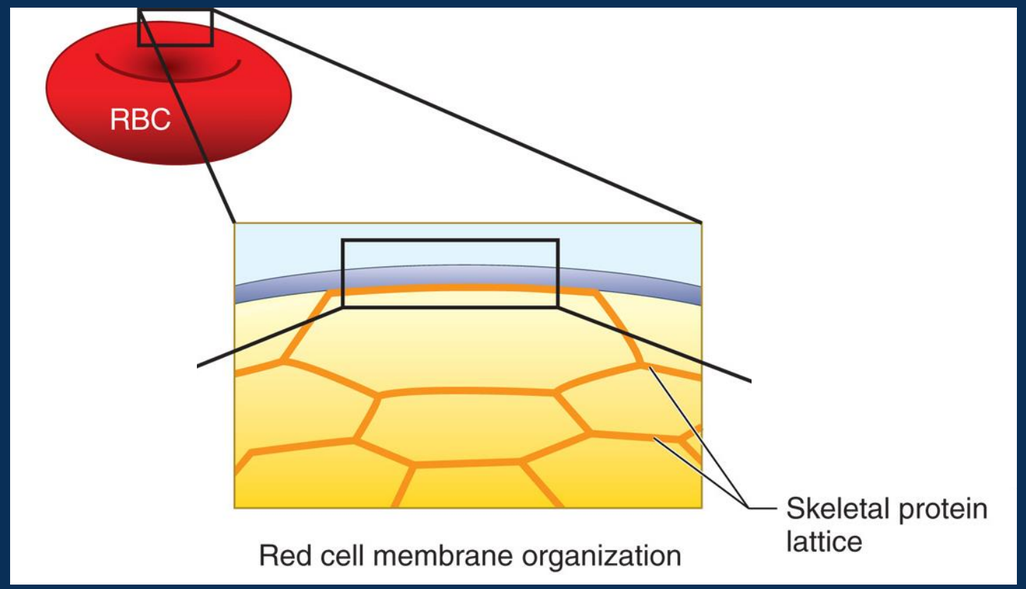


↓ OM : ↑ IM

Stomatocyte



Cytoskeleton



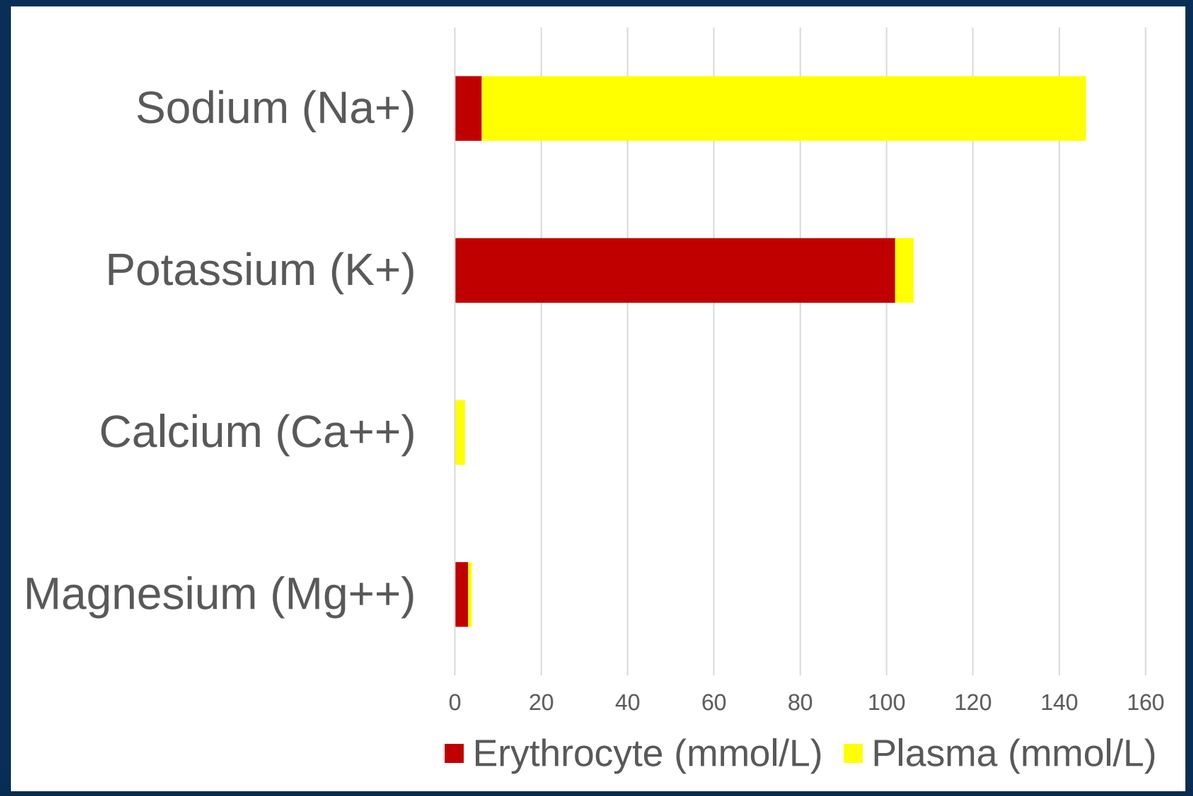
Red cell membrane organization

- Dynamic skeletal proteins
  - Continuous disassociation ↔ association equilibrium
  - Allows deformability
- ~80% intracellular Ca^{++} found associated with RBC membrane
  - Low intracellular concentration
  - [↑] reduces deformability
- Cytoskeleton + Membrane allows shape rebound

Membrane Permeability

- Freely permeable to
  - Water, anions
- Nearly impermeable (ATP required)
  - Mono/divalent cations
    - Na^+, K^+ / Ca^{++}, Mg^{++}
  - [Intracellular] ≠ [plasma]
- Glucose crosses via non-ATP transporter
  - Facilitated diffusion or passive transport

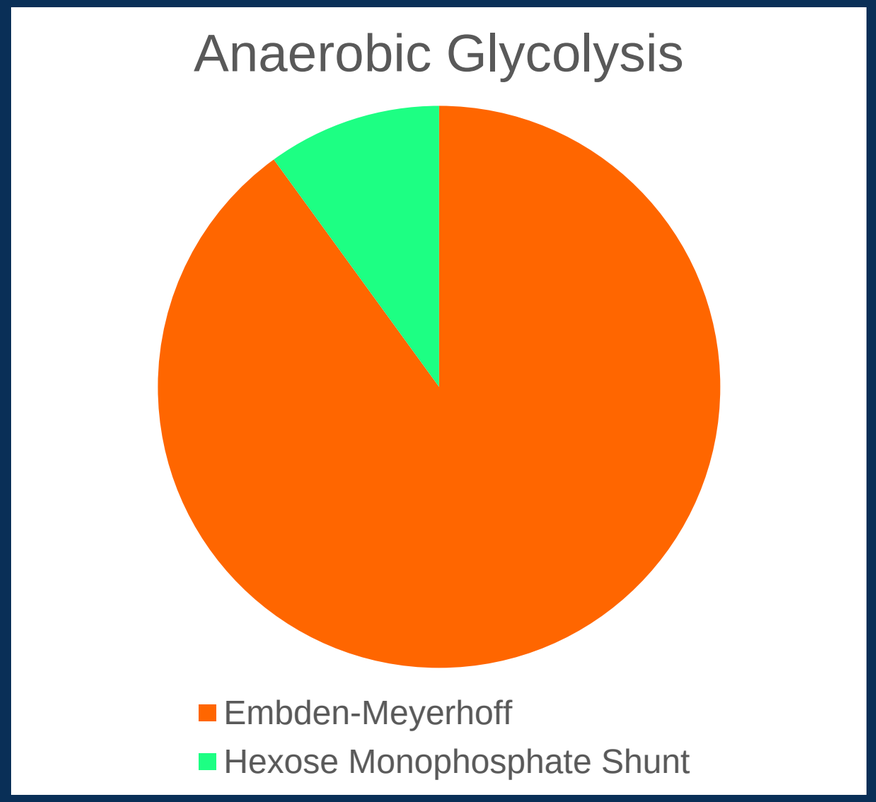
Cation	Erythrocyte (mmol/L)	Plasma (mmol/L)
Sodium (Na^+)	5.4–7.0	135–145
Potassium (K^+)	98–106	3.6–5.0
Calcium (Ca^{++})	0.0059–0.019	2.1–2.6
Magnesium (Mg^{++})	3.06	0.65–1.05



Membrane Permeability

- Osmotic equilibrium maintained by
  - Selective (low) permeability to cations
  - Cation pumps
    - Na^+/K^+ pump
      - 1 ATP = remove 3 Na^+, uptake 2 K^+
      - ↑ Ca^{++} allows Na^+/K^+ movement along gradients
    - Ca^{++} pump
      - Maintains low intracellular concentration of Ca^{++}
- Disruptions in permeability or pump failures?

Intracellular Biochemistry



- Anaerobic glycolysis (no mitochondria)
- ~90-95% glucose metabolized by glycolytic pathway
  - Embden-Meyerhof pathway
    - ATP
- ~5-10% glucose metabolized by
  - Hexose Monophosphate Shunt
    - HMP + G6PD → NADPH
    - Maintains stability of hemoglobin
    - Disruptions = Heinz bodies (denatured hemoglobin)

Intracellular Biochemistry

- Methemoglobin Reductase Pathway
  - O_2 dissociates from heme iron
  - Methemoglobin produced = iron in (ferric) state Fe^{+++}
  - Pathway + NADH maintains heme iron in reduced (ferrous) state Fe^{++}
- Rapoport-Leubering Shunt
  - Controls production of 2,3-BPG
  - Decreases O_2 affinity > releases O_2

Erythrocyte Destruction

- Mostly from senescence
  - ~90% Extravascular
    - Within macrophages of spleen, bone marrow, liver
      - Recycles RBC components
  - ~10% Intravascular
    - Trauma during circulation results in lysis
    - Plasma proteins (haptoglobin, hemopexin) bind free hemoglobin
      - Transport to liver

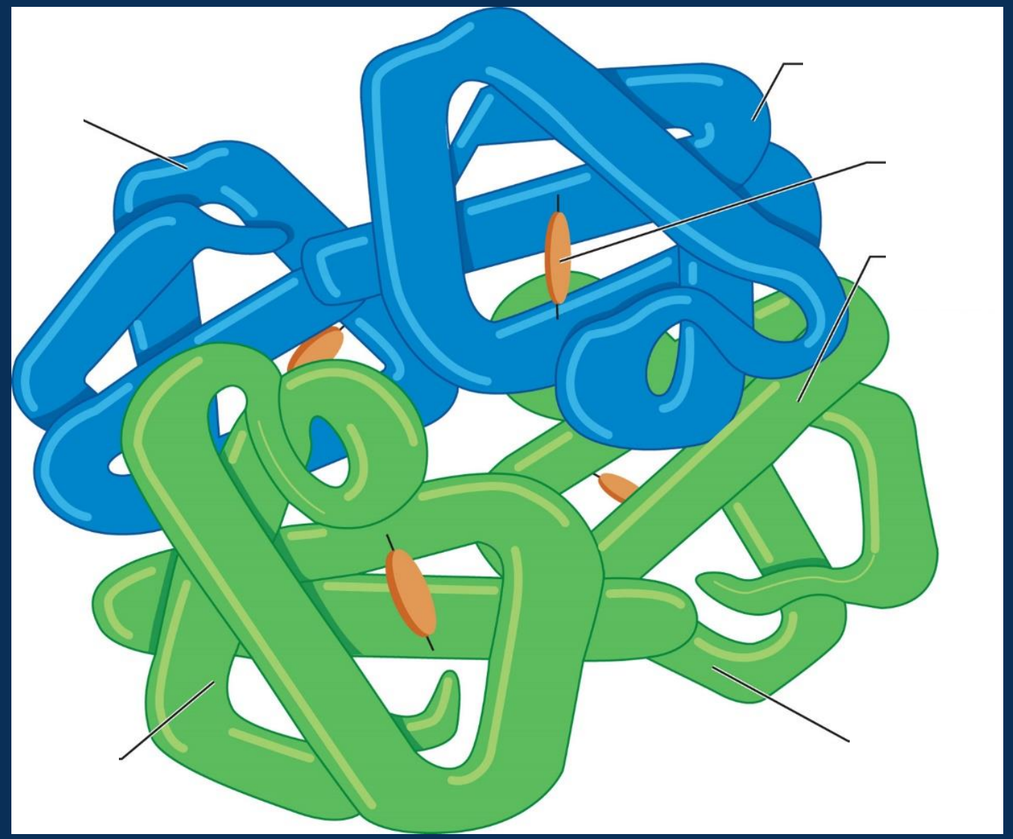
Hemoglobin

Objectives

- Hemoglobin and its structure
- Heme and globin synthesis
  - Synthesis regulation
- O_2 and CO_2 transport
  - Oxygen dissociation curve
- Hemoglobin end of life processes
- Acquired nonfunctional hemoglobins

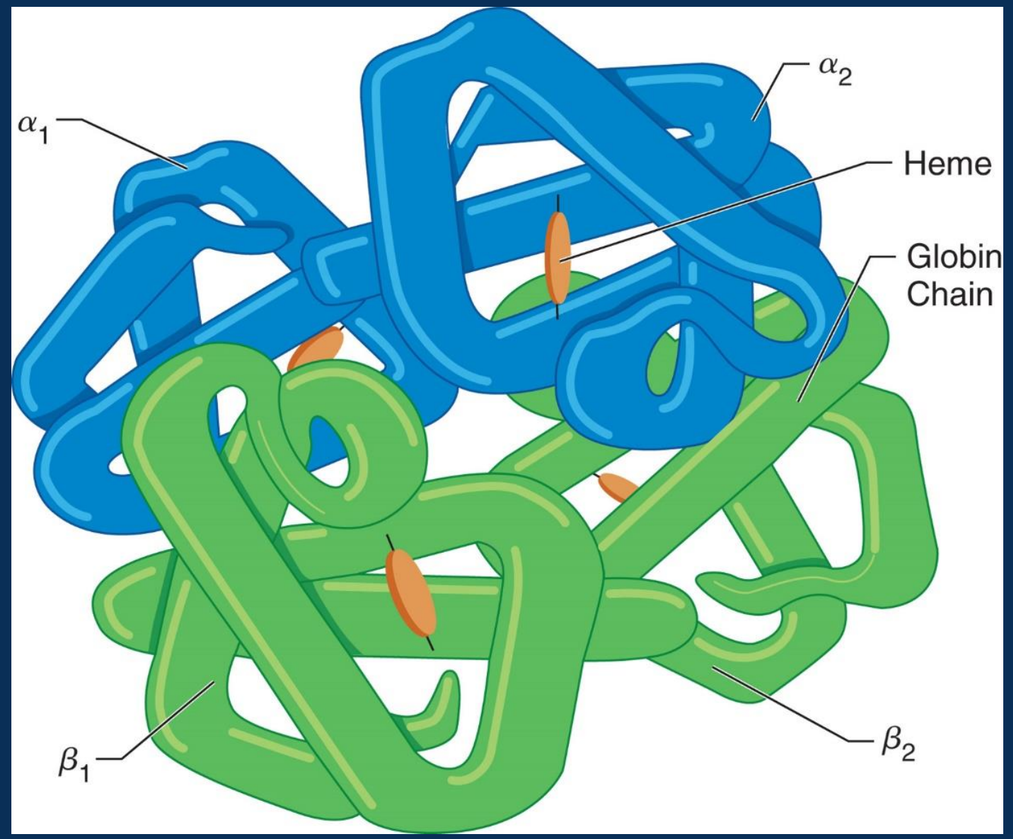
Hemoglobin

- Transports O_2 from lungs to tissues
- Facilitates CO_2 from tissues to lungs
- 33% volume of RBC
  - ~90% of RBC dry weight
  - MCH = 28 – 34 pg
  - MCHC = 32 -36 g/dL
- Total Hgb = 12 – 17 g/dL



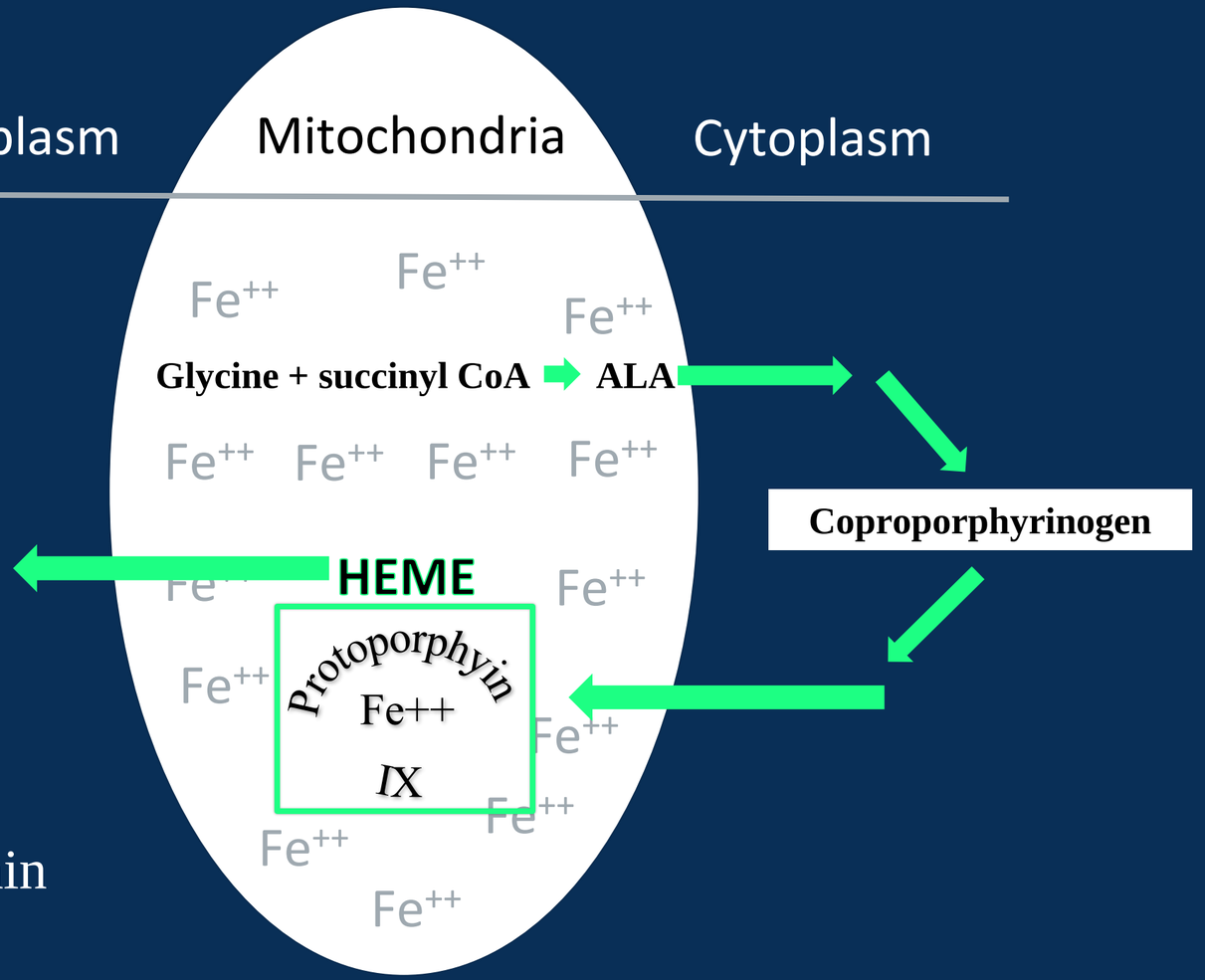
Hemoglobin Structure

- Four globular proteins
  - Two alpha-like
    - Alpha (α), zeta (ζ)
  - Two non-alpha
    - Epsilon (ε), beta (β), delta (δ), gamma (γ)
- Four subunits of heme
  - Iron-chelated porphyrin ring



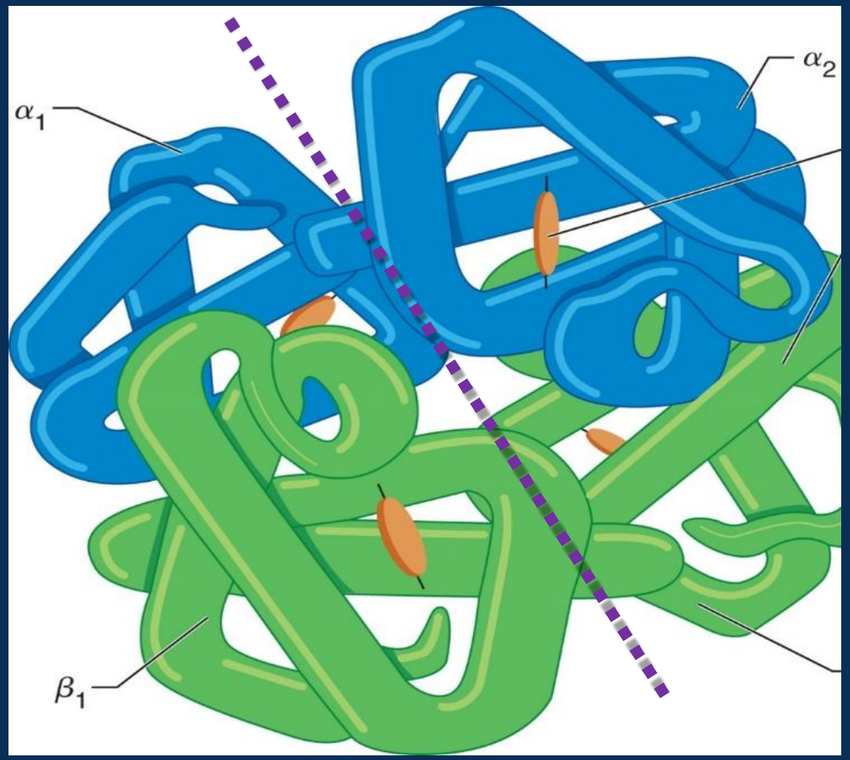
Heme Synthesis

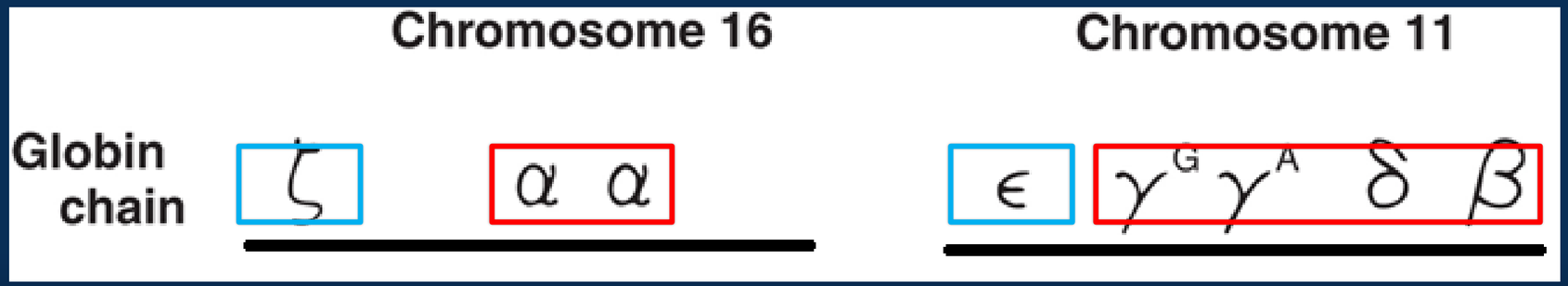
- Begins in mitochondria
  - Glycine + succinyl CoA => ALA
    - [iron] limits rate of ALA production
- Continues in cytoplasm
  - Eventual production of Coproporphyrinogen
- Renters and finishes in mitochondria
  - Protoporphyin IX chelates with iron => heme
- Heme enters cytoplasm to bind with a globin chain



Globin Synthesis

- ζ and ε found only in embryonic
- After birth, α- and β-chain production predominates
  - ~97% of adult hemoglobin
- Released from polyribosomes in cytoplasm
  - Heme falls into hydrophobic pocket
- Dimers form into tetrameric hemoglobin



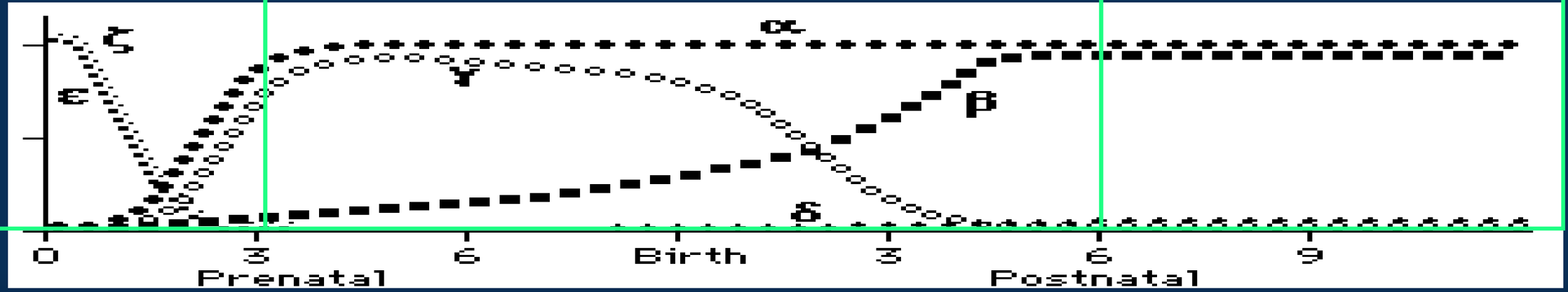


Hemoglobin Synthesis Regulation

- Normally equal production of α-, non-α-subunits, and heme
- Regulators
  - Concentration of iron
  - Concentrations of enzymes in heme production
    - Some depend on presence iron
  - Activity rate of DNA to mRNA
    - ↑ heme inactivates translation inhibitor

Hemoglobin Types

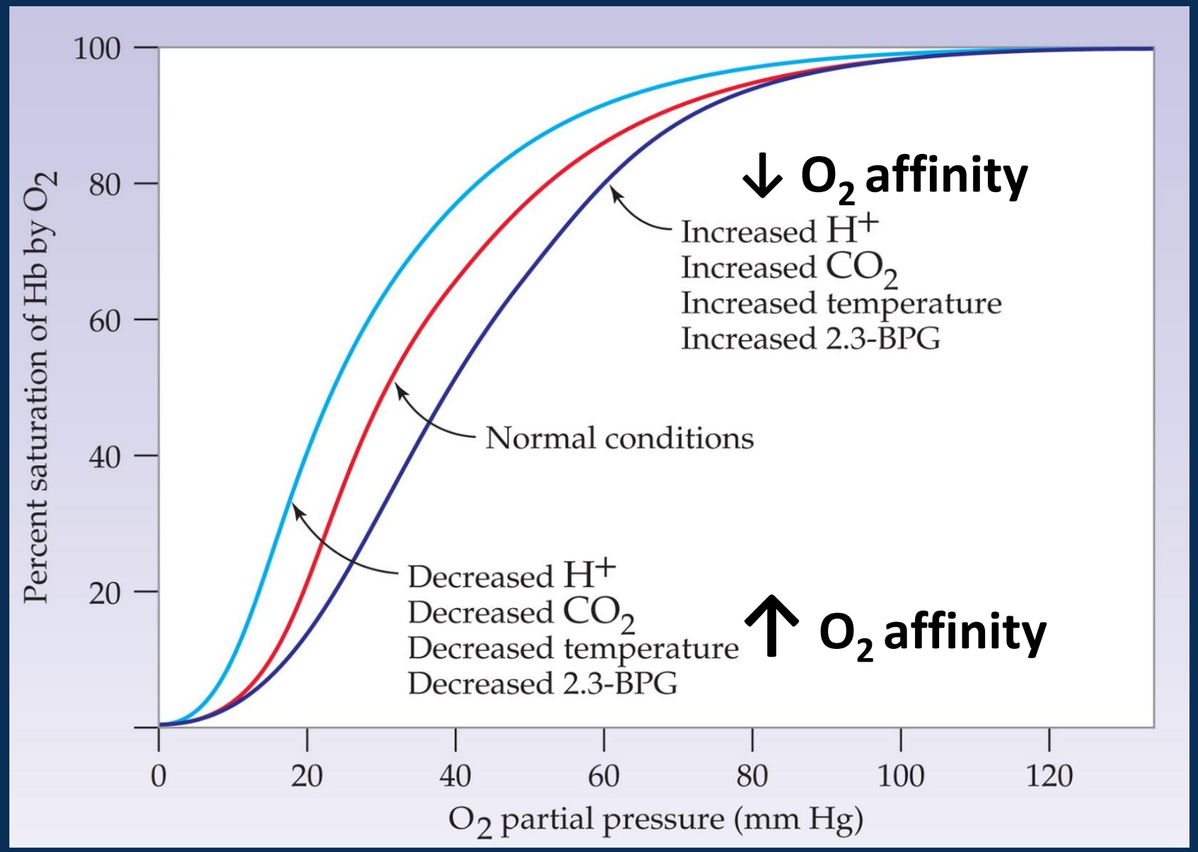
- Embryonic
  - Gower I ($\zeta_2\varepsilon_2$)
  - Gower 2 ($\alpha_2\varepsilon_2$)
  - Portland ($\zeta_2\gamma_2$)
- Fetal
  - HbF ($\alpha_2\gamma_2$)
- Adult
  - > 95% HbA ($\alpha_2\beta_2$), includes ~ 3.5% HbA_{1c}
  - < 2% HbF ($\alpha_2\gamma_2$)
  - 1.5–3.5% HbA_2 ($\alpha_2\delta_2$)



Oxygen Transport

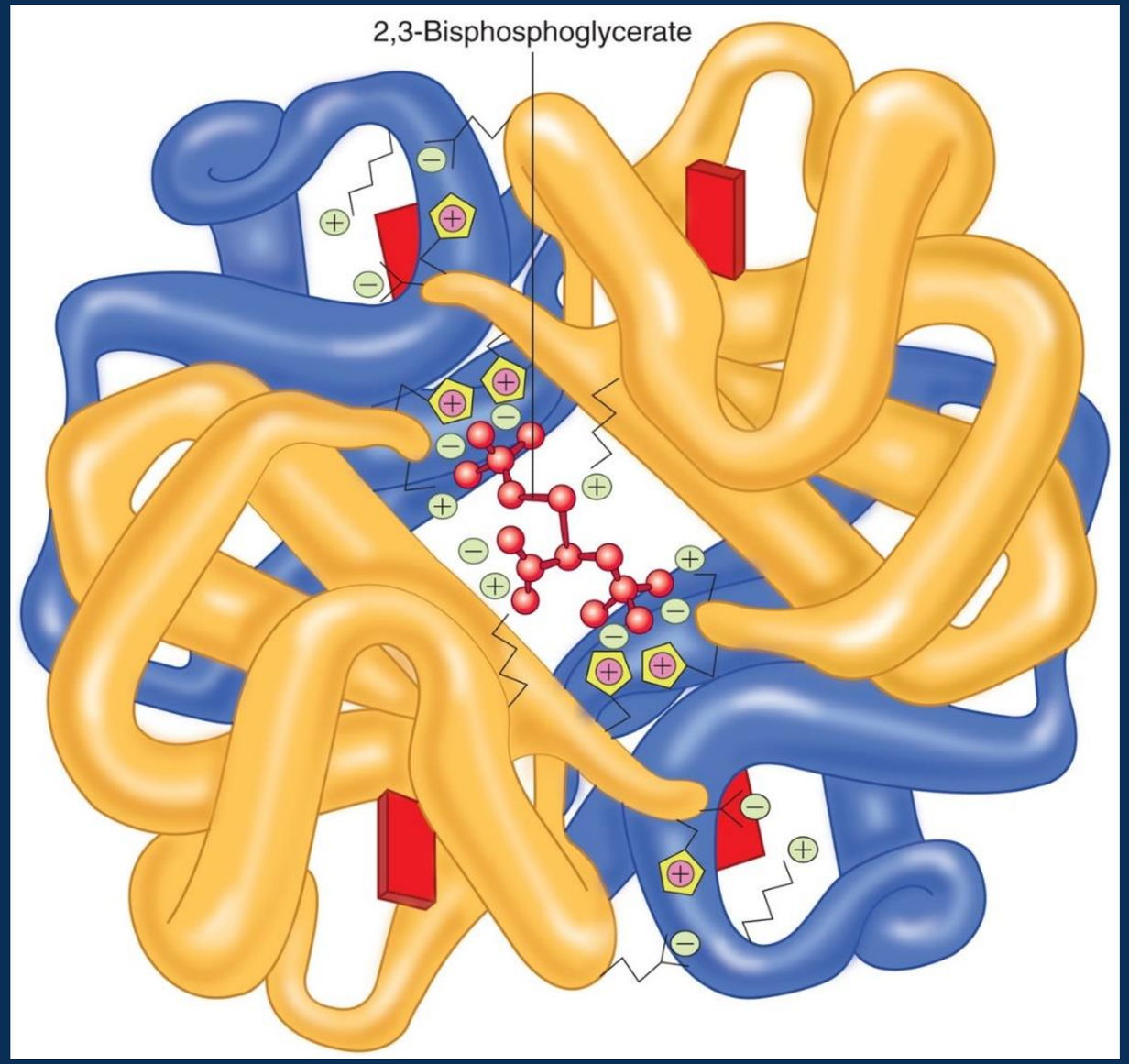
- Oxy (R)- vs Deoxy (T)- hemoglobin
- Amount of O_2 bound/released depends
  - PO_2, PCO_2, and Hgb affinity for O_2
    - P_{50} = PO_2 when 50% Hgb saturated with O_2,
      - Occurs at ~ 26 torr = 26 mm Hg
      - ↑ P_{50} => ↓ O_2 affinity
      - ↓ P_{50} => ↑ O_2 affinity
      - Oxygen Dissociation Curve

Oxygen Dissociation Curve



Allosteric Property

- 2,3-BPG (2,3-DPG)
  - Binds to T (↓ affinity) DeoxyHgb in 1:1
  - 3rd O_2 expels 2,3-BPG
    - ↑ O_2 affinity



CO_2 Transport

- CO_2 carried to lungs via
  - ~ 70% Formation of carbonic acid
  - ~ 23% Bound to Hgb
    - Binds deoxyhgb, expired in lungs
  - ~ 7% Dissolution into plasma

Hemoglobin Destruction

- Extravascular
  - Most efficient and recycles RBC components
  - Iron
    - Stored as ferritin or hemosiderin in macrophages
    - Transported to BM via transferrin (80% of pool)
  - Globin
    - Broken down into amino acids
  - Heme cleaved to CO + biliverdin > bilirubin

Hemoglobin Destruction

- Intravascular
  - Dissociates into dimers
    - Quickly binds to haptoglobin > liver
  - Acute hemolysis
    - Haptoglobin depleted
    - Globin dimers filtered by kidney
      - Reabsorbed in proximal tubules
        - » Hemosiderinuria

Acquired Nonfunctional Hgbs

- Patients present with hypoxia and/or cyanosis
- Methemoglobin (no O_2 affinity)
  - Hgb iron in ferric state (Fe^{+++}) = MetHgb
  - Blood appears chocolate brown
- Sulfhemoglobin
  - Sulfer atom binds to heme for life of RBC, no O_2 affinity
- Carboxyhemoglobin
  - Hgb 200x ↑ CO affinity compared to O_2
  - Cherry red blood/skin

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