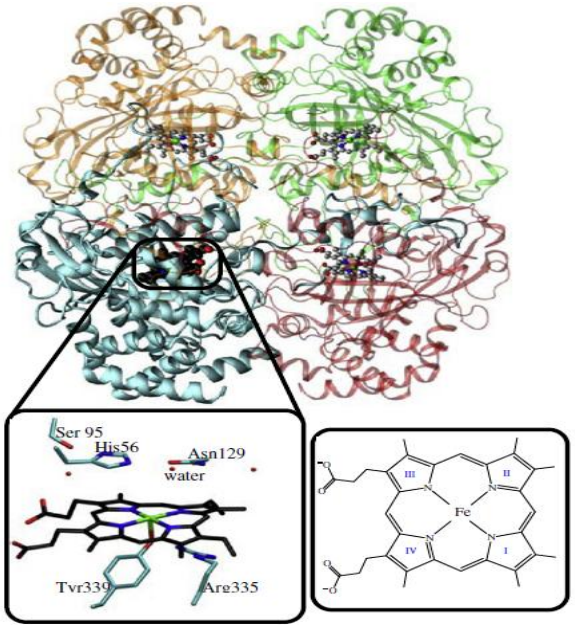
Hemoglobin and heme metabolism

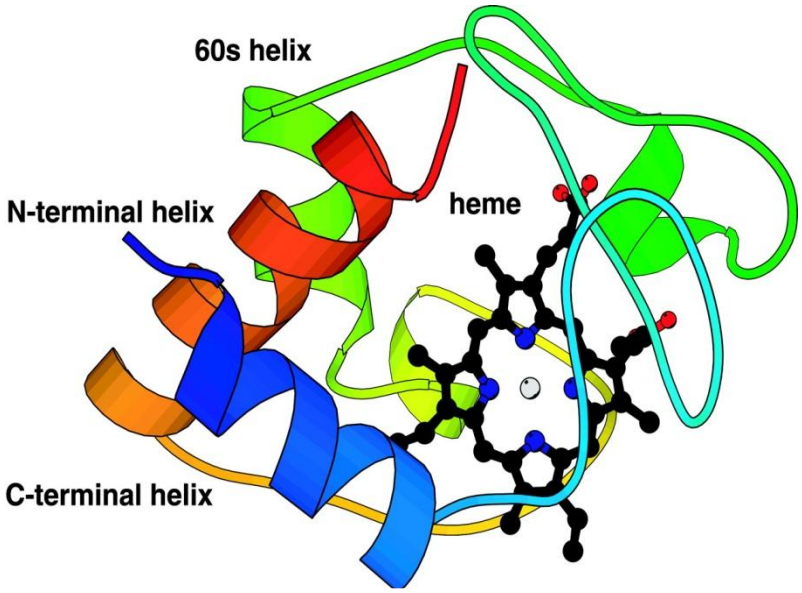
Basem Battah, Pharm, Msc, PhD

Hemoprotein

- **Hemoprotein** are group of functional protein contain the **heme** as a tightly bond prosthetic group.
- The role of the heme group differ depend on the 3D structure of the protein.
- **For example** the heme group in Cytochrome C serve as an electron carrier.
- Another example is the catalase enzyme where the heme group is a part of the active site that catalyze the breakdown of the hydrogen pyroxide.



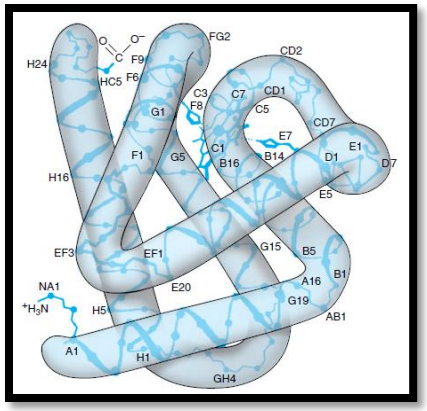
Catalase

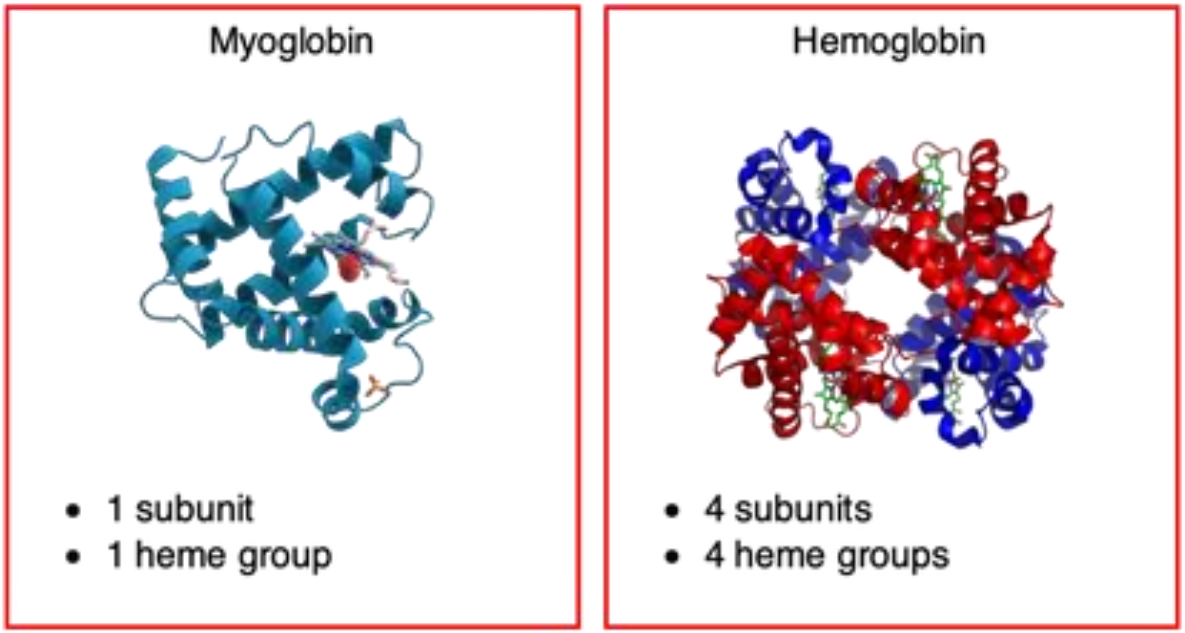


Cytochrome C

Hemoglobin and myoglobin

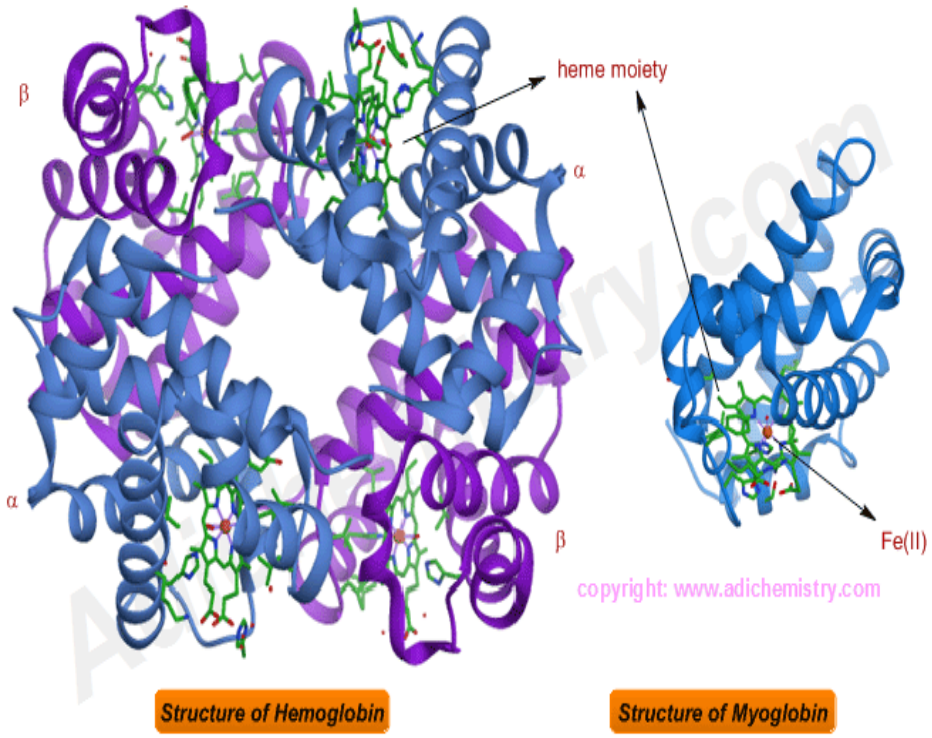
- The heme group in the hemoglobin and myoglobin serve to bind oxygen reversibly.

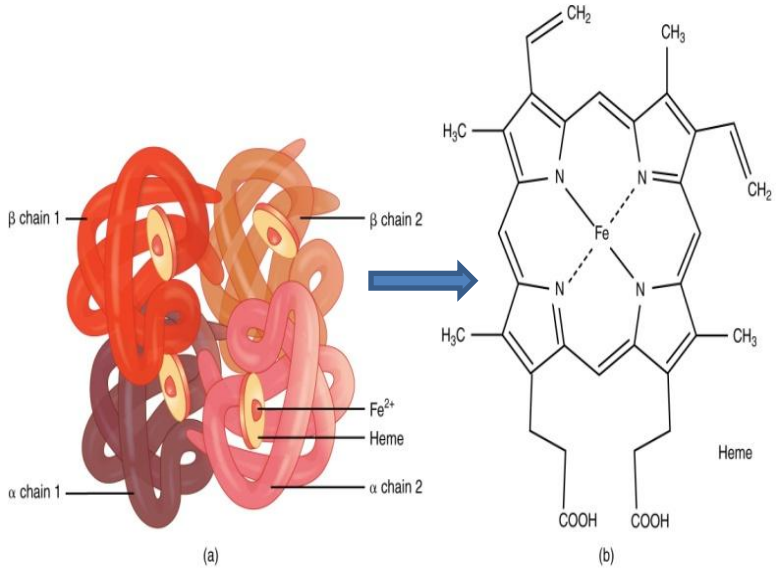




Heme structure

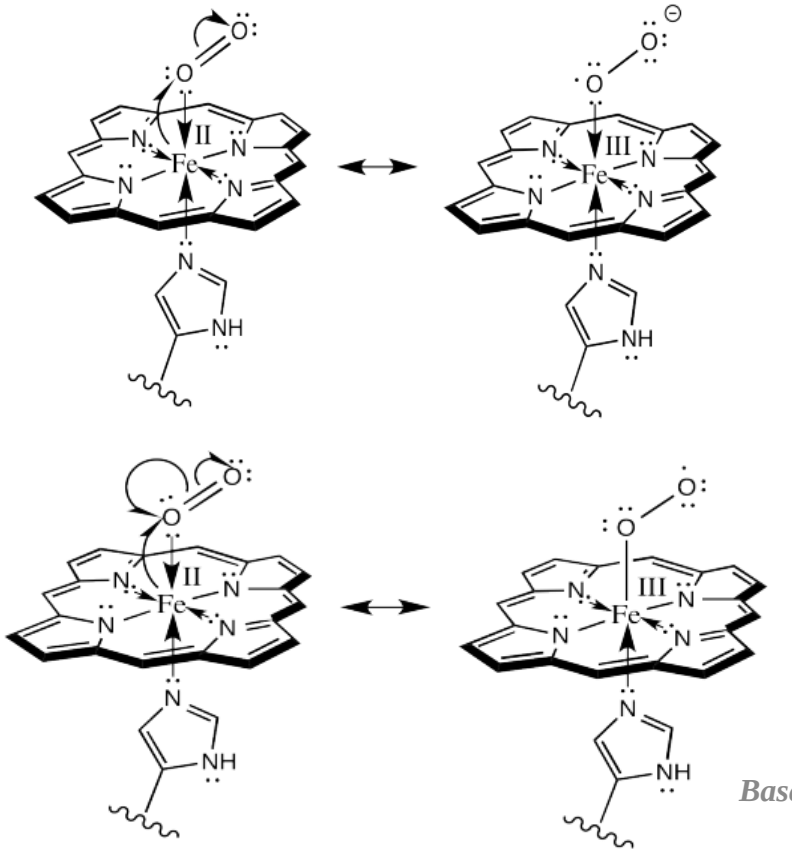
- **Heme** is a complex of protoporphyrine IX with ferrous iron Fe+2 in the centre of the ring and bind the four nitrogen atoms from the porphyrin ring.





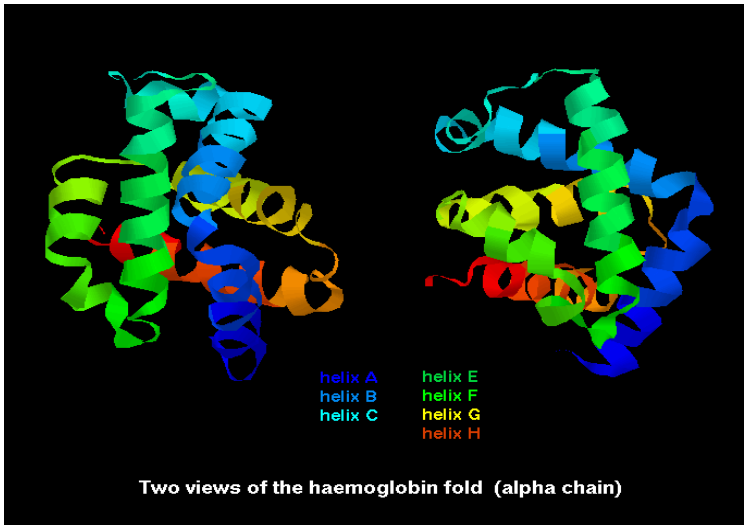
Heme structure

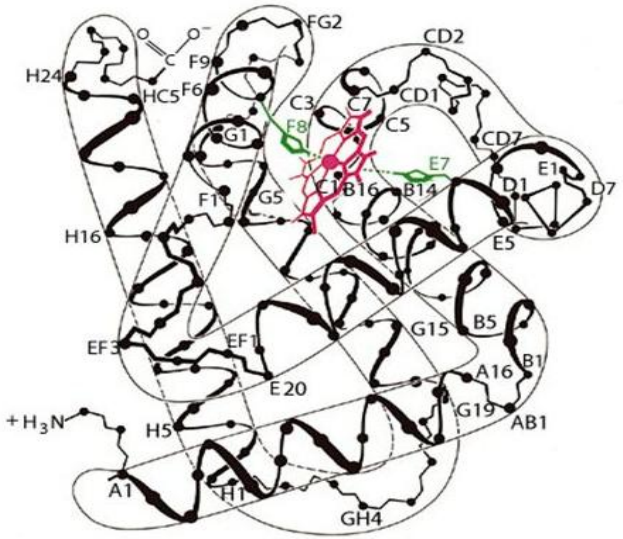
- The ferrous iron can form two bonds, on both side of the prophyrin ring plate, on with oxygen and the another one with histidine residue of the globin and from the oxyhemoglobin with Fe3+ (ferric iron).



Myoglobin structure

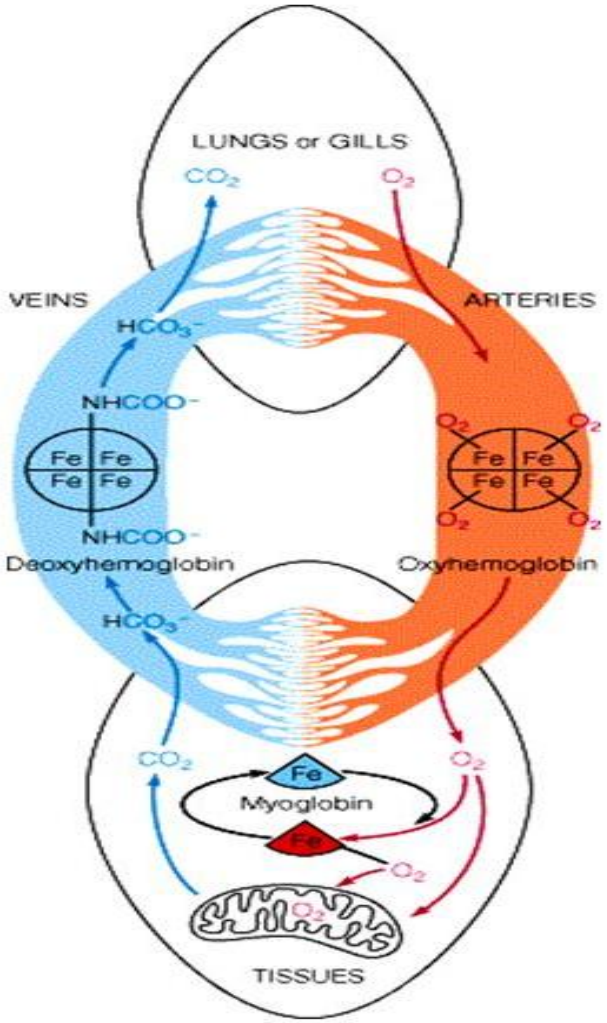
- 153 amino acids (16.7 Kda).
- Has 8 helices A-H terminated either by proline or by ß bends and loops stabilized by hydrogen and ionic bonds, heme F2+ is bound through the side group of N of His F8 (Proximal His).
- His E7 is close to other side of heme but doesn't coordinate (distal His) helps stabilize the binding of the O2 to ferrous iron.
- The globin the protein part of the myoglobin create the microenvironment for heme to bind oxygen reversibly .





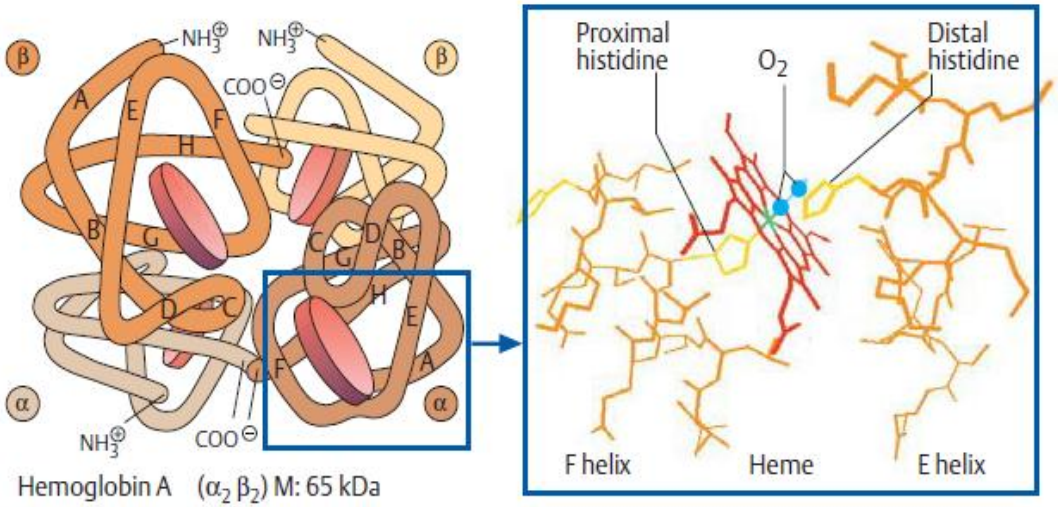
Myoglobin funtion and localization

- Myoglobin O2 storage protein present in the heart and and skeletal muscle and transport the O2 within the muscle cell.
- Hemoglobin transport O2 from lungs to tissue.
- Most tissues are constantly dependent on a supply of molecular oxygen (O2) to maintain their oxidative metabolism. Due to its poor solubility, O2 is bound to hemoglobin for transport in the blood.
- This not only increases the oxygen transport capacity, but also allows regulation of O2 uptake in the lungs and O2 release into tissues.



Hemoglobin structure

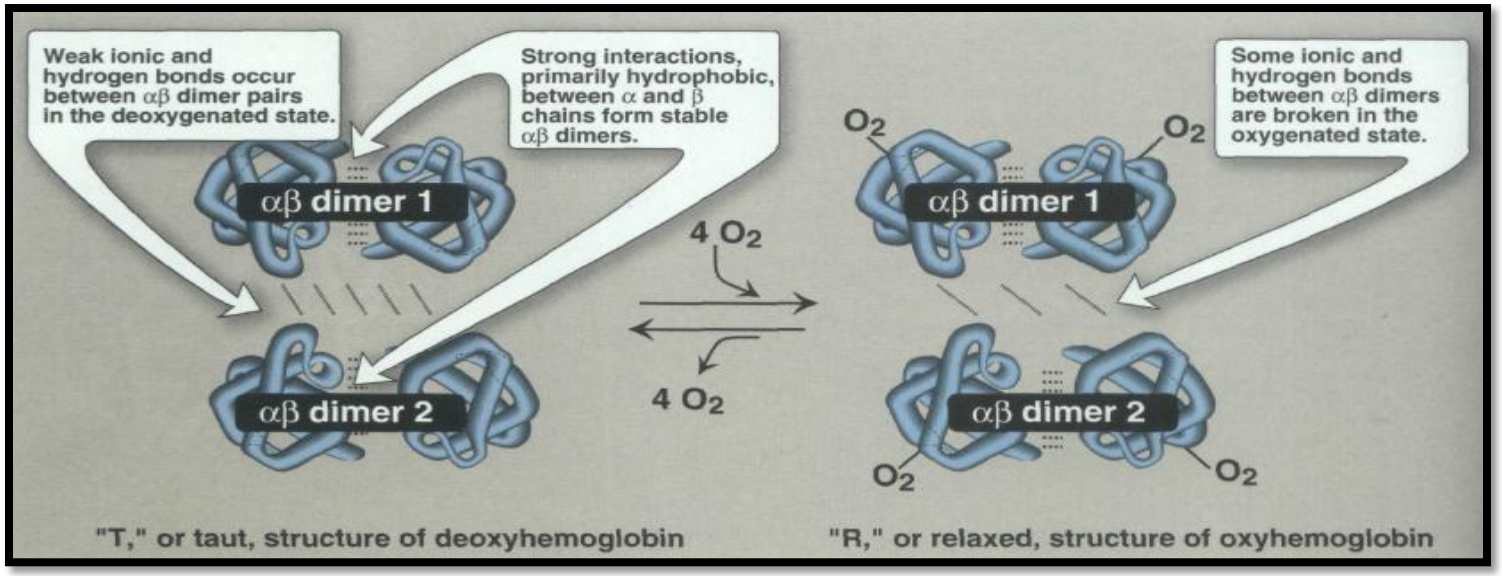
- Hemoglobin presents only in the red blood cells transports the oxygen from the lungs to the tissues.
- The major type of the hemogloin in the adults is the hemoglobin A.
- Hemoglobin A composed of four polyeptide chians 2α (141 AA each) and 2β (146 AA each) chians held together by noncovalent interaction.
- The hemoglobin molecule is structurlly and functionally more complex than the myoglobin which transport 4 molecule of the O2 from the the lungs to the tissue and the CO2 from the tissue to the lungs.



Hemoglobin A ($\alpha_2 \beta_2$) M: 65 kDa

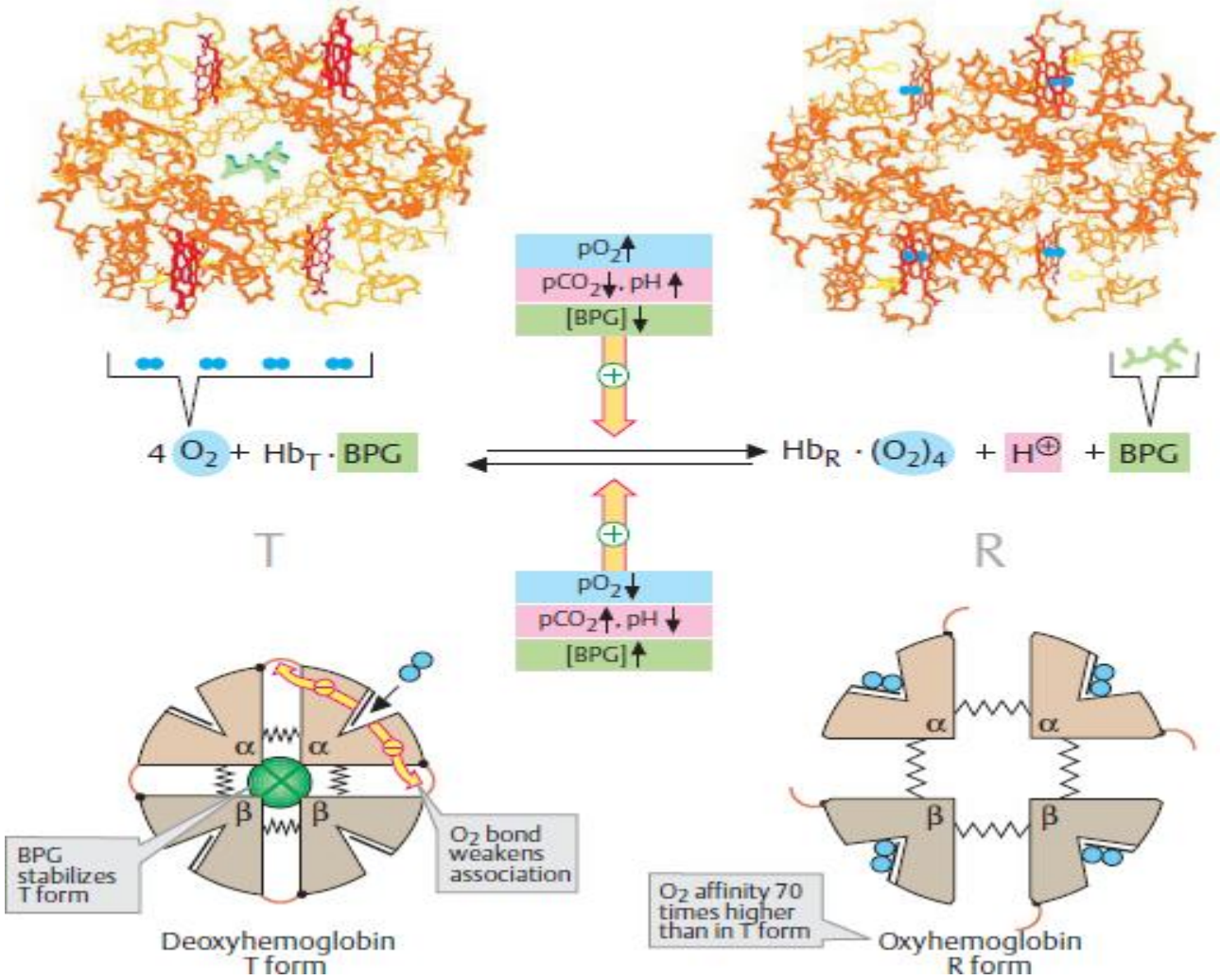
Hemoglobin structure & function

- The hemoglobin is tetramer composed of two identical dimers αß dimer 1 and αβ dimer 2.
- Oxygen binding make a structural changing between T and R form.
- The **T (tens)** form: is the deoxy form, the two dimers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T form is the **low oxygen-affinity form of hemoglobin.**
- The **R (Relax)** form**:** when the oxygen bind to the hemoglobin that leads to breakdown some ionic bonds and hydrogen bonds between the two dimers. Which gives the polypeptide chain more relaxed and freedom movement and this form is the **high oxygen-affinity form of hemoglobin**



Hemoglobin – oxygen binding properties

- Hemoglobn- O2 binding is regulated by allosteric effetors.

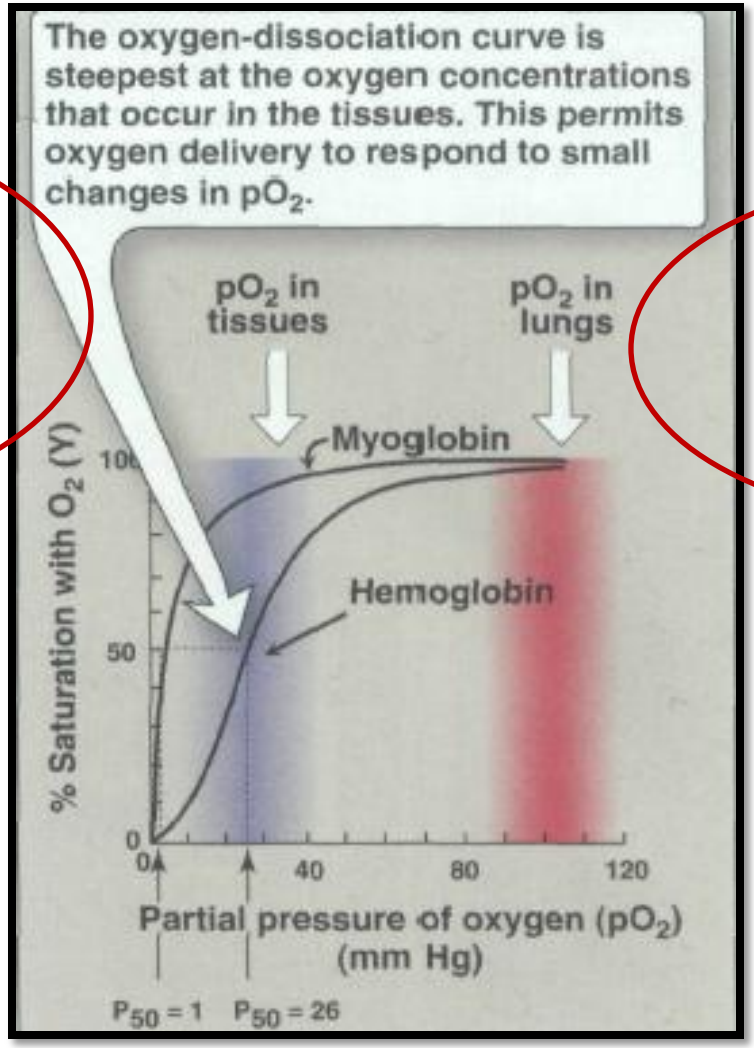


Regulation of O2 transport

- Haemoglobin is an allosteric protein release the oxygen without changing.
- The O2 molecule is a positive homotropic effector promotes its own binding by allosteric regulation.
- The structure of myoglobin is similar to that of a subunit of hemoglobin, but as a monomer it does not exhibit any allosteric behavior.
- CO2, H+, and a special metabolite of erythrocytes— **2,3-bisphosphoglycerate (BPG)—** act as heterotropic effectors of hemoglobin. BPG is synthesized from 1,3–bisphosphoglycerate, an intermediate of glycolysis.
- BPG binds selectively to deoxy–Hb, The result is increased O2 release at constant pO2. and the same for CO2 and H+.

Regulation of O2 transport

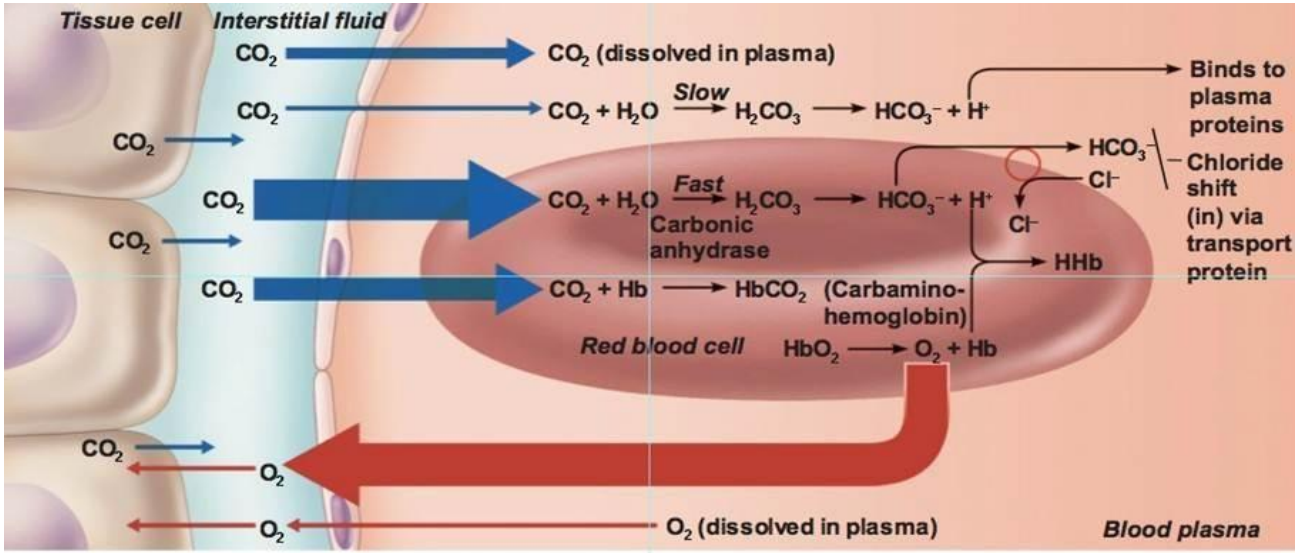
The O2 – Myoglobin binding is simple equilibrium, bind O2 released by the hemoglobin at low pO2 in the muscle and release the oxygen within muscle in response to the O2 demand.



The O2 – hemoglobin binding is cooperative binding one O2 molecule increase the affinity of other heme group to O2

Hemoglobin and CO2 transport

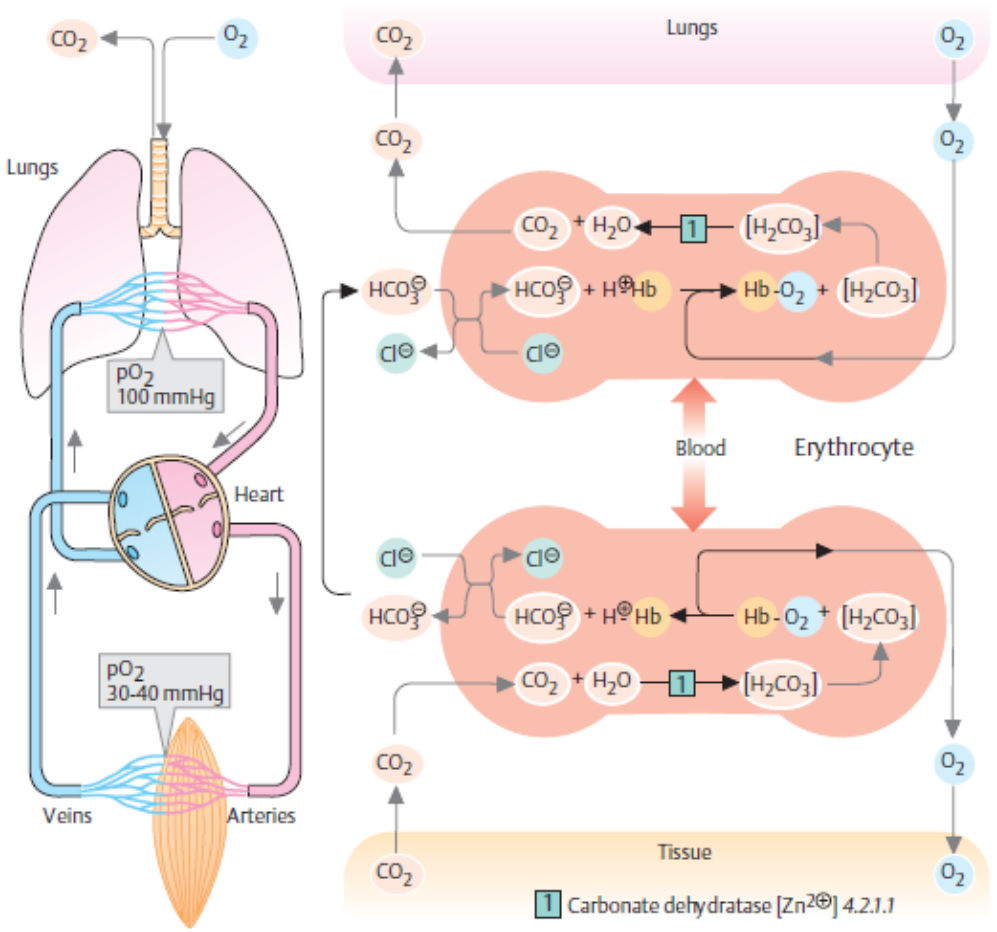
- The hemoglobin also transport the CO2 from the tissue to the lungs.
- 5 % of the CO2 in the tissue is transported as carbaminohemoglobin where the CO2 is bind to the N-terminus of the hemoglobin.
- About 90% of the CO2 is first converted in the periphery into hydrogen carbonate (HCO3–), which is more soluble. In the lungs the CO2 is regenerated again from the (HCO3-), and then exhaled



(a) Oxygen release and carbon dioxide pickup at the tissues

Hemoglobin and CO2 transport

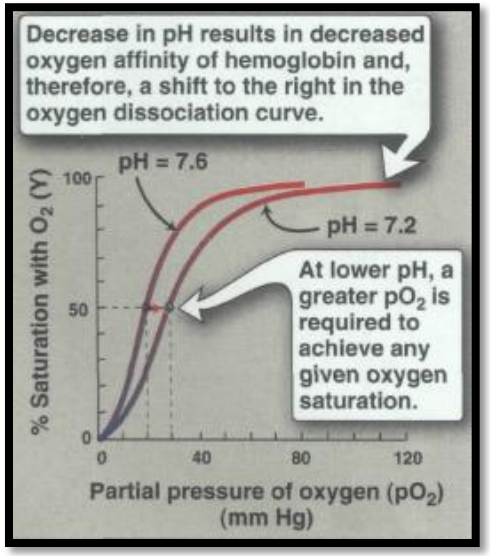
- Deoxy–Hb is a stronger base than oxy–Hb. therefore binds additional protons (about 0.7 H+ per tetramer), which promotes the formation of HCO3 – from CO2 in the peripheral tissues. The resulting HCO3 – is released into the plasma via an antiporter in the erythrocyte membrane in exchange for Cl–, an passes from the plasma to the lungs.

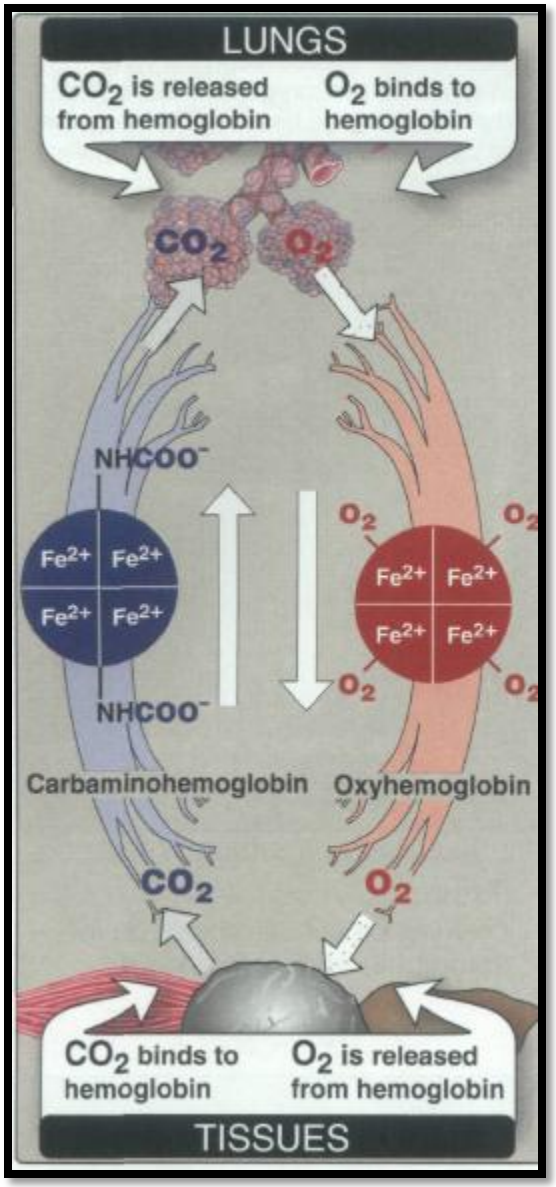


Hemoglobin and CO2 transport

- In the lung deoxy-Hb is oxygenated and releases protons. The protons shift the HCO3/CO2 equilibrium and thereby promote CO2 release.

- The O2 – hemoglobin binding is affected by PH, the high concentration of the CO2 in tissue increase the H+ concentration and thereby reduce the O2-Binding affinity (Bohr effect). These leads to O2 release. And improved oxygen supply.

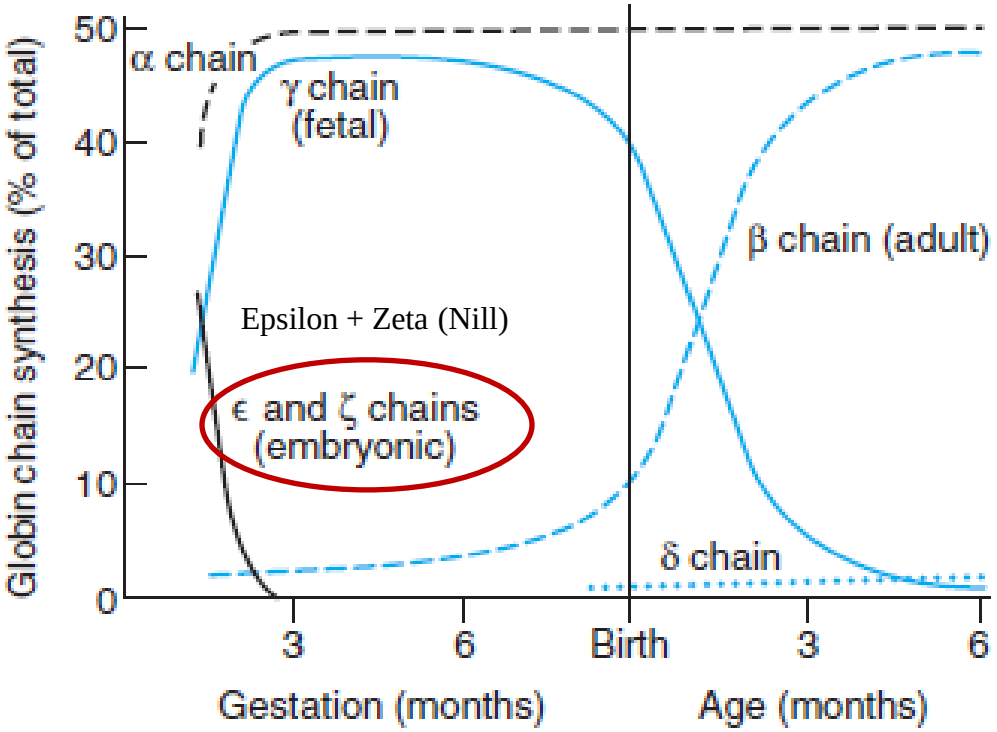




Types of hemoglobin

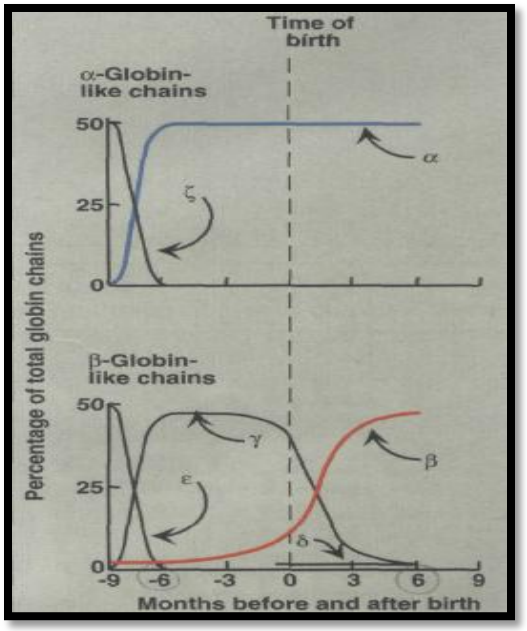
Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3–9%

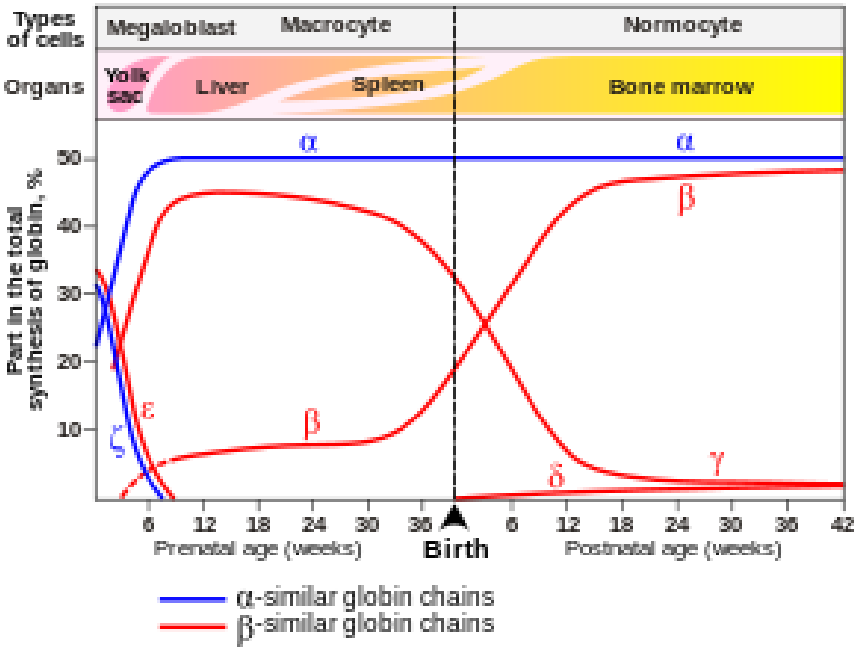
- All hemoglobins are oxygen carrier protein types are tetramer 90% of them composed of 2α and 2β chain HbA.
- Some kinds of hemoglobin are ony being synthesized during fetal development called HbF and other could be covallently bind to glucose and form the glucosylated Hemoglobin HbA1C.
- Globin chain synthesis percentages differ from fetal to the adult age.



Fetal hemoglobin (HbF)

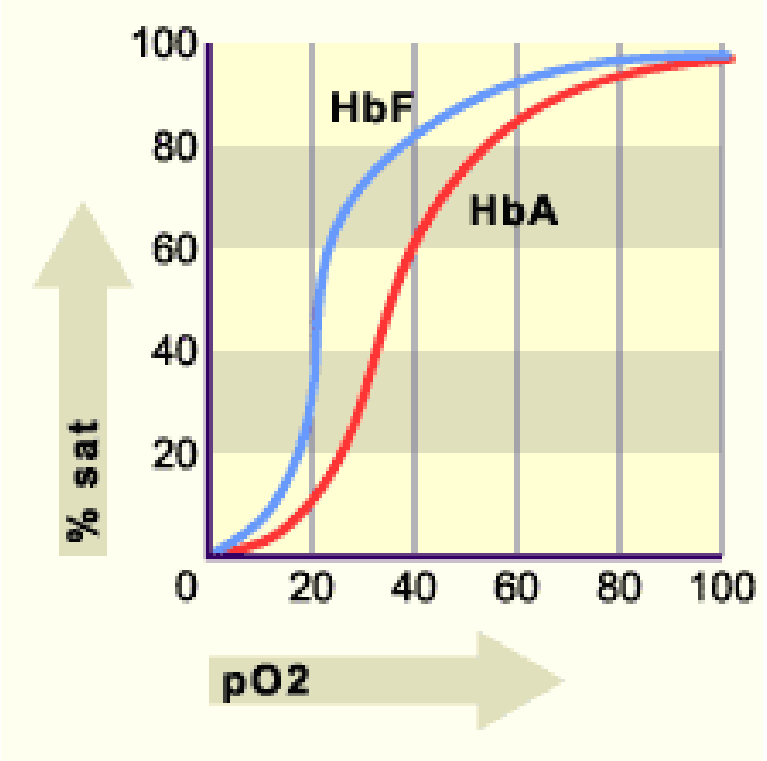
- HbF Consists of two α chain and two δ chain (α2, γ2).
- While the embryonic hemoglobin is synthesized by the embryonic yolk sac in the first few week of conception, the fetal liver start to synthesize HbF Within few weeks .
- The HbF remain the major type (60%) of the hemoglobin in the fetus and newborn.
- The synthesis of the HbA starts in the bone marrow eight month of pregnancy.





HbA Vs HbF

- The γ globin chain in the HbF lack some of the positively charged amino acids necessary to bind 2,3 BPG in HbA which serves to reduce the hemoglobin affinity to Oxygen.
- For this reason the Hbf has more affinity to O2 than the HbA.
- This ability helps the HbF to transfer oxygen from the maternal circulation across placenta to the fetus red blood cells.

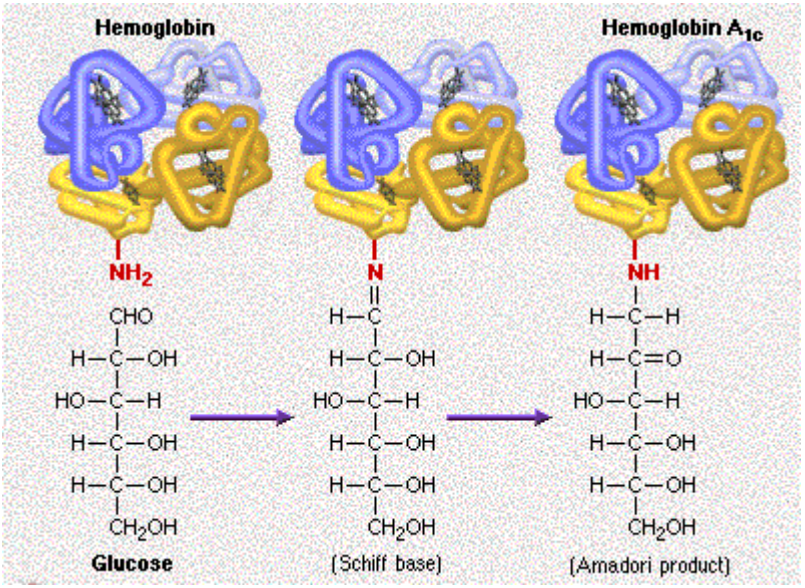


HbA 2

- Appears 12 weeks after birth and occupy only 2 % of the total hemoglobin and consists of 2 alpha globin chain and 2 gamma chian α2γ2

HbA 1c

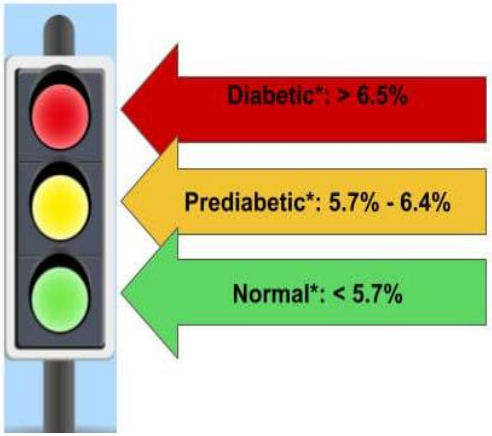
- The hemoglobin bind glucose under physiological condition (non enzymically).
- The hemoglobin glycolsylation depend on the glucose plasma level.
- In glucose molecule binds to the NH2 group of the N-terminal valine of the β globin group.
- In patients with Diabetes mellitus, there is increased amount of the HbA1c in the red blood cells which remain in the contact with the glucose during 120 days of the life span of the red blood cells .
- Indicated the disease progression.



HbA1c test measures the percentage of HbA1c in blood. It reflects the average blood glucose over a period of past two to three months (8 - 12 weeks)

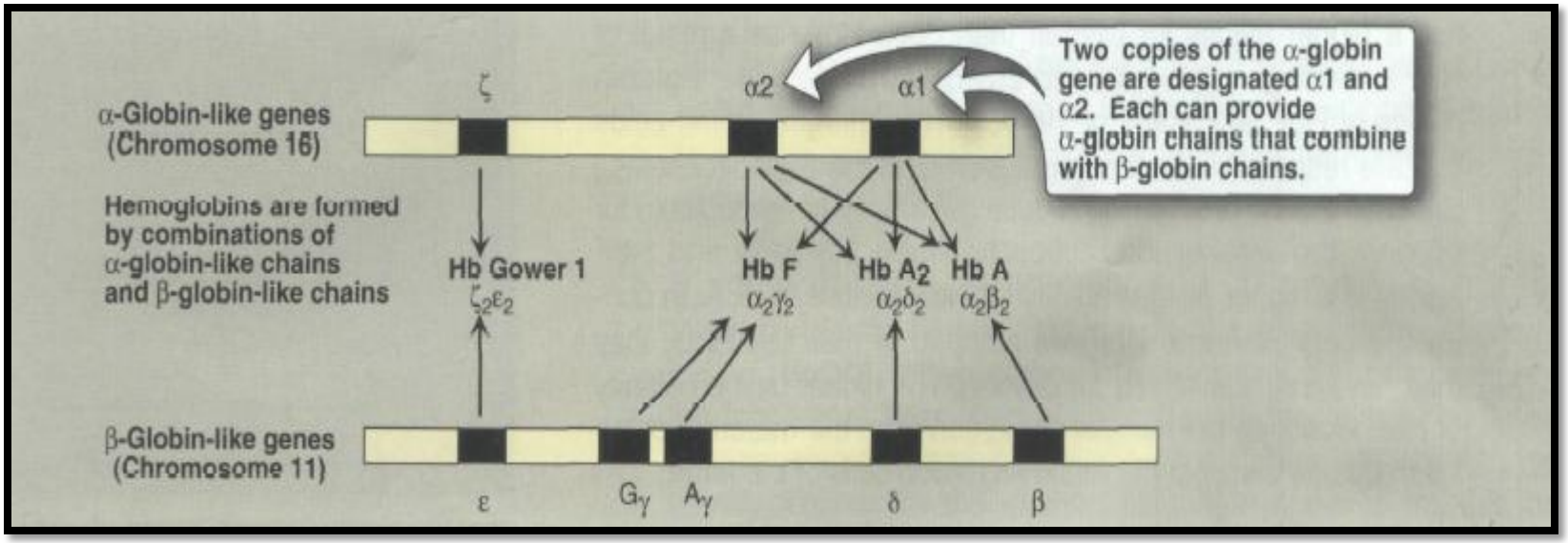
HbA1c refers to hemoglobin which is bound to glucose

HbA1c test is done to
- *Diagnose Diabetes*
- *To monitor Diabetes*



Globin gene

- the globin gene encodes for the α and ß subunits presents in two gene cluster located in two different chromosome



Hemoglobinopathies

- the different hemoglobin disorders called the hemoglobinopathies.
- manifest with structurally abnormal hemoglobin production. Or insufficient quantity of normal hemoglobin or both.
- Includes :
  - **Sickle - cell anemia HbS.**
  - **Hemoglobin C disease.**

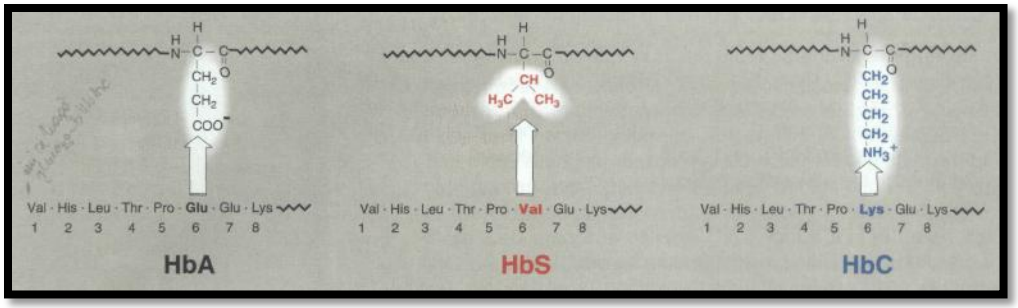
  } Altered aminoacid sequence

  - **Thalassemia**

  } Decreased production of normal hemoglobin

Sickle cell disease

- Hemoglobin S disease also called sickle cell anemia is a genetic disease caused by a single point mutation in the β globin gene where the glutamate at position six has replaced with valine .
- This disorder is a homozygous occurs in individuals who have inherited two mutant genes (one from each parent) that code for synthesis of the β-chains of the globin molecules.
- **affecting 80.000 Americans each years and 1 of 500 African Americans**
- The symptoms of this disease begins to appear when the HbF replaced with HbS.
- The symptoms includes :
  - Episode of pain
  - Hemolytic anemia
  - Increased susceptibility to infection

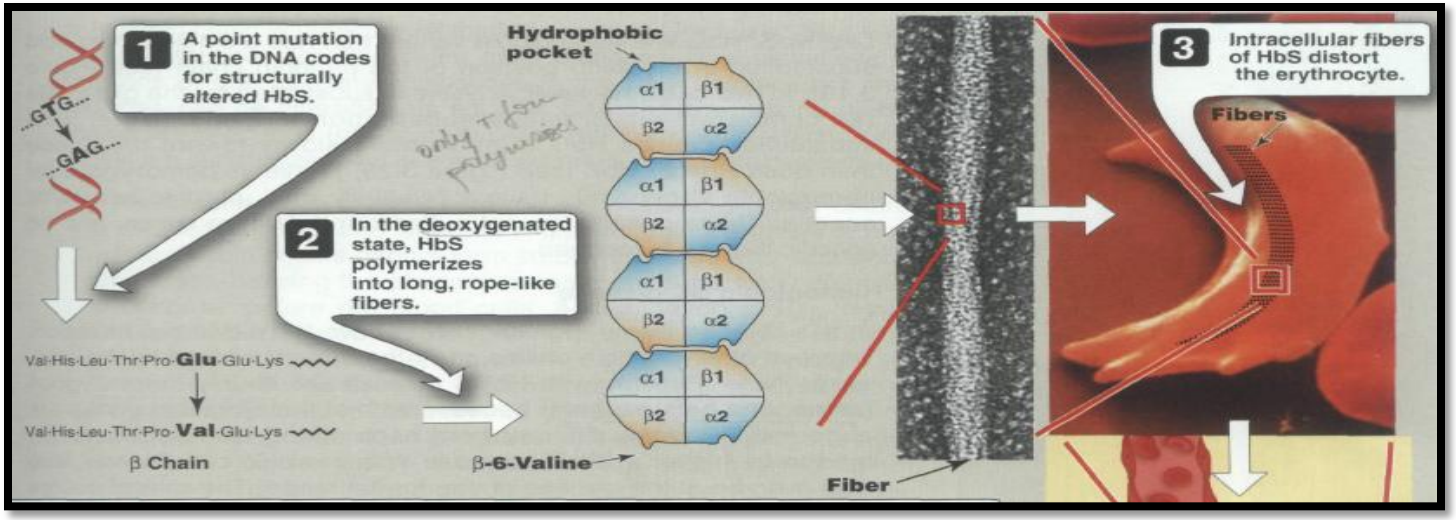


Other symtoms include splenic and renal dysfunction

- the lifetime of the red blood cells with homozygous HbS is around 20 days.
- Heterozygotes, representing one of ten African-Americans, have one normal and one sickle cell gene. The blood cells of such heterozygotes contain both HbS and HbA.they don’t show clinical symptoms and have normal life span.

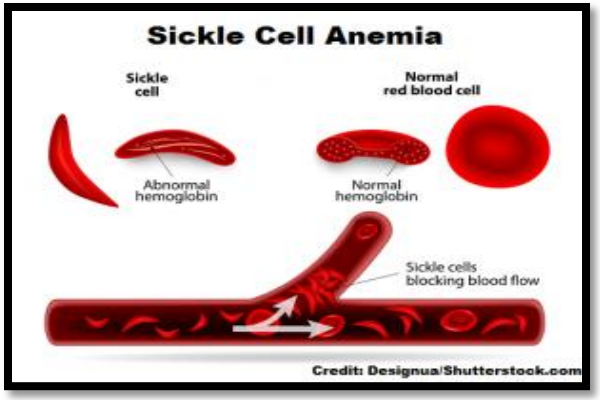
Sickel cell crisis

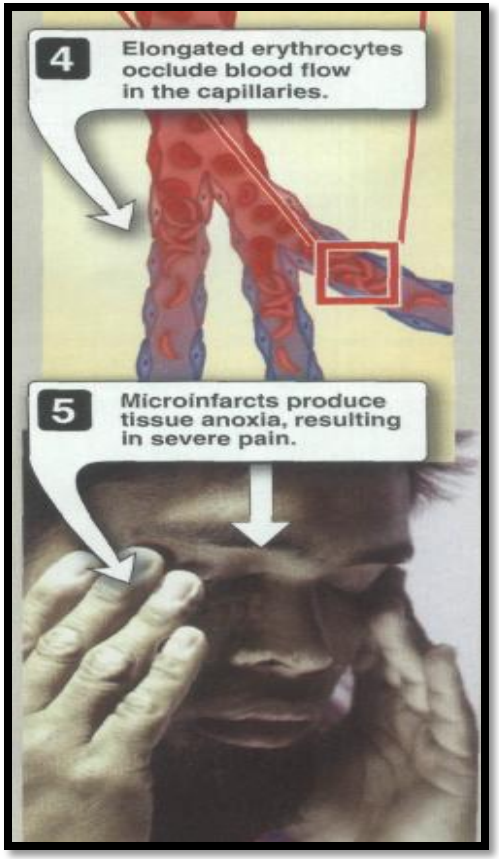
- The substitution of the nonpolar valine for a charged glutamate residue forms a protrusion on the β-globin that fits into a complementary site on the α-chain of another hemoglobin molecule in the cell.
- At low oxygen tension, HbS polymerizes inside the red blood cells, first forming a gel, then subsequently assembling into a network of fibrous polymers that stiffen and distort the cell, producing rigid, misshapen erythrocytes.
- Sickel cells block the flow of blood in the narrow capillaries. This interruption in the supply of oxygen leads to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually death **(infarction) of cells in the vicinity of the blockage.**



Sickel cell crisis

- Decreased oxygen tension, increased pCO2, decreased pH, and an increased concentration of 2,3-BPG in erythrocytes. Increase disease severity.
- Blood transfusion is recommended to treat at high risk patients. With caution from the high iron load and immunologic complication.
- Combined with antibiotic in case of infection



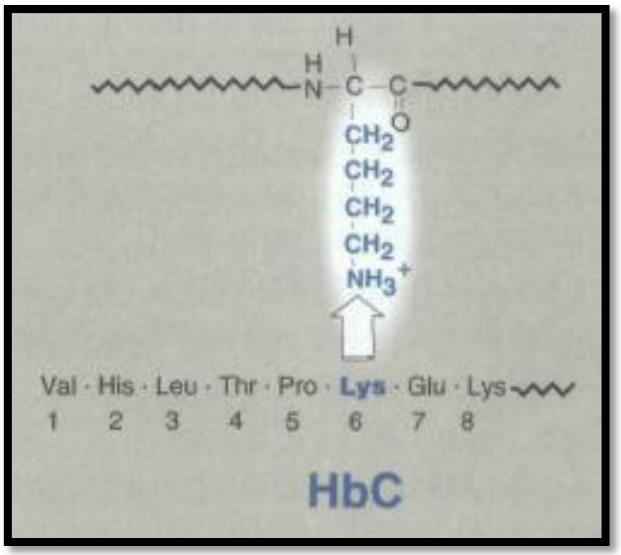


Hb C disease

•Point mutation leads to amino acid substitution in the sixth position on the β chain of the globin.

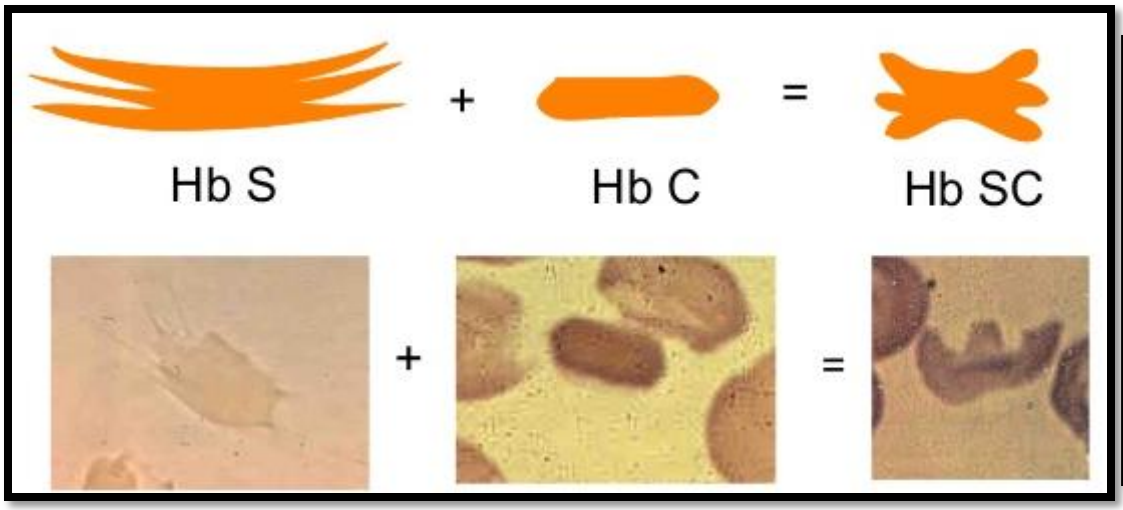
•in patients with homozygous HbC, have mild hemolytic anemia, no infarctive crisis and no therapy need .

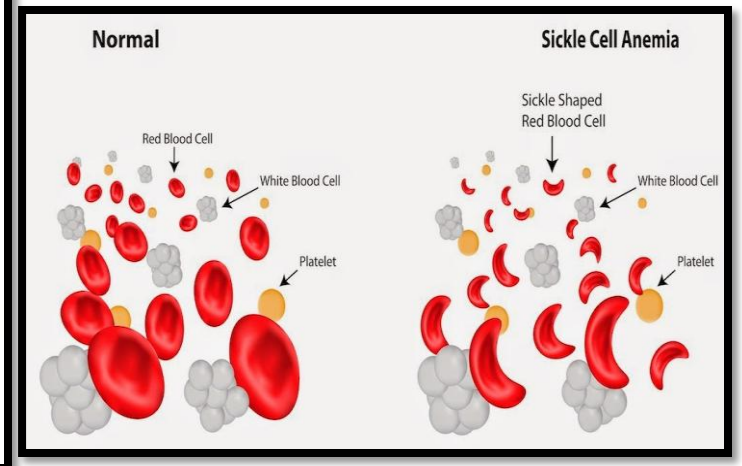
• in general is tolerated.



Hb SC

- This kind of Hb is mixed between the Hbs and HbC, hence some β chains have the mutation corrisponde to the HbS and the other have the mutation of the Hbc.
- So it is a compound heterozygous Hb.
- The Hb level is higher from Hbs but less than the normal range.
- The symptoms could manifest by painful crisis, starting from childhood.
- The patient could live a normal live.
- Infarctive crisis follow birth or during surgery could be fatal.

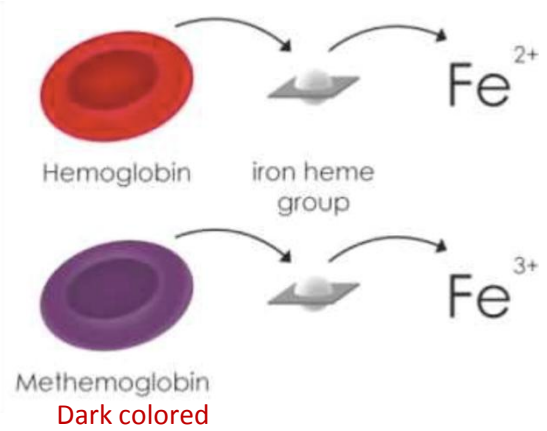


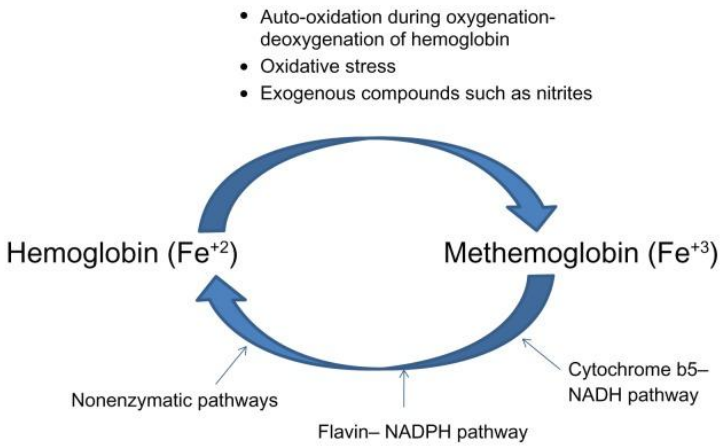


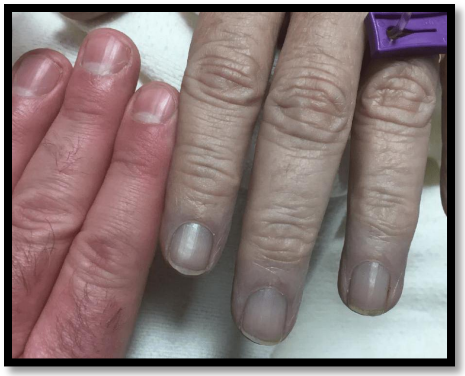
Methemoglobinemia

- It is one of the hemoglobin disorder occurs when the ferrous iron ion in the heme (Fe2+) being oxidized to the ferric (Fe3+). And loss its capability to bind O2.
- This oxidation could be stimulated by many factors:
  - Some drugs like nitrates. Endogenous reactive oxygen ROS.
  - Some mutation in the αβ globin chian promot formation of the HbM (methemoglobin).
  - Deficiency in the NADH-cytochrome reductase.

- **Symptoms:**
  - Skin color changing to brownish blue .
  - Chocolate colored blood.
  - tissue hypoxia, and include anxiety, headache, and dyspnea.
  - Rarely coma and death in some cases.







Thalassemias

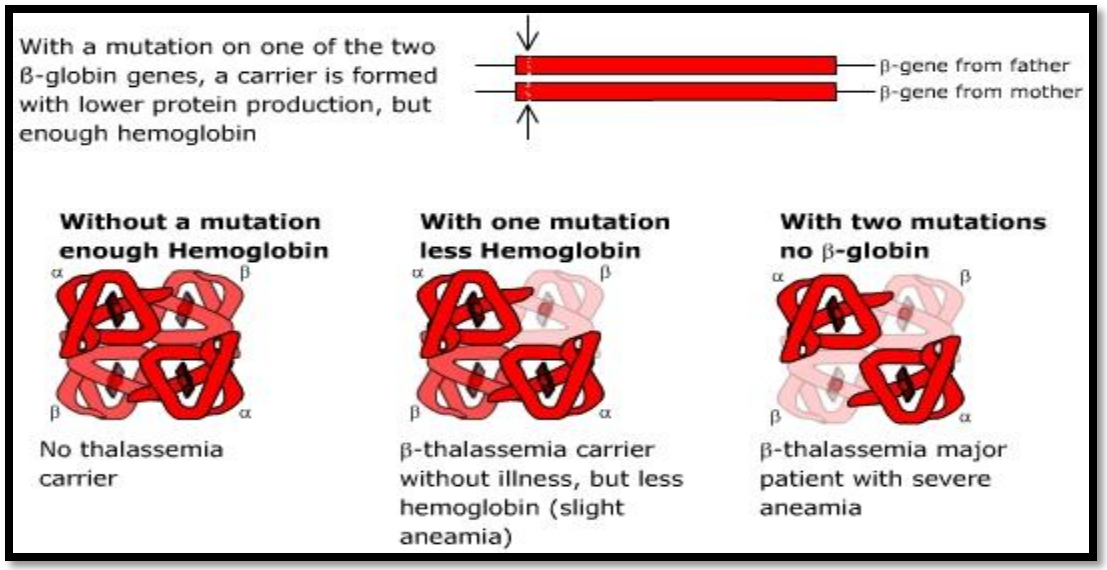
- Thalassemia is an inherited hemolytic disease. In thalassemia the globin chain synthesis is impaired or abnormal.
- Mutation in the globin chain genes (deletion –substitution) can occur.

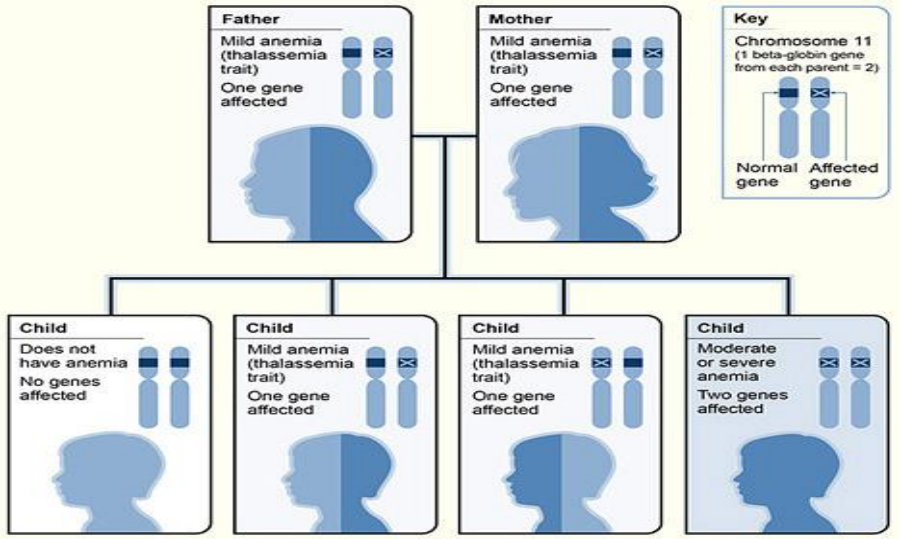
- This gene disorders can classify the thalassemia into :
  - β thalassemia (the disorder in the β chian)
  - α thalassemia (the disorder in the α chian)
  - Or rarely no globin chain are produced.

β thalassemia

- Only β chain are affected by decreasing or obscene of the synthesis and the α chain remain normal.
- Each cell have only two copy of the β chain gene. One on each chromosome 11.
- There is two types of the β thalassemia:
  - Minor β thalassemia when one gene is defective.
  - Major β thalassemia when the both of gene are defective.
- the symptoms occur only after birth.
- In the minor one no need to treatment.
- In the major thalassemia. The sever anemic symptoms start to appear in the second year of birth, and required a regular transfusion of blood but have a disadvantage of iron accumulation which leads to hemosiderosis syndrome and finally death at age 15-25years.
- Bone marrow transplantation could give a big chance for this patients

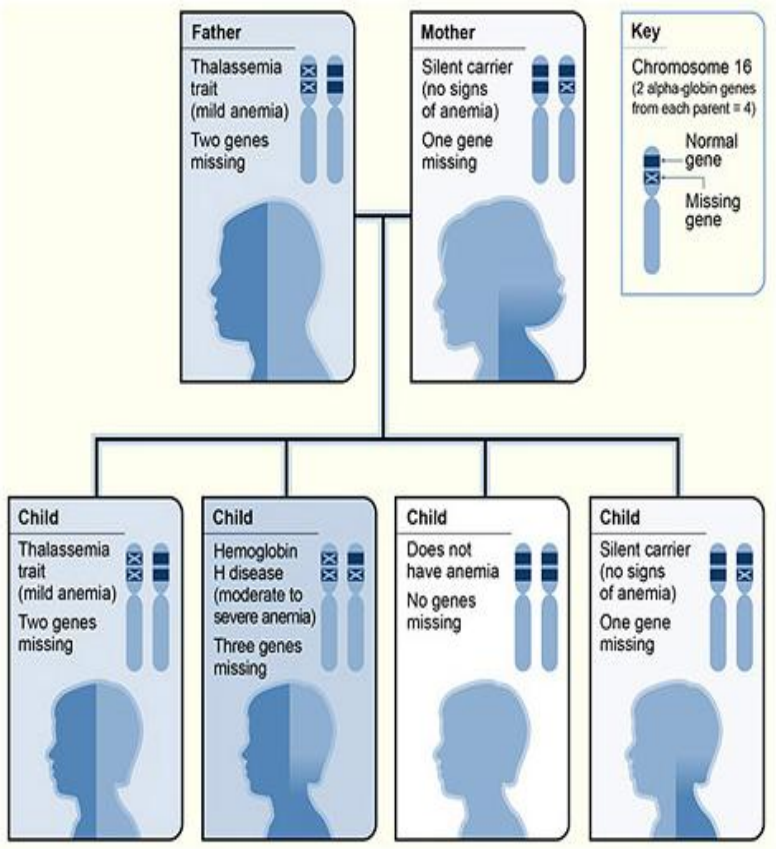
β thalassemia



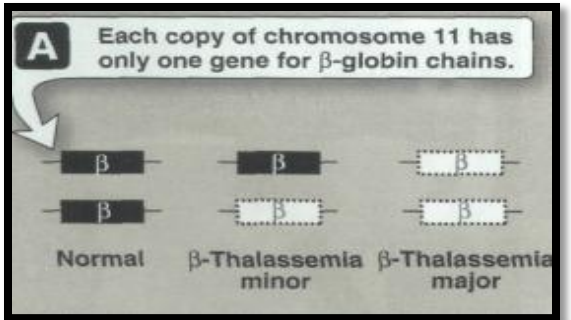


α thalassemia

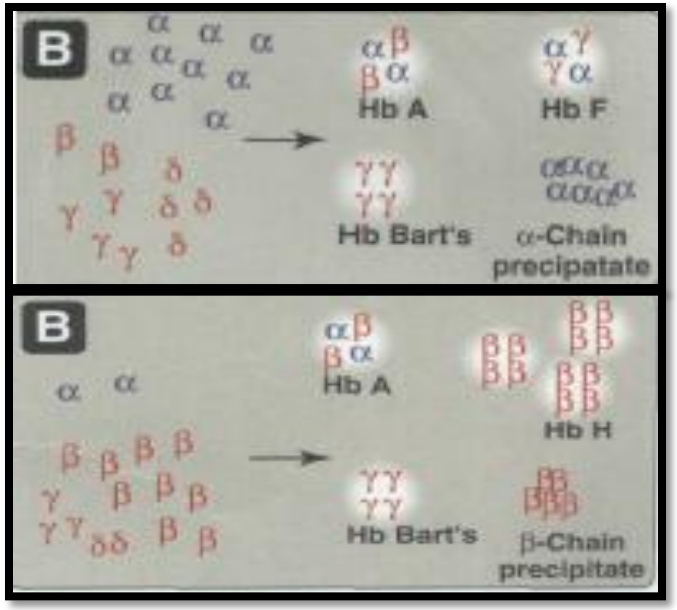
- Only α chain are affected by decreasing or obscene of the synthesis and the β chain remain normal.
- the α gene chain have two copy on each chromosomes 16 , so there is different level of this kind of deficiency.
- The patient could be a silent carrier when one of the four gene is defective.
- The patient could have α thalassemia trait when two genes of four are defective.
- When three of genes are defective the patient have hemoglobin H disease with sever to moderate hemolytic anemia.
- In the case of absence of all α chain the fetal death occur.



β thalassemia Vs α thalassemia

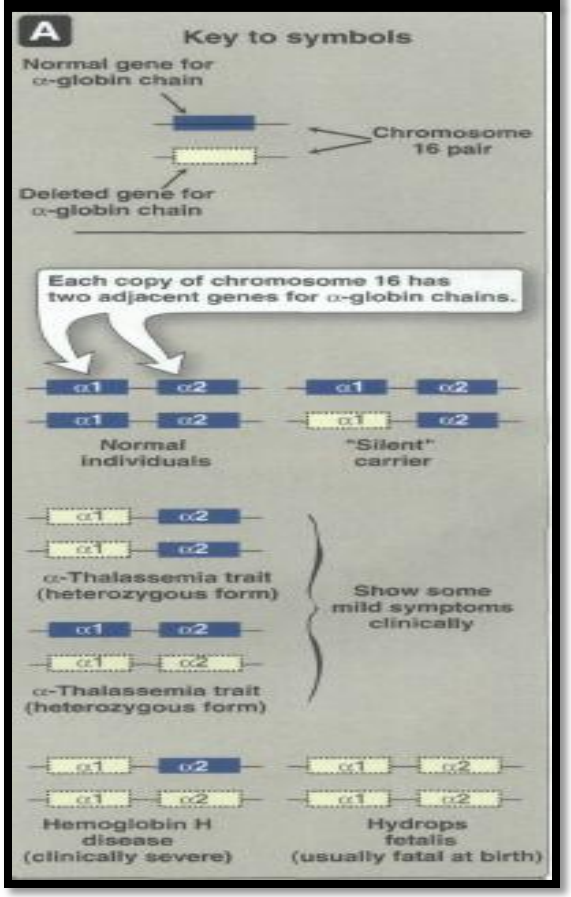


β thalassemia



β thalassemia

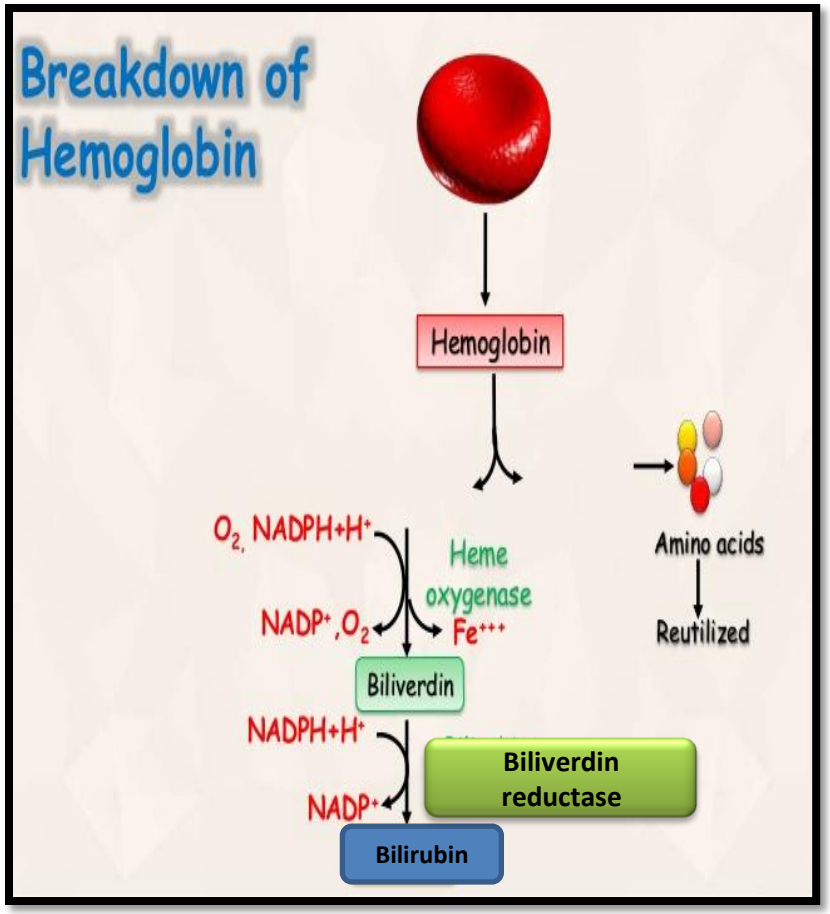
α thalassemia



α thalassemia

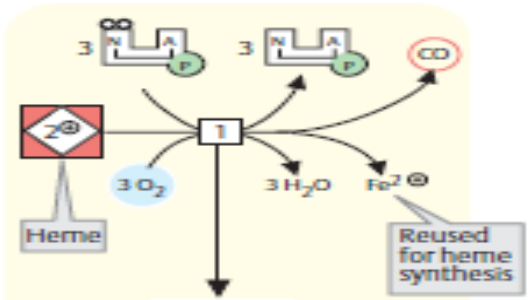
Breakdown of heme

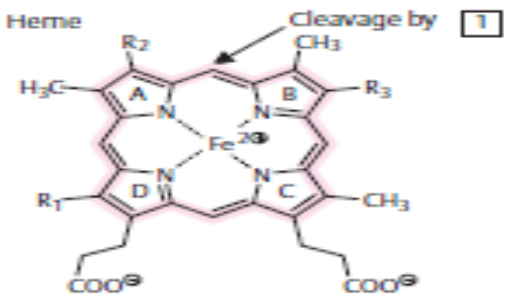
- The life span of the erythrocyte is 120 days. Then the red blood cells being degraded by the reticuloendothelial cells in the liver and spleen.
- The major part of the degraded heme comes from the red blood cells and the rest from the immature red blood cells and the cytochromes.
- First metabolite of the heme oxidation is the biliverdin formed by heme oxygenase.
- In the next step the biliverdin being redused by the bilverdin reductase to form bilirubin.

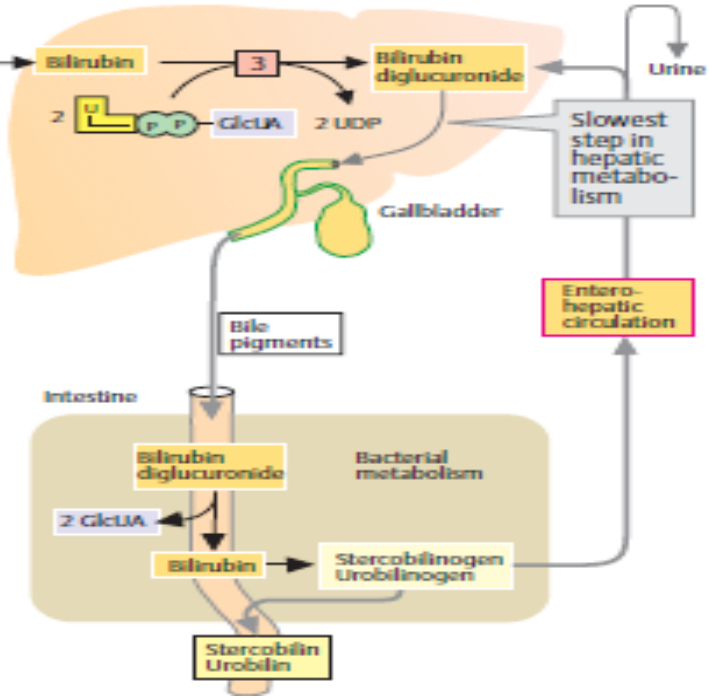


Breakdown of heme

- After the protein part (globin) has been removed, the tetrapyrrole ring of heme is oxidatively cleaved between rings A and B by heme oxygenase .
- This reaction requires O2 nd NADPH+H+, and produces green **biliverdin, as well as CO (carbon** monoxide) and Fe2+, which remains available for further use.
- In the next step the biliverdin is reduced to orange colored bilirubin.
- The color changing during heme degradation could observed during bruise or hematoma from purple to green to yellow.







1 Heme oxygenase (decyclizing) 1.14.99.3

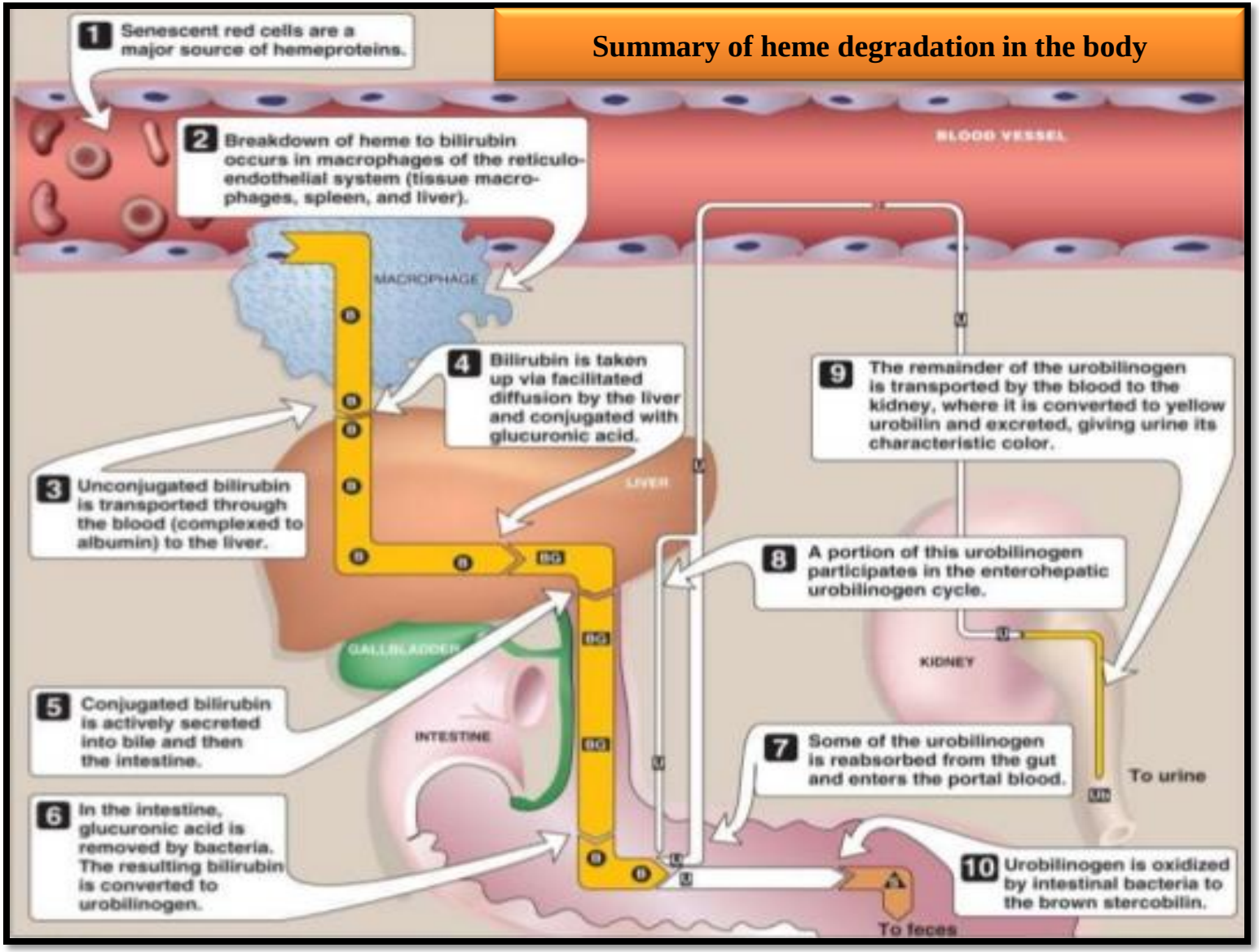
2 Biliverdin reductase 1.3.1.24

3 Glucuronosyl transferase 2.4.1.17

Bilirubin trasport to the liver

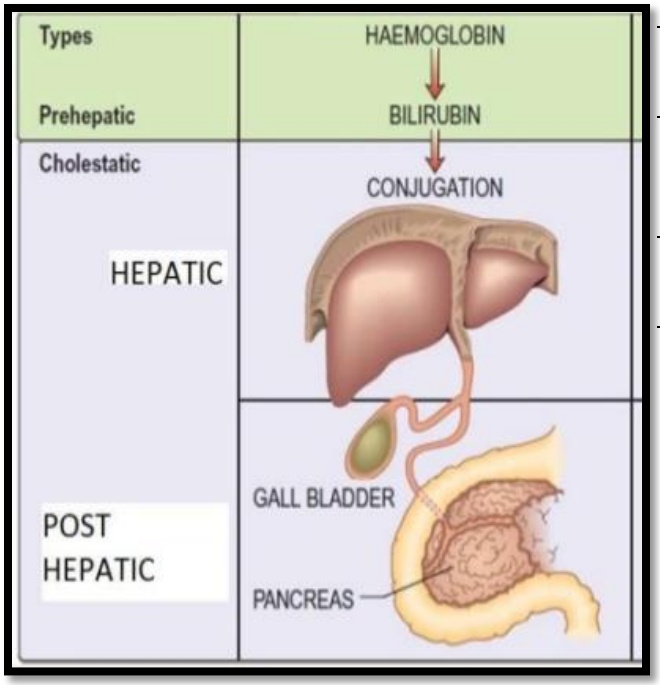
- The bilirubin being formed from the heme degradation is poorly soluble in the blood and transported to the liver by binding to the albumin as a carrier protein.
- Then the bilirubin dissociates from the albumin and binds to another protein in the liver cell called ligandin.
- In the liver the bilirubin conjugates to glucorunic acid by bilirubin glucuronyltransferase using 2 molecules of UDP glucoronic acid forms bilirubin diglucoronide more soluble than bilirubin.
- the congucated bilirubin then secreted to the bile and intestine.
- In the intestine the congugated bilirubin reduced by the bacteria and form the urobilinogen (colorless).
- In next step the urobilinogen oxidized to sterocobilin (brown) by intestinal bacteria.
- some of the urobilinogen is reabsorbed and enter the portal blood to the liver.
- The rest of the urobilinogen is transported to the kidney and transformed to urobilin (yellow) which give the urine its color.

The summary of heme breakdown



Jaundice

- **Jaundice** is bilirubin deposition as a result of incresed its plasma levels (greater than 2.5 to 3 mg per dL)
- Manifest by yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposition of bilirubin.
- The jaundice could be;
  - **Prehepatic** (hemolytic jaundice).
  - **Hepatic jaundice** (hepatocellular jaundice-jaundice in newborn).
  - **Post hepatic** (obstructive jaundice)



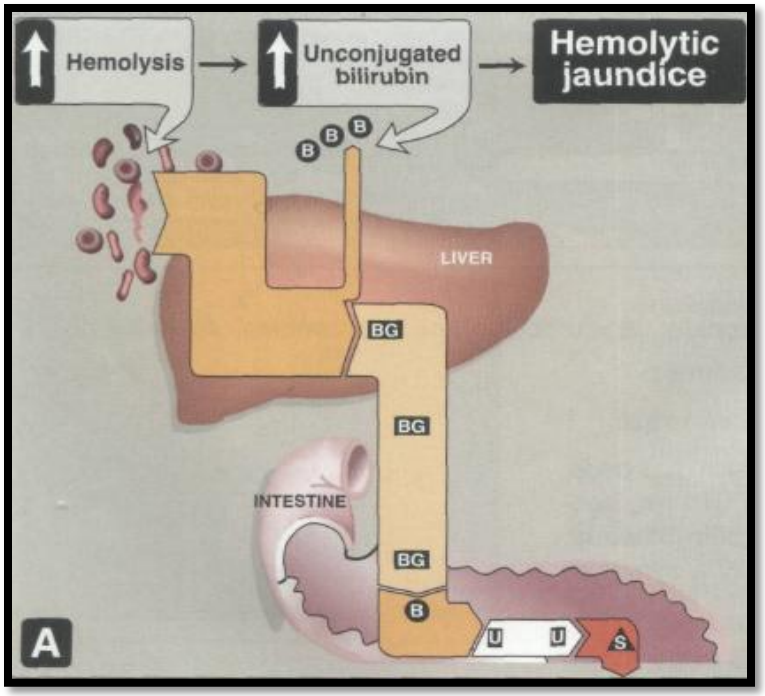
hymolysis

Viral hepatitis
Drugs
Alcoholic hepatitis
Cirrhosis
Congenital disorders

Duct stones
Carcinoma of the bile duct
Biliary stricture

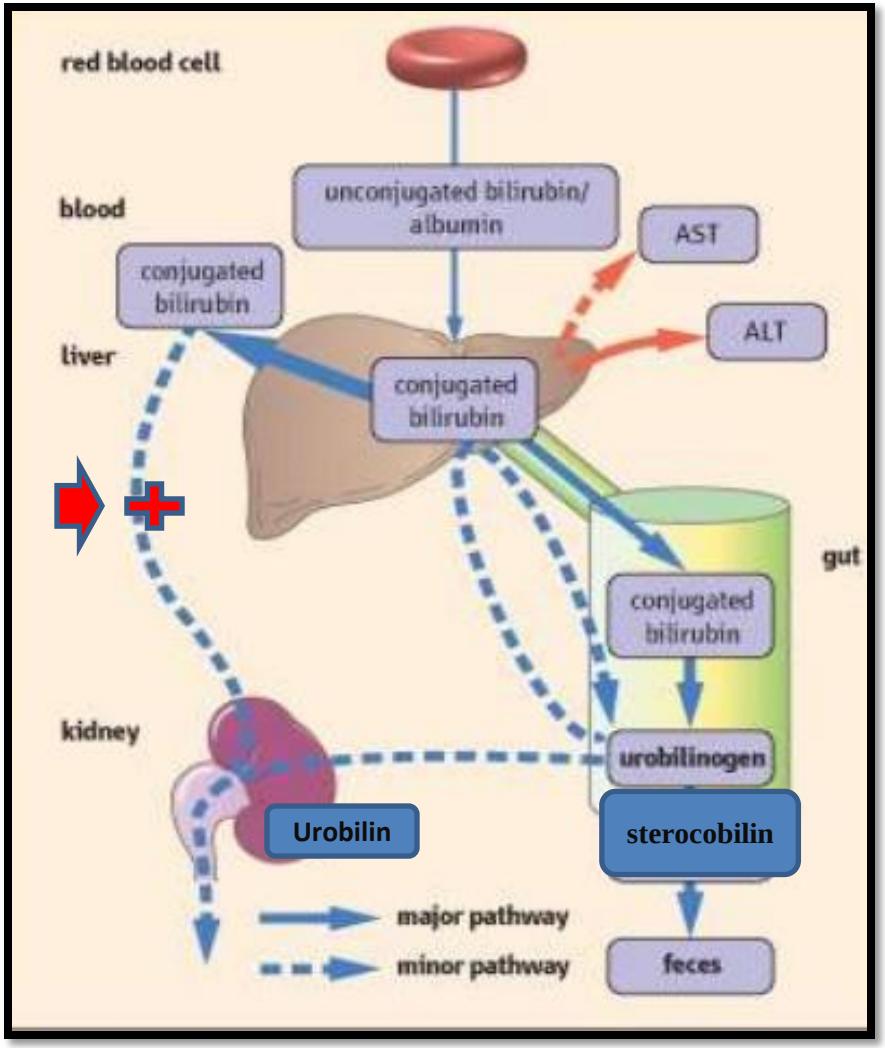
Hemolytic jaundice (prehepatic)

- Occur when the bilirubin levels increased.
- For example; in the cases of sickle cell anemia , G6PD deficiency, pyruvate kinases deficiency and malaria).
- The bilirubin blood level increased and the urobilinogen increased in both enterohepatic circulation and urinary tract.



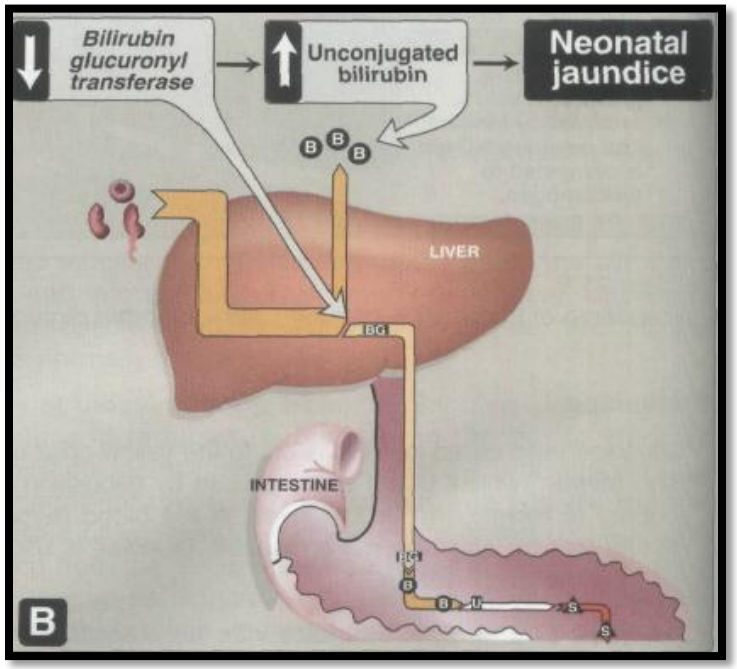
Hepatocellular jaundice (hepatic)

- This type of jaundice happens when the liver cell damaged because of hepatitis or cirrhosis.
- The unconjugated bilirubin levels is increased in the blood as a results of liver cell deficiency .
- Also the portion of the conjugated bilirubin diffuse to the blood instead of being secreted to the bile.
- Because of damage of the enterohepatic circulation the urobilinogen increased in the urine making the urine a dark color. While the stool with caly color.
- Increased level of the hepatic enzyme (AST, ALT).
- Symptoms of anorexia and nausea



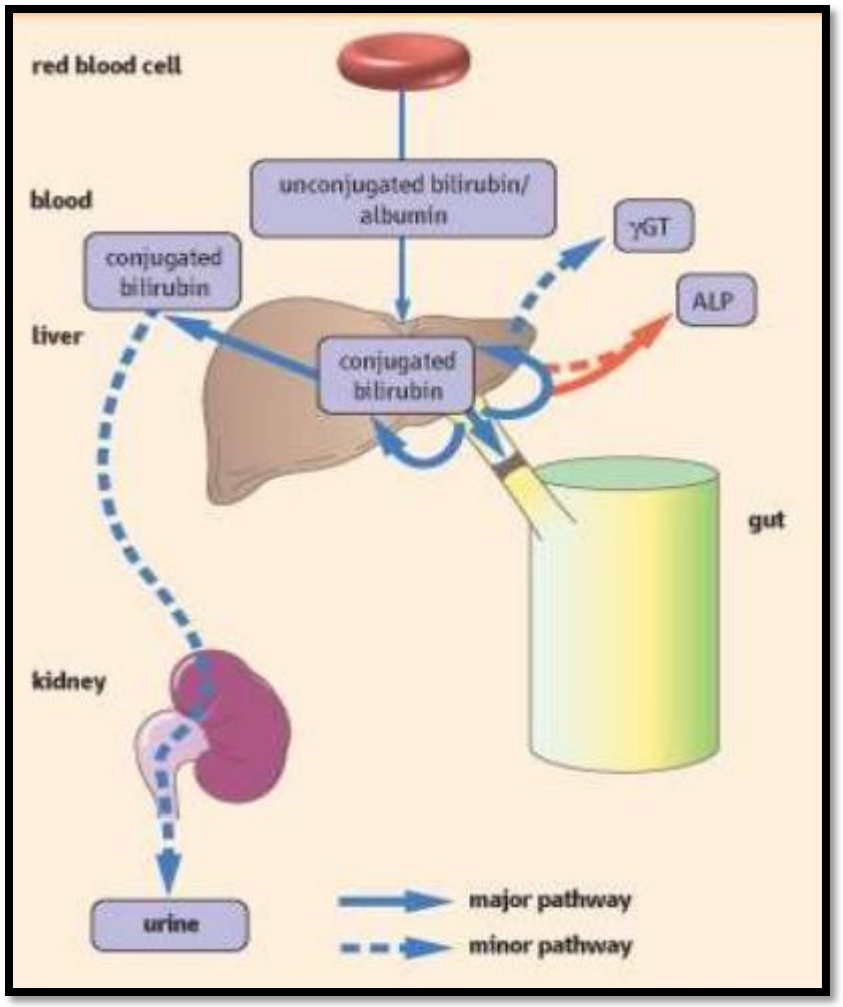
Neonatal jaundice (hepatic)

- Occurs often in the premature when the enzymatic activity of the hepatic bilirubin glucuronyl transferase is low then reach the adult levels after 4 weeks.
- The increased bilirubin level in the newborn can cause encephalopathy .
- For this reason its recommend to treat the newborns with high bilirubin levels with blue florescent light.
- The deficiency of the hepatic bilirubin glucuronyl transferase cause Crigler-Najjar syndrome



Obstructive jaundice (post hepatic)

- This type of jaundice caused by obstruction of the bile duct. Which could be as a result of a tumor or a bile stones that block the block passing of the bilirubin from the bile into the intestine.
- The conjugated bilirubin is excreted to the urine.
- Patients can suffer from gastrointestinal pain and nausea.
- Long term obstruction can leads to liver damage and rise of the unconjugated bilirubin blood level.



Thank you