Hemolytic Anemias Part I- Intrinsic Defects

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Objectives

- Identify general diagnostic findings of hemolytic anemia.
- Describe a classification system for hemolytic anemias.
- Identify laboratory findings in intravascular and extravascular hemolysis.
- Identify hemolytic anemias caused by red blood cell membrane defects.
- Identify hemolytic anemias caused by red blood cell enzyme defects.

Hemolytic Anemia

- Inadequate number of RBCs caused by premature destruction of RBCs
- Extreme bone marrow compensation for hemolysis
- ↑reticulocytosis may result in macrocytic anemia
- Normocytic anemia

Classification of Hemolytic Anemia

- Site of RBC destruction
  - Intravascular
    - Within the blood vessels
  - Extravascular
    - Within macrophages in liver, spleen and bone marrow
- Cause of destruction
  - Intrinsic defects (RBC membrane abnormalities, metabolic disturbances, hemoglobin disorders)
  - Extrinsic defects (abnormal elements in vascular bed that attack RBCs)

Defects is Intrinsic to RBC

- Defects in RBC membrane or internal contents

A. Hereditary

- Membrane defect (spherocytosis, elliptocytosis)
- Metabolic defect(G6PD deficiency, PK deficiency)
- Hemoglobinopathies(unstable hemoglobins, thalassemias, sickle cell anemia)

B. Acquired

- Paroxysmal nocturnal hemoglobinuria (PNH)

Defects is extrinsic to RBC

- Defects in RBC environment and often acquired conditions

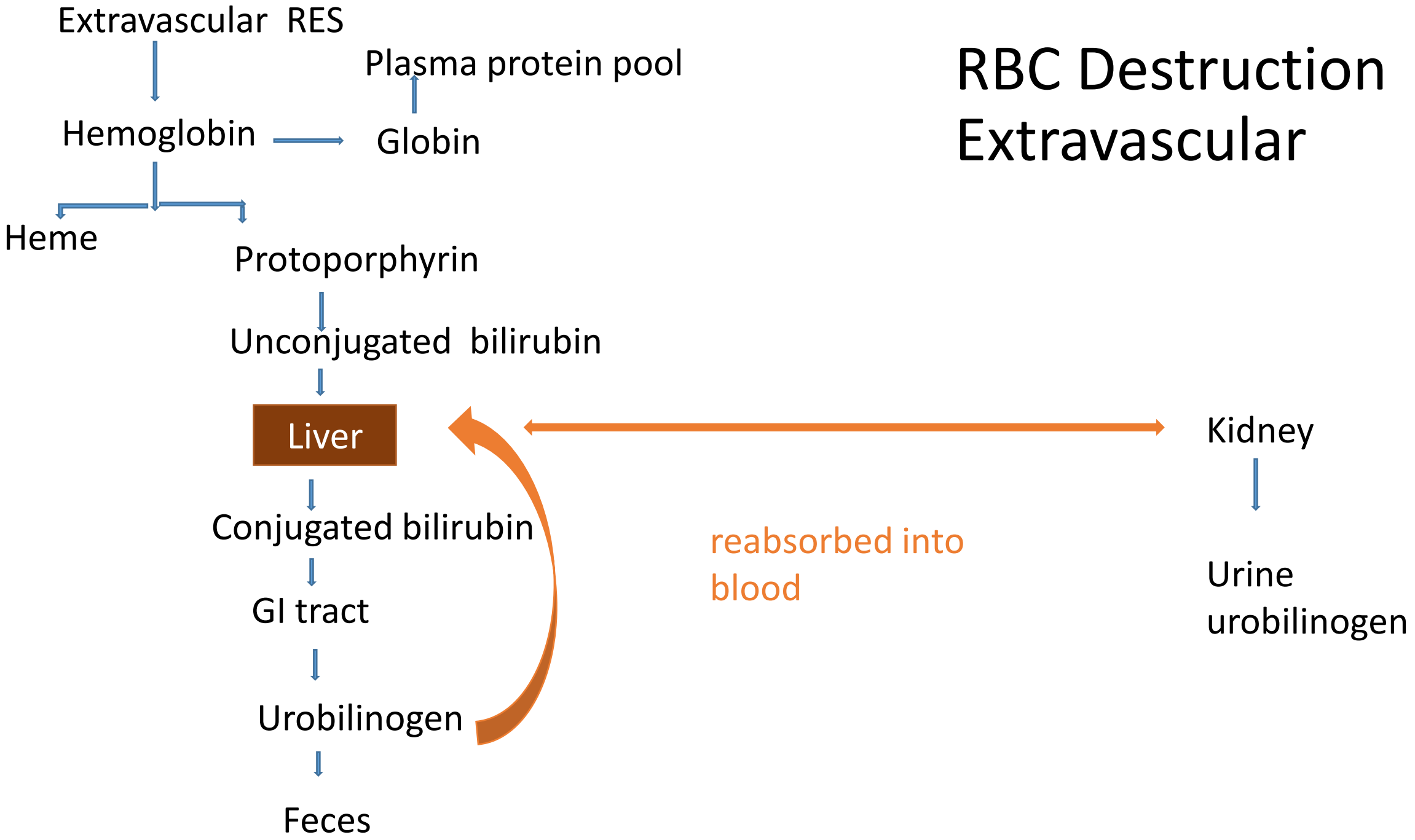
A. Immune hemolytic anemias
   - Autoimmune hemolytic anemia
     - Caused by cold reacting antibody
     - Caused by warm reacting antibody
   - Transfusion of incompatible blood

B. Non-immune hemolytic anemias
   - Microangiopathic hemolysis
   - Prosthetic heart valve
   - Infections
   - Drugs/chemicals
   - Thermal injury

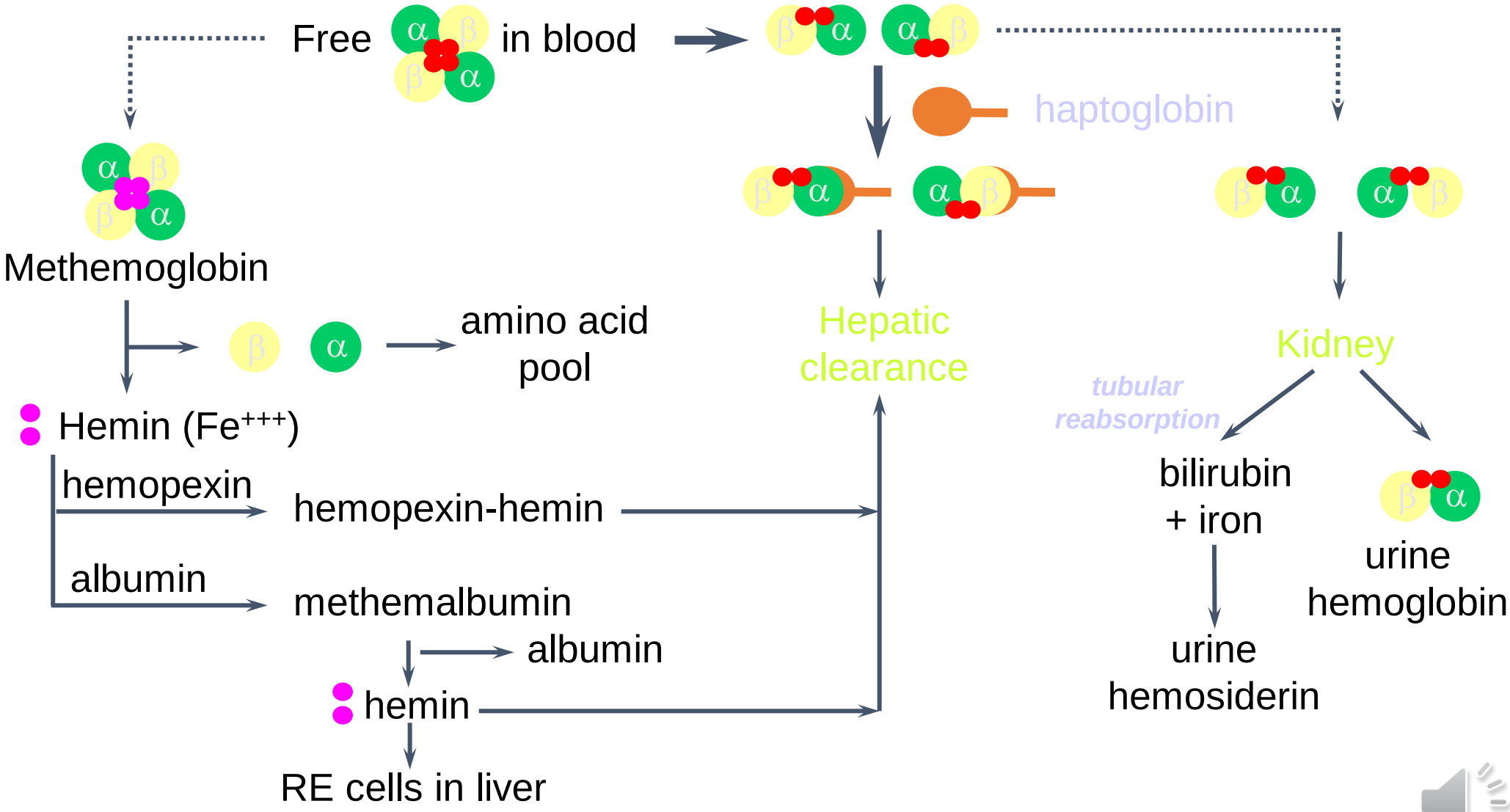
Clinical Findings

- Signs and symptoms of anemia
- Jaundice
- Splenomegaly
- Gallstones
- Brown urine
- Skeletal abnormalities

RBC Destruction Extravascular



Intravascular Hemolysis



Initial Laboratory Tests for Hemolysis

Test	Results in hemolysis	Cause
Haptoglobin	Decreased	Binds free hemoglobin
Lactate dehydrogenase	Increased	Released from lysis of red blood cells
Unconjugated bilirubin	Increased	Increased hemoglobin breakdown
Urinalysis	Increased urobilinogen, Chemistry positive for blood	Free hemoglobin and its metabolites
Direct antiglobulin test	Positive	Autoimmune cause
Stool	Increased urobilinogen	Increased RBC destruction
Peripheral blood smear	Increased polychromasia	Based on cause of anemia
Reticulocyte	Increased	Marrow's response to anemia

Common Laboratory Findings

- Increased bone marrow activity
  - Reticulocytosis (RPI >2)
  - Leukocytosis
  - NRBC in peripheral blood smear
  - Erythroid hyperplasia
- Increased RBC destruction
  - Hemoglobinemia*
  - Hemosiderinuria*
  - Methemoglobinemia*

(**associated with intravascular hemolysis)*

Extra vs Intravascular Hemolysis

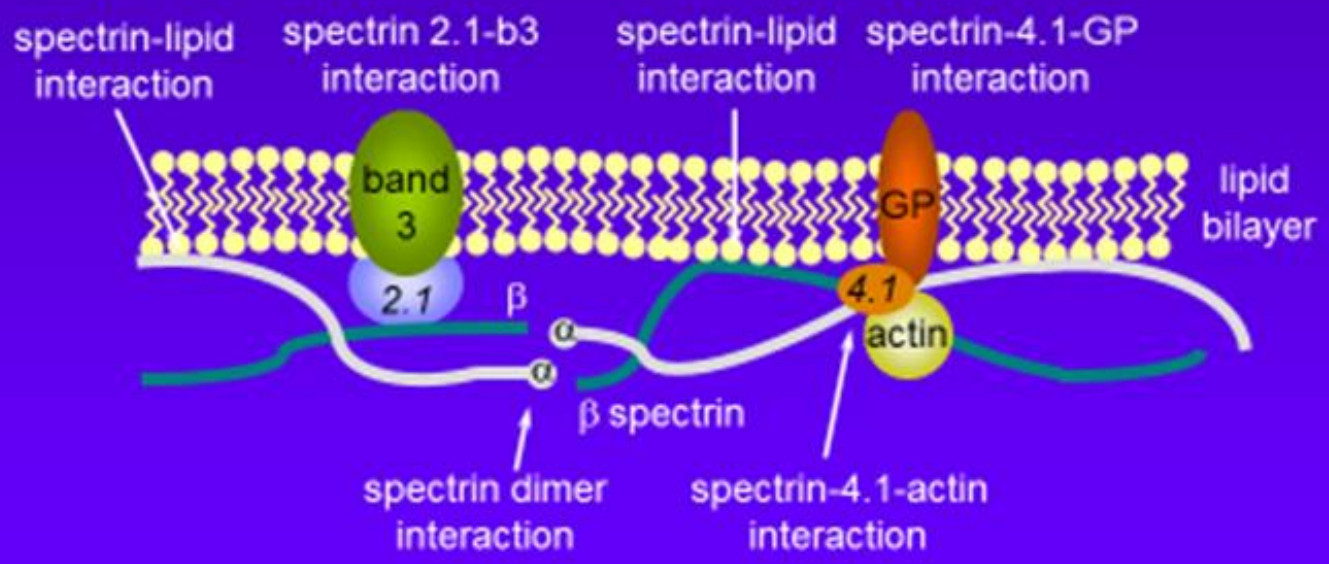
	Intravascular hemolysis	Extravascular hemolysis
Urine hemoglobin	Present	Usually absent
Urine hemosiderin	Present	Usually absent
Haptoglobin	Low	Usually normal
Peripheral smear	Schistocytes , Heinz bodies and bite cells in G6PD def.	Spherocytes
Methemoglobinemia	Present	NA
Hemopexin	Decreased	NA
Carboxyhemoglobin	NA	increased

Hemolytic Anemias

Intrinsic RBC Membrane Defects

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Hereditary pyropoikilocytosis
- Hereditary stomatocytosis
- Hereditary xerocytosis
- Paroxsymal noctural hemoglobinuria

RBC Membrane Structure



RBC Membrane Defects- Skeletal Protein Abnormalities

- Interactions between lipid bilayer and spectrin lattice internal to membrane act to stabilize lipid bilayer.
- Loss of portions of membrane causes spherocyte formation and hemolysis.
- Interactions between spectrin lattice proteins stabilize cell membrane.
- Disruption of skeletal lattice causes formation of poikilocytes.

Hereditary Spherocytosis

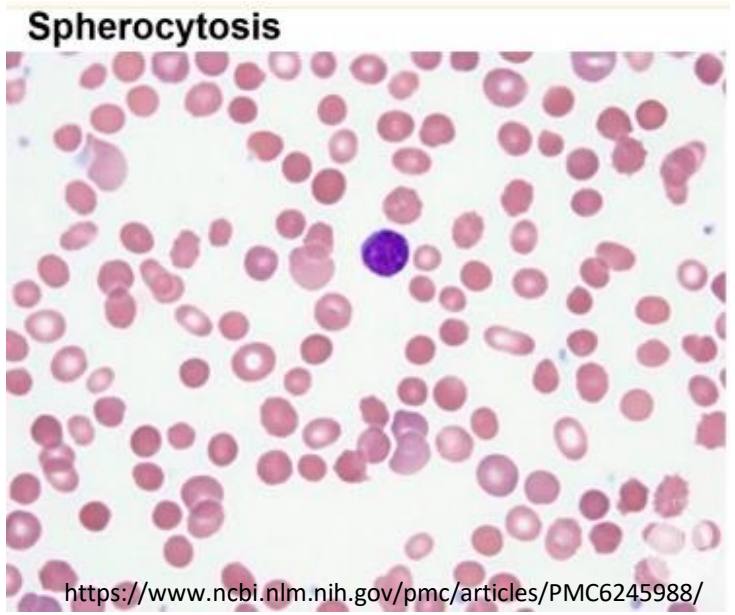
- 75% autosomal dominant fashion and expressed in heterozygotes
- Most commonly encountered in northern Europeans
- Anemia occurs due to alterations in one or more vital structural protein(s) leading to an imbalance in the membrane lipid content and cation transport
  - spectrin, ankyrin, band 3 and protein 4.2
- The defective proteins disrupt the vertical membrane interactions between lipid bilayer and the cytoskeletal network.

Hereditary Spherocytosis

- Specifically, these lead to:
  - Decreased lipid content
  - Decreased surface area to volume ratio and spherical shape
  - Less deformability and leads to decreased survival
- Clinical findings
  - Mild to moderate hemolytic anemia and if anemia is compensated , may be asymptomatic
  - Fatigue
  - Intermittent jaundice
  - Splenomegaly

Hereditary Spherocytosis Laboratory Findings

- Reticulocytes >8%
- Polychromasia and anisocytosis
- Increased MCHC
- Spherocytes in PB smear
- Increased bilirubin
- Increased LD
- Decreased haptoglobin
- Increased serum & fecal urobilinogen

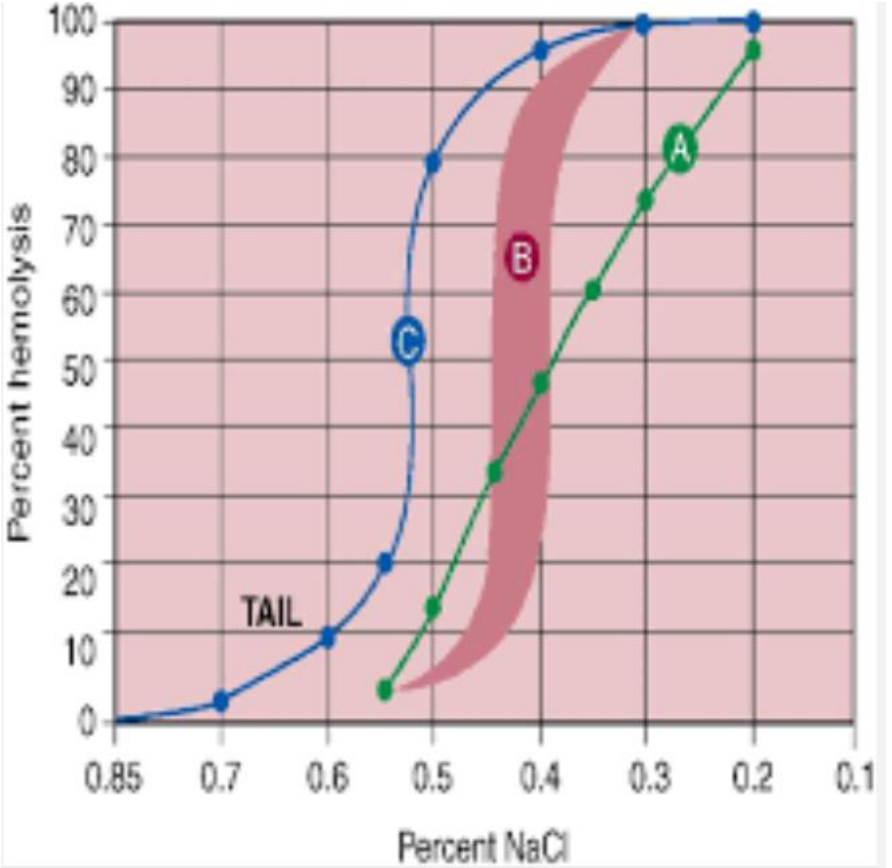


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6245988/

- Negative direct antiglobulin test (DAT)
- Additional tests
  - Increased osmotic fragility test
  - Incubated osmotic fragility

Osmotic fragility test

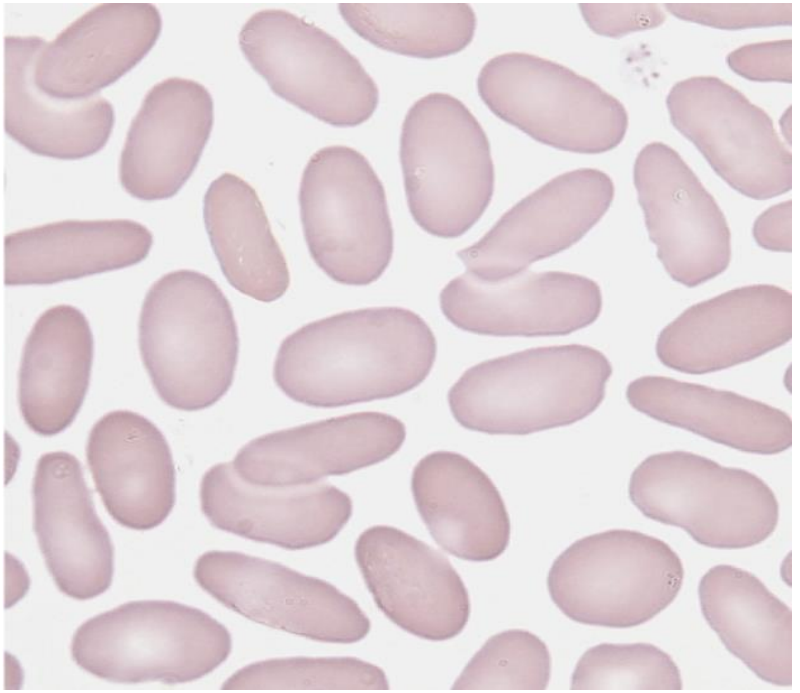
- Mixed heparinized blood with 0.9% NaCl solutions
- Normal RBC
  - Hemolysis onset at: 0.45% NaCl
  - Hemolysis complete at: 0.3-0.33% NaCl
- Incubated osmotic fragility test is more specific for hereditary spherocytosis
- Decreased osmotic fragility is seen with target cells (due to excess membrane)



Hereditary elliptocytosis

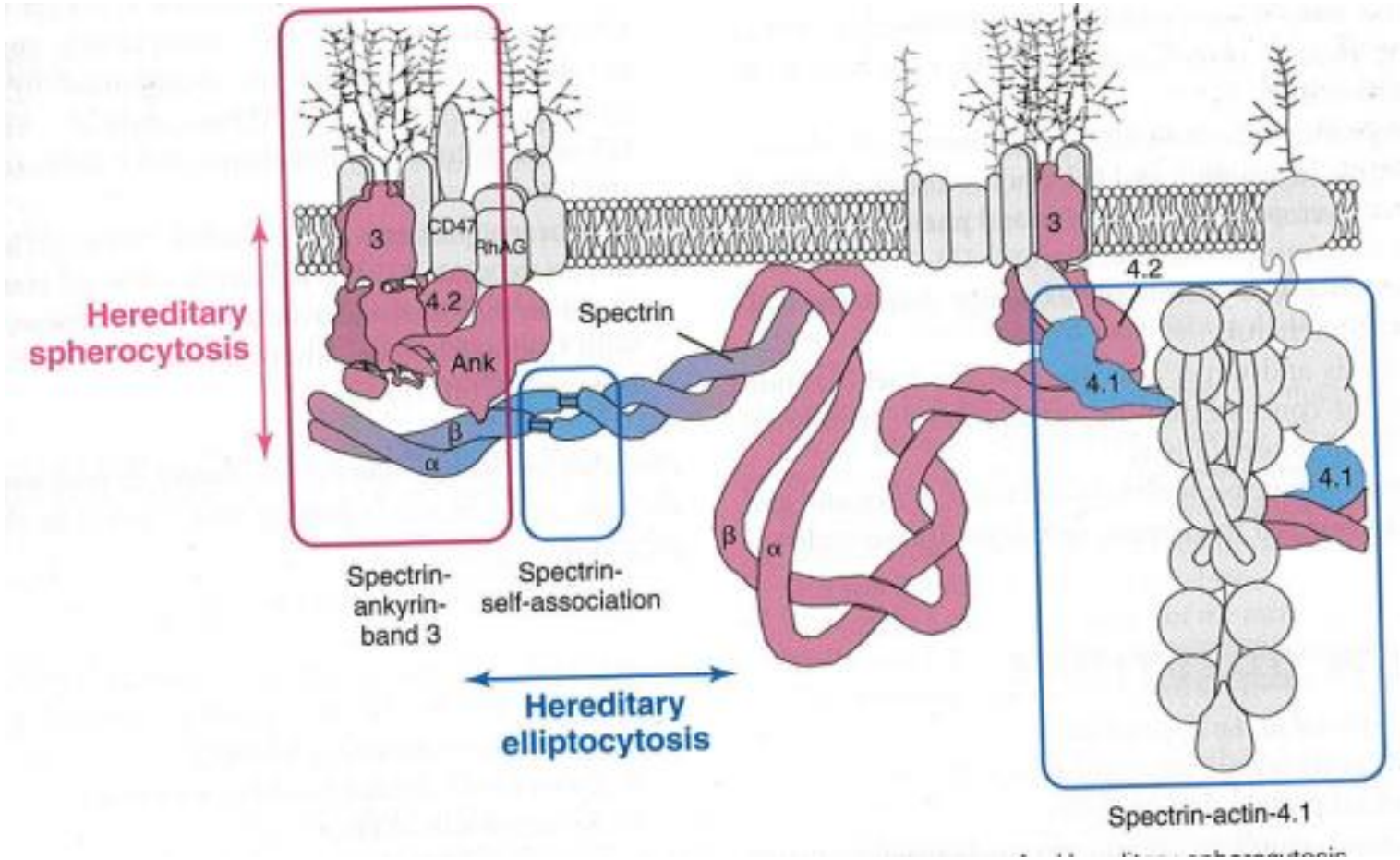
- Rare autosomal dominant disorder
- Genetic mutations in which defective protein disrupt the horizontal linkage and weaken the mechanical stability of the membrane.
  - Spectrin or protein 4.1
  - RBC are elliptical or oval
- Clinically heterogeneous; hemolysis is usually absent to mild

Hereditary elliptocytosis (HE)



- Mild reticulocytosis
- Prominent elliptocytosis in >25 % of RBC
- Erythroid hyperplasia with normal maturation
- More common in African-American population
- Natural protection against malarial parasites

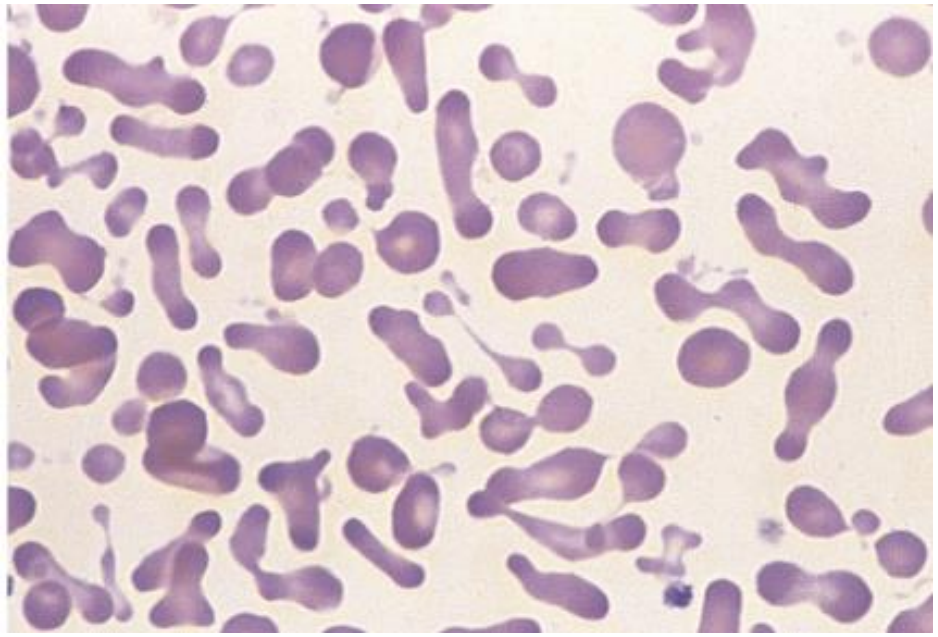
Membrane Defects in Hereditary Spherocytosis and Hereditary Elliptocytosis



Hereditary Pyropoikilocytosis (HPP)

- Severe hemolytic disease part of the HE group
- Patients with HPP are compound heterozygotes
- One membrane defect is inherited from each parent
- Presents in infancy as severe hemolytic anemia
- Deficiency of α- spectrin plus mutant spectrin that cannot associate to tetradimers.
- Poikilocytes are removed in spleen, and improvement may be noted after splenectomy.

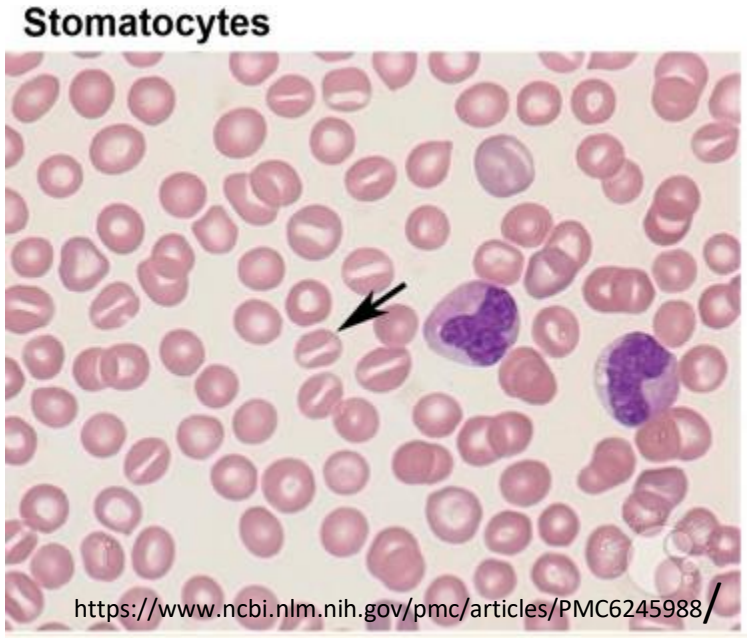
Hereditary Pyropoikilocytosis (HPP)



- Low MCV(50-65fL)
- Moderate to severe anemia
- Peripheral blood smear :
  - microspherocytes, extreme Poikilocytosis, fragments and few elliptocytes
- Increased osmotic fragility and biochemical evidence of excessive hemolysis
- RBC shows marked thermal sensitivity
- RBC fragments under heat stress at 41°- 45°C
- Normal RBC do not fragment until 49°-50 °C.

Hereditary Stomatocytosis

- Rare autosomal dominant disorder
- Increased red cell membrane cation permeability with a greater efflux of K^+ ions than Na^+ ions, with net gain of Na^+.
- Over-hydrated cells are stomatocytes
- Stomatocytes in peripheral blood smear (5-50 % RBC)



Hereditary Stomatocytosis

- MCHC is decreased and MCV may be increased.
- Moderate to severe hemolytic anemia
- Increased reticulocytes
- Increased bilirubin
- Increased osmotic fragility and autohemolysis
- Rh null disease is associated with stomatocytosis.
- Distinguish from acquired stomatocytosis

Hereditary Xerocytosis

- Autosomal dominant inheritance
- Net loss of K+ from cell
- Cellular dehydration
- Rigid cells are trapped in the spleen
- Anemia mild to moderate
- Laboratory findings:
  - ↑ MCHC and ↓MCV
  - ↑ retics and ↑bilirubin
  - Contracted, spiculated cell

Hereditary Acanthocytosis

- Abetalipoproteinemia: autosomal recessive disorder with decreased apolipoprotein B
- Decreased plasma cholesterol
- Preferential expansion of outer face of RBC lipid bilayer, leading to acanthocytosis.
- Associated with McLeod phenotype and neurologic syndrome (chorea).
- Acquired acnathocytosis can occur in severe liver disease.

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- A rare, **acquired** , clonal disorder of hematopoietic stem cells
  - RBC, WBC , and platelets are affected
- Genetic defect is in phosphatidylinositol glycan Class A molecule (PIG-A) gene.
- Glycosyl-phosphatidylinositol (GPI) is deficient.
- GPI-linked proteins: CD55, CD59,etc.
- Complement mediated hemolysis
- Intermittent intravascular hemolysis and nocturnal hemoglobinuria

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Occurs in children and adults
- Lab findings:
  - Biochemical evidence of hemolysis
  - Anemia
  - Bone marrow hypoplasia
- Associated with development of other hematologic abnormalities: MDS, AML, MPD, etc.
- Flow cytometry is the gold standard test to detect the absence cell markers on cell surfaces.

Hemolytic Anemia
RBC Enzyme Defects

- Mature RBC lose ability for protein synthesis and aerobic metabolism (no nucleus, no mitochondria).
- Enzymes present must last lifetime of cell.
- Glucose-6-Phosphate Dehydrogenase (G6PD)
- Pyruvate kinase (PK) deficiency

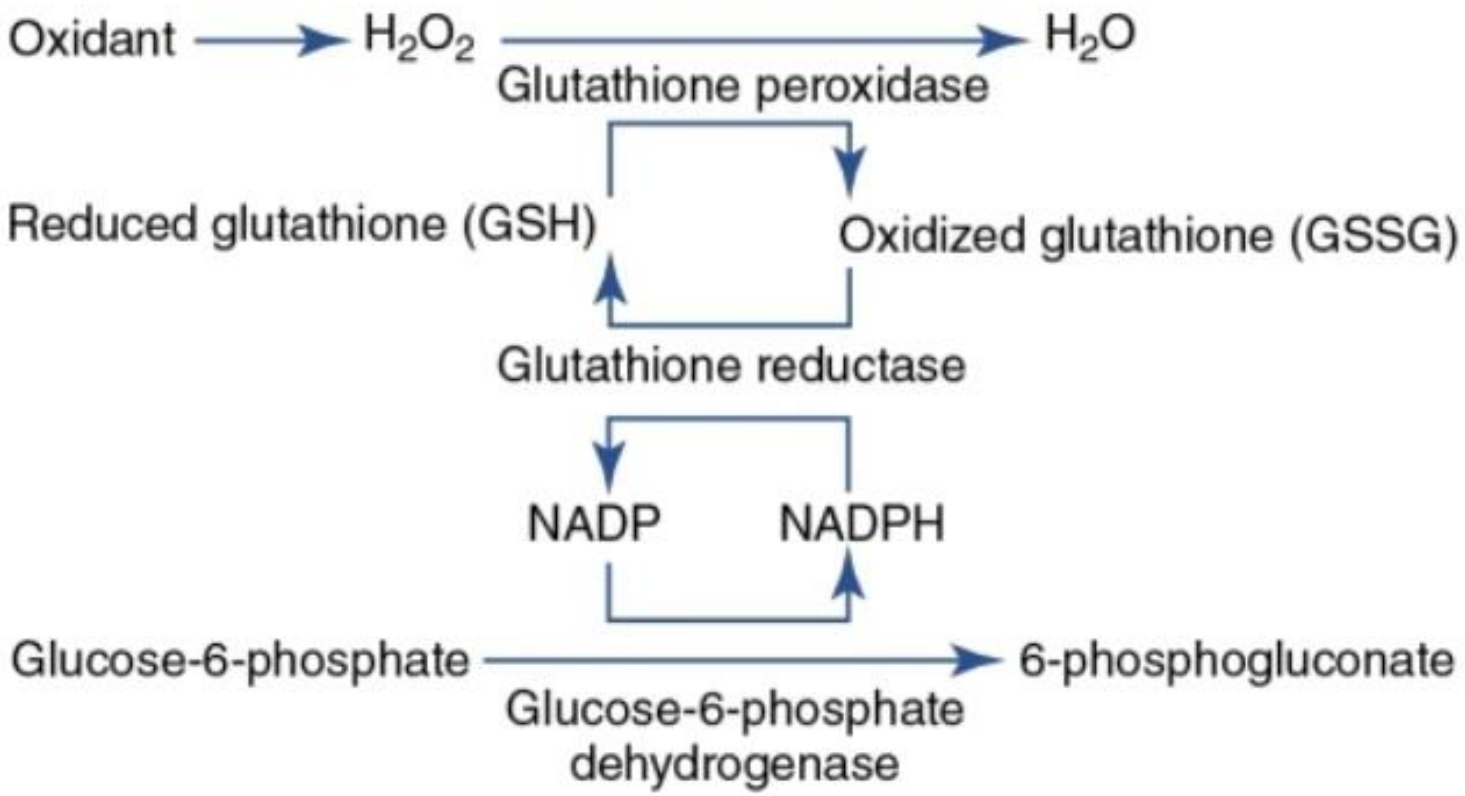
G6PD Deficiency

- Most common enzyme defect
- X-linked recessive inheritance disorder
- Affects 15% U.S black males
- Acute hemolytic crisis due to:
  - Oxidant drugs
  - Infections (ex. Viral hepatitis, pneumonia etc.)
  - Ingestion of fava beans

G6PD Deficiency

- G6PD deficient RBCs can’t generate sufficient NADPH to maintain reduced glutathione level
- Deficiency of the G6PD enzyme increases susceptibility of RBC to oxidative damage
- Oxidant damage leads to oxidation of hemoglobin to methemoglobin and formation of Heinz bodies which damage membrane.

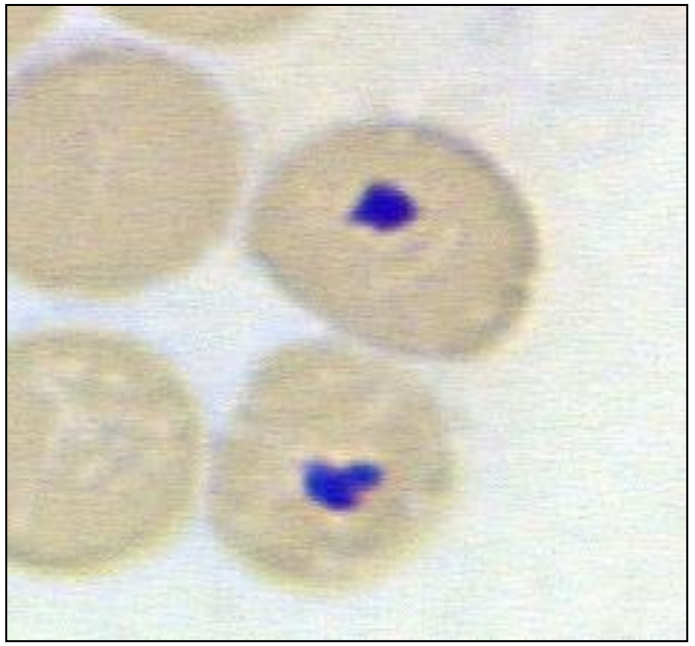
G6PD Deficiency



G6PD Deficiency

- Most are asymptomatic without anemia.
- Variable intravascular hemolysis.
- Peripheral smear and G6PD assay
  - Heinz bodies on peripheral blood smear with supravital stain
  - Presence of bite cells , spherocytes, fragments
- Hemoglobinuria, hemoglobinemia
- Reticulocytosis

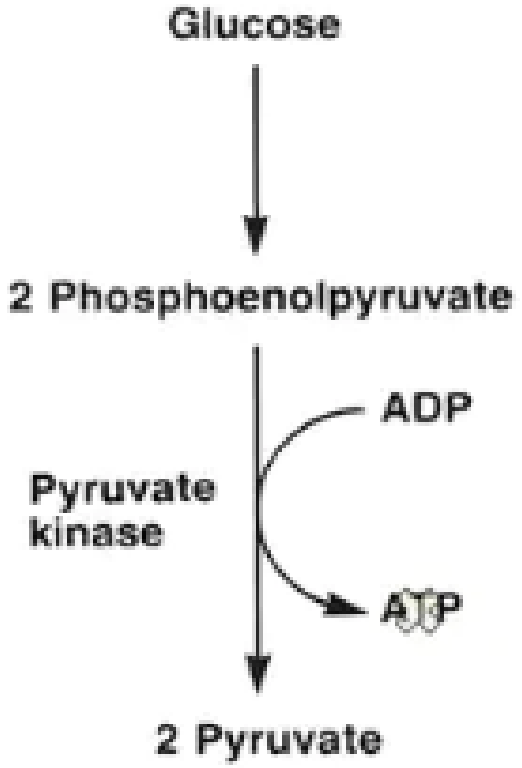
Bite cell
Wright-Giemsa stain



Heinz body
Supravital stain

Pyruvate Kinase Deficiency (PK Deficiency)

- PK catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, thereby producing ATP.
- Metabolic consequences of deficient PK:
  - Sufficient amount of ATP are not available for RBC survival
  - Buildup of 2-3- BPG shift oxygen dissociation curve to the right
- A mutation occurs in PKLR gene which lead to deficiency of the enzyme pyruvate kinase



Pyruvate Kinase Deficiency (PK Deficiency)

- An autosomal recessive disorder
- Most common hereditary chronic nonspehrocytic hemolytic anemia
- The deficiency occurs in severe (infancy) to mild (adult) forms
- RBC are removed by the RES particularly the spleen
- Lab findings:
  - ↑ Retic, abnormal contracted RBC , no Heinz bodies
  - ↓ haptoglobin , ↑ LD and bilirubin
  - ↓ pyruvate kinase in RBC

References

- Rodak’s Hematology, Clinical Principles and Applications 6th Edition
- Additional material courtesy of Dr. Karl Theil