Hematology lecture

Danil Hammoudi, MD
• **PFC stands for perfluorocarbons.**

Perfluorocarbons are a type of synthetic blood that helps carry dissolved gases in the blood. They are mixed with an emulsifier to create a liquid suspension that can be mixed with blood. PFCs can carry about 20 percent more gas than blood plasma.

**PFCs may be especially helpful in these areas:**

- Restoring the delivery of oxygen in the body
- Treating traumatic brain injury
- Treating anemia
- Increasing the effectiveness of chemotherapy
- Preventing the need for surgical blood transfusion
HEMOPOIESIS

- **Hemo**: Referring to blood cells
- **Poiesis**: “The development or production of”
- The word Hemopoiesis refers to the production & development of all the blood cells:
  - Erythrocytes: Erythropoiesis
  - Leucocytes: Leucopoiesis
  - Thrombocytes: Thrombopoiesis.

- Begins in the **20th week of life**
  - in the fetal liver & spleen
  - continues in the bone marrow till young adulthood & beyond!
SITES OF HEMOPOIESIS

- Active Hemopoietic marrow is found, in children throughout the:
  - Axial skeleton:
    - Cranium
    - Ribs.
    - Sternum
    - Vertebrae
    - Pelvis
  - Appendicular skeleton:
    - Bones of the Upper & Lower limbs

- In Adults active hemopoietic marrow is found only in:
  - The axial skeleton
  - The proximal ends of the appendicular skeleton.

- Before birth, blood cell formation takes place in
  - The fetal yolk sac,
  - Liver,
  - Spleen

- By the seventh month, red bone marrow is the primary hematopoietic area

- Blood cells develop from mesenchymal cells called blood islands

- The fetus forms HbF, which has a higher affinity for oxygen than adult hemoglobin
Hemopoietic cells (those which produce blood) first appear in the yolk sac of the 2-week embryo.

By 8 weeks, blood making has become established in the liver of the embryo.

By 12-16 weeks the liver has become the major site of blood cell formation.

It remains an active hemopoietic site until a few weeks before birth.

The spleen is also active during this period, particularly in the production of lymphoid cells, and the fetal thymus is a transient site for some lymphocytes.

At birth, active blood making red marrow occupies the entire capacity of the bones and continues to do so for the first 2-3 years after birth.

The red marrow is then very gradually replaced by inactive, fatty, yellow, lymphoid marrow.

The latter begins to develop in the shafts of the long bones and continues until, by 20-22 years, red marrow is present only in the upper ends of the femur and humerus and in the flat bones of the sternum, ribs, cranium, pelvis and vertebra.

However, because of the growth in body and bone size that has occurred during this period, the total amount of active red marrow (approximately 1000-1500 g) is nearly identical in the child and the adult.
1. Erythrocytes form in red bone marrow.

2. Erythrocytes circulate in bloodstream for 120 days.

3. Aged erythrocytes are phagocytized in the liver and spleen.

4. Heme components of blood are recycled.

5. Membrane proteins and globin proteins are broken down into amino acids, some of which are used to make new erythrocytes.

Heme

Heme is converted into biliverdin and then to bilirubin, which is secreted in bile from the liver.

Fe²⁺ Fe³⁺ (iron ions)

Iron is transported in the blood by the protein transferrin and stored by the protein ferritin in the liver.
Pluripotent stem cells: are mesenchymal cells representing less than 1% of Red Bone Marrow cells having the ability to develop into several other kinds of cells.

These further lead to 2 other stem cells known as Myeloid and lymphoid stem cells.

Myeloid Stem Cells lead to development of:
- RBCs (erythrocytes) (CFU-E)
- Platelets (thrombocytes)
- Eosinophils
- Neutrophils (CFU-GM)
- Basophils

Of the cell types listed above, all except eosinophils and basophils are produced by intermediate or Progenitor cells before fully developing.

Lymphoid Stem Cells lead to the development of T and B lymphocytes (T/B cells).

HGFs (Hemoipoietic Growth factors) control the production of progenitor cells. Examples include:
- erythropoietin (EPO)
- Thrombopoietin (TPO).

EPO cells are produced in the kidneys, therefore, renal failure contributes to insufficient production of RBCs.

TPO originates in the liver to promote the synthesis of platelets from Megakaryocytes.

Cytokines - Typically acting as autocrine/paracrine,
- cytokines are glycoproteins synthesized by RBM cells, leukocytes, macrophages, fibroblasts and endothelial cells.
- They promote the spread of progenitor cells.

2 Cytokine Families that stimulate production of WBCs:
- CSFs (Colony-Stimulating Factors)
- Interleukins
Erythropoietin PRODUCED BY THE KIDNEY, also known as *Epoetin alfa*, to treat

- Low RBC concentration during end-stage kidney disease.
- Patients going through chemotherapy are treated with Granulocytic-Macrophage CSF and Granulocyte CSFs to treat low WBC concentration.

- Thrombopoietin also produced by the kidneys is used to treat platelet depletion.
Hematopoiesis

B Cell
Lymphoid Stem Cell

T Cell

Pluripotent Stem Cell

Pluripotent Stem Cell multiplication

Orthochromatic erythroblast

Reticulocyte

Erythrocyte (Red Blood Cells)

Megakaryocyte

Thrombocytes (Platelets)

Monocyte

Eosinophilic myelocyte

Basophilic myelocyte

Neutrophil

Eosinophil

Monocyte/granulocyte progenitor

Basophil

Granulocytes

Megakaryocyte

Erythroid island

Trabecular bone
Def and Generality

1. Prick finger and collect a small amount of blood.
2. Place a drop of blood on a slide.
3. Using a second slide, pull the drop of blood across the slide surface, leaving a thin layer of blood on the slide. After the blood dries, apply a stain for contrast. Place a coverslip on top.
4. When viewed under the microscope, blood smear reveals the components of the formed elements.
• Blood is a connective tissue whose matrix is fluid.
• It is composed of:
  • red corpuscles,
  • white cells,
  • platelets,
  • blood plasma.
• It is transported throughout the body within blood vessels veins arteries and white cells within lymphatics and arteries, veins.

• In human adults about 5 liter of blood contribute 7-8% to the body weight of the individual.

• The contribution of red blood cells (erythrocytes) to the total volume of the blood (hematocrit) is about 43%.

• Erythrocytes are the dominant (99%) but not the only type of cells in the blood.

• Erythrocytes, leukocytes and blood platelets = formed elements of the blood.

• Erythrocytes and blood platelets perform their functions exclusively in the blood stream.

• In contrast, leukocytes reside only temporarily in the blood.
• Leukocytes can leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.
Whole blood is a living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body’s tissues.

Whole blood contains red blood cells, white blood cells, and platelets suspended in a fluid called plasma.
Differential WBC count
(All total 4800–10,800/μl)

- Granulocytes
  - Neutrophils (50–70%)
  - Eosinophils (2–4%)
  - Basophils (0.5–1%)
- Agranulocytes
  - Lymphocytes (25–45%)
  - Monocytes (3–8%)
Blood Plasma

- Blood Plasma - Representing 55% of whole blood
- Of plasma, water makes up 91.5%, while 7.5% makes up proteins
- 1% is a mix of other solutes including electrolytes, enzymes, hormones and waste products.

- Blood plasma contains over 100 solutes, including:
  - Proteins – albumin, globulins, clotting proteins, and others
  - Lactic acid, urea, creatinine
  - Organic nutrients – glucose, carbohydrates, amino acids
  - Electrolytes – sodium, potassium, calcium, chloride, bicarbonate
  - Respiratory gases – oxygen and carbon dioxide

- There are 3 basic types of plasma proteins synthesized by hepatocytes (liver cells):
  - Albumins (54%)
  - Globulins (38%)
  - Fibrinogen (7%)

- Gamma globulins are also known as immunoglobulins or antibodies.
- bacterial or viral invasion prompts the production of millions of antibodies, which bind to an antigen (invader).
**Physical characteristics**

- **Viscosity**: 4.5 - 5.5 (where $H_2O = 1.0$)
- **Temperature**: 100.4°F [38 degree Celsius]
- **pH**: 7.35 - 7.45
- **Salinity**: 85% - .90%
- **Volume**: 5 - 6 liters in males
  4 - 5 liters in females
- **Blood accounts for approximately 7-8% of body weight**

Blood is a sticky, opaque fluid with a **metallic taste (iron)**
Color varies from scarlet to dark red
Overview of Blood Circulation

- Blood leaves the heart via arteries that branch repeatedly until they become capillaries.
- Oxygen (O$_2$) and nutrients diffuse across capillary walls and enter tissues.
- Carbon dioxide (CO$_2$) and wastes move from tissues into the blood.

- Oxygen-deficient blood leaves the capillaries and flows in veins to the heart.
- This blood flows to the lungs where it releases CO$_2$ and picks up O$_2$.
- The oxygen-rich blood returns to the heart.
The volume of blood in an average-sized person (70 kg; 154 lbs) is approximately 5.5 L.

If we take the hematocrit to be 45 percent, then

**Erythrocyte volume = 0.45 x5.5 L = 2.5 L**

Since the volume occupied by the leukocytes and platelets is normally negligible, the plasma volume equals the difference between blood volume and erythrocyte volume; therefore, in our average person

**Plasma volume = 5.5 L - 2.5 L = 3.0 L**
Blood Volume

- Blood volume is about 8% of body weight.
- 1 kg of blood ≈ 1 L of blood
- \(70 \text{ kg} \times 0.08 = 5.6 \text{ Kg} = 5.6 \text{ L}\)
- 45% is formed elements
- 55% plasma
BLOOD VOLUME
7% body weight
60% plasma 40% cells

SYSTEMIC CIRCULATION 84%

Veins 64%
Arteries 13%
Arterioles & Capillaries 7%

HEART (7%) & LUNGS (9%)
Total 16%
Blood Volume

- Constitutes small fraction of total body fluid
- 2/3 of body $H_2O$ is inside cells (intracellular compartment)
- 1/3 total body $H_2O$ is in extracellular compartment
  - 80% of this is interstitial fluid; 20% is blood plasma
Day of blood collection <8 hours or after 24 hours at 22°C

After storage at 4°C for 24–72 hours

Whole Blood

Red Blood Cells

Platelet Rich Plasma

Plasma

Whole Blood

Buffy coat

Fresh-frozen plasma

Red cells

Platelets

Fresh-frozen plasma

Filtered Red Blood Cells

Frozen Red Blood Cells

Washed Red Blood Cells

Plasma

Platelet Concentrates

Frozen Plasma

Fresh Frozen Plasma

Fractionation

Plasma Protein Fraction

Albumin

Gamma Globulin

IVIG

Special Gamma Globulin Products

Lyophilized Factor VIII

Other Clotting Factor Derivatives

Source: Transfusion All Transfusion Med © 2008 Blackwell Publishing Ltd.
BLOOD PRODUCTS

• Blood-cells products
  ■ whole blood
  ■ packed red blood cells
  ■ leukocyte-poor (reduced) red cells
  ■ washed red blood cells
  ■ random-donor platelets concentrates
  ■ single-donor platelets concentrates [human leukocyte antigens (HLA)-matched platelets]
  ■ irradiated blood products (red blood cells and platelets concentrates)- after exposure 20 to 40 Gy
  ■ leukocyte (granulocyte) concentrates

• Plasma products
  ■ fresh-frozen plasma (FFP)
  ■ cryoprecipitate
  ■ factor concentrates (VIII, IX)
  ■ albumin
  ■ immune globulins
Blood volume and osmotic pressure are regulated by several negative feedback mechanisms.

Those mechanisms of specific interest involve:
- aldosterone,
- ADH
- atrial natriuretic peptide
Hematocrit

- **The hematocrit (Ht or HCT) or packed cell volume (PCV) or erythrocyte volume fraction (EVF)** is the proportion of **blood volume** that is occupied by red blood cells.

- The volume of RBCs refers to the amount of space that the RBCs occupy within the blood.

- **Males**: the average is slightly higher at 47% (40-54%) due to higher levels of **testosterone** in males.

- **Testosterone promotes synthesis of EPO (erythropoietin)**, which contributes to a higher RBC count.

| At sea level, the hematocrit of a normal adult male averages about 47 |
| which means that 47% of the blood volume is RBCs |
| while that of a normal adult female is 42. |

- **Ht=Hbx3**
The normal ranges for hematocrit are dependent on age and, after adolescence, the sex of the individual.

The normal ranges are:

- Newborns: 55%-68%
- One (1) week of age: 47%-65%
- One (1) month of age: 37%-49%
- Three (3) months of age: 30%-36%
- One (1) year of age: 29%-41%
- Ten (10) years of age: 36%-40%
- Adult males: 42%-54%
- Adult women: 38%-46%
Functions of Blood

- **Primary**
  - Transportation
  - Exchange

- **Secondary**
  - Immunity
  - Thermoregulation
  - Fluid volume balance
  - pH balance
Distribution

• **Blood transports:**
  – Oxygen (co2) from the lungs and nutrients from the digestive tract
  – Metabolic wastes from cells to the lungs and kidneys for elimination
  – Hormones from endocrine glands to target organs
Regulation

• **Blood maintains:**
  – Appropriate **body temperature** by absorbing and distributing heat
  – **Normal pH** in body tissues using buffer systems
  – Adequate **fluid volume** in the circulatory system
Protection

- **Blood prevents blood loss by:**
  - Activating plasma proteins and platelets
  - Initiating clot formation when a vessel is broken

- **Blood prevents infection by:**
  - Synthesizing and utilizing antibodies
  - Activating complement proteins
  - Activating WBCs to defend the body against foreign invaders
Components of Whole Blood

- Platelets
- Erythrocytes
- Monocyte
- Neutrophils
- Lymphocyte
Formed Elements

- Erythrocytes, leukocytes, and platelets make up the formed elements
  - Only WBCs are complete cells
  - RBCs have no nuclei or organelles, and platelets are just cell fragments

- **Most formed elements survive in the bloodstream for only a few days**

- Most blood cells do not divide but are renewed by cells in bone marrow
<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration</th>
<th>Characteristics</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (RBCs)</td>
<td>4.8 Million/microliter in females 5.4 Million/microliter in males</td>
<td>Biconcave disc no nucleus 120 day lifespan</td>
<td>Transports oxygen and carbon dioxide</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>60% - 70% WBC concentration</td>
<td>Has 2-5 lobes joined by chromatin with a fine, pale granular cytoplasm</td>
<td>Phagocytosis. Uses lysozyme, super oxides and peroxides to destroy antigens.</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2% - 4% WBC concentration</td>
<td>Has 2 - 3 lobes with large red/orange granules.</td>
<td>Responds to histamine in allergic reactions. Phagocytosis. destroys parasitic worms.</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.5% - 1% WBC concentration</td>
<td>2 lobes with large, deep blue/purple granules.</td>
<td>Intensify inflammatory response by liberating heparin, histamine and serotonin</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>20% - 25% WBC concentration</td>
<td>T/B cells. Cytoplasm forms ring around nucleus</td>
<td>Mediates immune response. B cells become plasma cells that form antibodies. T cells attack viruses, cancer cells and transplanted tissue cells.</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3% - 8% WBC concentration</td>
<td>kidney-shaped nucleus with blue/gray cytoplasm</td>
<td>Becomes macrophage before phagocytosis</td>
</tr>
<tr>
<td>Thrombocytes (Platelets)</td>
<td>150,000 - 400,000/microliter</td>
<td>cells fragments, no nucleus</td>
<td>Forms platelet plug in hemostasis, and releases chemicals to promote vascular spasms and blood clotting.</td>
</tr>
<tr>
<td>CELL TYPE</td>
<td>ILLUSTRATION</td>
<td>DESCRIPTION*</td>
<td>CELLS/µL (mm³) OF BLOOD</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agranulocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Lymphocyte</td>
<td><img src="image1.png" alt="Lymphocyte Illustration" /></td>
<td>Spherical, nucleated cells</td>
<td>4800–10,800</td>
</tr>
<tr>
<td>· Monocyte</td>
<td><img src="image2.png" alt="Monocyte Illustration" /></td>
<td>Nuclear spherical or indented; pale blue cytoplasm; diameter 5–17 µm</td>
<td>1500–3000</td>
</tr>
<tr>
<td>Platelets</td>
<td><img src="image3.png" alt="Platelets Illustration" /></td>
<td>Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 µm</td>
<td>150,000–400,000</td>
</tr>
</tbody>
</table>

*A Appearance when stained with Wright’s stain.

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Erythrocytes

Life cycle

1. erythropoiesis
   a. negative - feedback regulation
      1. erythropoietin
2. 120 days ---> destruction
3. recycling bilirubin; jaundice
Erythrocytes (RBCs)

- Erythrocytes are an example of the complementarity of structure and function

- **Structural characteristics contribute to its gas transport function**
  - **Biconcave** shape has a huge surface area relative to volume
  - Erythrocytes are more than 97% hemoglobin
  - ATP is generated anaerobically, so the erythrocytes do not consume the oxygen they transport

- Biconcave discs
- anucleate
- essentially no organelles
- Filled with hemoglobin (Hb), a protein that functions in gas transport
- Contain the plasma membrane protein **spectrin** and other proteins that:
  - Give erythrocytes their flexibility
  - Allow them to change shape as necessary
A schematic diagram of **spectrin** and other cytoskeletal molecules

The erythrocyte model demonstrates the importance of the spectrin cytoskeleton in that mutations in spectrin commonly cause hereditary defects of the erythrocyte, including

- **hereditary elliptocytosis**
- **Hereditary spherocytosis**
Erythrocytes (RBCs)
Spherocytosis

hereditary elliptocytosis
Erythropoiesis-Brief

- **Bone marrow**
  - Pluripotent stem cells
  - Chemical regulation
    - Cytokines
    - Erythroid specific growth factor
    - Erythropoietin (EPO)
  - Life span
    - Reticulocyte - 4 days
    - RBC - 120 days
Production of Erythrocytes

- **Hematopoiesis** – blood cell formation
- Hematopoiesis occurs in the red bone marrow of the:
  - Axial skeleton and girdles
  - Epiphyses of the humerus and femur
- **Hemocytoblasts** give rise to all formed elements

**ERYTHROPOIESIS: SITES/PHASES**

- **INTRAUTERINE LIFE:**
  - **INTRAVASCULAR PHASE:** Upto 3rd month of Intra Uterine Life.
    - Endothelial cells = = = RBCs
  - **HEPATIC PHASE:** 3rd to 5th month IUL
    - Liver & Spleen
    - nRBCs from Mesenchymal cells.
  - **MYELOID PHASE:** From 5th month of IUL onwards.

- **POST NATAL LIFE:**
  - **CHILDREN:**
    - Predominantly Red Bone Marrow of skeleton:
      - Axial &
      - Appendicular.
  - **ADULTS:**
    - Red Bone Marrow of Axial Skeleton.
Production of Erythrocytes: Erythropoiesis

- A hemocytoblast is transformed into a proerythroblast
- Proerythroblasts develop into early erythroblasts
- **The developmental pathway consists of three phases**
  - 1 - ribosome synthesis in early erythroblasts
  - 2 - Hb accumulation in late erythroblasts and normoblasts
  - 3 - ejection of the nucleus from normoblasts and formation of reticulocytes
- Reticulocytes then become mature erythrocytes
Yolksac or Fetal Liver in Fetus
Bone Marrow in Adults

Phase - I: Generation of Erythroid committed blast cells
- Stem cell
- Multi Potential Progenitor
- Committed Progenitor

Phase - II: Division and Differentiation of Erythroid Progenitors
- Precursor Cells
  - EPO Responsive
  - Basophilic erythroblast
  - Orthochromatophilic erythroblast
  - Proerythroblast
- Polychromatophilic erythroblast
  - Orthochromatophilic erythroblast
- Reticulocyte

Phase - III: Terminal Maturation
- Erythrocyte

Lymphoid Stem cell
- CFU-GEMM
- Platelets & White Cells

Stages of Erythropoiesis

<table>
<thead>
<tr>
<th>Stage of Erythropoiesis</th>
<th>Important Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proerythroblast</td>
<td>Synthesis of hemoglobin starts</td>
</tr>
<tr>
<td>Early normoblast</td>
<td>Nucleoli disappear</td>
</tr>
<tr>
<td>Intermediate normoblast</td>
<td>Hemoglobin starts appearing</td>
</tr>
<tr>
<td>Late normoblast</td>
<td>Nucleus disappears</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>Reticulum is formed. Cell enters capillary from site of production</td>
</tr>
<tr>
<td>Matured RBC</td>
<td>Reticulum disappears. Cell attains biconcavity</td>
</tr>
</tbody>
</table>

Basophilic erythroblast = Early normoblast
Polychromatophilic erythroblast = Intermediate normoblast
Orthochromatophilic erythroblast = Late normoblast
Reticulocyte = Polychromatophilic erythrocyte
Ribosome = Basophilic (Blue)
Hemoglobin = Acidophilic (Pink)
Erythropoiesis

Proerythroblast
Basophilic erythroblast
Polychromatic erythroblast
Acidophil erythroblast
Reticulocyte
Erythrocyte

Stem cell
Committed cell
Developmental pathway

Phase 1
Ribosome synthesis
Phase 2
Hemoglobin accumulation
Phase 3
Ejection of nucleus

Hemocytoblast
Proerythroblast
Early erythroblast
Late erythroblast
Normoblast
Reticulocyte
Erythrocyte
Red Bone Marrow - H&E

- Normoblasts
- Erythroblast
FACTORS REGULATING ERYTHROPOIESIS

- SINGLE MOST IMPORTANT REGULATOR: “TISSUE OXYGENATION”
- BURST PROMOTING ACTIVITY
- ERYTHROPOIETIN
- IRON
- VITAMINS:  
  - Vitamin B$_{12}$
  - Folic Acid
- MISCELLANEOUS

- Circulating erythrocytes – the number remains constant and reflects a balance between RBC production and destruction
  - Too few RBCs leads to tissue hypoxia
  - Too many RBCs causes undesirable blood viscosity

- Erythropoiesis is hormonally controlled and depends on adequate supplies of iron, amino acids, and B vitamins
Hormonal Control of Erythropoiesis

- **Erythropoietin (EPO) release by the kidneys is triggered by:**
  - Hypoxia due to decreased RBCs
  - Decreased oxygen availability
  - Increased tissue demand for oxygen

- **Enhanced erythropoiesis increases the:**
  - RBC count in circulating blood
  - Oxygen carrying ability of the blood
**Erythropoietin Mechanism**

**Homeostasis:** Normal blood oxygen levels

- **Stimulus:** Hypoxia due to decreased RBC count, decreased amount of hemoglobin, or decreased availability of O\(_2\)

- **Start:** Kidney (and liver to a smaller extent) releases erythropoietin

- **Erythropoietin stimulates red bone marrow**

- **Enhanced erythropoiesis increases RBC count**

- **Reduces O\(_2\) levels in blood**

- **Increases O\(_2\)-carrying ability of blood**

Figure 17.6
Erythrocyte Function

- RBCs are dedicated to **respiratory gas transport**

- Hb reversibly binds with oxygen and most oxygen in the blood is bound to Hb

- Hb is composed of the protein globin, made up of two alpha and two beta chains, each bound to a heme group

- Each heme group bears an atom of iron, which can bind to one oxygen molecule

- Each Hb molecule can transport four molecules of oxygen
Decomposition of Hemoglobin Molecule

Heme

Biliverdin \((C_{33}H_{34}O_6N_4)\)

Bilirubin \((C_{33}H_{36}O_6N_4)\)
Hemoglobin (Hb)

- **Oxyhemoglobin** – Hb bound to oxygen
  - Oxygen loading takes place in the lungs
- **Deoxyhemoglobin** – Hb after oxygen diffuses into tissues (reduced Hb)
- **Carbaminohemoglobin** – Hb bound to carbon dioxide
  - Carbon dioxide loading takes place in the tissues

### Name of Hemoglobin
<table>
<thead>
<tr>
<th>Name of Hemoglobin</th>
<th>Subunit Structure</th>
<th>Time of Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin Portland</td>
<td>$\zeta_2\gamma_2$</td>
<td>Embryonic</td>
</tr>
<tr>
<td>Hemoglobin Gower I</td>
<td>$\zeta_2\varepsilon_2$</td>
<td>Embryonic</td>
</tr>
<tr>
<td>Hemoglobin Gower II</td>
<td>$\alpha_2\varepsilon_2$</td>
<td>Embryonic</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>$\alpha_2\gamma_2$</td>
<td>Fetal</td>
</tr>
<tr>
<td>Hemoglobin Barts</td>
<td>$\gamma_4$</td>
<td>Fetal (pathologic Hb secondary to absence of all 4 $\alpha$ globulin genes; fatal in utero)</td>
</tr>
<tr>
<td>Hemoglobin $A_2$</td>
<td>$\alpha_2\delta_2$</td>
<td>Minor adult hemoglobin</td>
</tr>
<tr>
<td>Hemoglobin $A$</td>
<td>$\alpha_2\beta_2$</td>
<td>Major adult hemoglobin</td>
</tr>
</tbody>
</table>

- Men: 13.5 to 16.5 g/dl
- Women: 12.1 to 15.1 g/dl
- Children: 11 to 16 g/dl
- Pregnant women: 11 to 12 g/dl
ERYTHROPOIETIN

- A hormone produced by the Kidney.
- A circulating Glycoprotein
- Nowadays available as Synthetic Epoietin
- Acts mainly on CFU – E.
- Increases the number of:
  - Nucleated precursors in the marrow.
  - Reticulocytes & Mature Erythrocytes in the blood.
VITAMINS

• B_12: Cyanocobalamine & Folic Acid:[dna formation]
  – Is also called Extrinsic Factor of Castle.
  – Needs the Intrinsic Factor from the Gastric juice for absorption from Small Intestine.
  – Deficiency causes Pernicious (When IF is missing) or Megaloblastic Anemia.
  – Stimulates Erythropoiesis
  – Is found in meat & diary products.

Iron functions

• Oxygen carriers
  – hemoglobin
• Oxygen storage
  – Myoglobin
• Energy Production
  – Cytochromes (oxidative phosphorylation)
    – Krebs cycle enzymes
• Other
  – Liver detoxification (cytochrome p450)
IRON

• Essential for the synthesis of Hemoglobin.

• Deficiency causes Microcytic, Hypochromic Anemia.

• The MCV, Color Index & MCH are low.
Iron Distribution

- 35 - 45 mg / kg iron in adult male body
- Total approx 4 g
  - Red cell mass as hemoglobin - 50%
  - Muscles as myoglobin - 7%
  - Storage as ferritin - 30%
    - Bone marrow (7%)
    - Reticulo-endothelial cells (7%)
    - Liver (25%)
  - Other Heme proteins - 5%
    - Cytochromes, myoglobin, others
  - In Serum - 0.1%
Iron Transport in Blood

- **Red cells**
  - As hemoglobin
  - Cannot be exchanged

- **Plasma**
  - Bound to Transferrin
  - Carries iron between body locations
    - eg between gut, liver, bone marrow, macrophages
  - Iron taken up into cells by transferrin receptors

**Transferrin**

- Synthesised in the liver.
- Each molecule binds can bind two Fe$^{3+}$ molecules (oxidised)
- Contains 95% of serum Fe.
- Usually about 30% saturated with Fe.
- Production decreased in iron overload.
- Production increased in iron deficiency.
- Measured in blood as a marker of iron status.
Transferrin Testing

- A routine blood test used for iron status
- Also known as TIBC (total iron binding capacity)
- High levels:
  - Low body iron stores.
- Low levels:
  - High body iron stores.
- Other conditions
  - Increase: high oestrogen states (pregnancy, OCP)
  - Decrease: malnutrition, chronic liver disease, chronic disease (e.g., malignancy), protein-losing states, congenital deficiency, neonates, acute phase (negative reactant).
Dietary Requirements of Erythropoiesis

- **Erythropoiesis requires:**
  - Proteins, lipids, and carbohydrates
  - Iron, vitamin $B_{12}$, and folic acid

- The body stores iron in Hb (65%), **the liver, spleen, and bone marrow**

- Intracellular iron is stored in protein-iron complexes such as **ferritin and hemosiderin**

- Circulating iron is loosely bound to the transport protein transferrin
Regulation of Erythropoiesis

- CFU - E → Proerythroblasts → Mature Erythrocytes
- Erythropoietin stimulates CFU - E
- Erythropoietin decreases tissue oxygenation
- Factors decreasing tissue oxygenation:
  - Hypovolemia
  - Anemia
  - Poor blood flow
  - Pulmonary Disease

An example of a Negative feed back mechanism
Factors That Influence Iron Absorption

Physical State (bioavailability): heme > Fe2+ > Fe3+

Inhibitors: phytates, tannins, soil clay, antacids, iron overload

Competitors: cobalt, lead, strontium, manganese, zinc

Facilitators: ascorbate, citrate, amino acids, iron deficiency
Fate and Destruction of Erythrocytes

- **The life span** of an erythrocyte is 100-120 days
- Old RBCs become rigid and fragile, and their Hb begins to degenerate
- Dying RBCs are engulfed by macrophages
- Heme and globin are separated and the iron is salvaged for reuse
- Heme is degraded to a yellow pigment called **bilirubin**
- The liver secretes bilirubin into the intestines as bile
- The intestines metabolize it into urobilinogen
- This degraded pigment leaves the body in feces, in a pigment called stercobilin
- 75% is derived from RBCs
- In normal adults this results in a daily load of 250-300 mg of bilirubin
- Normal plasma concentrations are less than 1 mg/dL
- Hydrophobic – transported by albumin to the liver for further metabolism prior to its excretion
Handling of Free (Intravascular) Hemoglobin

Purposes:
1. Scavenge iron
2. Prevent major iron losses
3. Complex free heme (very toxic)

- **Haptoglobin**: hemoglobin-haptoglobin complex is readily metabolized in the liver and spleen forming an iron-globin complex and bilirubin. Prevents loss of iron in urine.

- **Hemopexin**: binds free heme. The heme-hemopexin complex is taken up by the liver and the iron is stored bound to ferritin.

- **Methemalbumin**: complex of oxidized heme and albumin.
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Enzymes</th>
<th>Role</th>
<th>Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMP</td>
<td>Phosphofructokinase, Pyruvate kinase (PK)</td>
<td>Produce ATP, RBC Shape (ion pumps)</td>
<td>Hemolytic Anemia, PK Deficiency</td>
</tr>
<tr>
<td>HMS</td>
<td>Glutathione Reductase, G6PD</td>
<td>NADPH Production, OXY-METH HB Balance</td>
<td>Hemolytic Anemias</td>
</tr>
<tr>
<td>RLB</td>
<td>DPG Synthetase</td>
<td>2,3-DPG Production, HB Oxygen Affinity</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>MHBR</td>
<td>Methemoglobin reductase</td>
<td>Protects HB from Oxidation via NADH</td>
<td>Hemolytic Anemia, Hypoxia</td>
</tr>
</tbody>
</table>