HLS

MODIFIED NO. 3

PHYSIOLOGY

كتابة: زين مالك وآية فريحات

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Introduction: Red Blood Cells, Anemia and Polycythemia

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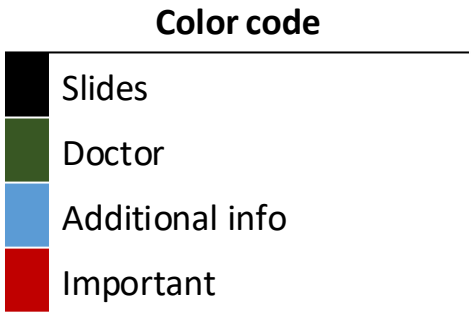


Erythropoietin (EPO)

-We will complete our talk about Erythropoiesis requirements.
-Previouslywe talked about erythropoietin role –how does it response to low and high level of oxygen –and now we will talk about erythropoietin features.

- **Circulating hormone, mw ~34,000**
- **Necessary for erythropoiesis in response to hypoxia**
- **~90% made in the kidney**
- **Cells of origin not established**

If EPO isn't secreted hypoxia will have no effect on RBCs production.
The cells that synthesize erythropoietin are not very well known, but there are reports indicating interstitial cells or epithelial renal tubular cells that can release erythropoietin upon hypoxia.

Hypoxia ➡ *HIF-1* ➡ binds hypoxia response element ➡ ⬆EPO transcription

The signaling behind the stimulation of erythropoietin starts from stimulus hypoxia, which will increase the level of hypoxia-inducible factor one (HIF-1 in the cell, this transcriptional factor binds to the genes that have the hypoxia response elements and that will increase expression of erythropoietin.

Erythropoietin (cont'd)

- **Extra-renal hypoxia can stimulate Epo production… also there are some hormones can promote EPO production as:**
  - **epinephrine, norepinephrine, and some prostaglandins can promote Epo production**

- **In anephric or in kidney failure; severe anemia ????**
  - Anephric: absence of kidney congenitally or surgically .

- Hypoxia in other tissues rather than the kidney can stimulate erythropoietin release by some sensors that connect to kidney receptors to stimulate it to release EPO.

No kidney, no enough erythropoietin production, mostly 90% of RBCs will be lost.

- **In anephric individuals, 10% residual Epo (mainly from liver), supports 30-50% needed RBC production…**
  - **Hematocrit (packed cell volume) ~23-25% rather than 40- 45%**

%10residual EPO is not enough to produce the required number of RBCand this percent of EPO can provide half or less than half the required number of RBCs %(30-50) and so the hematocrit becomes %25-23

Additional explain:
There are 3routes to stimulate EPO synthesis in the kidney:
)noitalumits-otua(aixopyh laner 1)
2)Etra renal hypoxia
3)Hormones

That's why the kidney is responsible to 90% of EPO prduction.

Response to Hypoxia

- Minutes to hours… ⬆ Erythropoietin
- New circulating reticulocytes…~ 3 days 
- Erythropoietin…
  - drives <u>production</u> of proerythroblasts from HSCs
    Hematopoietic Stem Cells
  - <u>accelerates their maturation</u> into RBCs
- Can increase RBC production up to 10-fold 10 times over a normal number of RBCs.
- Erythropoietin remains high until normal tissue oxygenation is restored.

- Then the negative feedback will decrease the EPO
- ↑ Oxygen ↓ EPO

- This slide is not explained in the lecture.

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

- This slide is not explained in the lecture.

- **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

- This slide is not explained in the lecture.

- **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

- The formation of hemoglobin occurs from the stage of proerythroblasts and the whole process or the stages of RBC formation until the reticulocyte stage.

- **Occurs from proerythroblast through reticulocyte stage**

- **Reticulocytes retain a small amount of endoplasmic reticulum and mRNA, supporting continued hemoglobin synthesis ,and then when it becomes a mature RBC, all hemoglobins that have been synthesized will be present for the lifespan of RBC with no more hemoglobin production.**

Shapes of RBC and Hemoglobin

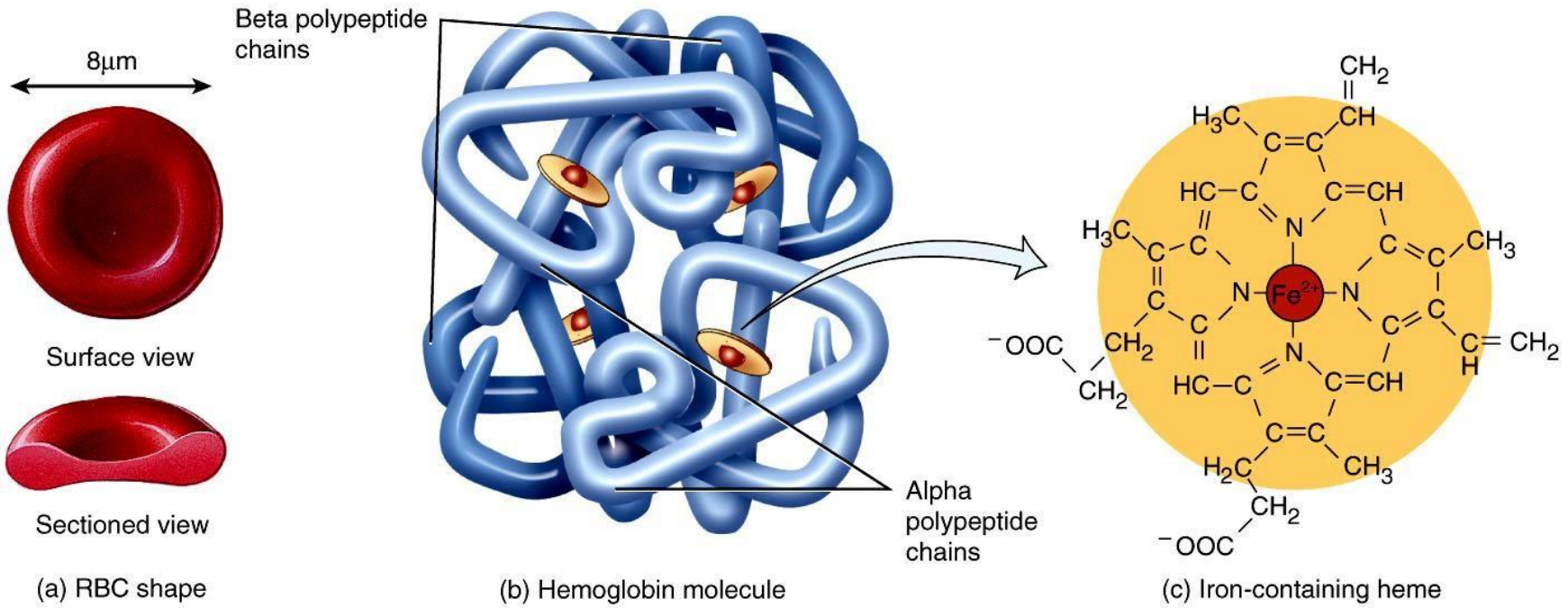


Figure 19.04 Tortora - PAP 12/e

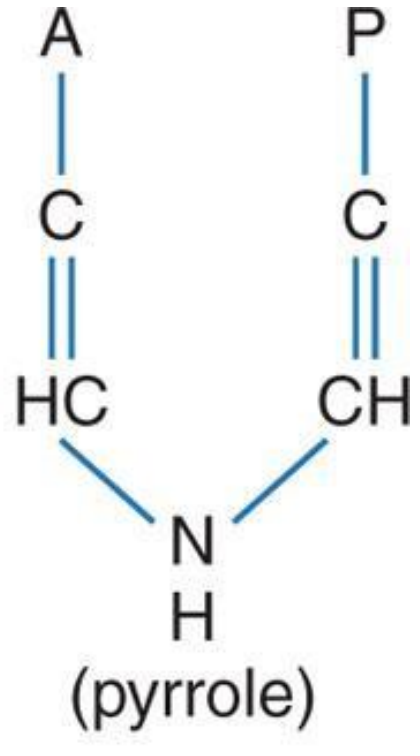
- The Hb molecule is present inside the RBC where it is composed of 4 polypeptide chains which we call them a globin chains.

The heme group is iron containing group)prosthetic group)
Which has an iron in the center of it.

Formation of Hemoglobin

Formation of Hemoglobin is composed of many steps

The first three steps to form the Heme group.

I. 2 succinyl-CoA + 2 glycine → 

(pyrrole)

II. 4 pyrrole → protoporphyrin IX

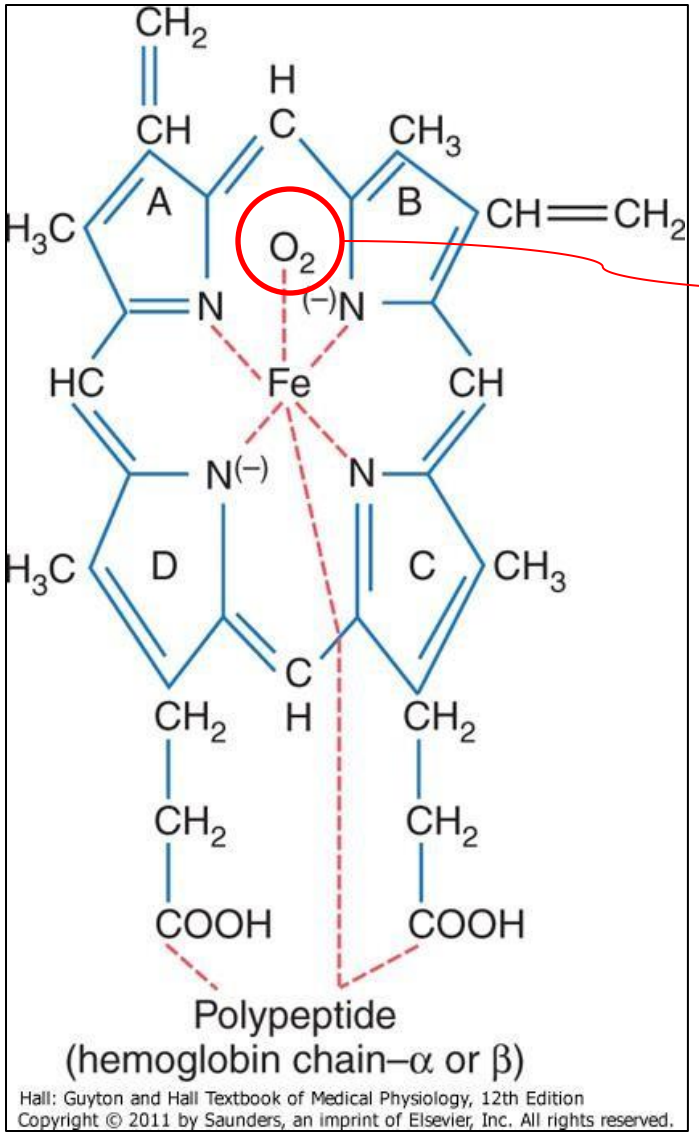
III. protoporphyrin IX + Fe^{++} → heme

IV. heme + polypeptide → hemoglobin chain (α or β)

V. 2 α chains + 2 β chains → hemoglobin A

Adult hemoglobin, recall there are other types of hemoglobin

Hemoglobin Structural Units



- The oxygen binds to iron as a molecule not as an ion so, the bond is coordination bond not an ionic bond.
- The oxygen forms a H bond with distal Histidine.

Types of Globin Chains

- Several types of globin chains resulting from gene duplication – α, β, γ, δ; MW ~ 16,000KDalton.
- Predominant form in adults is Hemoglobin A, with 2 α and 2 β chains; MW 64,458KDalton
- 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

- The variation in globin chains will result in differences in the oxygen binding affinity and sometimes the variation is mutation as sickle hemoglobin.
- We talk about the proteinous part of Hb that consists of amino acids, not the heme group.

- **Modest differences in O_2 binding affinities**

- **Sickle hemoglobin:**

  Glutamic acid ⟶ Valine at AA 6

It is a genetic disease where Glutamate amino acid is constituted with valine amino acid at amino acid number six. Therefore, in low oxygen saturation, Hb will convert into long crystals, causing rupture of RBCs and decreasing their flexibility to be squeezed in narrow cappillaries, resulting in RBCs rupture as well.

- **Hemoglobin of homozygous individuals (“SS”) forms elongated crystals when exposed to low O_2**

⟶ hemolysis, vascular occlusion

If the patients have homozygous genes)SS(they will have more extreme complications of sickle cell anemia as hemolysis and vascular occlusion.

Oxygen Binding to Hemoglobin

- **Must be <u>loosely bound</u> – binding in settings of higher O_2 concentration, releasing in settings of lower concentration))Diffusion movement)**

- **Binds loosely with one of the coordination bonds of iron**

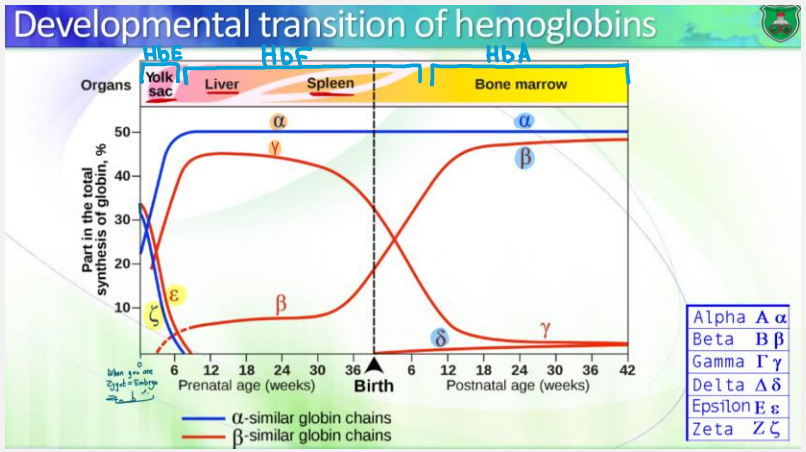
- **Carried as molecular oxygen (not as ionic oxygen)**

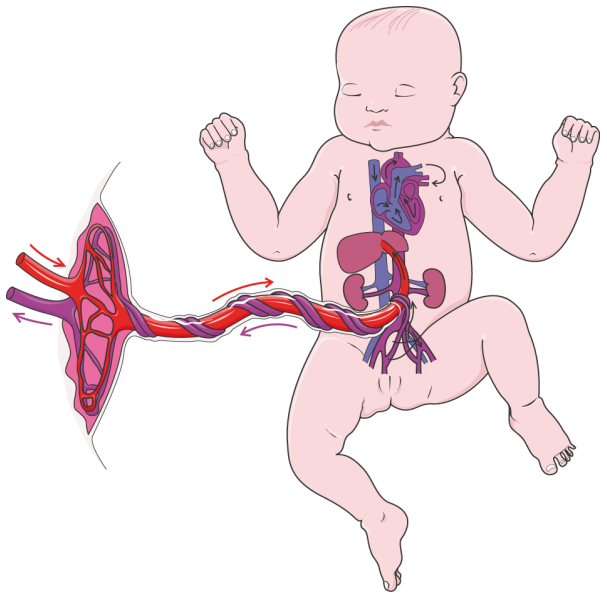
That why it is loosely bond between oxygen and iron.

Fetal hemoglobin

The main characteristic of fetal hemoglobin, it has the highest affinity to oxygen and presents an efficient extraction of oxygen from the mother's circulation during pregnancy.

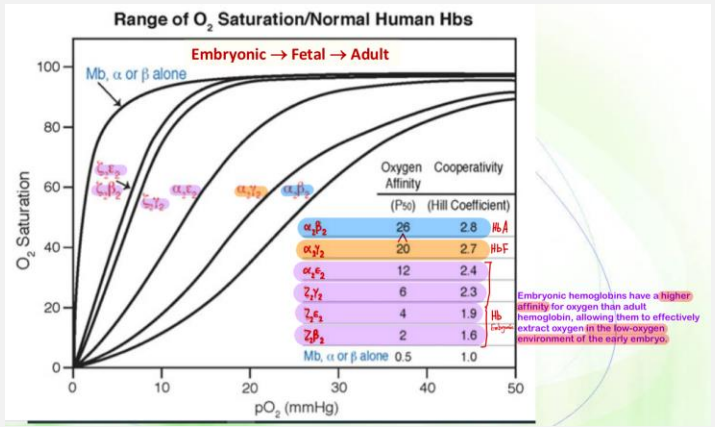
Extra image from biochemistry





Q:why does it have the highest affinity as biochemical view?
Because of its different globin chains.
If we compared the fetal Hb with adult Hb, we found that fetal Hb composed of 2alpha and 2gamma globin chains.

Extra image from biochemistry:



هاي أجمل جزئية في المحاضرة بس بدها شوية تركيز وبعدها رح تستمتع 😊
مرتبطة جدا في محاضرة البيوكيم الأولى ف ممكن تدرسها بعدها ترجع ل هاي السلايد.
اهم معلومة لازم تعرفها من البيوكيم انه في ثلاث أنواع او اشكال اساسية من الهيموغلوبين خلال حياة الإنسان من النطفة الى الممات وهي:

1)Embryonic Hb = HbE
2)Fetal Hb = HbF
3)Adult Hb = HbA

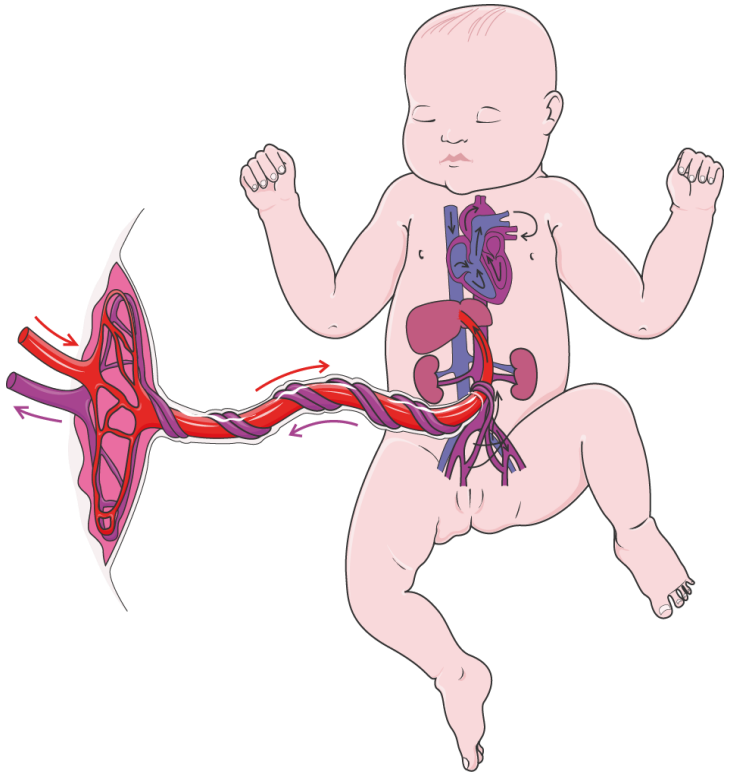
قال تعالى:{أولم يرَ الإنسانُ أنَّا خلقناهُ من نطفةٍ فإذا هو خصيمٌ مبين}

Fetal hemoglobin

- Fetal hemoglobin is a principal oxygen carrier in blood in the fetus and neonate.

  It has a higher affinity to oxygen and the OHD curve is shifted to the lift. (efficient extraction of oxygen from mothers circulation.

  It is composed of 2 alpha and 2gamma subunits.



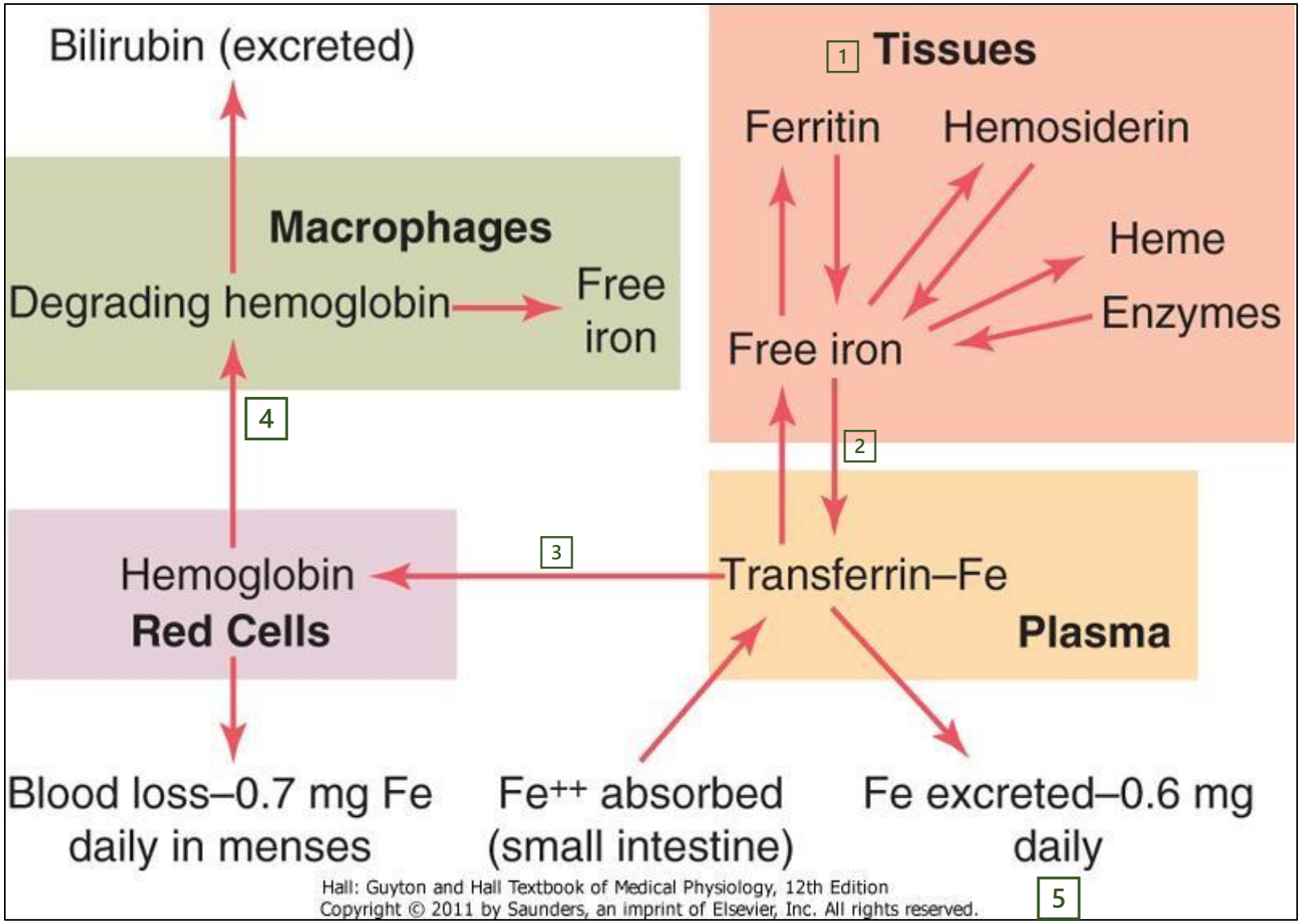
Iron Metabolism

Iron is one of the main requirements to produce enough hemoglobin and other proteins as oxidation enzymes…

- **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 and it is distributed as:**
  - **65% in hemoglobin**
  - **4% in myoglobin(muscle cells)**
  - **1% in intracellular heme compounds**
  - **0.1% associated with circulating transferrin**
  - **15 – 30% stored mainly as ferritin in RES**

- Stored as protein bound mainly in liver and other organ of reticular endothelial system RES.

Iron Transport and Metabolism



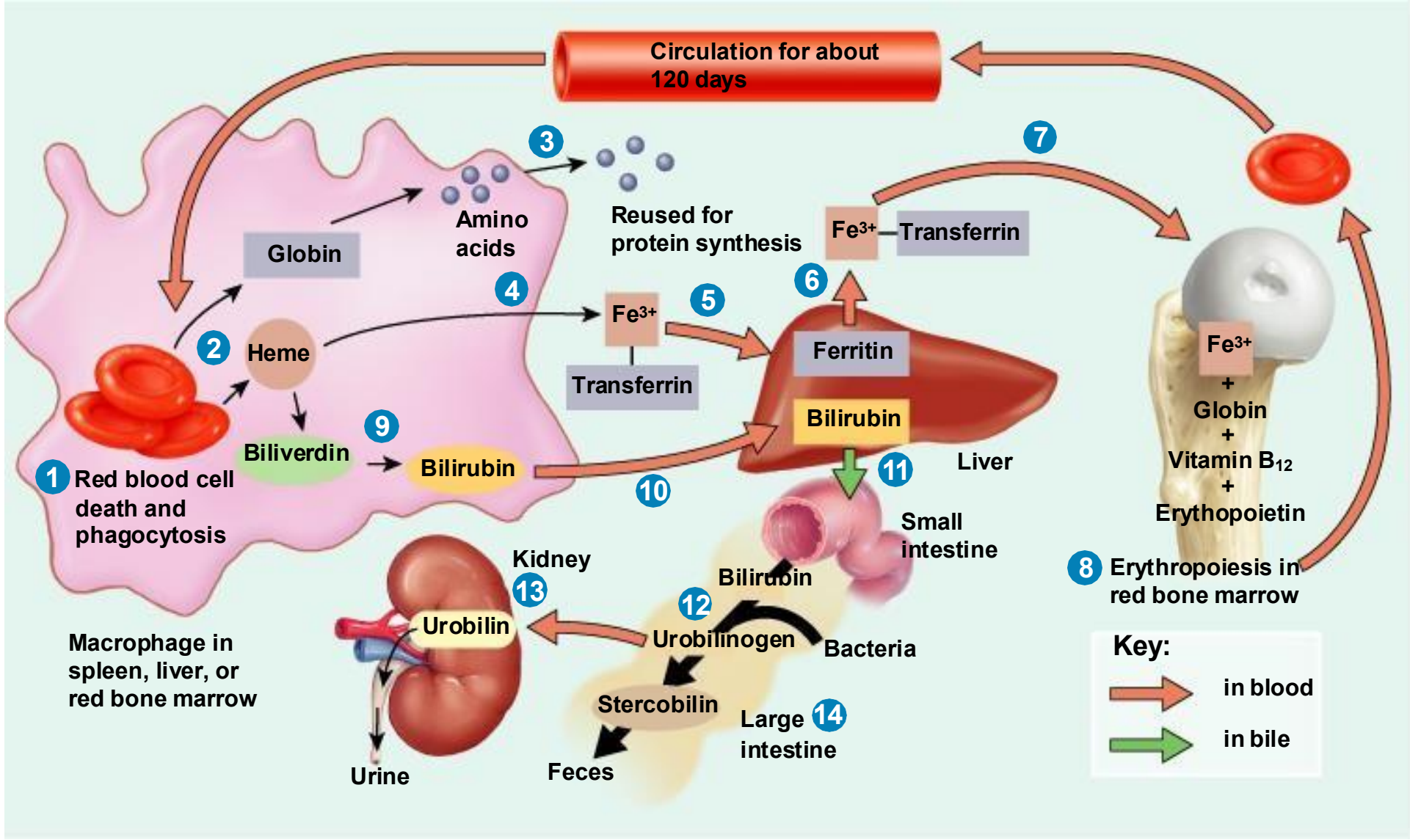
[1] Iron present in tissues is either bound to the storage protein ferritin, If there is excess iron that isn't bound to ferritin, it will accumulate as hemosiderin (clusters of iron inside the cells) when there aren't enough ferritins to bind with iron. Iron is also bound to heme in various types of cells, as well as bound to enzymes.

[2] Free iron outside the cell should be bound to the transport protein transferrin in the plasma.

[3] This compound is continuously renewed by hemoglobin degradation, which occurs during the life cycle of red blood cells (RBCs)->[4] Therefore, free iron released from hemoglobin that has degraded from aged RBCs in such locations as the liver, spleen, and bone marrow will be transported via plasma to where it will be stored or used.

[5] The excretion of iron is about 0.6 mg daily through the gastrointestinal (GI) system. Additionally, females lose approximately 0.7 mg during menstruation(menses).
Iron absorption from the diet occurs in the small intestine, and only what is needed is absorbed, the rest is going to be excreted by the digestive system.

Formation and Destruction of RBC's



Let's discuss the **life cycle of RBCs** and the turnover of iron during this cycle …

1. First of all, aged RBCs that have been destroyed and damaged in organs (spleen, liver, and red bone marrow) are engulfed by macrophages. For example, in the liver, we have Kupffer cells.

2.After phagocytosis, the components related to hemoglobin are reused.
3. The **globin part** is broken down into amino acids, which are then reused for **protein synthesis.**

4. As for the **heme part**, the iron in heme is transported by a plasma protein called transferrin.
5. The iron can be taken up by the liver and bound to a storage protein called ferritin.

6.7. If there is a need for RBCs production and hemoglobin synthesis, iron is released from these stores and transported by transferrin to the bone marrow. Where other components necessary for RBCs production, such as **amino acids** for the globin chains, **vitamin B12**, **erythropoietin**, and **folic acid**, are all essential for new RBCs production and erythropoiesis in the red bone marrow.

8.After that, RBCs circulate for roughly **120 days** before beginning a new cycle that begins with macrophage engulfment.

9. For the **heme part**, after iron is taken by transferrin molecules, the remaining part of heme is converted into **biliverdin**, which is then converted into **bilirubin** inside the macrophages.
10. Bilirubin is transported via the blood to the liver, 11. where it is excreted in bile and released into the duodenum of the small intestine.

12.In the intestine, bilirubin undergoes further changes, being converted by bacteria into **urobilinogen**, and then into **stercobilin**, which gives feces its brown color.

13.Urobilinogen can be reabsorbed into the bloodstream and excreted by the kidneys as **urobilin**, which gives urine its yellow color.

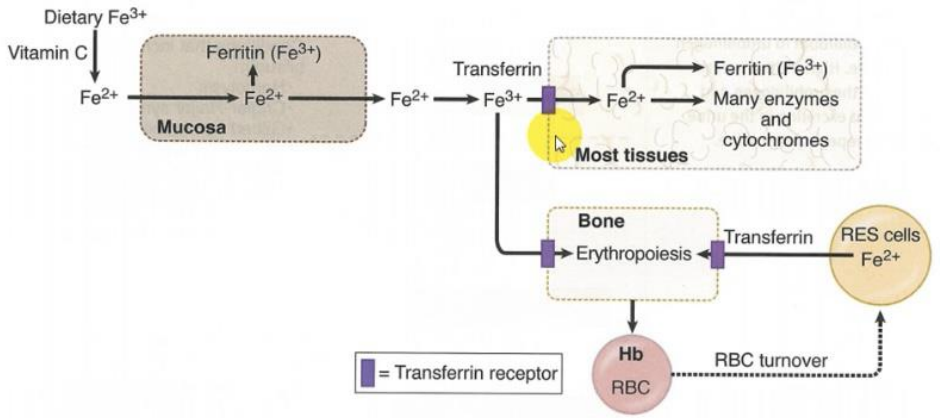


Figure I-17-7. Iron Metabolism

Extra image

Iron Absorption, Transport & Storage

- **Absorbed from small intestine, combines with *apotransferrin* ⟶ *transferrin* (transport iron)**

-> Transferrin is a transport protein that has the important function of transporting iron.

- **Iron can be released to any cell**

-> To be used intracellularly.

- **RBC precursors have <u>transferrin receptors</u> and actively accumulate iron**

->Because it is required for erythropoiesis and the generation of RBCs, particularly hemoglobin in the RBCs.

- **Particularly in hepatocytes and reticulo-endothelial cells, iron combines with *apoferritin* ⟶ *ferritin* (MW 460,000)** -> is a very large protein.

- **Ferritin is variably saturated (storage iron).**
  - ***Hemosiderin* is quite insoluble excess iron.**

Ferritin is sometimes <u>completely saturated</u> with iron and sometimes <u>not</u>. When it is completely saturated with iron, the excess iron inside the cells will accumulate in clusters and will <u>appear</u> under a <u>light microscope</u> as a large entity called **hemosiderin**, which is an insoluble excess of iron. However, ferritin in cells **cannot** be seen in light microscopy; it can only be detected inside cells using an electron microscope.

Iron Exchange

- **When iron in the plasma is low, iron is released from ferritin and bound to transferrin for transport.**
  ->From stores"liver".
- **It is delivered to the bone marrow, bound by transferrin receptors on erythroblasts, internalized, and delivered directly to the mitochondria for incorporation into heme.**
  Or apotransferrin
- **Deficiency of transferrin can result in severe *hypochromic* anemia.**
  ->As we know, **transferrin** is a carrier for transporting iron, so without it, there wouldn't be enough iron transported to the bone marrow.
- **Hemoglobin released from senescent aged RBCs is ingested by macrophages and stored as ferritin.**
  after being recycled, it is stored as **ferritin** in the liver.

This induces the endocytosis of iron into the cytosol of RBCs, where it is taken up by the **mitochondria**, which is the site of heme production. In the mitochondria, iron is used to produce heme.

Iron Balance

- **Daily iron loss of ~ 0.6 mg/day in men (GI) or ~1.3 mg/day in women (GI and menses)** ~> the monthly menstrual cycle .
- **Iron is absorbed throughout the small intestine**

- **Liver secretes *apotransferrin* into the bile, which binds with free iron and some iron compounds to become *transferrin***

Apotransferrin, which is a protein, becomes transferrin once it binds with iron. It is synthesized and secreted by the liver, and released with bile into the intestine. In the intestine, it binds to free iron. Some iron compounds present in food, such as myoglobin from meat, also bind to transferrin.

- **Binds to *transferrin receptors* on intestinal epithelium, Transcytosed into the blood as plasma transferrin.**

- **Transferrin** is important because it binds to transferrin receptors in the intestinal epithelium. Once transferrin is bound to the receptors, it is transcytosed along with iron into the bloodstream, becoming plasma transferrin.

- **Maximal absorption of a few mg per day, modulated over 5 – 6-fold range based on body stores**

Any changes in the erythropoiesis process **or** in the body's iron stores can affect the rate of absorption. Iron absorption varies according to the level of iron stores. If the stores are fully saturated, iron absorption will not be as high as it could be.

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**

These enzymes are important for metabolizing glucose anaerobically to provide **ATP.**

- **As enzymes deplete with age, RBCs become fragile and rupture in small passages, often in the spleen**

Especially in the trabeculae of the spleen, because the space is very narrow for aged RBCs and their membranes are fragile, RBCs are most likely to be damaged in these areas.

Degradation of Hemoglobin

- **When RBCs rupture, hemoglobin is phagocytosed by macrophages, particularly in the liver and spleen**

  - When iron is released from phagocytes, it will either be transported by transferrin into the blood to support erythropoiesis, or it will be stored as ferritin in the liver.

- **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** -> to the small intestine…

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!