Med Chem 535P ~ Diagnostic Medicinal Chemistry

Hematology ~ Erythrocytes (Red Blood Cells, RBCs)

I. Tests

A. Red Blood Cell Count (RBC)*
B. Hemoblobin (Hb or Hgb)*
C. Hematocrit (Hct)*
D. Wintrobe Indices (Indices)
   1. *Mean Corpuscular Volume (MCV)*
   2. *Mean Corpuscular Hemoglobin (MCH)*
   3. *Mean Corpuscular Hemoglobin Concentration (MCHC)*
E. Red Cell Distribution Width (RDW)
F. Reticulocyte Count (Retics)*
G. Erythrocyte Sedimentation Rate (ESR or Sed Rate)
H. Serum Ferritin
I. Transferrin and Serum Iron
J. Haptoglobin
K. Zinc Protoporphyrin Hemoglobin (ZPPH)

II. RBC Disorders

A. Polycythemia
B. Anemias
   1. Acute Blood Loss
   2. Hemolytic Anemia
   3. Anemia of Chronic Disease
   4. Nutritional
C. Hemoglobinopathies
   1. Sickle Cell Anemia
   2. Thalassemia
   3. Methemoglobinemia/G6PD Deficiency

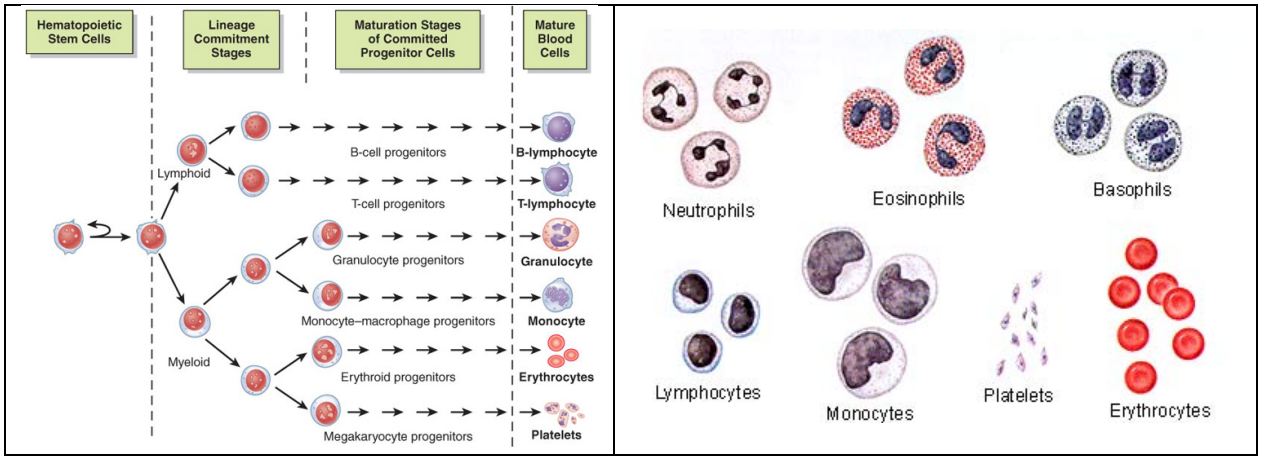
III. Drug Induced Anemia

A. Drug-Induced Oxidative Stress
B. Cyanide Poisoning
C. Immune Hemolytic Anemia

HEMATOLOGY ~ ERYTHROCYTES

HEMATOLOGY ~ the study of blood and its cellular elements: ***Erythrocytes*** (a.k.a., red blood cells), ***Leukocytes*** (a.k.a., white blood cells), and ***Platelets***.

The bone marrow typically produces ~ 2.5 x 10^9 RBCs, 1 x 10^9 granulocytes and 2.5 x 10^9 platelets per kg/day.

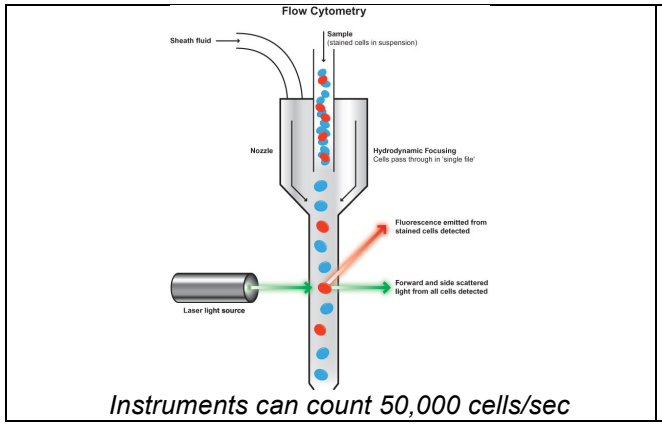


CBC ~ *Complete Blood Count* is one of the most commonly ordered lab tests. It typically includes an erythrocyte count (RBC), leukocyte count (WBC), hemoglobin concentraton (Hgb), hematocrit (Hct), RBC indices, reticulocyte count, and platelet count.

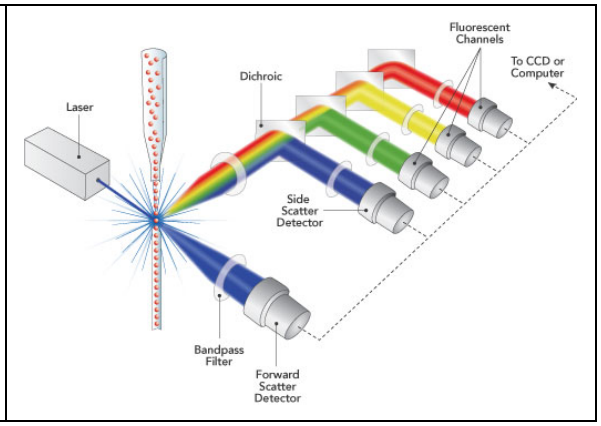
When a **CBC *with differential*** (Dif) is ordered, the WBC subtypes are *quantified*.

Absolute Neutrophil Count (ANC) ~ neutrophil absolute number vs. relative percent

Flow Cytometry



Instruments can count 50,000 cells/sec



When a particle passes through the detector it scatters light.

Forward scattering is proportional to the size of the particle (cell):

Particles between 2 fL and 20 fL are platelets;
Particles > 36 fL are RBCs and WBCs;
Analysis of samples with lysed RBCs affords WBCs.

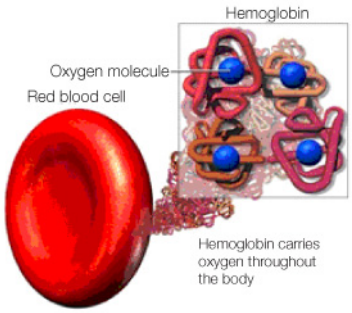
Side-scattering light is a reflection of cellular complexity; lymphocytes, monocytes, and neutrophils have increasing amounts of DNA and granules and increasing amounts of side scattered light.

Cells have specific “surface markers” that can be tagged with specific fluorescent antibodies. This allows identification of very specific cell types in the blood.

I. Tests

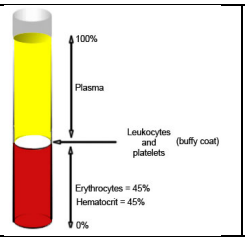
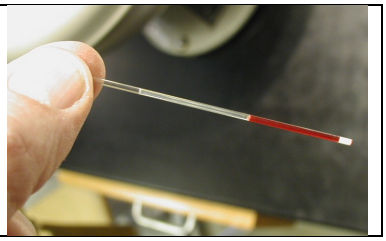
A. Red Blood Cell Count (RBC). *Reference Range:* *4.5 - 5.7 x 10^6 cells/μL (male)**
*3.9 - 5 x 10^6 cells/μL (female)**

B. Hemoglobin Concentration (Hb, Hgb). *Reference Range:* *14 – 18 g/dL (male)**
*12 – 16 g/dL (female)**

This is a quantitation of the *concentration of hemoglobin* in the blood. It is measured spectrophotometrically. Note that the normal range is different between males and females; a number to keep in mind is 15 g/dL.	

C. Hematocrit (Hct). *Reference Range:* *42% – 50% (male)**
37% - 47% (female)

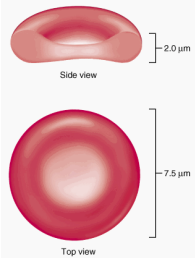
Hct is the volume of cells that is composed of erythrocytes. It is usually reported as a percentage.

Reference ranges differ between males and females; a number to keep in mind is 45% for males.		

Rule of 3: *Hgb= 3*RBC*
*Hct= 3*Hgb*

Rule of Thumb: Female values ~ 87% those of males

D. RBC Indices (Wintrobe Indices). These represent red blood cell characteristics, essentially the size and Hb content of the RBCs.



These are derived values, calculated from Hgb, Hct, and RBC count.

1. *Mean Corpuscular Volume* (MCV). *Reference Range:* 80 - 95 fL/cell

   This is the average volume of an individual RBC in the blood (*normocytic*)

$$\text{MCV (fL)} = \frac{\text{Hct (\%)}}{\text{RBC } (10^6/\mu l)} \times 10$$

   microcytic ~ decrease in MC (iron deficiency, thalassemia)

   macrocytic ~ increase in MCV (folate, B12 deficiency, alcoholism)

   normocytic ~ normal MCV (hemorrhage, chronic disease)

2. *Mean Corpuscular Hgb* (MCH). *Reference Range:* 27 – 31 pg/cell

   This is the average *amount* of hemoglobin in an individual RBC:

$$\text{MCH (pg/cell)} = \frac{\text{Hgb (g/dL)}}{\text{RBC } (10^6/\mu l)} \times 10$$

   A low MCH is associated with *hypochromic* cell and is seen with iron deficiency anemia.

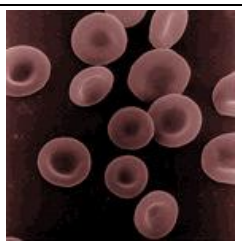
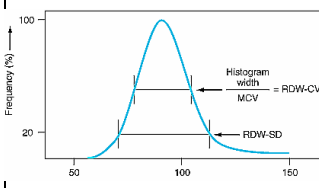
3. *Mean Corpuscular Hgb Conc.* (MCHC). *Reference Range:* 32 – 36 g/dL

   This is the average *concentration* of Hb in an individual RBC

$$\text{MCHC (g/dL)} = \frac{\text{Hgb (g/dL)}}{\text{Hct (\%)}} \times 100$$

   As with MCH, a low MCHC is associated with *hypochromic* cell and is seen with iron deficiency anemia.

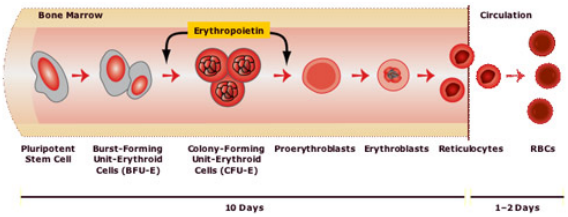
E. RBC Distribution Width (RDW). *Reference Range:* 11% - 16%

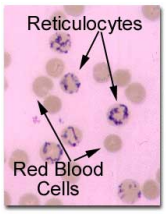
This is the variation in RBC *size;* an increase is called *anisocytosis* Anemia can result in RDW > 16% Zidovudine (AZT) therapy causes an increase in RDW without symptoms of anemia. RDW is sometimes used to monitor compliance.		

F. Reticulocyte Count (Retics). *Reference Range:* 0.5% - 2%*

Reticulocytes are immature RBCs. They still contain some DNA and RNA, which can be visualized with Wrights stain. They typically persist in circulation for ~ 2 days before fully maturing into erythrocytes.

An increase in retics is associated increased RBC production





An increase is observed with chronic hemorrhage, hemolysis, and with the initiation of iron therapy.

Decreases are observed with chronic anemia due to iron, folate, B12 deficiency

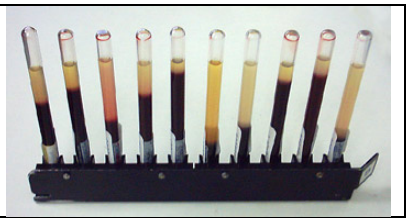
In anemia the Hct drops, which can artificially raises the retic count; this can be corrected using the Reticulocyte Index:

$$RI = \%\ Retics * \left(\frac{Actual\ Hct}{Normal\ Hct}\right)$$

An increase in retic count can artificially increase the MCV.

G. Erythrocyte Sedimentation Rate (Sed Rate, ESR).

Reference Range: 1 – 10 mm/hr (males)
1 – 20 mm/hr (females; increases with age)

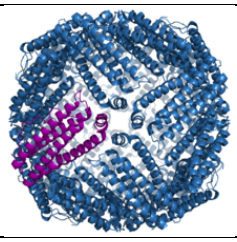
ESR is the rate that RBCs settle to the bottom of a standardized tube under specific conditions. Normal RBCs settle slowly, but this rate can increase when the cells aggregate. It is a rapid (but non-specific) test that can suggest some occult disease, typically inflammatory.	

Increases are observed in the active phase of an inflammatory disease such as rheumatoid arthritis, infection (often respiratory), and pregnancy.

Decreases are observed with congestive heart failure, sickle cell anemia, and carcinoma; high dose glucocorticoid therapy can also decrease ESR.

ESR is most useful to monitor the course of a disease as it is being treated.

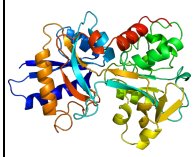
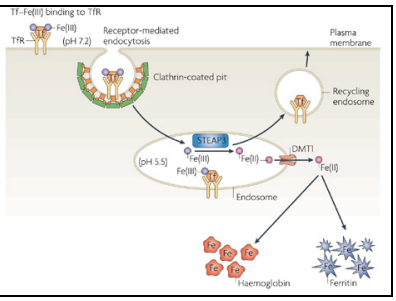
H. Serum Ferritin. *Reference Range:* 12 - 300 ng/mL (males)
12 - 150 ng/mL (females)

Ferritin is an *intracellular* protein that *stores* iron (Fe^{3+}; liver, marrow, and spleen macrophages). It is composed of 24 subunits and can store about 4500 iron atoms. Accumulation of ferritin within the cell generates *hemosiderin.*	

The concentration of ferritin in the serum is directly related to the amount stored in cells, and thus *total iron storage*. Serum ferritin levels are markedly reduced in iron deficiency anemia.

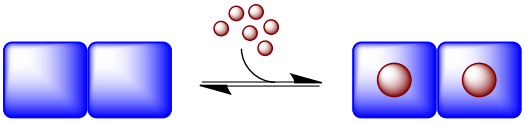
Elevated levels can be observed with a chronic disease or an inflammatory condition. A value > 300 ng/ml means a severe tissue iron overload and can often lead to a heart attack.

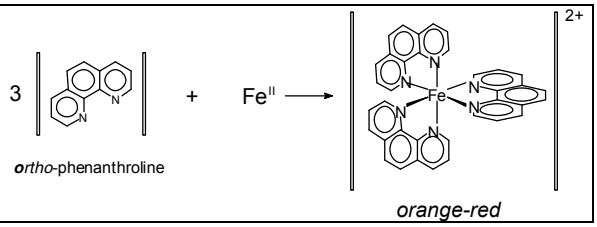
I. Transferrin and Serum Iron

Transferrin is a plasma protein that transports free iron in solution; the protein reversibly binds two Fe^{3+} ions. The concentration of Fe regulates transferrin biosynthesis.		

1. *Serum Iron* (Fe). *Reference Range: 50 – 160 µg/dL (males)*
40 – 160 µg/dL (females)

Note that all circulating iron is bound to transferrin:





2. *Total Iron Binding Capacity* (TIBC). *Reference Range:* 250 – 400 µg/dL

This test measures the iron binding capacity of transferrin and is an *indirect* measure of transferrin concentration. Iron is added to the sample to saturate the transferrin binding sites. The excess is removed and iron content quantified.

An ELISA assay is now available for direct quantitation of transferrin.

3. *Total Transferrin Saturation (TSAT):* $\text{TSAT} = \left(\frac{\text{Serum Iron}}{\text{Total Iron Binding Capacity}} \right)$

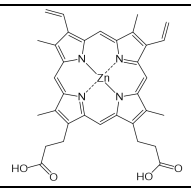
Typical value is ~ 30%

Condition	[Fe]	TIBC	TSAT
Iron deficiency	↓	↑	↓
Anemia of chronic disease (carcinoma, infections)	↓	↓	normal
Iron overload (hemolysis)	↑	↓	↑
Pregnancy, oral contraceptives	normal	↑	↓

J. Haptoglobin (HPT) is a plasma protein that binds free Hb when RBCs are lysed *intravascularly*. The Hb•HPT complex is degraded in the marrow and spleen.

**Plasma HPT less than 30 mg/dL indicates RBC hemolysis.*

K. Zinc Protoporphyrin Hemoblobin (ZPPH) is contains a zinc atom in place of the heme iron.

Normally 30-80 µmole/mole Hb. Elevated values usually indicate iron deficiency anemia; lead poisoning can also lead to an elevated ZPPH; Thus, this test can be used as a screen for iron deficiency anemia and lead poisoning.	

II. RBC Disorders

A. Polycythemia is an increase in RBC greater than 6 $X10^6$/dL, Hct > 55%. This can be a secondary effect of chronic hypoxemia from chronic lung diseases, from certain types of thallasemias, living at elevated altitude, or bone-marrow related disorders (e.g., polycythemia vera).

B. Anemia is a pathological condition where there is a decrease in RBC, Hb, and Hct leading to pallor of the skin, weakness, lethargy, and dizziness.

Anemia is not a disease in and of itself, but rather a manifestation of an underlying problem.

This can be caused by:

1. Acute Blood Loss. This situation typically results in normocytic, normochromic RBCs. Tachycardia, breathlessness, hypovolemia is observed.

2. Hemolytic Anemia ~ the process of premature RBC destruction.

Intravascular hemolysis occurs in the bloodstream. Haptoglobulin levels drop and an increase in serum LDH is observed in this situation.

Extravascular hemolysis results from ingestion of RBCs by macrophages in the speen and liver.

Hemolysis is associated with genetic defects in Hb (sickle cell, thalassemia), metabolic disorders (G6PD deficiency), and with certain drugs *(discussed further below*).

**Similar labs as with acute blood loss, but with reticulocytosis.*

3. *Anemia of Chronic Disease* may be associated with chronic infection (TB), chronic inflammatory illness (rheumatoid arthritis), and hematological malignancies (leukemia, Hodgkin's disease). This is typically a normocytic, normochromic anemia with a low reticulocyte count.

4. *Nutritional: Lack of Factors for Proper RBC Maturation*

i. Macrocytic Anemia typically results from folate or vitamin B12 (cobalamin) deficiencies.

•*Cobalamin* (B12) is required for DNA synthesis and the erythrocyte maturation is defective. The cells are large (*megalocytes*), deficient in number, and Hb content. Reticulocyte count is low.

B12 deficiencies typically result from decreased absorption, which can occur in the elderly, in alcoholics, and in malabsorption syndromes such as Crohn's disease, celiac disease, and *pernicious anemia* (an autoimmune disorder where antibodies are directed against "intrinsic factor" required for B12 absorption). Vegans and vegetarians are at risk.

Colchicine can decrease vitamin B12 absorption.

Treatment is B12 administration. Unfortunately, neurological symptoms are most troublesome and may persist even after therapy.

•*Folic acid* (B9) is required in a variety of biochemical reactions, including nucleotide, amino acid, and neurotransmitter biosynthesis.

Effects on DNA synthesis result in a presentation similar to B12 deficiency (macrocytic, megaloblastic anemia).

Deficiencies are typically due to inadequate dietary intake and from drug interactions; phenytoin and penobarbital can decrease folate absorption; methotrexate, trimethoprim, trimetrexate, and sulfamethoxazole can inhibit folate metabolism.

Treatment is folate administration; *note that folate may reverse the anemia in B12 deficiency, but will not stop the neurological sequelae!*

ii. Microcytic Anemia is predominantly a result of iron deficiency; the cells are hypochromic as well.

The first sign of iron deficiency is an increase in RDW. Patients present with a decrease in iron and serum ferritin levels, which triggers an increase in transferrin biosynthesis (increase in TIBC).

With severe deficiency, erythropoiesis is affected -> microcytic (low MCV), hypochromic (low MCH), anemia (low RBC).

Common causes are poor nutrition, pregnancy (increased demand), slow and continuous loss of blood, as seen in GI ulcers, hemorrhoids, menstruation, and celiac disease.

Tetracyclines and many antacids bind to iron and interfere with absorption; H2 antagonists decrease the acidity of the upper GI tract, which also decreases absorption.

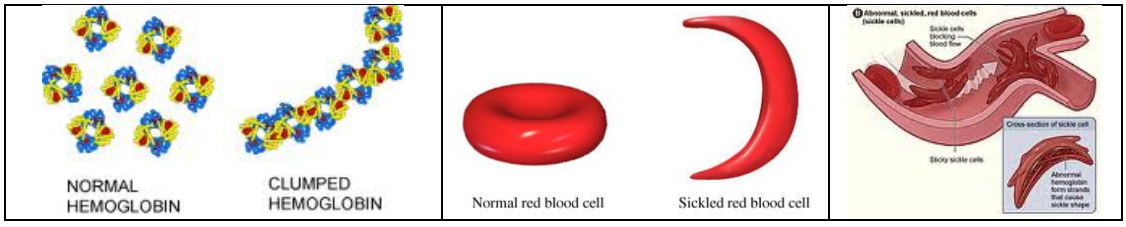
Treatment is iron replacement therapy.

C. Hemoglobinopathies result from hereditary mutations in the Hb genes. Normal adult hemoglobin (HbA) is a heterotetramer that contains two α-chains and two β-chains. Over 1000 mutations of the Hb chains have been identified; not all are pathologic.

1. *Sickle Cell Trait* results from an E->V mutation in the Hb β-chains. This leads to aggregation of the HbS tetramer *at low oxygen tension* and "sickling" of the cells.

Poikilocytosis: abnormal RBC shape (*poikilos*, ancient greek for "varied")

The cells are also "sticky" and rigid, and they form microinfarcts in the capillaries. Hemolytic anemia and spleenomegaly is observed.



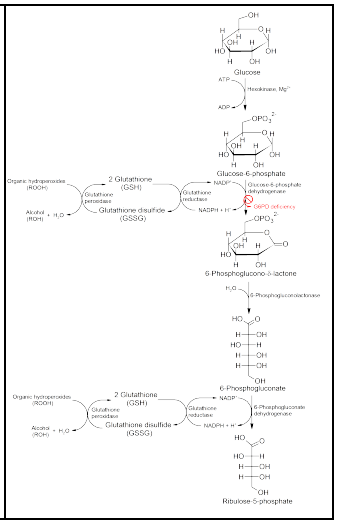
2. *Thalassemias* result from a defect or relative deficiency in of one of the Hb chains (thalassemia major vs. thalassemia minor). α-thalassemia is most common in peoples from southeast Asia, the Middle East, China, and in those of African descent; β-thalassemia are most common in persons of Mediterranean origin, and to a lesser extent, Chinese, other Asians, and African Americans.

The RBCs of these patients are often microcytic and hypochromic due to a decrease in Hb content. Anisocytosis and poikilocytosis may also exist.

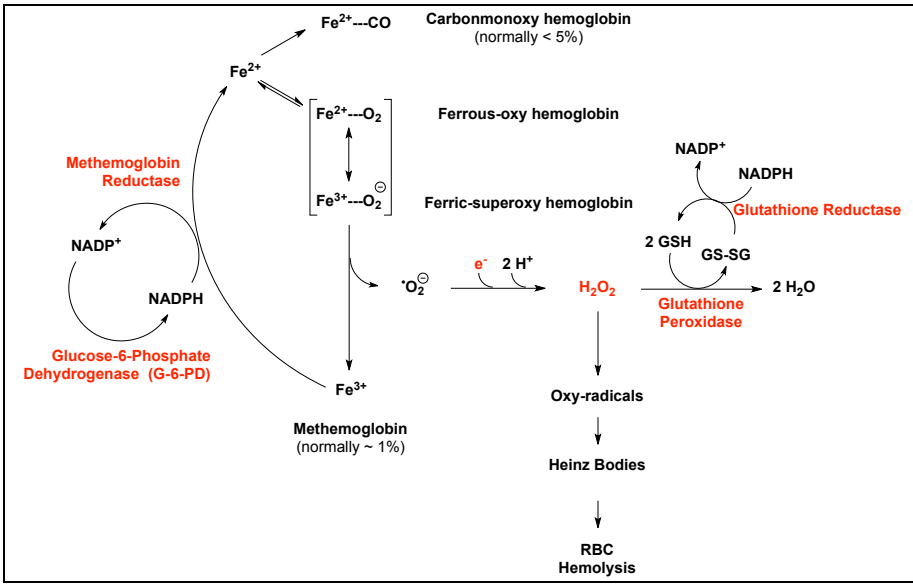
3. Methemoglobinemia and Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency.

Glucose-6-phosphate dehydrogenase is a key enzyme in the pentose phosphate pathway, which is the only source of NADPH, and thus reduced glutathione in the red blood cell. Thus, patients with G6PD deficiencies are thus at risk for oxidative damage to RBCs under conditions of oxidative stress.

In addition, patients with a genetic deficiency in G6PD are predisposed to methemoglobinemia; this occurs in 10% African Americans, 30% Mediterranean/Asian descent.



Methemoglobinemia. Hemoglobin normally binds oxygen reversibly but a small percentage (~ 1%) is reduced to superoxide ($^{\bullet}O_2^-$) with concomitant oxidation of the ferrous heme to the ferric state (metHemoglobin). MHb is reduced back to the ferrous state by the enzyme methemoglobin reductase, which requires NADPH as a co-factor.

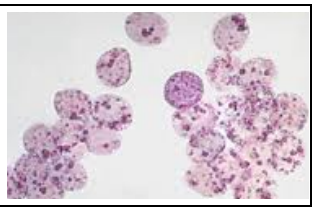


"*Hemoglobin M Disease*" refers to mutations in hemoglobin that predispose the protein to oxidation (which affords MHb and $^{\bullet}O_2^-$) and/or resistance to reduction of MHb back to Hb by methemoglobin reductase.

This results in *methemoglobinemia*.

III. Drug-Induced Anemia

A. Drug-Induced Oxidative Stress. Some drugs and/or their metabolites can promote hemoglobin oxidation leading to the formation of methemoglobin and oxidative stress. Patients with G6PD are extra sensitive to RBC oxidative stress, which can cause drug-induced hemolytic anemia.

Heinz-Ehrlich Bodies are small inclusions within a RBC. They are the result of MHb formation and damage by oxidation. The damaged Hb aggregates and deforms the cell membrane, which can lead to hemolysis; alternatively, the damaged cell is removed from circulation by the spleen.	

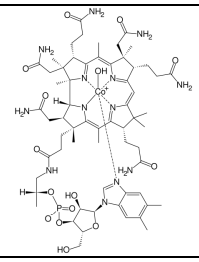
Common drugs include: catechol's (L-dopa, α-methyldopa)
nitrates (nitroglycerine, isosorbide dinitrate)
sulfa drugs (sulfonamides, trimethoprim), dapsone
anti-malarials (chloroquine, primaquine, quinine)
benzocaine, **lidocaine**

B. Cyanide Poisoning. Cyanide (CN^-) inhibits cytochrome C oxidase in the mitochondrial respiratory chain and causes a "histotoxic hypoxia" because cells are unable to use oxygen.

The historical cyanide antidote kit contains amyl nitrate (inhalation), sodium nitrite (infusion) and sodium thiosulfate (infusion):

$$Fe^{2+} \xrightarrow{\text{Amyl nitrate}} Fe^{3+} \xrightarrow{\text{Cyanide}} Fe^{3+}\text{---}CN$$

Sodium thiosulfate enhances the conversion of cyanide to thiocyanate, which is excreted. Ferric Hb can then be reduced to Ferrous Hb with methylene blue.

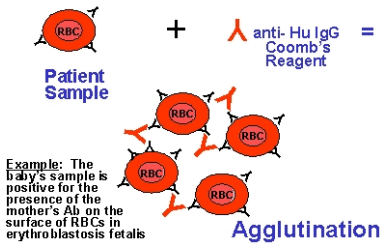
A newer antidote, hydroxocobalamin (vitamin B12), binds circulating and cellular cyanide molecules to form cyanocobalamin, which is excreted in the urine.	

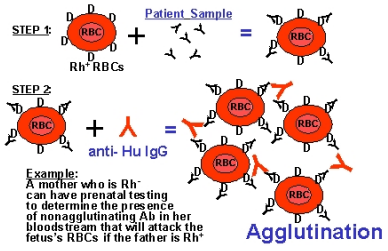
C. Immune Hemolytic Anemia

This is caused by drugs or their metabolites that bind to proteins in the RBC membrane to present a "*hapten*" complex.

This triggers γ-globulin and complement binding to the RBC, which leads to extravascular hemolysis.

Examples Include: **methyldopa**, procainamide, quinine/quinidine, sulfonamides, **penicillins**, cephalosporins, NSAIDS, and anti-tumor alkylating agents (especially platinum agents).

The *Direct Antiglobulin Test (DAT, a.k.a., direct Coomb's test)* is used to detect antibodies *bound to patient RBCs*. This test uses rabbit or goat anti-human globulin. If the patient's RBC's have bound γ-globulins they will agglutinate (Coomb's Test positive). *The DAT is most often used to test for autoimmune hemolytic anemia.*	

The *Indirect Antiglobulin Test (IAT, a.k.a., indirect Coomb's test)* is used to detect antibodies *in the patient's serum*. The patient's serum is mixed with RBCs that contain the antigen in question. Anti-human globulin is then added and agglutination will occur if the Abs are present. *This test is used in prenatal blood testing (Rh antibodies) and in blood testing prior to a transfusion.*	

These tests are used in the investigation of transfusion reactions, auto-immune hemolytic anemia, hemolytic disease of the newborn, and for drug-induced hemolysis.

Hematology, RBC Study Guide

Terms You Need to Know

Anemia
Anisocytosis
Heinz-Ehrlich Bodies
Hematocrit
Hypochromic
Macrocytic
Microcytic
Normocytic
Poikilocytosis
Polycythemia

You should be prepared to describe the basic principle behind flow cytometry.

You need to know the reference ranges for the lab tests indicated with a red asterisk in the lecture notes.

You should be prepared to describe exactly what hematocrit is and how it is measured. How does this differ from ESR? What is the utility of each test?

The "Rule of Three" would be handy to know.

While you do not need to memorize how they are calculated, you should be prepared to describe what the RBC indices and RDW actually measure. How do they change in relation to physiological conditions such megaloblastic vs. iron deficiency anemia, and other conditions discussed in class.

You should be prepared to describe what a reticulocyte is and how blood levels change with specific physiological conditions. What is the significance of the reticulocyte index?

You should be prepared to describe the role of ferritin, hemosiderin, and transferrin in iron transport and storage. What tests are used to "quantify" each? Know the location of each protein within the organism and how their serum concentrations change in response to physiological conditions as discussed in the notes.

Be prepared to describe how plasma iron content is measured (what is the assay) and how this is used to determine the difference between TIBC and TSAT.

Be prepared to describe the biological role of haptoglobin and zinc protoporphyrin and how their blood levels may be affected by physiological conditions, such as hemolysis and iron deficiency anemia as discussed in the notes.

You should be prepared to describe the physiological conditions that can lead to polycythemia and anemia. Be prepared to discuss the different types of anemia (hemorrhagic, hemolytic, chronic disease, nutritional) and to use CBC results to define which specific type of anemia is present in a patient, as discussed in the notes.

What is the outcome and significance of treating B12-deficiency anemia with folic acid?

You should be prepared to describe the difference between intra- and extra-vascular hemolysis, and which lab values are useful in distinguishing between them.

Be prepared to describe the basic biological defect in thalassemia, sickle cell anemia, glucose-6-phosphate dehydrogenase deficiency, and methemoglobinemia. What is the biological consequence of each defect? What is their relationship to malaria?

Be prepared to describe the mechanisms of immune hemolytic anemia and drug-induced oxidative stress. Know which drugs are commonly associated with each (as indicated in the lecture and in the notes).

Be prepared to discuss the relationship between G6PD deficiencies, met-hemoglobinemia, and drug-induced oxidative stress?

Be prepared to describe the difference between the direct and indirect antiglobulin (Coombs) tests and what each test actually measures.

Bugs, drugs, disease states!