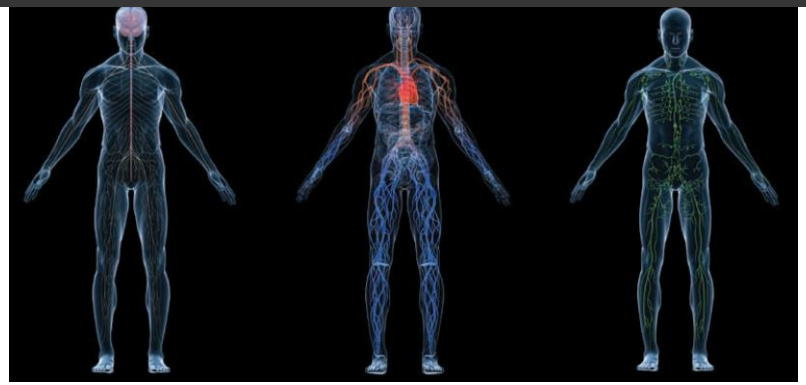
UNIT VI

Chapter 33

GUYTON AND HALL
TEXTBOOK OF MEDICAL PHYSIOLOGY



Erythropoiesis requirements Part I and Part II Pathophysiology of Anemia 2nd week Lab tests Theory

Ebaa M Alzayadneh, PhD

Associate Professor of Physiology

Response to Hypoxia

- Minutes to hours… ⬆ Erythropoietin
- New circulating reticulocytes…~ 3 days 
- Erythropoietin…
  - drives production of proerythroblasts from HSCs
  - accelerates their maturation into RBCs
- Can increase RBC production up to 10-fold
- Erythropoietin remains high until normal tissue oxygenation is restored.

Vitamin B_{12} and Folic Acid

- **Rapid, large-scale cellular proliferation requires optimal nutrition**
- **Cell proliferation requires DNA replication**
- **Vitamin B_{12} and folate both are needed to make thymidine triphosphate (thus, DNA)**
- **Abnormal DNA replication causes failure of nuclear maturation and cell division…**

➡ ***maturation failure*** ➡ ***large, irregular, fragile "macrocytes"***

Clinical Perspective

Pernicious Anemia

- Failure to absorb vitamin B_{12}
- Atrophic gastric mucosa…
  - Failure to produce *intrinsic factor*
- Intrinsic factor binds to vitamin B_{12}
  - Protects it from digestion
  - Binds to receptors in the ileum
  - Mediates transport by pinocytosis
- Vitamin B_{12} - stored in liver, released as needed
- Usual stores: 1 – 3 mg Daily needs: 1 – 3 µg
- Thus normal stores are adequate for 3 – 4 years

Clinical Perspective

Folic Acid Deficiency

- **Folic acid is present in green vegetables, some fruits, and meats**
- **Destroyed during cooking**
- **Subject to dietary deficiencies**
- **May also be deficient in cases of intestinal malabsorption**
- **Maturation failure may reflect combined B_{12} and folate deficiency**

Formation of Hemoglobin

- Occurs from proerythroblast through reticulocyte stage
- Reticulocytes retain a small amount of endoplasmic reticulum and mRNA, supporting continued hemoglobin synthesis

Shapes of RBC and Hemoglobin

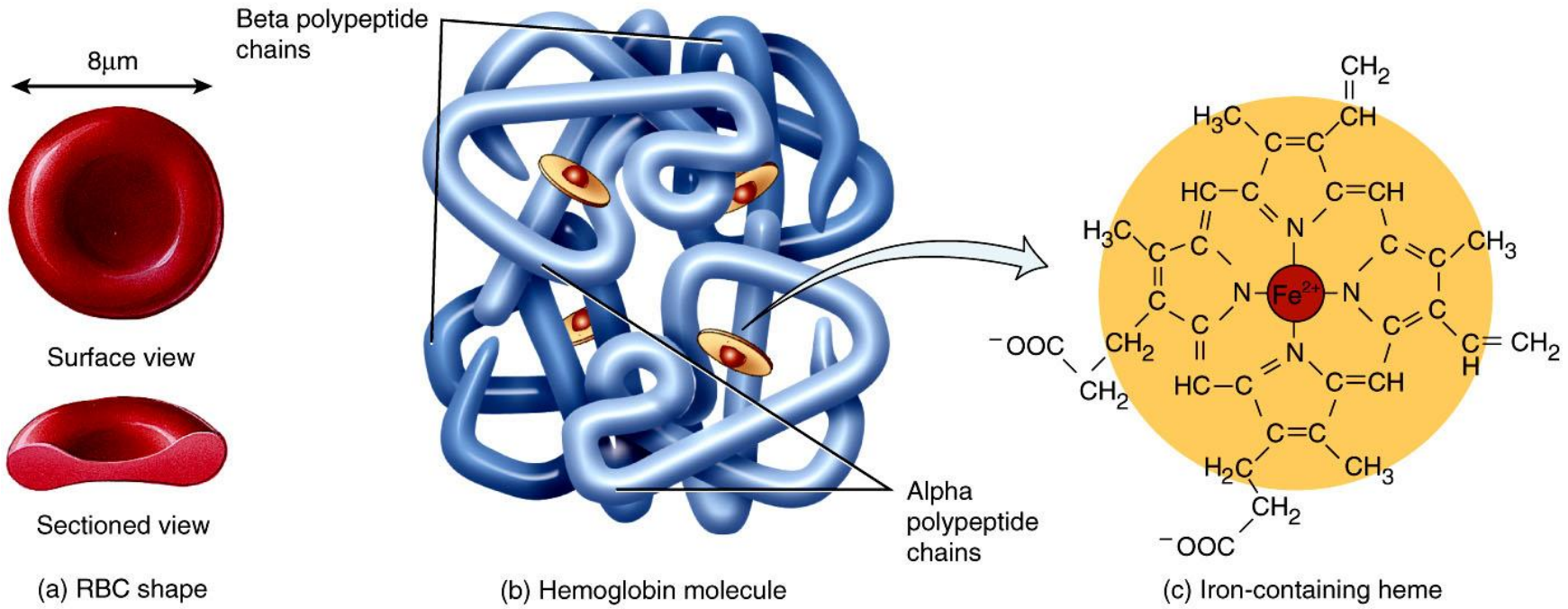
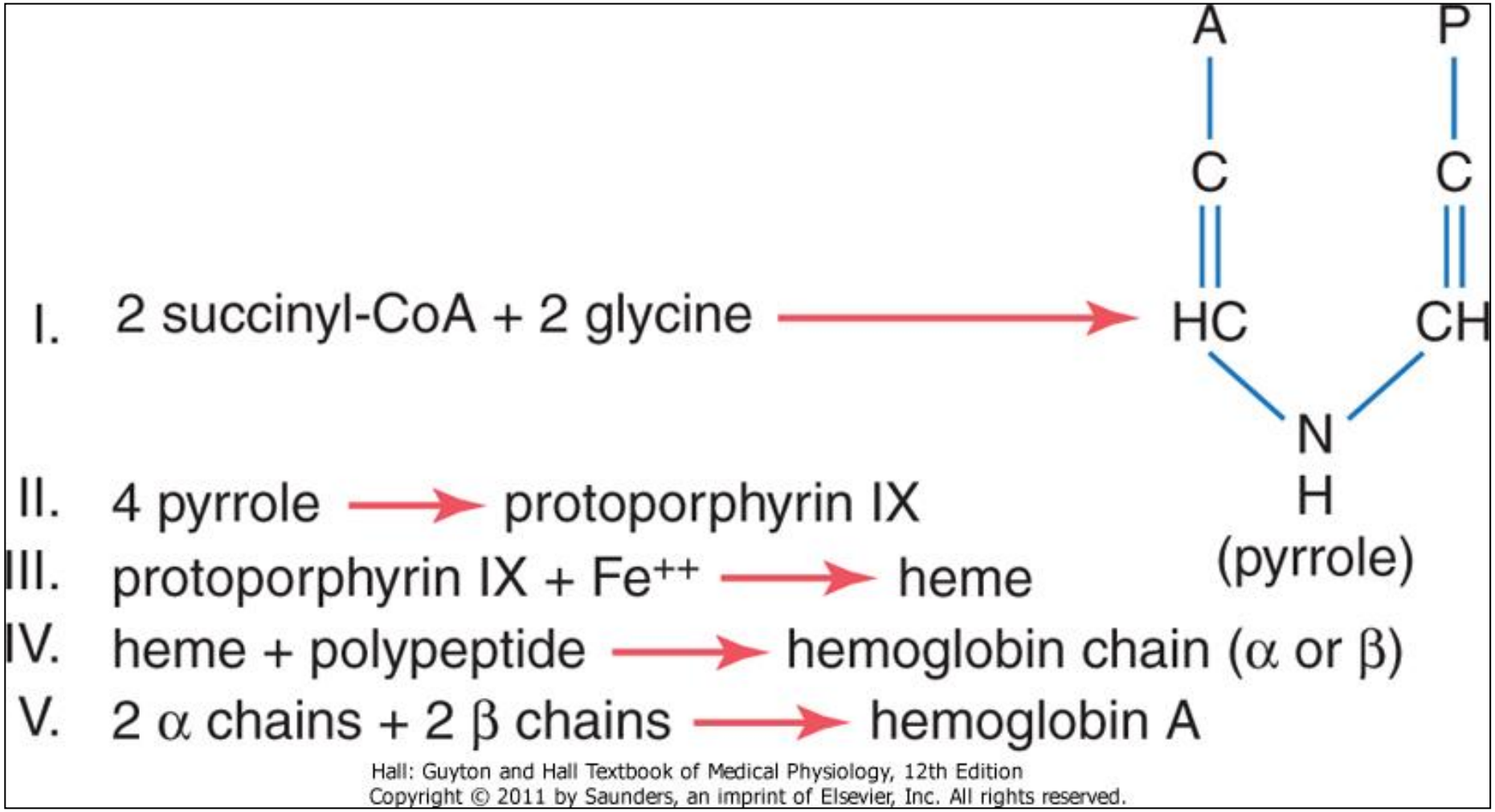
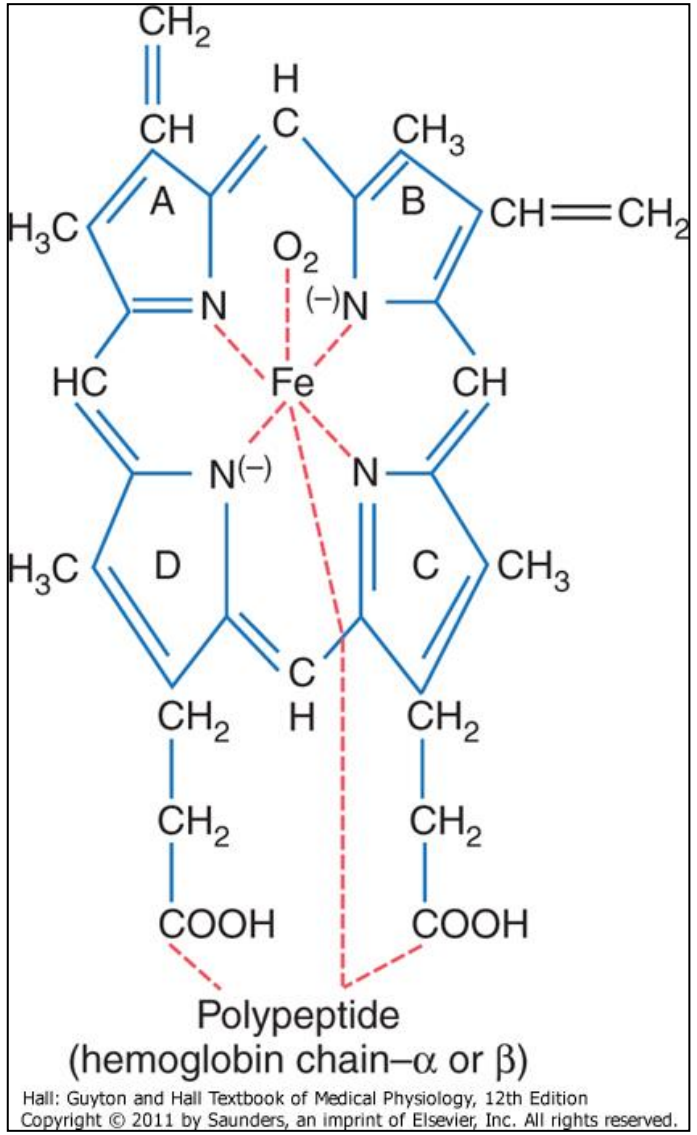


Figure 19.04 Tortora - PAP 12/e

Formation of Hemoglobin



Hemoglobin Structural Units



Types of Globin Chains

- Several types of globin chains resulting from gene duplication – α, β, γ, δ; MW ~ 16,000
- Predominant form in adults is Hemoglobin A, with 2 α and 2 β chains; MW 64,458
- Each globin chain is associated with one heme group containing one atom of iron
- Each of the four iron atoms can bind loosely with one molecule (2 atoms) of oxygen
- Thus each hemoglobin molecule can transport 8 oxygen atoms

Clinical Perspective

Variation in Globin Chains

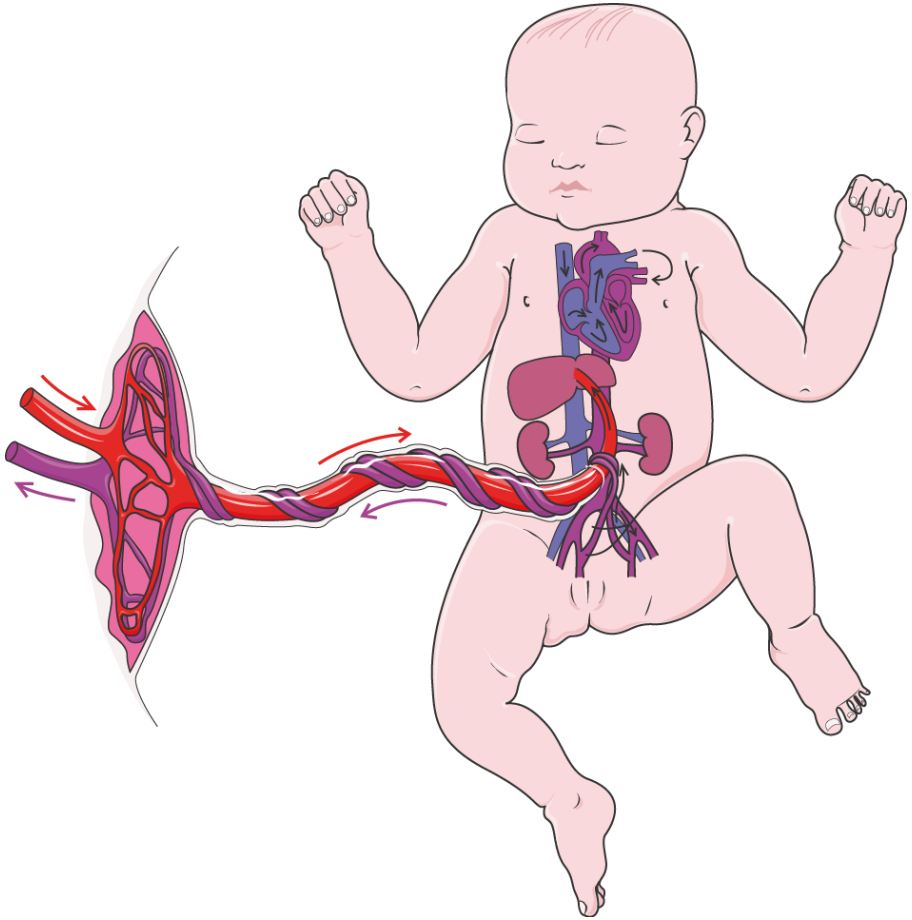
- **Modest differences in O_2 binding affinities**
- **Sickle hemoglobin:**
  Glutamic acid ➡ Valine at AA 6
- **Hemoglobin of homozygous individuals ("SS") forms elongated crystals when exposed to low O_2**

  ➡ hemolysis, vascular occlusion

Oxygen Binding to Hemoglobin

- **Must be loosely bound – binding in settings of higher O_2 concentration, releasing in settings of lower concentration**
- **Binds loosely with one of the coordination bonds of iron**
- **Carried as molecular oxygen (not as ionic oxygen)**

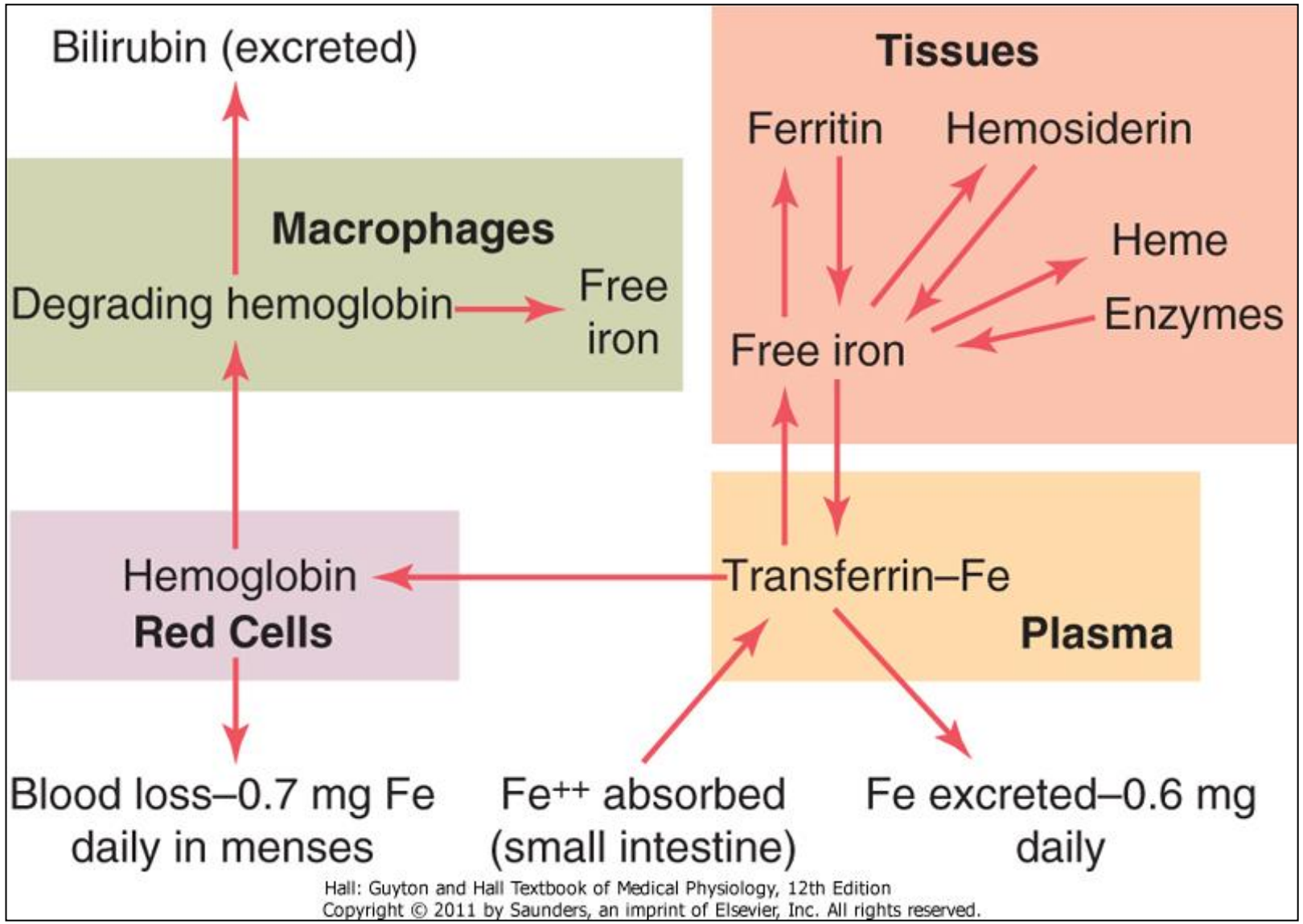
Fetal hemoglobin



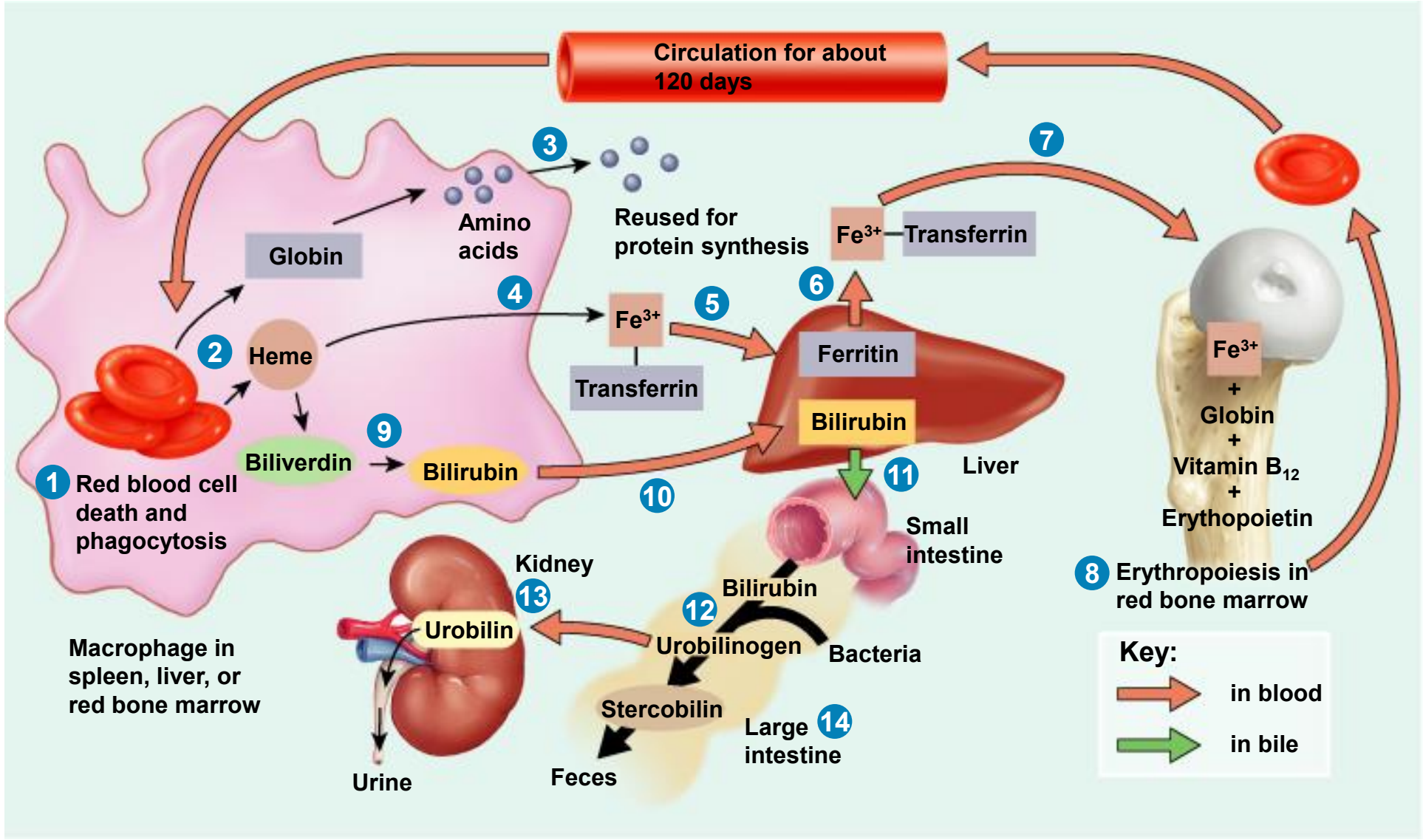
Iron Metabolism

- Iron is a key component of hemoglobin, myoglobin, and multiple enzymes (cytochromes, cytochrome oxidase, peroxidase, catalase)
- Thus iron stores are critically regulated
- Total body iron ~ 4 – 5 g
  - 65% in hemoglobin
  - 4% in myoglobin
  - 1% in intracellular heme compounds
  - 0.1% associated with circulating transferrin
  - 15 – 30% stored mainly as ferritin in RES

Iron Transport and Metabolism



Formation and Destruction of RBC's



Iron Absorption, Transport & Storage

- **Absorbed from small intestine, combines with *apotransferrin* ⟶ *transferrin* (transport iron)**
- **Iron can be released to any cell**
- **RBC precursors have transferrin receptors and actively accumulate iron**
- **Particularly in hepatocytes and reticulo-endothelial cells, iron combines with *apoferritin* ⟶ *ferritin* (MW 460,000)**
- **Ferritin is variably saturated (storage iron)**
- ***Hemosiderin* is quite insoluble excess iron**

Iron Exchange

- When iron in the plasma is low, iron is released from ferritin and bound to transferrin for transport.
- It is delivered to the bone marrow, bound by transferrin receptors on erythroblasts, internalized, and delivered directly to the mitochondria for incorporation into heme.
- Deficiency of transferrin can result in severe *hypochromic* anemia.
- Hemoglobin released from senescent RBCs is ingested by macrophages and stored as ferritin.

RBC Senescence & Destruction

- RBC life span is ~120 days
- Though lacking a nucleus, mitochondria, and endoplasmic reticulum, RBCs have enzymes that can metabolize glucose and make small amounts of ATP. These enzymes…
  - Maintain membrane pliability
  - Support ion transport
  - Keep iron in the ferrous form (rather than ferric)
  - Inhibit protein oxidation
- As enzymes deplete with age, RBCs become fragile and rupture in small passages, often in the spleen

Degradation of Hemoglobin

- When RBCs rupture, hemoglobin is phagocytosed by macrophages, particularly in the liver and spleen
- Iron is released back to transferrin in the blood to support erythropoiesis or be stored as ferritin
- Macrophages convert the porphyrin portion, stepwise, into bilirubin, which is released into the blood and secreted by the liver into the bile

Clinical Perspective

Anemias (Reduced Hgb in Blood)

- **Blood loss (acute, chronic)**

- **After hemorrhage…**
  - **Fluid volume restored in 1 – 3 days**
  - **RBC concentration restored in 3-6 weeks**

- **Chronic blood loss can lead to iron deficiency, with hypochromic, microcytic anemia.**

Clinical Perspective

Aplastic Anemia

- **Bone marrow failure caused by…**
  - **Radiation**
  - **Chemotherapy**
  - **Chemical toxins**
  - **Auto-immune**
  - ***Idiopathic***

- **Supported by transfusions or treated by bone marrow transplantation**

Clinical Perspective

Megaloblastic Anemia

- Deficiency of Vitamin B_{12} and / or Folic Acid
  - Pernicious anemia
  - Dietary deficiency
  - Malabsorption
- Impairs DNA replication, causing maturation failure
- Formation of large, fragile cells with bizarre shapes, which rupture easily, potentially causing profound anemia

Clinical
Perspective

Hemolytic Anemia

- **Hereditary conditions causing fragility**
  - **Hereditary spherocytosis**
  - **Sickle cell anemia**

- **Immune-mediated destruction**
  - **Erythroblastosis fetalis**

Clinical
Perspective

Circulatory Effects of Anemia

- **Anemia**
  - **Decreased viscosity**
  - **Decreased O_2 - carrying capacity**

➡ **Increased cardiac output**

- **Markedly decreased exercise capacity**

Clinical Perspective

Secondary (RBC ~30%; 6-7 million/mm^3)

- - Chronic hypoxemia (heart or lung disease)
- - *Physiologic polycythemia*
  - - Living at 14 - 17,000 feet
  - - Markedly enhanced exercise capacity at altitude

Polycythemia Vera

- Clonal abnormality causing excessive proliferation
- Usually all lineages
- 7- 8 million RBCs / mm^3; Hematocrit 60-70%
- Blood volume increased almost two-fold
- Hyperviscosity, up to 3-fold normal (10 x water)

Clinical Perspective

Polycythemia & Circulation

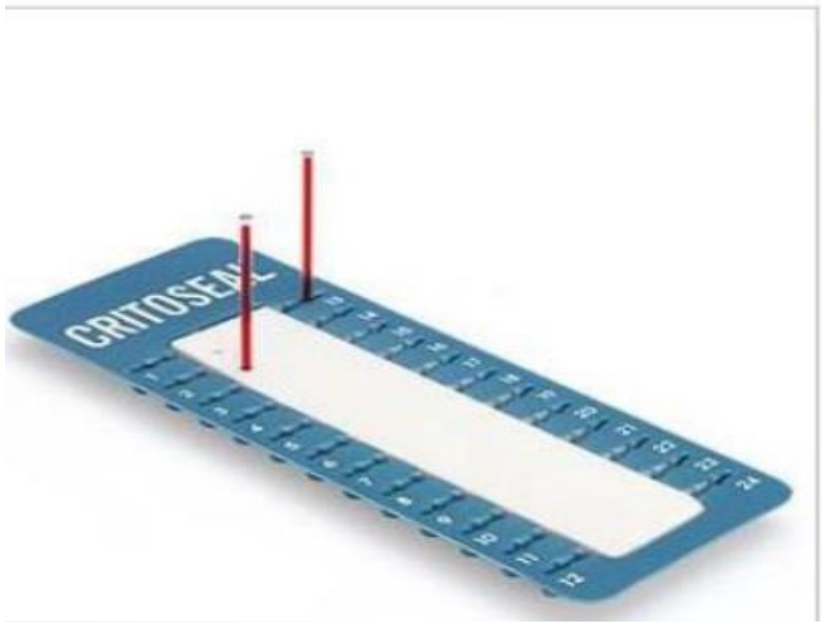
- Increased viscosity decreases venous return
- Increased blood volume increases venous return
- 2/3 normotensive, 1/3 hypertensive
- The subpapillary venous plexus under the skin becomes engorged with slow-moving, de-saturated blood, producing a ruddy complexion with a bluish tint to the skin

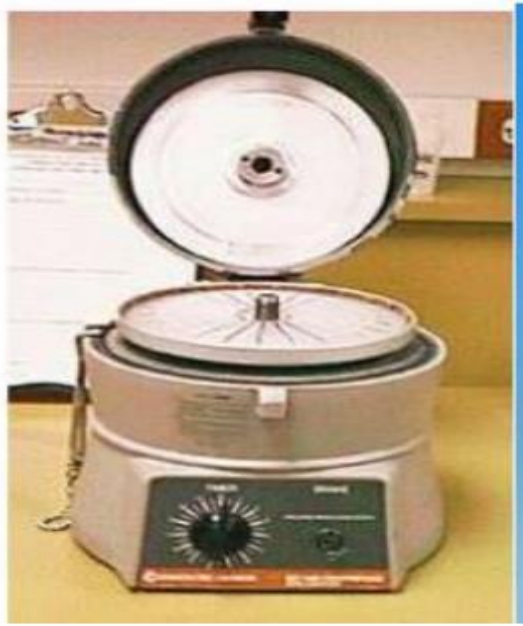
LAB TESTS

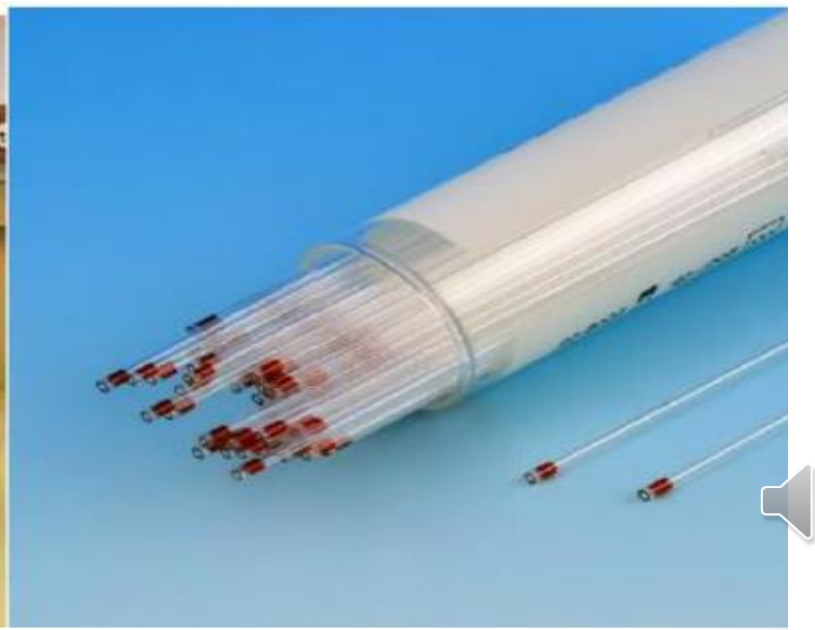
- Packed Red Blood Cell Volume PCV
- Erythrocytes Sedimentation Rate ESR
- Red Blood Cell Osmotic Fragility Test

Packed Cell Volume (PCV)

- PCV is the ratio of the volume of packed red cells to the total blood volume.
  - Adult males: 40–54% (avg = 47%).
  - Adult females: 38–46% (avg = 42%)
- It decreases in cases of anemia and increases in polycythemia and dehydration.







Erythrocyte Sedimentation Rate (ESR)

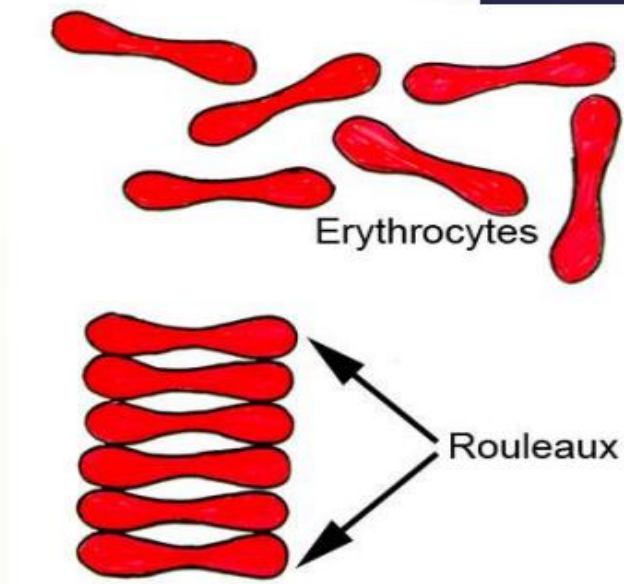
- The rate at which red blood cells settle out when anticoagulated whole blood is allowed to stand for a period of one hour.

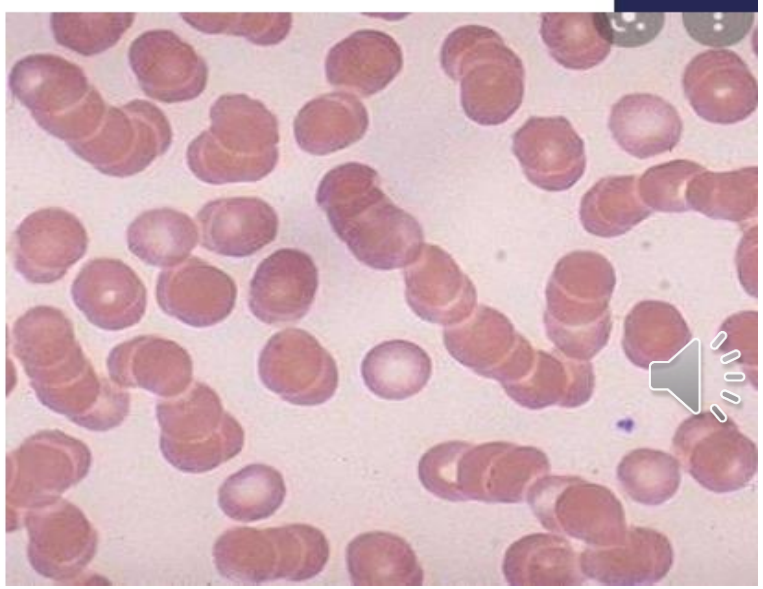
- The ESR is a simple, sensitive but **non-specific** screening test that indirectly measures the presence of inflammation in the body.

- It's increase reflects the tendency of red blood cells to settle more rapidly in the presence of inflammatory conditions, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins.
- Changes in red cell shape or numbers may also affect the ESR.

RBCs sedimentation

- The RBCs sediment because their density is greater than that of plasma. The sedimentation increases if stacking of RBCs (rouleaux formation) happens.
  - Rouleaux formation is possible because of the discoid shape of RBCs
- Normally, RBCs have negative charges on the outside of the cells, which cause them to repel each other and decreases or prevents rouleaux formation.
- Many plasma proteins have positive charges and can neutralize the negative charges of the RBCs, which allows for the formation of the rouleaux.
- Therefore, an increase in plasma proteins (present in inflammatory conditions) will increase the rouleaux formations, which settle more readily than single red blood cells leading to increased ESR during inflammation





- # Normal ESR values
  - Adult males < 15mm/hr
  - Adult females < 20mm/hr

- High ESR
  - Inflammation
  - Anemia
  - Old age
  - Pregnancy
  - Technical factors: tilted ESR tube, high room temperature.

- **Some interferences which decrease ESR:**
- Abnormally shaped RBC (sickle cells and spherocytosis)
- Polycythemia
- Technical factors: low room temperature, delay in test performance (>2 hours), clotted blood sample

Osmotic fragility

- When RBCs reside in an isotonic medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water.

- When RBCs reside in a hypertonic media , a net efflux of water occurs so the cells lose their normal biconcave shape, undergoing collapse.

- When RBCs reside in a hypotonic medium, a net influx of water occurs so the cells swell and the integrity of their membranes is disrupted resulting in **hemolysis**

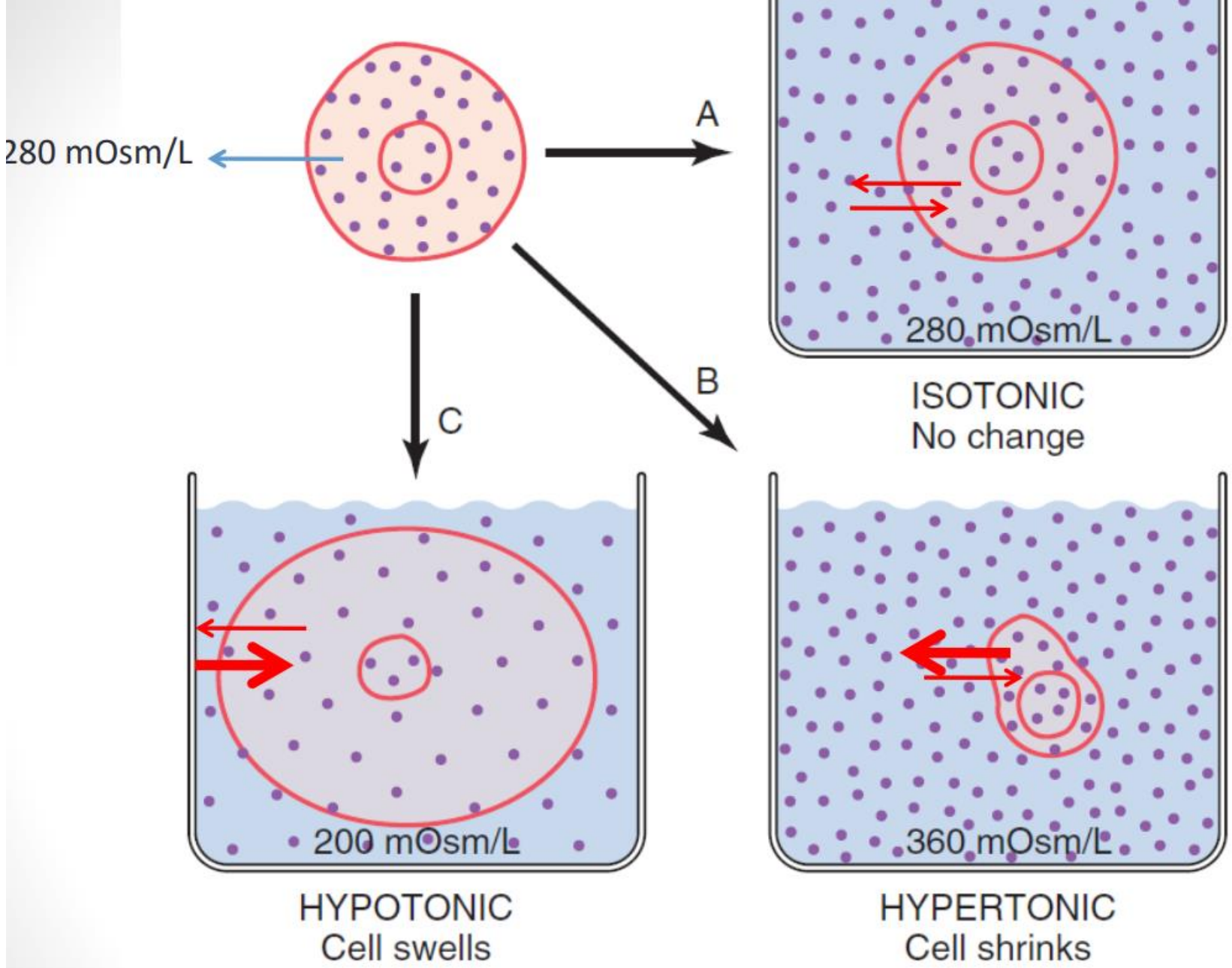


Figure 25-5. Effects of isotonic (*A*), hypertonic (*B*), and hypotonic (*C*) solutions on cell volume.

Osmotic fragility test

- A test designed to measures red blood cell's resistance to hemolysis when exposed to a series of increasingly dilute saline solutions.

- The susceptibility of RBCs to hemolysis is determined by:
  - **Surface area to volume ratio.**
  - Cell membrane composition and integrity

- This test is mainly used to diagnose hereditary spherocytosis.

Osmotic Fragility Test

- From 0.7% to 0.5% there is no hemolysis.
- At the concentration of 0.48% hemolysis starts and the solution becomes red in color, but there are some settled RBCs in the tube.
- At the concentration of 0.36%, the solution is bright red and there are no settled RBCs (complete hemolysis).
- With spherocytosis hemolysis starts at the concentration of 0.68% which means RBCs can't resist hemolysis as they normally do (they are more fragile)

RBC Osmotic Fragility

- Increased red cell fragility (increased susceptibility to hemolysis) is seen in the following conditions:
  - Hereditary spherocytosis
  - Autoimmune hemolytic anemia
  - Toxic chemicals, poisons, infections, and some drugs.
  - Severe burns.

✓ These cells have a low surface area: volume ratio

- Decreased red cell fragility (increased resistance to hemolysis) is seen with the following conditions:
  - Thalassemia.
  - Iron deficiency anemia.

✓ These cells have a high surface area: volume ratio