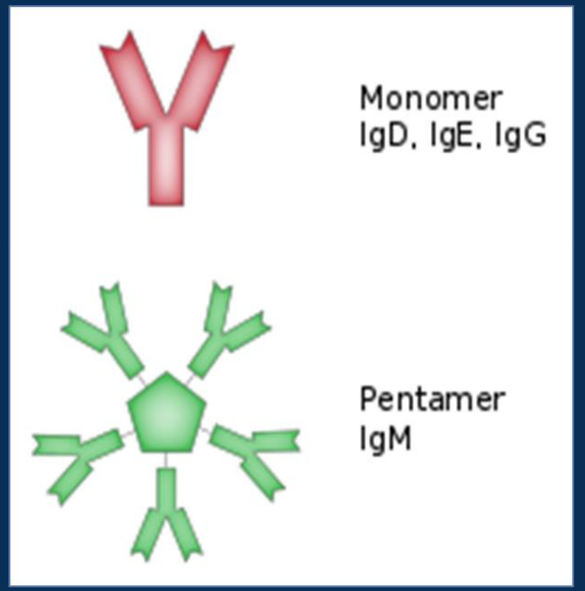
Hemolytic Anemias

Objectives

- Understand mechanisms of hemolysis due to antibodies
- Compare and contrast pathophysiology and laboratory findings for hemolytic anemias due to
  - Membrane Defects
  - Enzyme Deficiencies
  - Immune Defects
  - Nonimmune Defects

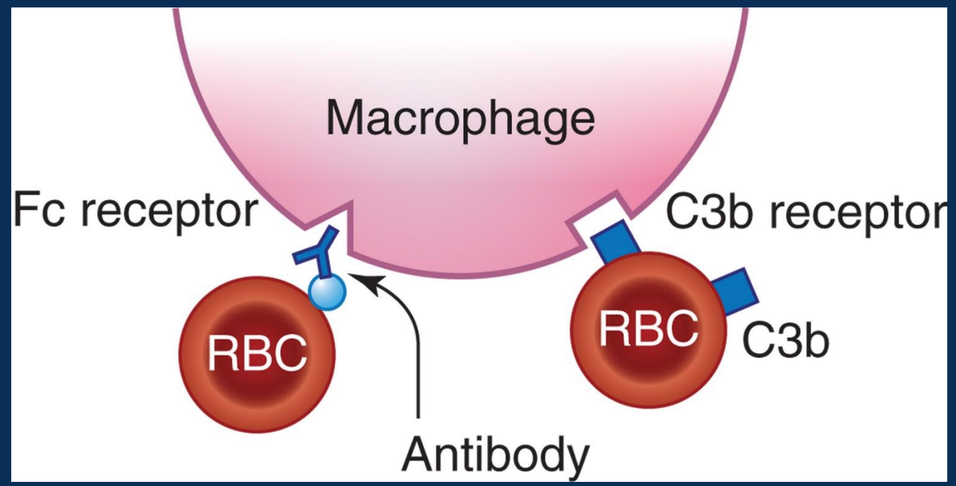
Mechanisms of Ab Hemolysis

- Intravascular or extravascular Ab hemolysis depends on
  - Class of Ab
  - Ability to fully activate complement cascade
- Based on whether IgM, IgG, and/or complement are present on RBC
  - IgG-mediated
  - Complement-mediated
  - IgM-mediated



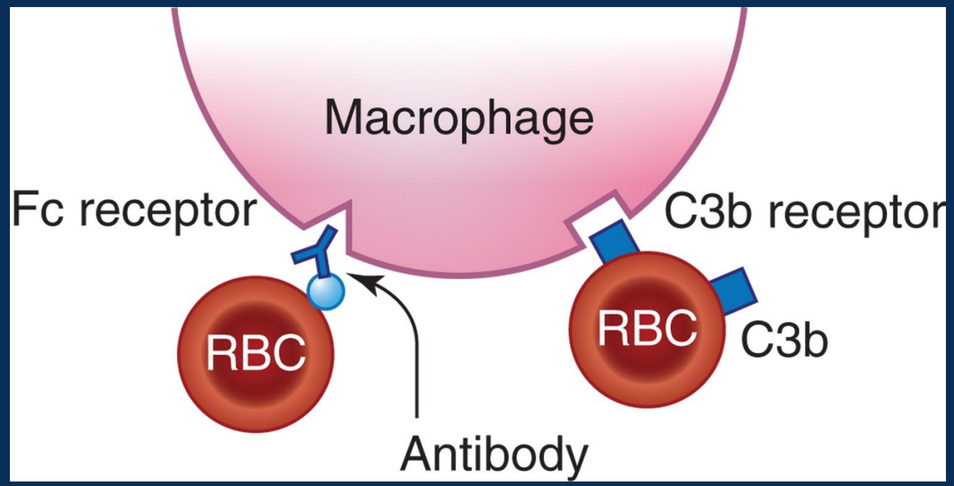
Mechanisms of Ab Hemolysis

- Extravascular hemolysis
  - Most common
  - RBC sensitized with Ab or complement
    - Removed by RES
- Intravascular hemolysis
  - Complement cascade activated → RBC lysis



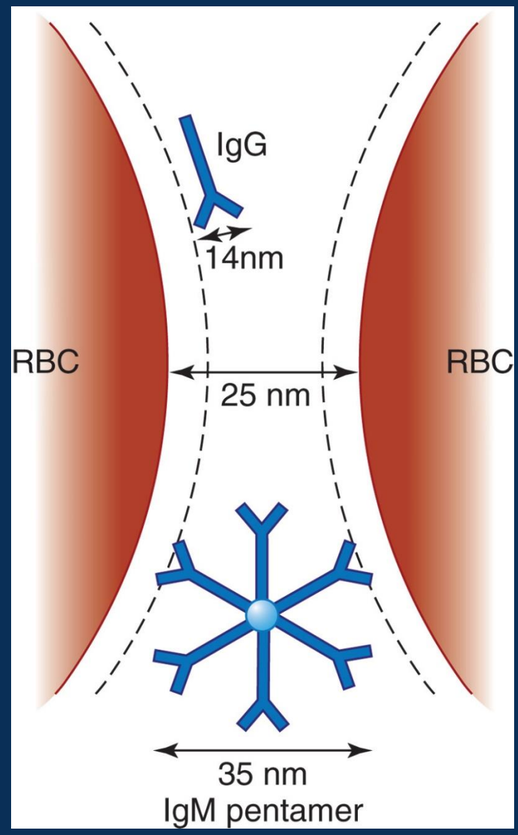
IgG Mediated Hemolysis

- IgG Ab attaches to RBC membrane Ag's
- Macrophages of RES pits the Ag/Ab complex
  - Or culls the RBC
- Pitted scenario
  - RBC membrane reseals itself
  - Repeated splenic passage – continues to lose membrane
    - forms spherocyte
  - Phagocytized by splenic macrophages



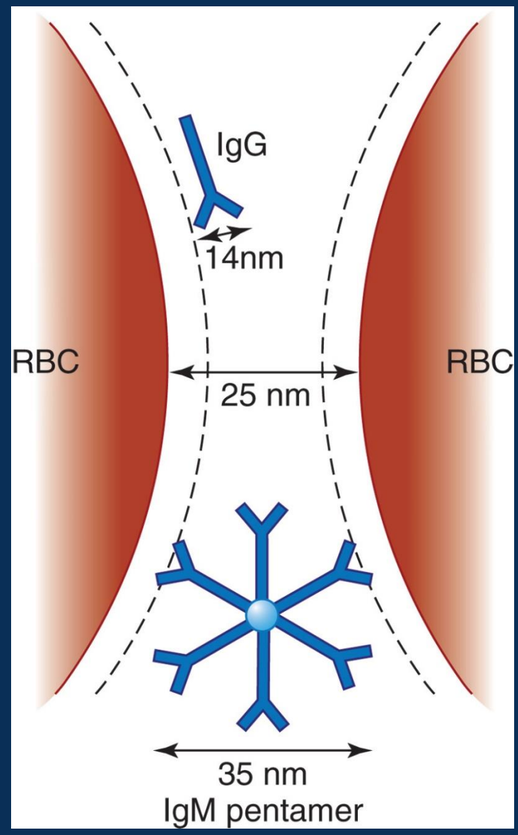
Complement Mediated Hemolysis

- Role of complement cascade
  - Sensitization (partial activation of complement on RBC)
  - Lysis of RBCs (full activation on RBC)
- Initiated by Ag/Ab reaction
  - IgM (activation more efficient, requires one IgM)
  - IgG (activation less efficient, requires two IgG)
- Activation ends with membrane attack complex (MAC)
  - Lytic attack to RBC membrane



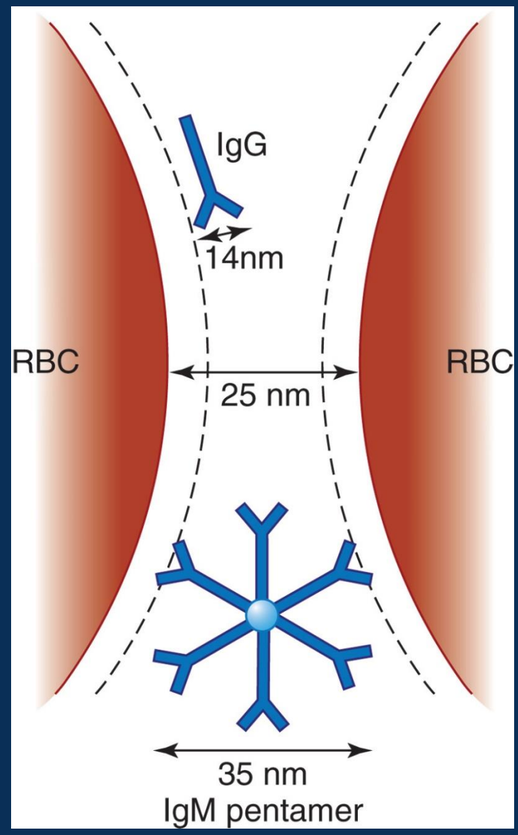
IgM Mediated Hemolysis

- Macrophages do not recognize IgM
- Efficient activator of complement
  - Full activation = intravascular hemolysis
  - Partial activation = extravascular hemolysis



Laboratory Detection

- Sensitized RBC's serologically detected
  - DAT (direct antiglobulin test)
    - Polyspecific AHG (antihuman globulin)
      - anti-IgG + anti-C3 (complement)
    - Monospecific AHG
      - anti-IgG
      - anti-C3

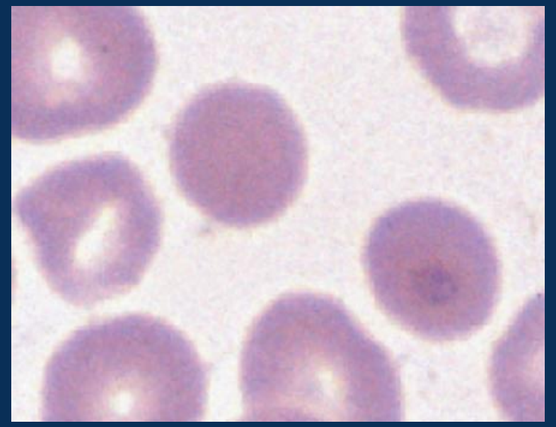


Membrane Defects

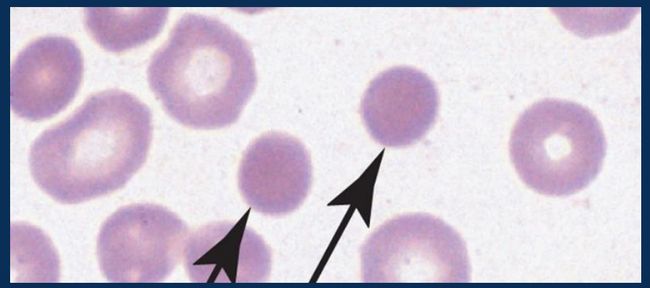
Membrane Defects

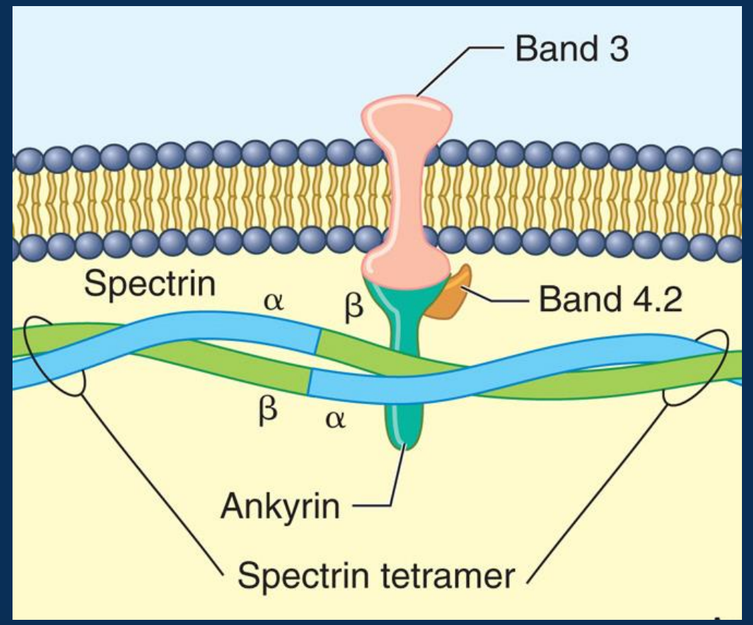
- Hereditary Spherocytosis (HS)
- Hereditary Elliptocytosis (HE)
  - Hereditary Pyropoikilocytosis (HPP)
- Overhydrated Hereditary Stomatocytosis (OHS)
- Dehydrated Hereditary Stomatocytosis (DHS)
- Acanthocytosis
- Paroxysmal Nocturnal Hemoglobinuria (PNH)

Hereditary Spherocytosis



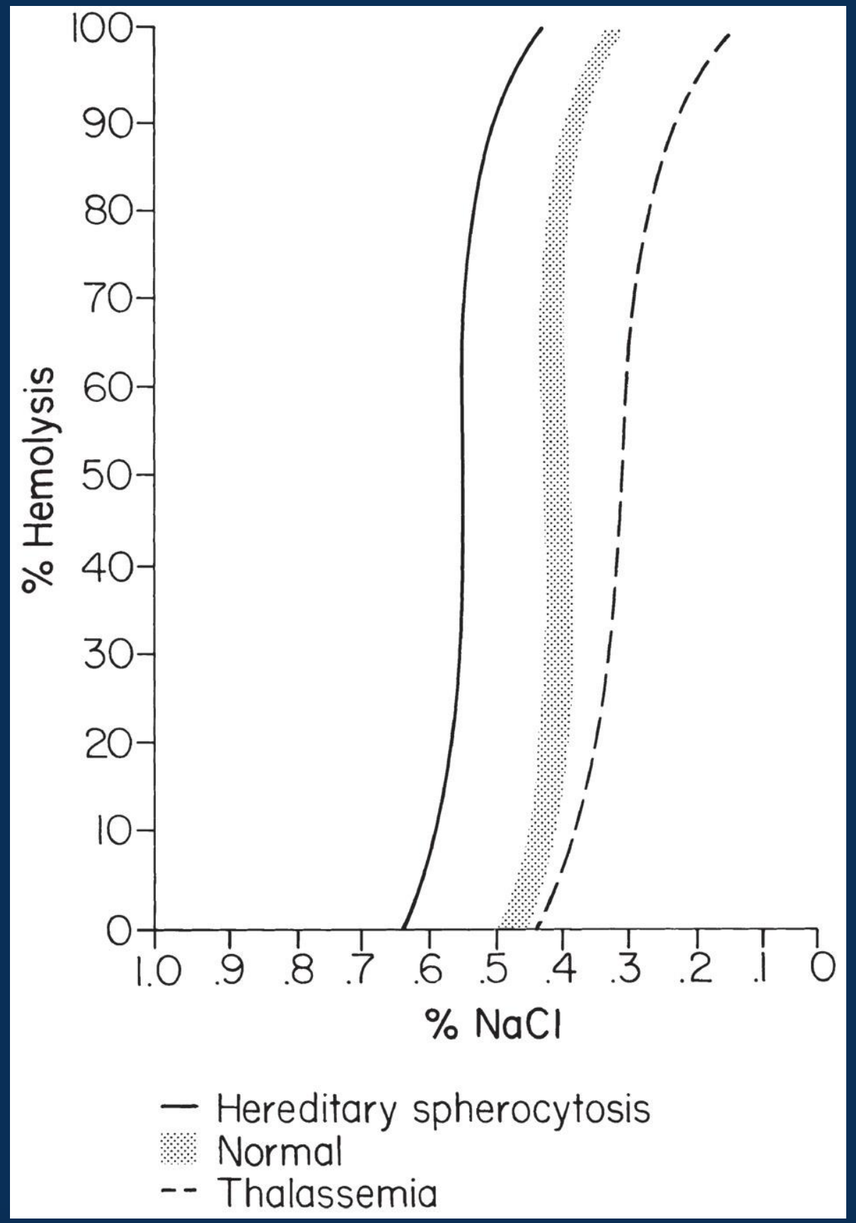
- Disorder of vertical protein interactions
  - Combined deficiency of spectrin and ankyrin
  - Weakening of vertical connections
  - Uncoupling between inner membrane skeleton and outer lipid bilayer
  - Shedding of lipid bilayer, forms microvesicles





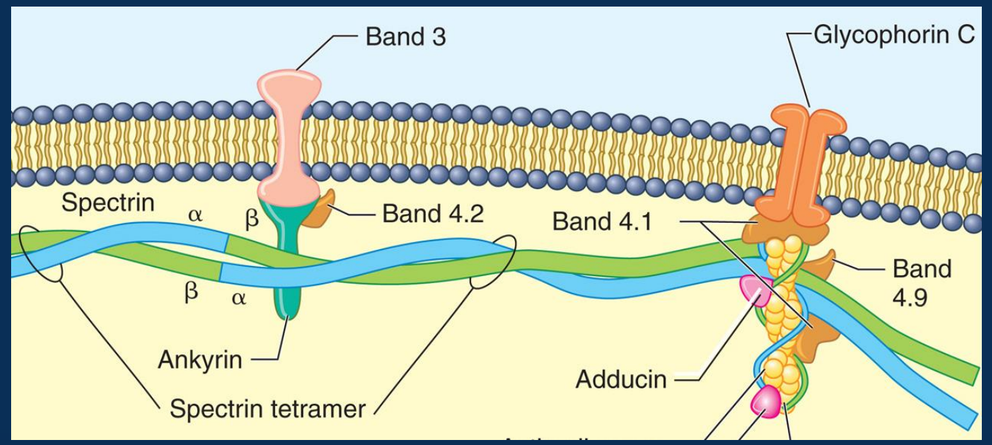
Hereditary Spherocytosis

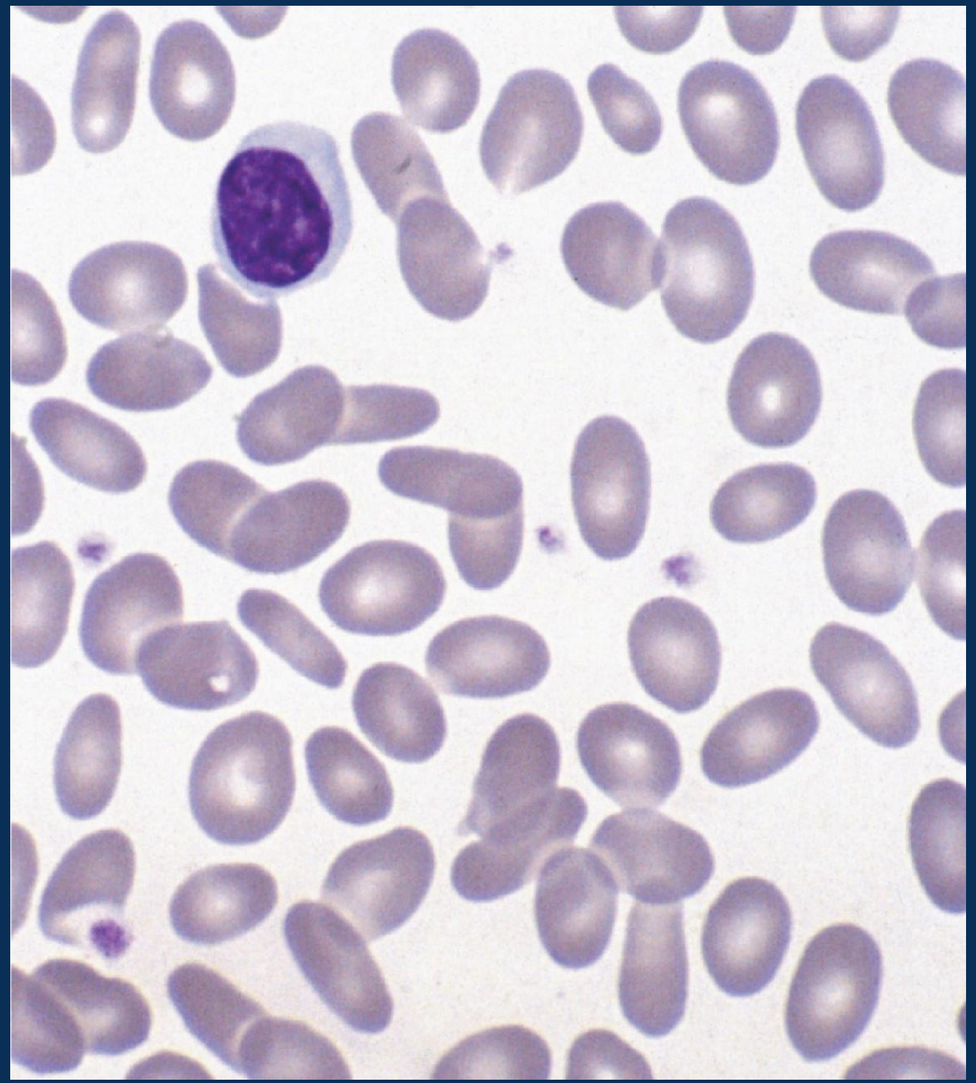
- Hgb and MCV – N or ↓
- MCHC > 36 g/dL (characteristic)
- ↑ serum bilirubin, ↓ haptoglobin
- Osmotic fragility +
- Autohemolysis test +
- Antihuman globulin test (DAT) -



Hereditary Elliptocytosis

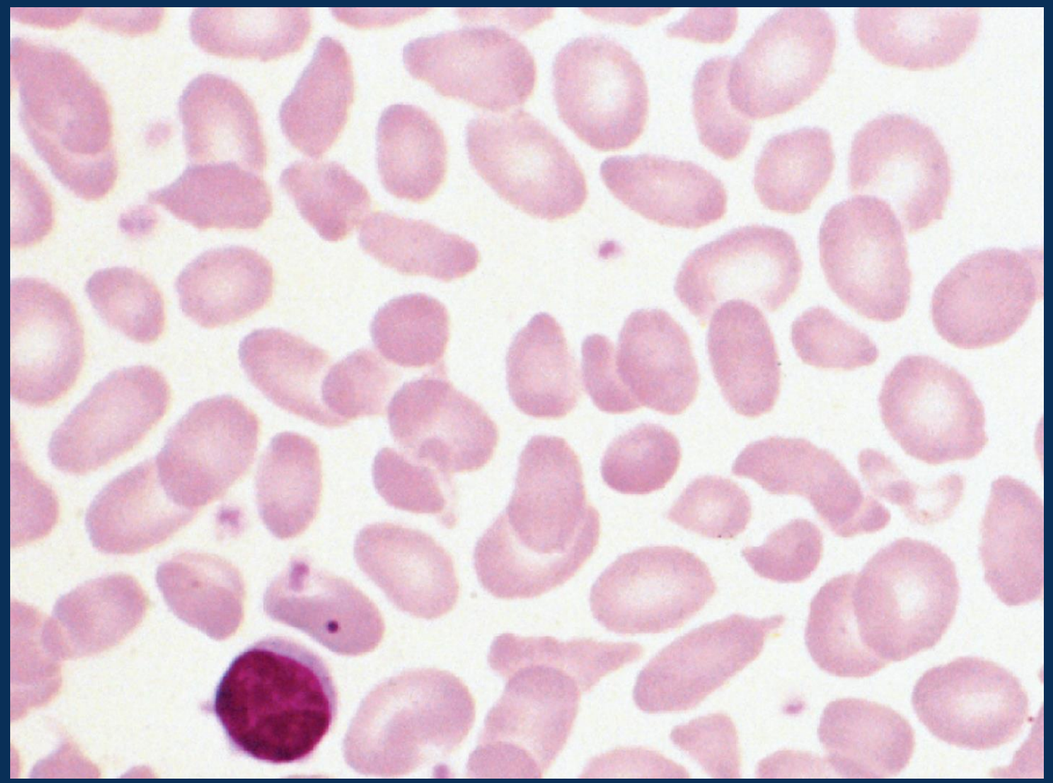
- Defect in horizontal skeletal proteins
  - ↓ association of spectrin dimers to form tetramers
  - Deficiency or defect in band 4.1
  - Abnormalities of the integral proteins





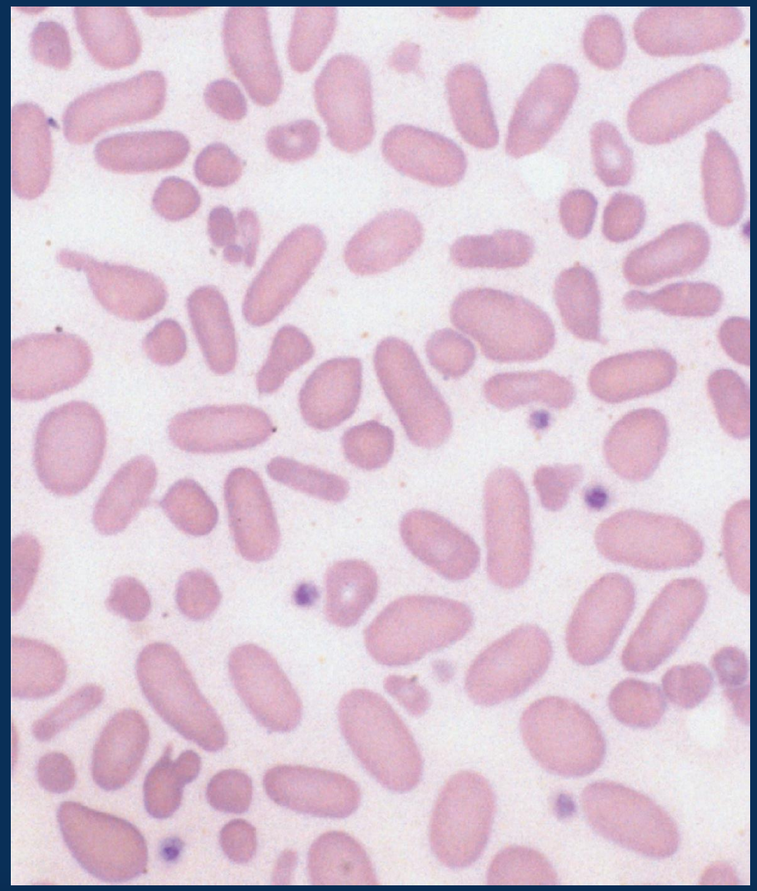
Hereditary Elliptocytosis

- Elliptocytes > 25%, usually > 60%
- Asymptomatic variants
  - Hgb levels usually > 12 g/dL
  - Reticulocyte mildly elevated
- Hemolytic HE variants
  - Hb 9-10 g/dL
  - Reticulocyte as high as 20%
  - Microelliptocytes, bizarre poikilocytes, schistocytes, spherocytes



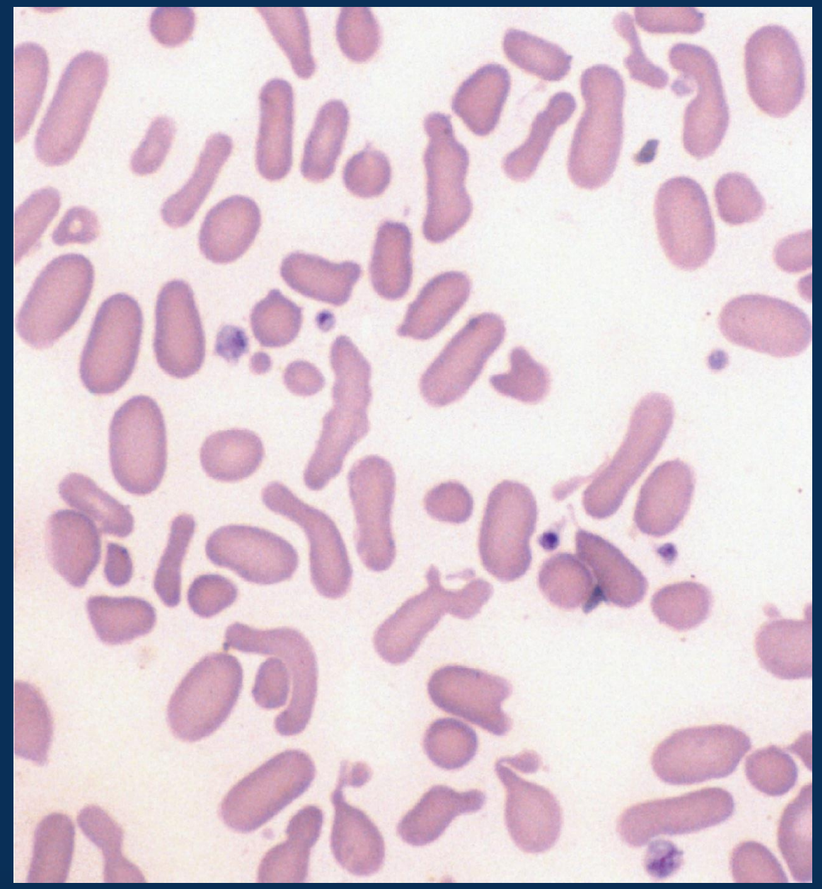
Hereditary Pyropoikilocytosis

- Severe subtype of HE
- Two defects (one from each parent)
  - Deficiency of α-spectrin
  - Mutant spectrin
- Defects cause
  - Disruption of the membrane skeletal lattice
  - RBC destabilization → fragmentation and poikilocytes



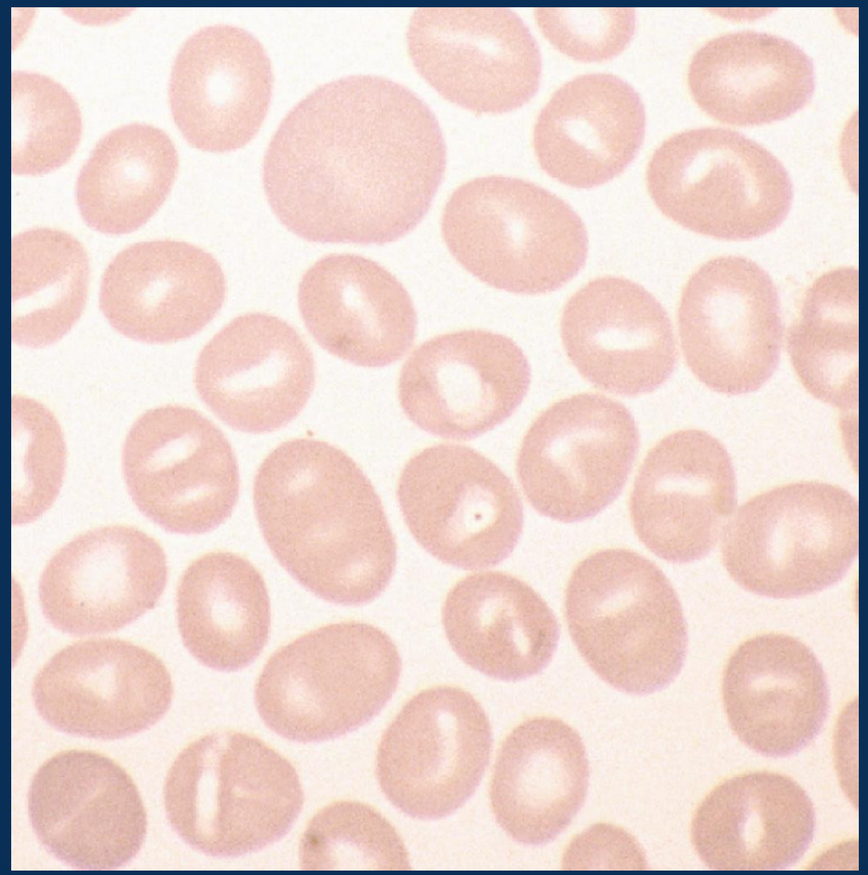
Hereditary Pyropoikilocytosis

- Striking poikilocytes
  - Budding, fragments, microspherocytes, elliptocytes, triangulocytes, bizarre forms
- MCV ↓ 25–55 fL due to RBC fragments
- Osmotic fragility +
  - Abnormal especially after incubation
- Thermal sensitivity test
  - Normal cells fragment at 49–50°C
  - HPP cells fragment at 45–46°C



Hereditary Stomatocytosis

- Overhydrated Hereditary Stomatocytosis
  - Abnormally permeable to Na+ and K+
  - Intracellular concentration of cations increase
  - Stomatocytes
- Dehydrated Hereditary Stomatocytosis
  - Net loss of K+ exceeds net gain of Na+
  - Targeted, contracted, or spiculated

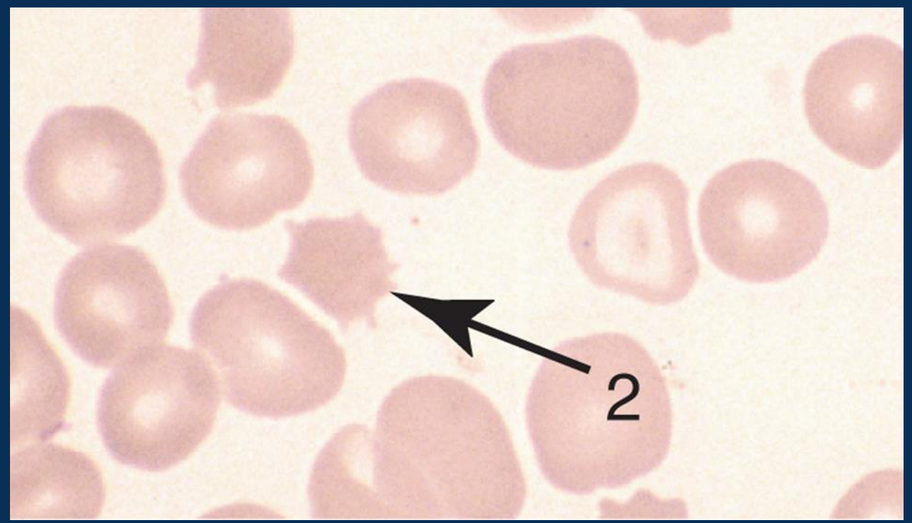


Hereditary Stomatocytosis

- Overhydrated Hereditary Stomatocytosis
  - ↓ MCHC, ↑ MCV
  - 10–50% stomatocytes
  - ↑ osmotic fragility and autohemolysis
- Dehydrated Hereditary Stomatocytosis
  - Target cells, RBCs with Hb puddled at periphery
  - Slight ↑ MCV, ↑ MCHC (can be >37 g/dL)
  - ↓ osmotic fragility

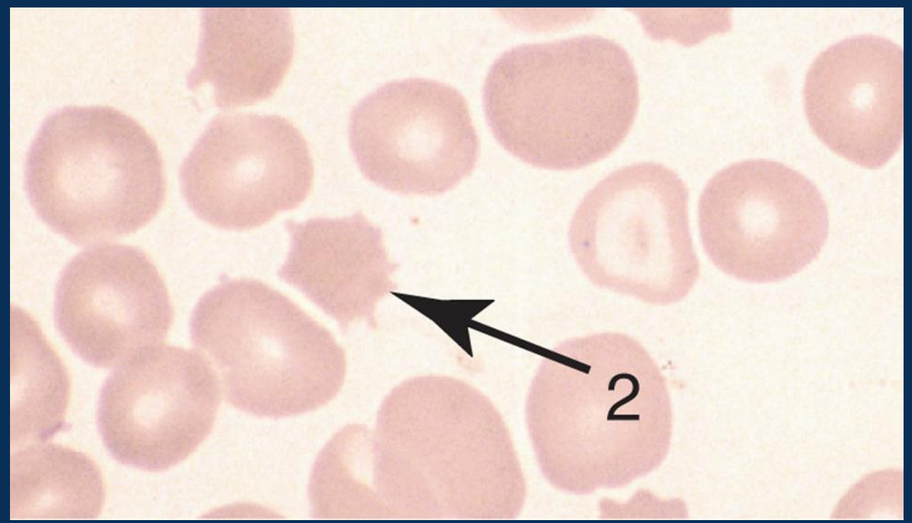
Acanthocytosis

- Abnormalities of lipid membrane
  - Acquired or hereditary
  - Liver disease
  - Spur cell Anemia (↑ C:P, excess cholesterol)
  - Abetalipoproteinemia (↑ C:P, decrease phospholipids)
- Concentration of plasma lipids increase
  - RBCs acquire excess lipids
  - Expand RBC membrane
    - Target cells, acanthocytes, echinocytes



Acanthocytosis

- Moderate to severe normocytic/normochromic anemia
- Hb 5–10 g/dL, reticulocytes 5–15%
- 20–80% acanthocytes
- May see spherocytes and echinocytes
- Normal permeability = normal osmotic fragility
- ↑ autohemolysis at 48 hrs
- ↑ unconjugated bilirubin, liver enzymes



PNH

- RBC abnormally sensitive to lysis by complement
- Acquired stem cell somatic mutation
  - RBCs, platelets, neutrophils
  - Bind abnormally large amounts of complement
  - Abnormally sensitive to complement lysis
- Deficient on PNH cells
  - CD55 (DAF = decay accelerating factor)
  - CD59 (MIRL = membrane inhibitor of reactive lysis)

PNH

- Pancytopenia
- Normocytic or macrocytic anemia
- Reticulocyte count 5–10%
- Normal osmotic fragility
- Autohemolysis ↑ after 48 hours
  - Addition of glucose hemolysis ↑
- Immunophenotyping
  - CD55, CD59

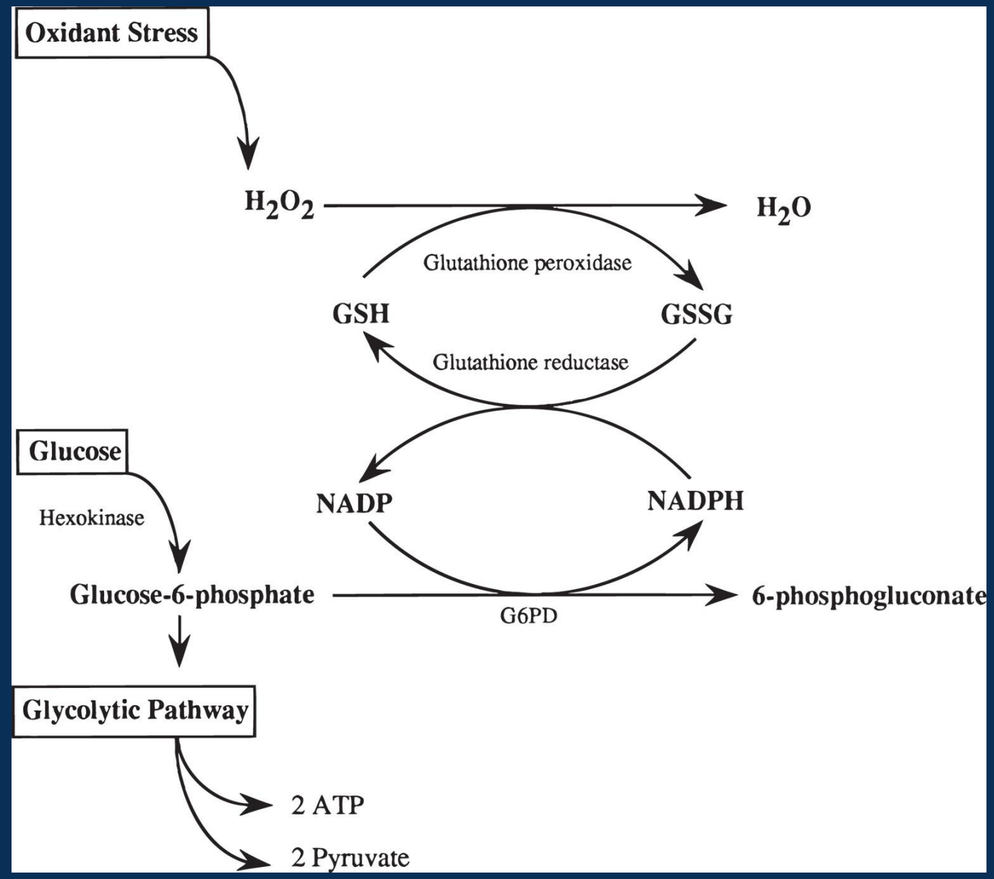
Enzyme Deficiencies

Enzyme Deficiencies

- Glucose-6-phosphate dehydrogenase (G6PD)
  - Most common
  - Affects hexose monophosphate shunt
- Pyruvate kinase (PK)
  - Second most common
  - Affects glycolytic pathway (Embden-Meyerhof)

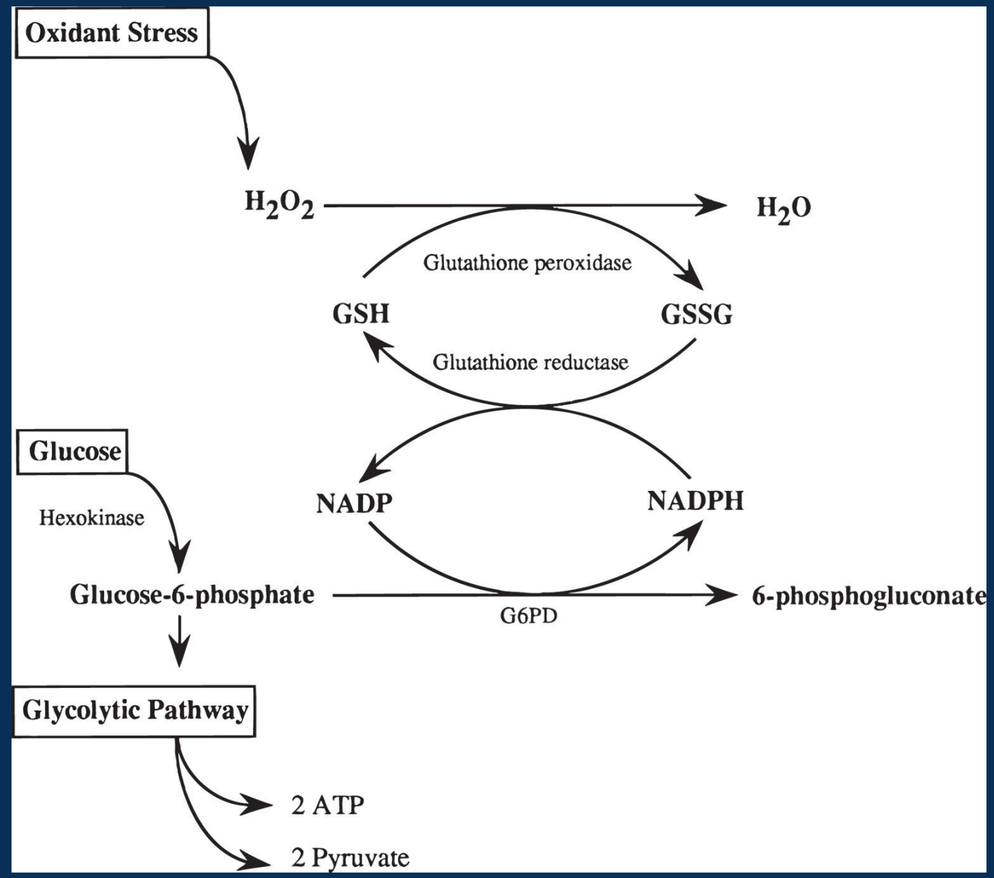
Hexose Monophosphate Shunt

- Catabolizes 10% of glucose
- Maintains adequate levels of reduced glutathione, GSH
  - Protects RBC from oxidant damage
  - Maintains HB in the reduced functional state
    - Nonreduced → Heinz bodies
- GSH levels maintained by G6PD
  - G6PD reduces NADP → NADPH



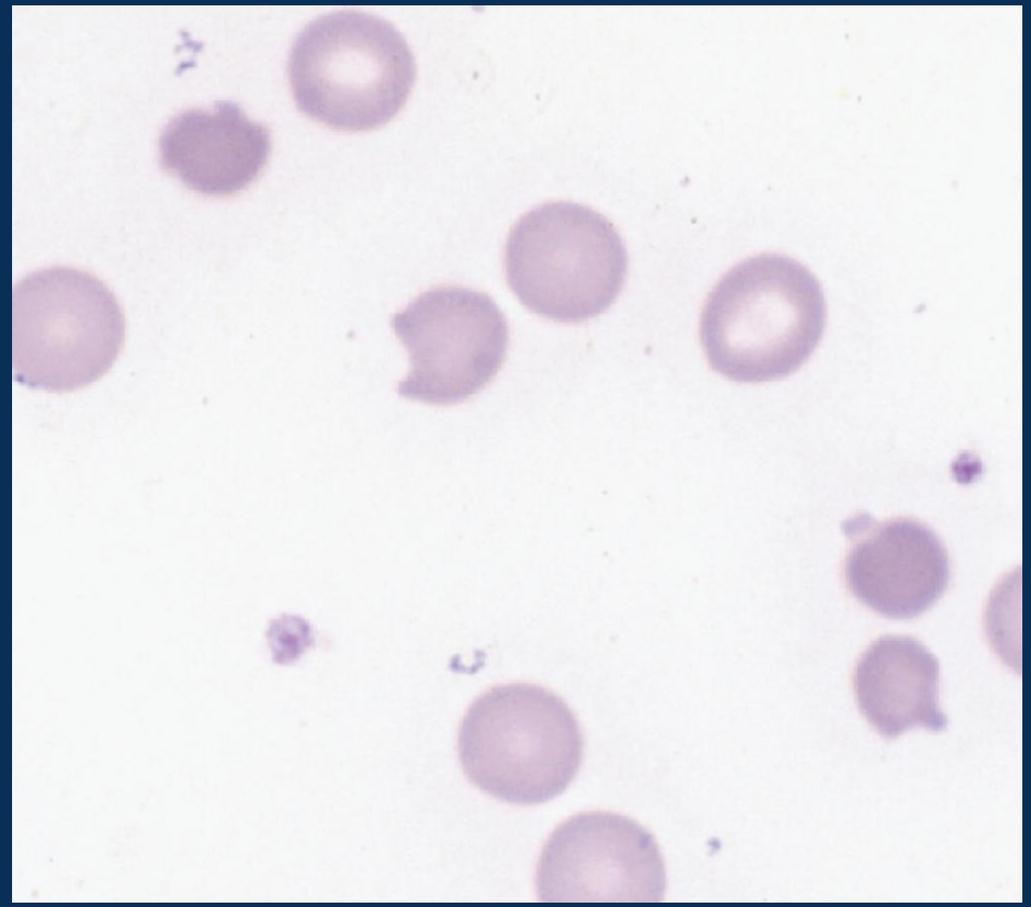
G6PD Deficiency

- X-linked inheritance
  - Fully expressed in males
  - Only in females with homozygous inheritance
- G6PD deficiency
  - Generation of NADPH and GSH impaired
  - Cellular oxidants accumulate ↓ Hgb solubility
    - Heinz bodies (supravital stain)
- Acute acquired
  - Favism (fava beans), infection, drug-induced



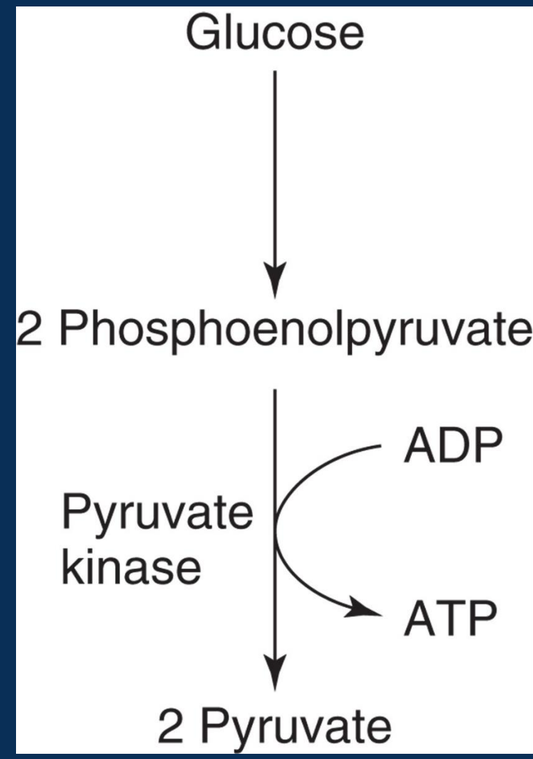
G6PD Deficiency

- Post hemolytic episode
  - Reticulocytosis: 4–35%
    - 5x more activity = N-↑ GSH
  - Bite cells, blister cells, spherocytes
- Definitive diagnosis
  - Demonstrate a ↓ in erythrocytic G6PD activity
  - Perform assays 2–3 months after hemolytic episode



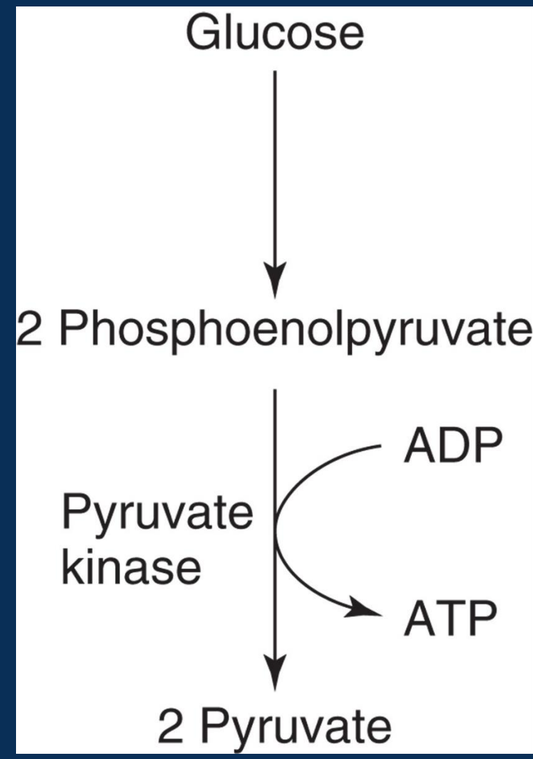
Embden-Meyerhof

- Maintains adequate levels of ATP for
  - Active cation transport across the cell membrane
  - Maintaining membrane deformability
  - Maintaining RBCs' bioconcave shape
- Rapoport-Luebering shunt
  - Maintains 2,3-BPG levels
    - Stimulates O2 delivery to tissues



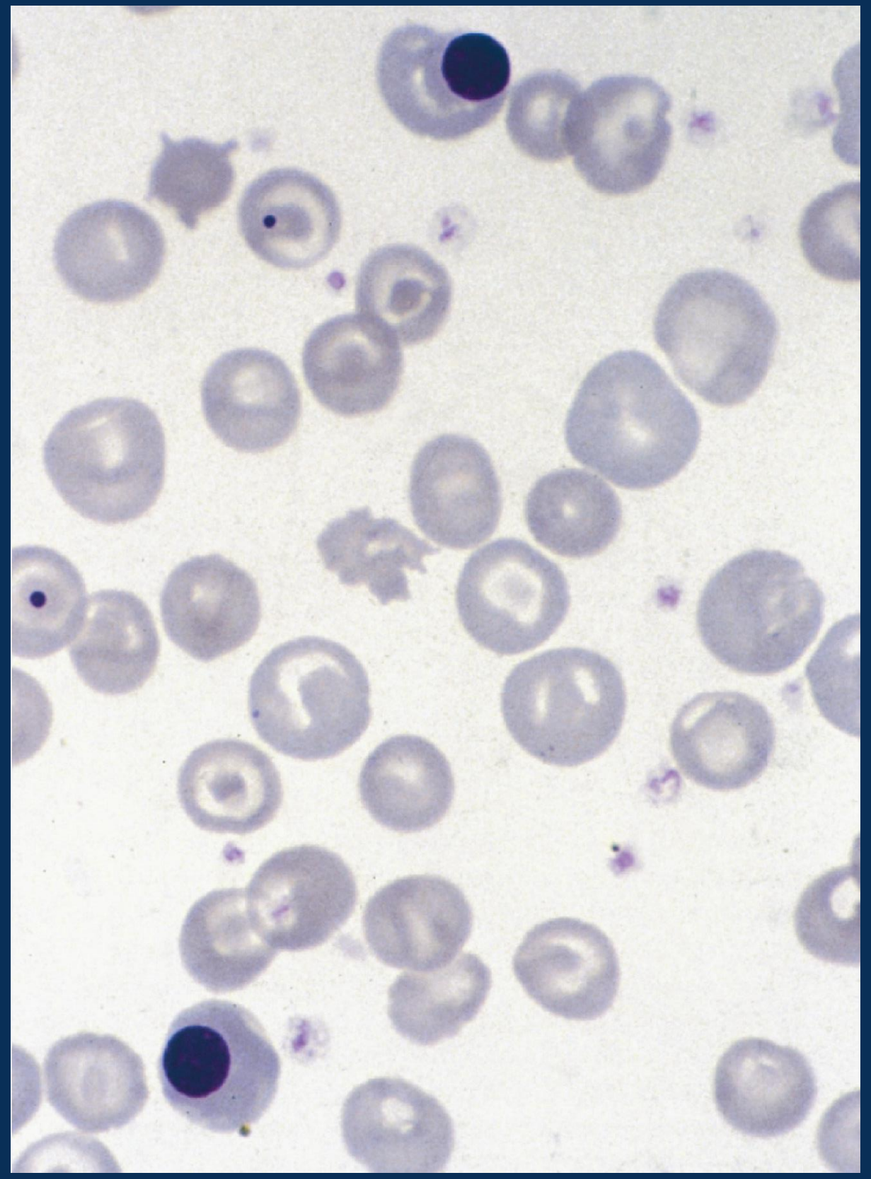
PK Deficiency

- Pyruvate kinase
  - Conversion of ADP to ATP
- PK deficiency (block in glycolysis)
  - ↓ ATP production
    - Cell membrane damage
    - Failure of cation pump = loss of Na+, K+, gain Ca++
    - Cell dehydration (echinocytes, target cells)
- Accompanied by 2–3x ↑ 2,3-BPG



PK Deficiency

- Normocytic/normochromic anemia
- Reticulocytosis from 2–15%
- Fluorescent screening test
  - RBCs incubated with PEP, LD, ADP, NADH
    - PEP + ADP →(PK)→ Pyruvate + ATP
    - Pyruvate + NADH + H + →(LD)→ Lactate +NAD+
    - Based on disappearance of fluorescence



Immune Defects

Immune Defects

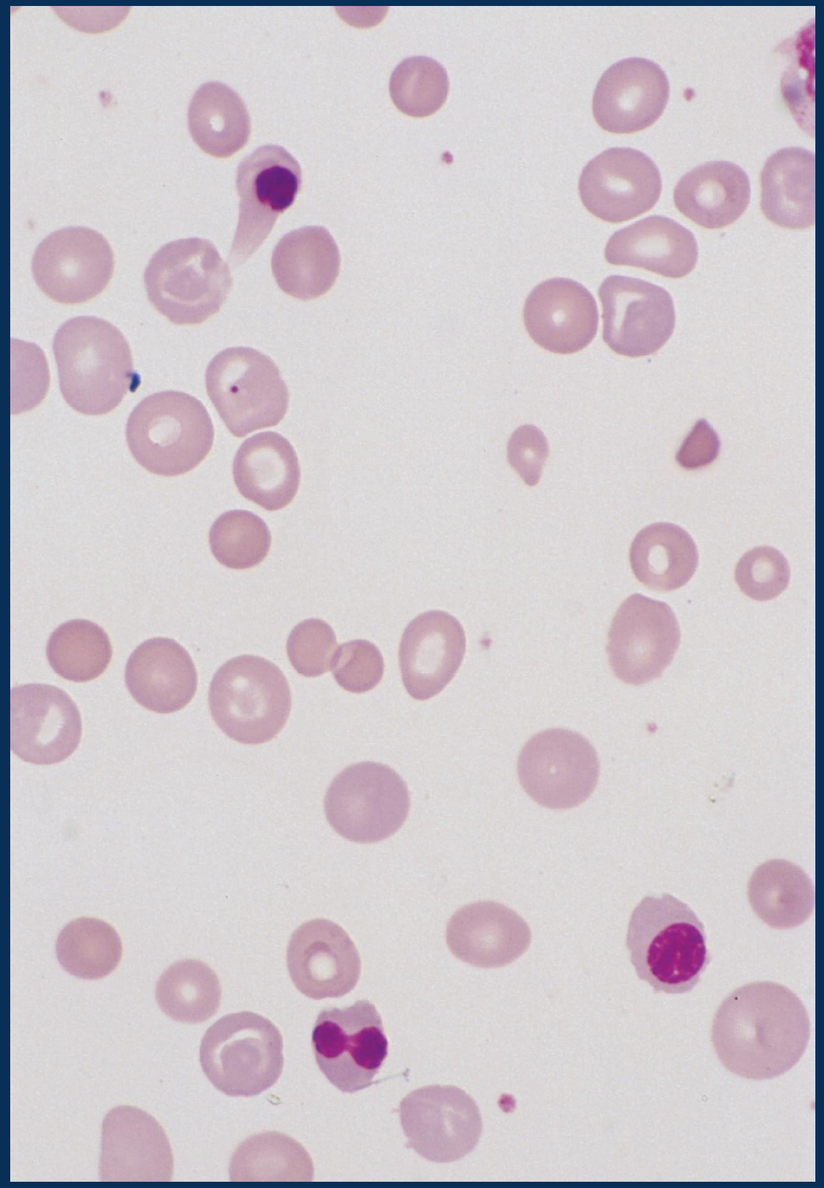
- Immune hemolytic anemia (IHA)
  - Autoimmune hemolytic anemia (AIHA)
    - Paroxysmal Cold Hemoglobinuria
  - Drug-induced hemolytic anemia
    - Immune response to drug-induced alteration of RBC
  - Alloimmune hemolytic anemia
    - Transfusion reactions
    - Hemolytic Disease of the Fetus and Newborn (HDFN)
      - ABO, Rh incompatibilities

Autoimmune Hemolytic Anemia

- Defects in regulation of immune tolerance against self
  - Genetic predisposition
  - Exposure to infectious agents (molecular mimicry)
- Warm AIHA
  - Optimal reactivity at 37°C
  - Usually IgG
- Cold AIHA
  - Optimal reactivity < 37°C
  - Usually IgM

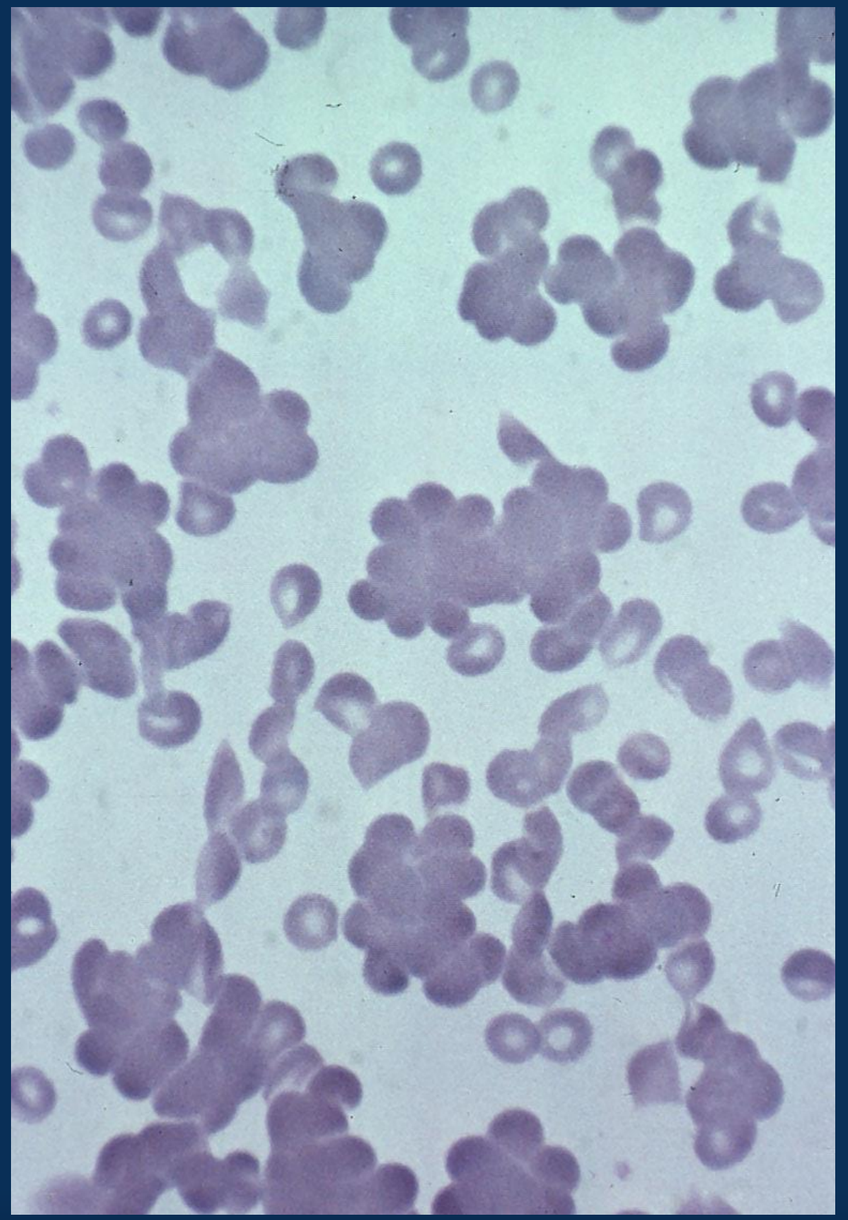
Warm AIHA

- ~ 70% of cases AIHA
- Most Abs react with "Rh protein" complex
- Most hemolysis is extravascular
- Moderate to severe normocytic/normochromic anemia
- Reticulocytosis, nRBCs, spherocytes, schistocytes
- + DAT (direct antiglobulin test)
  - + polyspecific AHG and anti-IgG monospecific AHG
  - 30% + anti-C3



Cold AIHA

- ~ 16-30% of cases AIHA
- Most Abs react with I/i Ag’s
- Most hemolysis due to complement-mediated lysis
- Mild to moderate normocytic/normochromic anemia
- RBC clumps, reticulocytosis, nRBCs, spherocytes, schistocytes
- + DAT (direct antiglobulin test)
  - + polyspecific AHG and anti-C3 monospecific



Paroxysmal Cold Hemoglobinuria

- Bi-phasic complement fixing IgG antibody
  - Donath Landsteiner antibody
    - Binds RBCs at low temps (< 20°C)
      - activates complement
    - Upon warming to 37°C, Ab detaches
    - RBC lysed by complement activation
  - Usual reactivity = P-antigen
- DAT negative for antibodies
- DAT weakly + for complement

Paroxysmal Cold Hemoglobinuria

Patient's Whole Blood[a]	Control	Test
Incubate for 30 min at	37°C	4°C
Incubate for 30 min at	37°C	37°C
Centrifuge: observe plasma for presence of hemolysis		
Interpretation		
D-L antibodies present	No hemolysis	Hemolysis
No D-L antibodies present	No hemolysis	No hemolysis

[a]Two tubes of patient's whole blood are used; one tube serves as the control and the other as the test.

Drug-induced

- Drug binds to RBC membrane
  - Abs produced to react with epitopes specific to drug
  - Combination of drug and RBC proteins
  - Epitopes primarily on RBC membrane
- Lab testing
  - Drug dependent vs drug independent
- + DAT

Alloimmune

- Ab develops to a RBC Ag that the individual lacks
  - Patient transfused foreign RBC's
  - Ag's on transfused cells stimulate production of Ab (alloAb)
- Seen in:
  - Transfusion reactions
  - Hemolytic disease of the fetus or newborn (HDFN)

Transfusion Reactions

- Patient’s Ab’s recognize foreign Ag’s on transfused RBC’s
- Two types of transfusion reactions
  - Immediate (IgM)
    - Occurring within 24 hr
    - intravascular hemolysis
  - Delayed (IgG)
    - Occurring 2–14 days after transfusion
    - extravascular hemolysis

HDFN

- Mother forms alloAb's against fetal RBC Ag's
  - IgG Ab's cross placenta → destroys fetal RBCs in utero
- Three ABO, Rh incompatibility categories:
  - Rh(D) caused by anti-D (more severe)
    - Immune IgG = no spherocytes or schistocytes
  - ABO caused by anti-A and/or anti-B (more common)
    - Nonimmune IgG = spherocytes and schistocytes present
  - "Other" caused by Ab's to other blood group system Ag's

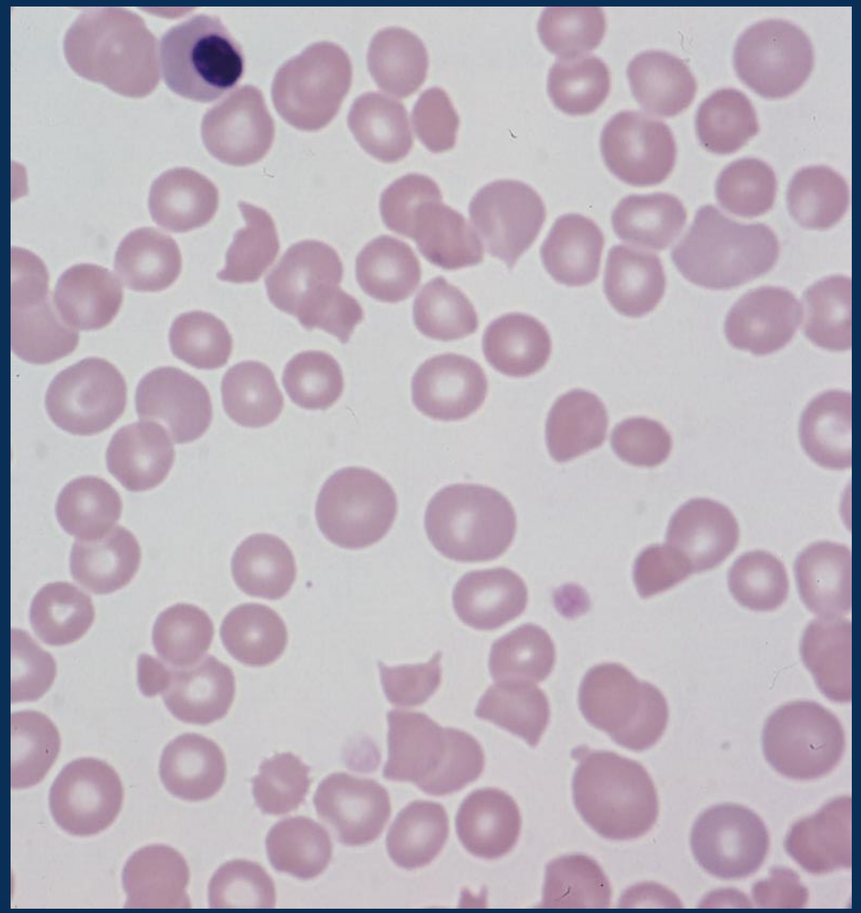
Nonimmune Defects

Nonimmune Defects

- Microangiopathic Hemolytic Anemia (MAHA)
  - Hemolytic Uremic Syndrome (HUS)
  - Thrombotic Thrombocytopenic Purpura (TTP)
  - Disseminated Intravascular Coagulation (DIC)
- Hemolytic Anemias from Antagonists
  - Malaria
  - Babeosis

Microangiopathic Hemolytic Anemias

- Hemolytic process caused by microcirculatory lesions
  - Damage to endothelial lining of the small vessels
  - Deposition of platelets and fibrin in microvasculature
- Schistocytes, keratocytes/helmet cells, ↑ reticulocytes
- Hemolysis may be intravascular and/or extravascular
- Disorders associated with MAHA
  - HUS, TTP, DIC

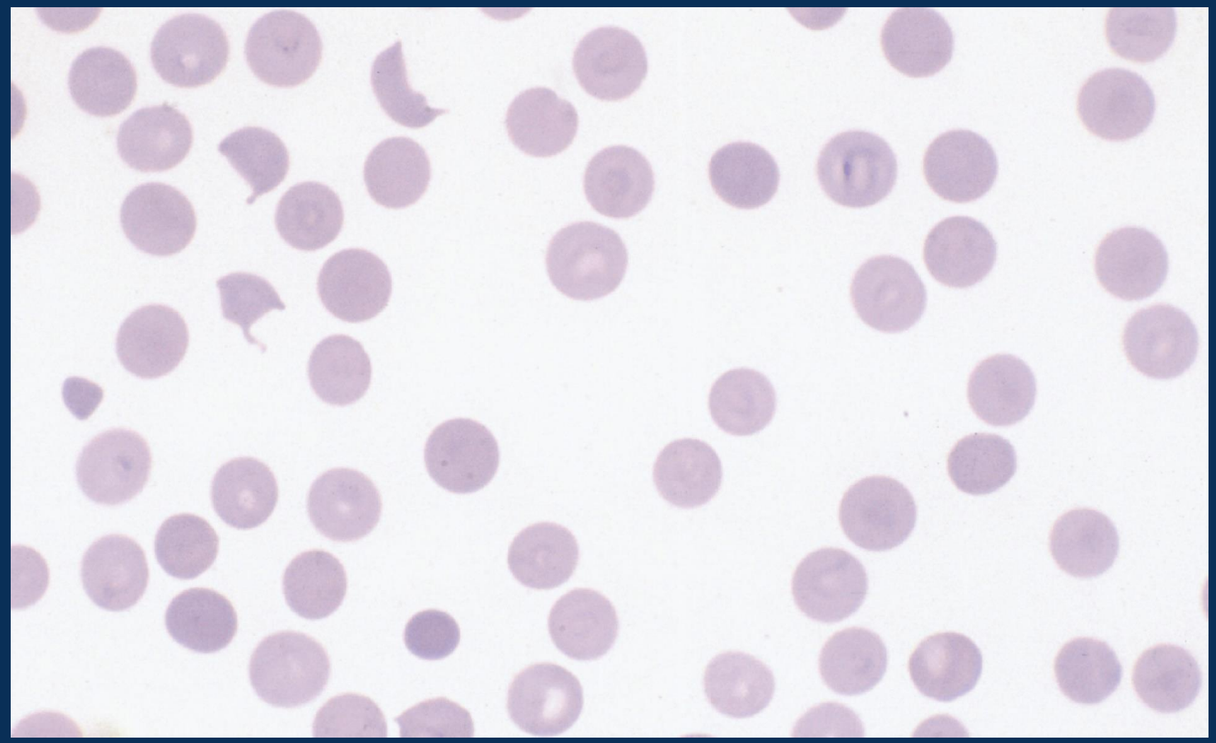


Hemolytic Uremic Syndrome

- D+ HUS (diarrhea-associated)
  - 90% of cases, most in children ≤ 5
  - GI infections – Shiga toxin producing E. coli
    - Damage to intestinal mucosa
    - Endothelial cells of glomerulus microvasculature
- D– HUS (nondiarrhea-associated) / Atypical HUS (aHUS)
  - Associated with lupus, cancer, diabetes, Streptococcus pneumoniae infections, immunosuppressive therapy
  - Observed in children and adults

Hemolytic Uremic Syndrome

- Moderate to severe normocytic, normochromic anemia
- Schistocytes, helmet cells, spherocytes, echinocytes/burrs
- Polychromasia, occasional nRBC
- Leukocytosis with left shift
- Platelet counts: low normal to markedly
- D-dimer ↑



Thrombotic Thrombocytopenic Purpura

- Acute disorder with platelet aggregation on microvascular endothelium
- Affects young adults (20–50 yrs)
- Mostly precipitated from infections
- Microthrombi
  - Composed of platelets and large vWF multimers
  - Occlude capillaries and arterioles in organs
    - Kidneys, heart, brain, pancreas
  - Deficiency in ADAMTS13 is cause of TTP

Thrombotic Thrombocytopenic Purpura

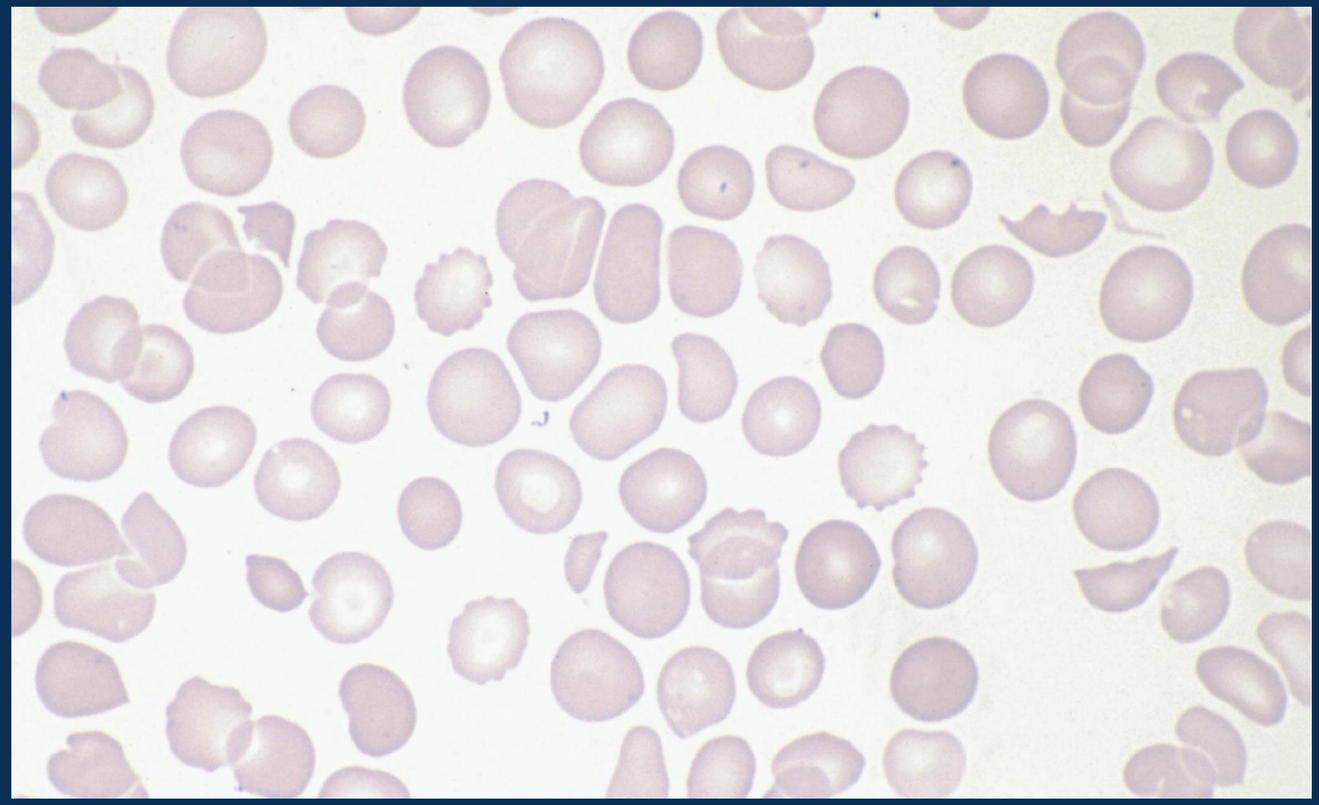
- Normocytic, normochromic anemia
- Polychromasia, nRBC’s
- ↑↑ schistocytes
- Leukocytosis with left shift
- Severe thrombocytopenia

Disseminated Intravascular Coagulation

- Normal coagulation process altered
  - Bacterial sepsis, Neoplasms, Immunologic disorders, Trauma
- Damage to endothelial lining of vessels
  - Release of thromboplastic substances
  - Activate coagulation mechanism
    - Platelet activation and aggregation

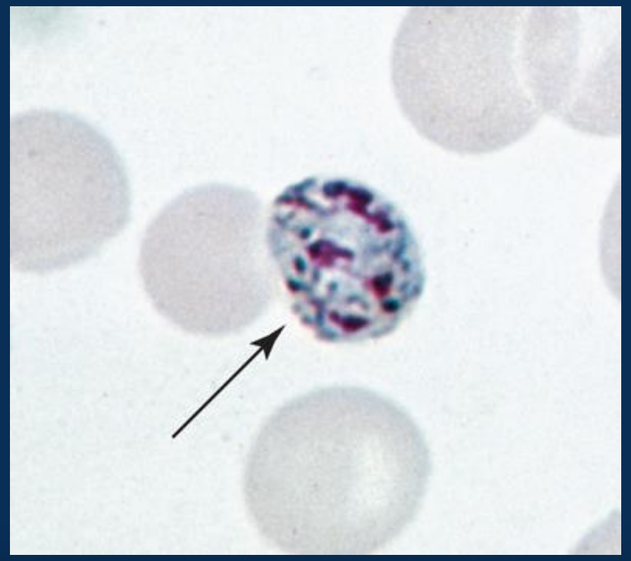
Disseminated Intravascular Coagulation

- Consumptive coagulopathy
  - Severe thrombocytopenia
  - Decreased coagulation factors
- Schistocytes
- Abnormal coagulation
  - Prolonged PT, APTT, TT
  - ↑ D-dimer, FDPs
  - ↓ fibrinogen



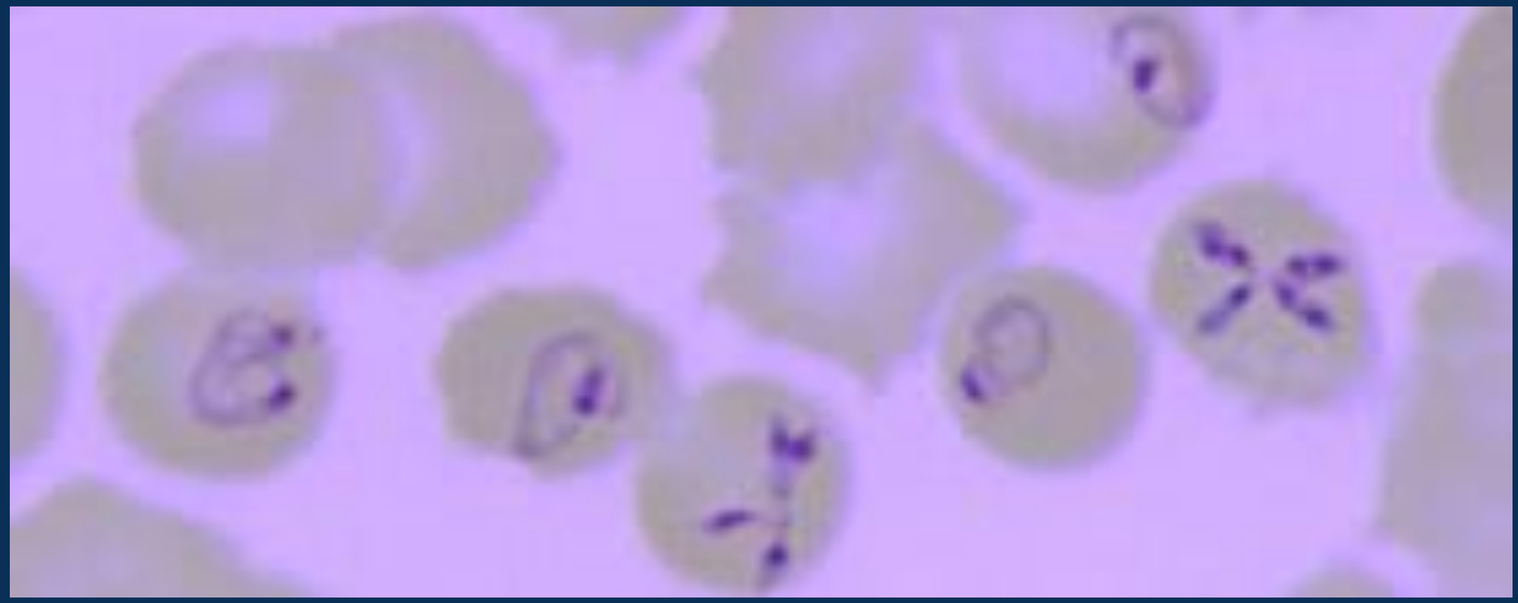
Malarial parasites

- Mild normocytic, normochromic anemia
- Extravascular destruction of parasitized RBCs
- Diagnosis is finding life cycle stage within RBCs



Babesiosis

- Mild to moderate anemia, ↑ reticulocytes, ↓ platelets
- Extravascular destruction of parasitized RBCs
- Diagnosis is finding life cycle stage within RBCs
  - Maltese cross



References

American Society for Clinical Laboratory Science. (2016). Hematology and Hemostasis Medical Laboratory Scientist Entry Level Curriculum. American Society for Clinical Laboratory Science.

American Society for Clinical Pathology. (2021). Medical Laboratory Scientist, MLS(ASCP) Examination Content Guideline. American Society for Clinical Pathology.

Greer, J. (2014). Wintrobe's clinical hematology (Thirteenth ed.). Philadelphia, Pennsylvania: Lippincott Williams & Wilkins.

Kaushansky, Kenneth. (2016). Williams hematology (9th ed.). New York: McGraw Hill Education.

McKenzie, S. B., & Williams, J. L. (2015). Clinical laboratory hematology (3rd ed.). Boston: Pearson.

McPherson, R., & Pincus, M. (2017). Henry's clinical diagnosis and management by laboratory methods (23rd ed., ClinicalKey). St. Louis, Mo. : Elsevier.

Rodak, B. F., & Carr, J. H. (2015). Clinical Hematology Atlas. Elsevier Health Sciences.