PATHOLOGY OF ANEMIA I

I. HEMATOPOIESIS - series of events in which pluripotent hematopoietic stem cells mature into functional blood cells.

A. Pluripotent stem cell is the precursor of all blood cell lines: red blood cells (RBCs), white blood cells (WBCs), platelets.
   1. Red blood cells (erythrocytes) – bulk of the formed elements in blood; anucleate biconcave discs that carry oxygen to the tissues and return to the lungs carrying carbon dioxide.
   2. White blood cells (leukocytes) – defend against infection, function mainly in tissues.
      a. Phagocytes
      b. Lymphocytes
   3. Platelets – anucleate fragments of a larger cell called a megakaryocyte; work with coagulation proteins to defend the blood vessels in case of injury.

Pluripotent stem cells

\[ \text{Committed stem cells} \]

\[ \text{Morphologically identifiable immature cells} \]

\[ \text{Mature cells} \]

B. Formed blood elements are visualized by means of peripheral blood smear.
   1. Drop of blood smeared out on glass slide.
   2. Dried smear stained with Wright’s stain.

C. Evaluation of hematopoiesis is accomplished by bone marrow biopsy and aspirate.
II. RED BLOOD CELLS (RBCs)

A. MATURATION SEQUENCE
1. Reticulocyte is usually the first stage of the RBC released from marrow into peripheral blood; cytoplasm may be slightly bluish-pink due to residual RNA (polychromasia).
2. Majority of RBCs in peripheral blood are mature biconcave discs with a normal life-span of about 120 days.

B. RBC MEMBRANE
1. Phospholipid bilayer combined with glycolipids and cholesterol; integral proteins span the bilayer (act as sites for RBC antigen formation).
2. Membrane cytoskeleton controls biconcave shape of cell deformability (qualities essential for travel through microcirculation).

C. HEMOGLOBIN
1. Hemoglobin synthesis involves 2 biosynthetic pathways.
   a. Synthesis of heme.
   b. Synthesis of globin chains.
2. Heme consists of 4 pyrrole groups joined into large ring with ferrous ion incorporated into center.
3. a. Globin chains contain approximately 150 amino acids and each chain is arranged in a “knotted sausage” fashion; heme binds to a specific region of globin chain.
   b. Two globin dimers form hemoglobin. Most important hemoglobin is hemoglobin A \( (\alpha_2\beta_2) \), comprising more than 95% of the hemoglobin normal adult red cell.

D. RBC CATABOLISM
1. Aging RBCs are largely removed by mononuclear phagocytic engulfment in the spleen. Heme and globin chains are separated; heme is divided into iron (which is recycled) and porphyrin rings (eliminated as bilirubin), and globin is dismantled into amino acids.

Accelerated RBC destruction is called “hemolysis”
III. INTRODUCTION TO ANEMIAS

A. Physiologic Definition: Reduction in the oxygen transport capacity of the blood.

B. Anemia may also be defined as reduction below normal limits of the total circulating red cell mass.
   1. Reduction below normal in volume of packed cells (hematocrit).
   2. Reduction in hemoglobin concentration of blood.

C. Many classifications of anemia have been proposed. A classification of anemia based on underlying mechanisms is shown below.

1) Blood loss – acute and chronic

2) Increased rate of destruction (hemolytic anemias)
   a) Intrinsic abnormalities of RBCs
      Hereditary
      Red cell membrane disorders (Hereditary spherocytosis)
      Red cell enzyme deficiencies (G-6-PD deficiency)
      Disorders of hemoglobin synthesis
      Abnormal globin production (Sickle cell anemia)
      Deficient globin production (thalassemia)
      Acquired e.g. Paroxysmal nocturnal hemoglobinuria (PNH)
   b) Extrinsic abnormalities
      Antibody-mediated (Immune hemolytic anemias)
      Mechanical trauma (microangiopathic hemolytic anemia)
      Infections (malaria)
      Chemical injury (lead poisoning)
      Sequestration (hypersplenism)

3) Impaired red cell production (diminished erythropoiesis)
   a) Disturbances of proliferation / differentiation of erythroblasts
      Deficient heme synthesis (iron deficiency anemia)
      Deficient DNA synthesis (megaloblastic anemia: B12/folate)
      Multiple mechanisms (anemia of chronic disease)
   b) Disturbances of proliferation / maturation of stem cells
      aplastic anemia
D. Red cell indices.
   1. **Mean cell volume (MCV)**: Measures the average volume of a red blood cell.
   2. Mean cell hemoglobin (MCH): Measures the average mass of hemoglobin per red blood cell
   3. Mean cell hemoglobin concentration (MCHC): Measures the hemoglobin concentration in a given volume of red blood cells.
   4. Red blood cell distribution width (RDW): coefficient of variation of red blood cell volume, measures *anisocytosis*.

E. Classification based on MCV.
   1. Microcytic Anemia (MCV <80 fL): iron deficiency, thalassemias, anemia of chronic disease.
   3. Normocytic Anemia (MCV 80-100 fL).

F. “Sideroblastic anemia”
   1. Not a specific entity—outdated term
   2. Presence of abnormal erythroid precursors in the bone marrow: “ring sideroblasts”
   3. Seen in anemias due to many different causes including hereditary ALAS2 mutation (rare), lead poisoning, Vitamin B6 (pyridoxine) deficiency, drugs (e.g. Isoniazid), chronic alcoholism, myelodysplastic syndrome
   4. Microcytic, normocytic or macrocytic depending on cause

G. Hemolysis.
   1. Hemolytic anemias are characterized by: 1) Shortening of normal RBC life span (premature destruction of RBCs); 2) Accumulation of products of hemoglobin catabolism; 3) Marked compensatory increase in erythropoiesis within the bone marrow.
   2. **Intravascular hemolysis**: RBCs are destroyed within the vascular compartment resulting in hemoglobinemia, hemoglobinuria, and
hemosiderinuria. Free hemoglobin complexes mostly with haptoglobin (levels usually become low) and is removed by the liver.

3. **Extravascular hemolysis**: RBCs are destroyed primarily by tissue macrophages (as in normal RBC catabolism, but exaggerated). Does not usually result in hemoglobinemia or hemoglobinuria, but often the reticuloendothelial system is hyperactive, resulting in splenomegaly. More common than intravascular hemolysis.

### IV. HEMOLYTIC ANEMIA DUE TO INRINSIC (INTRACORPUSCULAR) ABNORMALITIES OF RED CELLS

#### A. **Hereditary spherocytosis** – a disorder of red cell membrane cytoskeleton.

1. Caused by a (usually) hereditary defect in the red cell membrane which renders the red cell spheroidal, less deformable, and, therefore, more likely to be sequestered and destroyed in the spleen. Autosomal dominant disorder in 75% of cases.

2. Defect in the red cell membrane seems usually related to spectrin deficiency, leading to reduced membrane stability. As the cells pass through the circulation, membrane fragments are lost. The reduction in membrane substance, and therefore, surface area, forces the red cells to assume the smallest possible diameter for a given volume – a sphere.

3. Patients may be asymptomatic or may be anemic; the course is highly variable. Characteristic features are anemia, splenomegaly and jaundice. Splenectomy is generally beneficial, emphasizing the important role of the spleen in this disorder. Increased osmotic fragility of the red cells is a characteristic finding that is helpful in diagnosis.

#### B. **G-6 phosphate dehydrogenase deficiency** – a red cell enzyme deficiency

1. Most important of the enzyme abnormalities is deficiency of G-6-PD, which participates in the
hexose monophosphate shunt pathway; abnormalities in the enzymes involved in the HMP shunt or glutathione metabolism may reduce the ability of RBCs to avoid oxidative injury.

2. Considerable polymorphism exists and there are more than 250 variants. Deficiency of G-6-PD may protect against malaria due to *Plasmodium falciparum*. Inheritance is X-linked; all red cells of affected males are deficient in enzyme activity, so men with G-6-PD deficiency are more susceptible to oxidant injury than women. Mainly seen in the black population in the U.S.; up to 10% of black U.S. males are affected.

3. No symptoms unless red cells are subjected to oxidant injury following exposure to offending drugs or toxins:
   a. drugs, such as antimalarials, sulfonamides, nitrofurantoin, and others.
   b. infections, probably acting by generation of oxidant free radicals in macrophages.

4. Pathophysiology
   a. Oxygen is usually carried inert by the red cell. Occasionally oxygen may be converted to a reactive oxidizing species (such as superoxide [O₂] or hydrogen peroxide [H₂O₂]). Superoxide may be produced when oxyhemoglobin is converted to methemoglobin. Hydrogen peroxide is produced from aberrant dissociation of oxyhemoglobin and other less well-defined mechanisms, e.g., following exposure to oxidant drugs or infection.
   
   b. The red cell can deal with oxidizing species through a variety of mechanisms, the most important being the glutathione system. Hydrogen peroxide is destroyed by a reaction utilizing glutathione peroxidase; glutathione is oxidized in this reaction (GSH→GSSG). NADPH is necessary to reduce glutathione again; NADPH is regenerated via the hexose monophosphate shunt in which G-6-PD plays a critical role.

   c. If enough NADPH is not available to regenerate reduced glutathione, H₂O₂ accumulates, oxidizing sulfhydryl groups of globin chains. Hemoglobin denatures,
forming precipitates know as Heinz bodies which attach to the RBC membrane, decreasing deformability.

d. As RBCs with Heinz bodies pass through splenic cords, macrophages bite out Heinz bodies, with ensuing loss of membrane, forming spherocytes.

5. Drug-induced hemolysis is acute with variable severity. Intravascular hemolysis, characterized by hemoglobinemia, hemoglobinuria and decreased hematocrit, typically develops after two or three days. Spleen also plays a role. Older red cells are more susceptible to lysis, in the more common form of disease seen in the U.S. in blacks. The bone marrow begins to produce new red cells to compensate; these cells, being younger, are less susceptible to hemolysis.

C. Sickle cell anemia – a disorder of hemoglobin synthesis, producing a structurally abnormal hemoglobin

1. Most common form of familial hemolytic anemia worldwide; about 8% of U.S. blacks are heterozygous for HbS (sickle cell trait) and about 1 in 600 is homozygous.

2. Sickle hemoglobin (HbS) results from a point mutation at the sixth position of the β-globin chain, resulting in an amino acid substitution of valine for glutamic acid, leading to abnormal physiochemical properties. On deoxygenation, HbS molecules aggregate and polymerize (gelation or crystallization). The red cell becomes distorted into the sickle shape. The distortion is initially reversible with oxygenation, but continued injury to the RBC membrane causes eventual irreversible sickling.

3. Sickling of the red cells is influenced by many factors, importantly:
   a. Amount of HbS and its interaction with other hemoglobin chains in the red cell
   b. The mean corpuscular hemoglobin concentration
4. Two major clinical consequences of sickling are chronic hemolytic anemia and occlusion of small blood vessels.
   a. The anemia is usually severe and chronic hemolysis results in reticulocytosis and chronic increased bilirubin. Intravascular hemolysis may occur due to increased mechanical fragility of severely damaged RBCs. Extravascular hemolysis occurs from rigid sickled RBCs becoming sequestered in the spleen. Children may have moderate splenomegaly caused by the red pulp congestion. Over time, hypoxic tissue damage occurs and the spleen becomes small and fibrotic; “auto- or functional splenectomy”.
   b. Widespread micro-vascular obstruction from the rigid red cells and other abnormalities may result in ischemic damage.
   c. Intermittent sudden episodes are called vasoocclusive, or painful, crises. Aplastic crises, usually triggered by parvovirus infection of erythroid precursor cells, may also occur in which bone marrow activity suddenly ceases temporarily. Increased susceptibility to infections may be seen.
   d. An ordinary peripheral blood smear can show sickled cells or a blood sample can be treated in such a way as to induce sickling in susceptible cells, to suggest diagnosis. The disease in usually diagnosed definitely by hemoglobin electrophoresis
   e. In contrast to these major clinical manifestations of sickle cell anemia (homozygous for HbS), patients with sickle cell trait (heterozygous for HbS) usually have an uneventful clinical course (but with rare cases of exertional rhabdomyolysis, splenic infarction, renal medullary carcinoma).