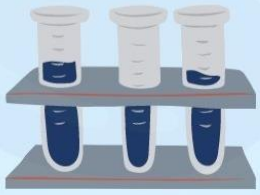
Sheet No.
3

Biochemistry



hematolymphatic system

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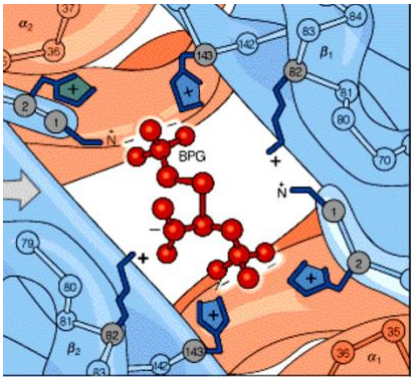
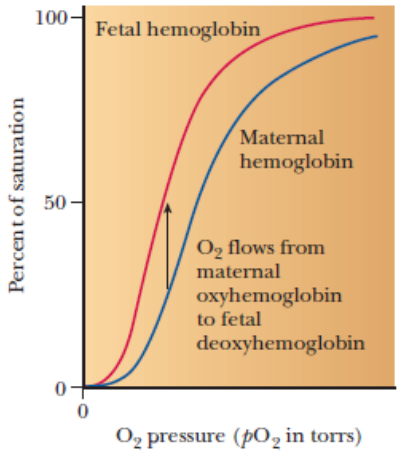
There are 4 remaining slides from the last file then we gonna start in the new one

- Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA), so fetus can steal oxygen from his mother





β and γ are highly similar to each other, but there are some differences. The most significant one is:

His143 residue in the β subunit is replaced by a Ser in the γ subunit of HbF.

HbA = α2β2 HbF = α2γ2

Since Ser cannot form a salt bridge with 2,3-BPG, it binds weaklier to HbF than to HbA, so in HbF, the hemoglobin stays in the R state for longer time

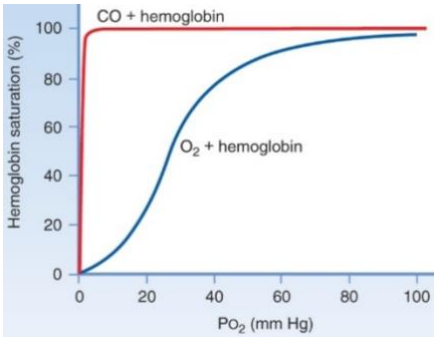
Notice the curve: HbF curve is shifted to left (higher affinity / lower P_{50})

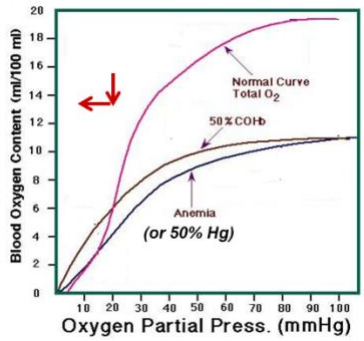
- Effect of CO (we have 2 effects)

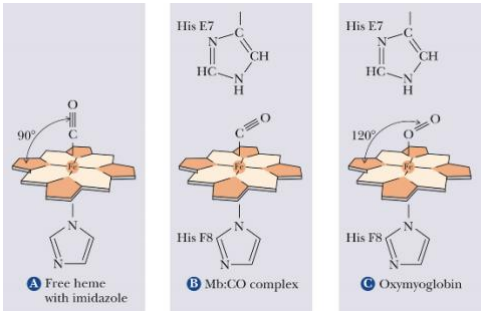
1. Competing with O2: CO has stronger affinity to heme than oxygen by 40000 folds. However, the bond between heme and globin reduces this affinity to 40-200 folds

Oxygen usually wins the competition because the higher concentration

2. affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues. O2 affinity is increased (the first thing comes to your mind it is good thing but indeed it is not) when the O2 affinity is increased, it will be harder to be released from Hb in the tissues







Notes: CO curve is hyperbolic not sigmoidal (left photo)

(middle photo) the maximum saturation that can 50%COHb (brown) reaches is less than max saturation in the normal Oxygen curve (pink). However, P50 is less in the brown curve (has higher affinity)

CO is a linear compound. CO-heme bond permits the CO to conserve this lineage (that's why it has high affinity

Hemoglobin cannot bind CO with its lineage. However, it can bind with it with changing the bond in the CO compound to bent bond which is not preferred by CO molecule, so the affinity is reduced

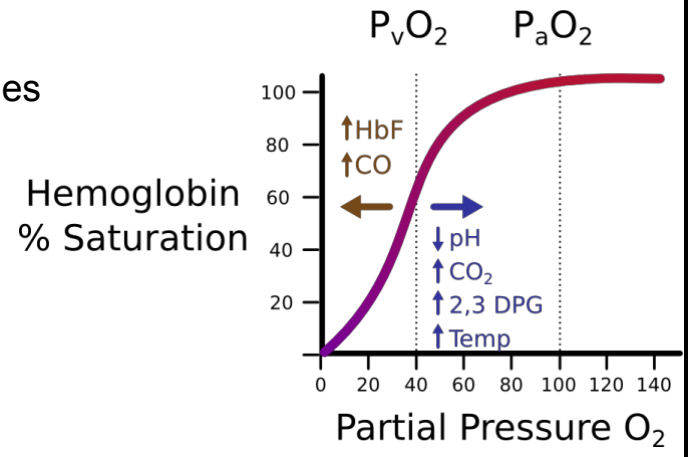
Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes.

Due to pollutants, the concentration of COHb in the blood is usually 1% in a nonsmoker.

In smokers, COHb can reach up to 10% in smokers. The body will start forming new hemoglobin and the patient will have higher amounts of Hb than normal people, but high amount of them aren't functional

If this concentration of COHb in the blood reaches 40% (as is caused by 1% of CO in inspired air), it would cause unconsciousness initially, followed by death.

The picture on the right is a summary



Hemoglobinopathies

Hemoglobinopathies are basically disorders of human hemoglobin, so we have deficiency in oxygen transport system, these are really serious diseases for 2 reasons:

- They are the most common genetic disease in the world {5% of people are carriers} with morbidity 300,000 each year.
- They account for 3.4% deaths in children younger than 5. The distribution of Hemoglobinopathies around the world and it is concentrated in the old world (the Middle East has significant share including Jordan) so it's a quite serious problem.

Hereditary hemoglobins disorders are classified into: (breifly)

1. Qualitative abnormalities: mutations resulting in structural variants (every single AA in hemoglobin has a chance to be mutated and as a result it will form defective hemoglobin), over 700 variants have been identified.

We have normal amount of Hb. However, the problem here is in the efficiency of hemoglobin

2. Quantitative abnormalities: when the molecule is fine in structure but there are less quantities in alpha & beta chains like (Thalassemias),

Jordan has a high prevalence of thalassemia.

Note: we can have both quantitative and qualitative disorders

3. Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from gamma to beta globin (has no symptoms or significance)

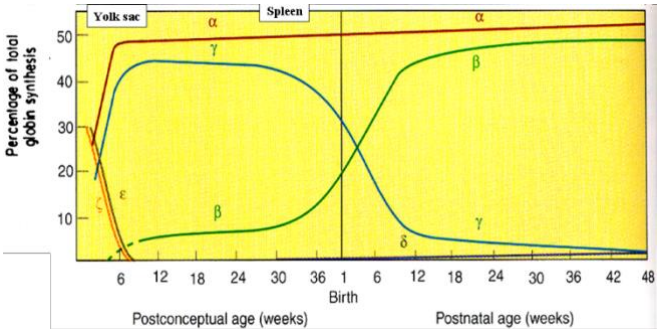
Now let's talk about each one in further details:

Quantitative abnormalities (Thalassemias): are the most common human single gene disorder they are prevalent in the old world including the Middle East.

Basically, it's a disease caused by a destruction in the α:β ratio. In normal RBC what we have is equal amount of alpha and beta chains, but in thalassemia we would have either less alpha (α thalassemia) or less beta (β thalassemia).

1. Alpha thalassemia It is caused mainly by a deletion mutation (rarely point mutations) of one or more of α genes which will result in underproduction of the α-globin chains (α:β ratio is decreased). As a result, HbA (α2β2), HbF (α2γ2), and HbA2 (α2δ2) are all affected.

Remember that alpha chain is produced early on and it continues throughout life on the other hand beta chains start to be produced slowly in the early fetal stage, but there is a big jump right before birth and it continues throughout life in equal quantities

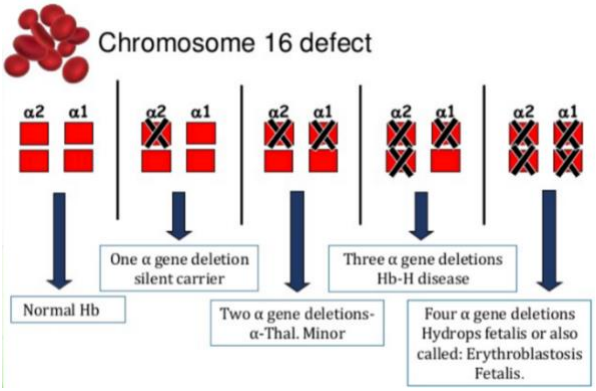


People with α thalassemia will be affected faster than β thalassemia

Reduction of α chains as well as accumulation of β chains will increase the probability of forming homotetramer of β which in known as HbH

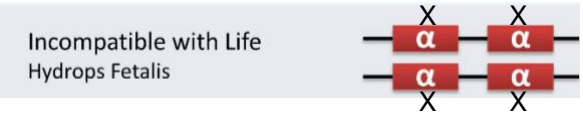
This HbH tetramers have a markedly reduced oxygen carrying capacity.

In α-thalassemia, the level of α-globin production can range from none to very nearly normal levels. This is since each individual has 4 functional genes for α-globin, so it depends on the number of deleted genes (variable severity)



We have 4 genes of α "2 each chromosome" and 2 genes of β "1 each chromosome"

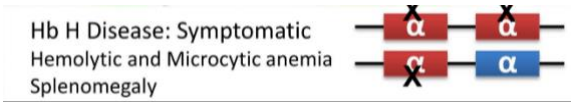
Hydrops fetalis (α thalassemia major) is a case where all 4 alpha genes are deleted



In this case the predominant fetal Hb is homotetramer of γ chains (known as Hb Bart or γ4) which has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues. Resulting in a fetus that is basiclly dead so you would have still birth or death shortly after birth.

They may have some Hemoglobin H

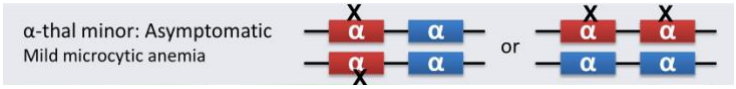
HbH disease is a case where 3 alpha genes are deleted



In this case the predominant Hb is HbH and it is less severe than hydrops fetalis (not fatal (because patients have enough amount of HbA to survive) but it is symptomatic)

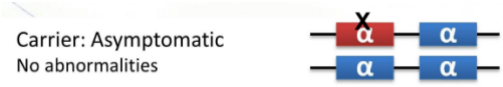
It is classified as mild to moderate hemolytic anemia in adults

Minor α thalassemia in this case, we have 2 deleted genes



It is generally asymptomatic

Silent carrier one gene is deleted and these people are completely asymptomatic



Genotype	α-globin gene number[a]	Name	Phenotype
αα / αα	4	Normal state	None
αα / α–	3	Silent carrier	None (values for Hb and MCV may be near the lower limits of normal)
– – / αα or α– / α–	2	Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic anemia
– – / α–	1	Hb H disease	Thalassemia intermedia: mild to moderate microcytic anemia
– – / – –	0	Alpha thalassemia major	Thalassemia major: hydrops fetalis

[a]Number of normal alpha globin genes

2. β thalassemia is caused mainly by point mutations in one or more of β genes which will result in underproduction of the β-globin chains (α:β ratio is increased). As a result, excess α globins will form α homotetramers which is extremely insoluble and leads to premature red cell destruction in the BM and spleen

Mutations can be in any amino acid in the gene, it can be within the promoter, translation initiation codon, splicing positions, or poly-adenylation termination signal.

As alpha thalassemia, we have variable severity here. There are different types of beta thalassemia depending on the severity of the mutation (number of genes affected and the place)

A complete lack of HbA is denoted as β^0-thalassemia or β-thalassemia major.

Affected individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.

Long-term transfusions lead to the accumulation of iron in the organs (hemochromatosis), particularly the heart, liver and pancreas and , finally, death in the teens to early twenties.

Individuals heterozygous for β-thalassemia is termed β-thalassemia minor (β/β^0). (Affected individuals carry one normal β-globin gene and a mutated gene)

Individuals with beta-thalassemia minor are generally asymptomatic (good production of beta globin but not optimal, they may have some symptoms depending on the severity)

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β^+/β^+ β^+/β^0 β^E/β^+ β^E/β^0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

β^0:complete lack

β^+: some expression

β: normal

β^E: HbE

Qualitative abnormalities: we are basically talking about point mutations which can take place anywhere in the Hb molecule, and these abnormalities (mutations) are divided into:

1. Mutations in surface residues: Usually asymptomatic (e.g. HbE), an exception is HbS which is produced in people with sickle cell disease. (has dramatic effect)

2. Mutations in internal residues: Hb is a globular protein with hydrophilic AA's outside & hydrophobic AA's inside so any change in the environment will result in unstable Hb Heinz bodies and causing hemolytic anemia (e.g. Hb Hammersmith, Hb CS),

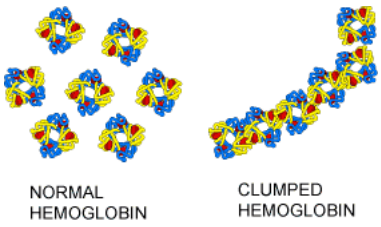
3. Mutations stabilizing methemoglobin: In which heme is bound to ferric iron [heme-Fe^{+3}] decreasing its capacity to bind oxygen and resulting in cyanosis, cyanosis is (a bluish-purple hue to the skin due to shortage of oxygen).

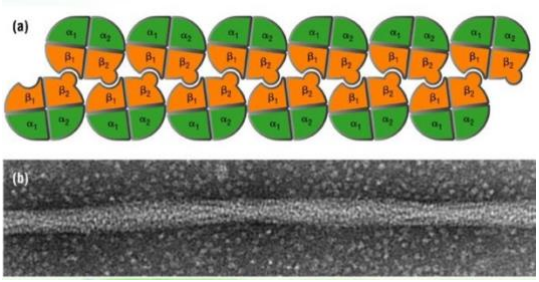
4. Mutations at α1-β2 contacts: Which alter the equilibrium of the T-state and R-state, so this affects oxygen affinity (mainly becomes higher, a condition known as polycythemia).

Some qualitative hemoglobinopathies:

NOTE: the doctor said that he doesn't require from you remember the mutation (changed amino acids)

1-**Sickle cell hemoglobin (HbS):** is a genetic disorder of the blood caused by a single nucleotide substitution (a point mutation) in the β-globin gene, resulting in a change of the amino acid in the 6th codon (Glu "negative, polar AA" to Val "nonpolar") alters the shape of β chains. The mutant hemoglobin is designated as α2βs2 or HbS. This mutation will lead to aggregation of hemoglobin tetramers into arrays upon deoxygenation in the tissues (deoxygenated HbS is poorly soluble). As a result, this fibrous aggregation (clumping) leads to deformation of the red blood cell (forming crescents), it can also cause hemolytic anemia (destruction of RBCs) in which the life span of RBCs is reduced from 120 days to <20

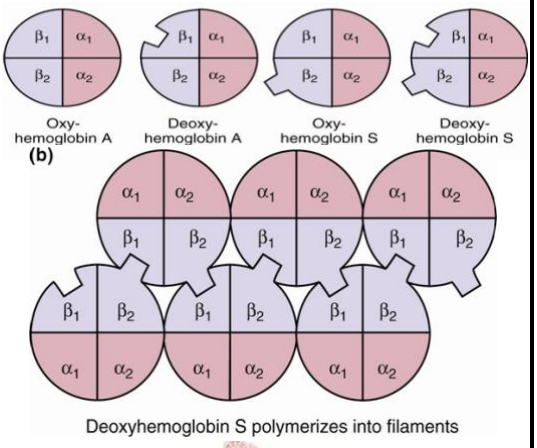




Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling so cells cannot squeeze though capillaries in a single file and therefore block blood flow causing local hypoxia. Long-term recurrent clogging of the capillary beds leads to damage to internal organs, in particular the kidneys, heart and lungs.

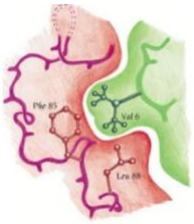
WHY DOES THIS AGGREGATION HAPPEN?

Fiber formation (aggregation) only occurs in the deoxy or T-state. There are two things that make this possible. First, in any deoxygenated hemoglobin molecule (whether normal or mutated), a region of the protein creates a hydrophobic pocket in β1 chain. Secondly, in HbS, the mutated valine (Val6) of the β-2 chain forms a hydrophobic protrusion on the surface.



Consequently, the protrusion of one Hb will fit in a pocket of another Hb

Remember: valine is hydrophobic



Variables that increase sickling:

• Decreased oxygen pressure (high altitudes) • Increased pCO2

• Decreased pH • Increased 2, 3-BPG • Dehydration

Note: these variables increase the proportion of HbS in the deoxy state (so, reduces the affinity of HbS for O2 or stabilizes the T-state) so increases the extent of sickling.

Sickle cell trait

Heterozygotes have a normal gene and a sickle cell one

The blood cells of such heterozygotes contain both HbS and HbA. These individuals are said to have sickle cell trait. They usually do not show clinical symptoms, but their cells sickle when subjected to low oxygen.

Indeed, these people have an advantage which is selective resistance from plasmodium falciparum that infects RBC and causes malaria

RBC in heterozygous individuals has shorter life span than normal people around (40 days), the reproductive duration of malaria is around 60 days, so having shorter life span will prevent malaria from completing its life cycle inside RBCs

2-HbC: is a genetic disorder of the blood caused by a point mutation in β-globin gene, resulting in a change of the amino acid in the 6th position of beta globin (from Glu to lysine) both are polar, but one is negative and the another is positive, so it doesn't cause a dramatic effect

This hemoglobin (HbC) is less soluble than HbA, so it crystallizes in RBCs, reducing their deformability in capillaries (i.e. reducing their ability to squeeze through). It also leads to water loss from cells leading to higher hemoglobin concentration. This problem causes only a minor hemolytic disorder

3, HbSC disease: If HbC is combined with HbS gene will cause (HbSC) which will cause mild hemolytic anemia. The individual has both of their β-globin alleles mutated: but differently

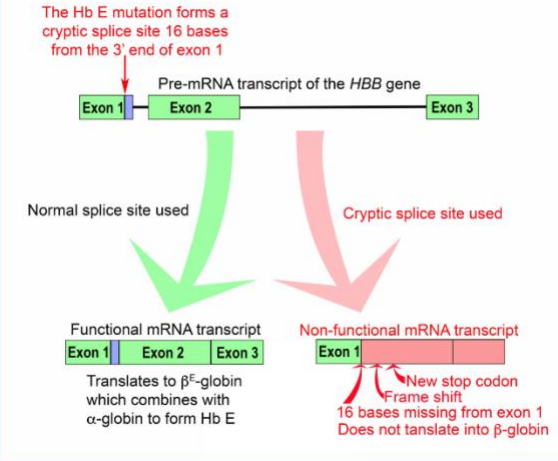
may have no clinical consequences, but it is clinically variable.

4, HbE

It is common in Southeast Asia

It is caused by a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein. Normally the mRNA will have exons 1/2/3 completely

In HbE disease there will be a missing part from exon 1 which will alter the codons after it



Individuals with this mutation make only around 60% of the normal amount of β- globin protein.

It has both quantitative and qualitative characteristics. How?? Amino acid substitution caused a genetic change (structural) which will create alternative RNA splicing site causing defective protein (qualitative) This protein once formed it's unstable as the normal protein leading it to be degraded, so there is less amount of protein (quantitative)

5-Hb Hammersmith: results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein

The most common point mutation of Hb Hammersmith substitutes an internal (around heme) phenylalanine (hydrophobic) with a serine (hydrophilic) within the beta globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and oxygen binding affinity causing cyanosis

More explanation: phenylalanine exists in the heme pocket and forms hydrophobic interactions with heme stabilizing the positioning of it into the pocket now if this phenylalanine changes to serine that will cause destabilization interaction between heme & hemoglobin protein which creates a hydrophilic environment that affects the oxidation state of the iron

6. Hb Constant spring HbCS: please differentiate between HbCS and HbSC

Hemoglobin Constant Spring (Hb CS) is an abnormal Hb caused by a mutation at the termination codon of α2-globin gene leading to the production of unstable mRNA and protein products. (changing the stop codon to another one that encodes certain amino acid, so the translation won't stop as it should, so many amino acids will be added in the terminus)

It has both quantitative and qualitative characteristics. The anemia is usually moderate.

Heterozygotes have the genotype ($\alpha\alpha/\alpha\alpha^{CS}$) and have α°-thalasaemia trait phenotype.

It is commonly found among Southeast Asian and Chinese people.

If co-inherited with α-thalassemia, it leads to an α°-thalassemia intermedia syndrome.

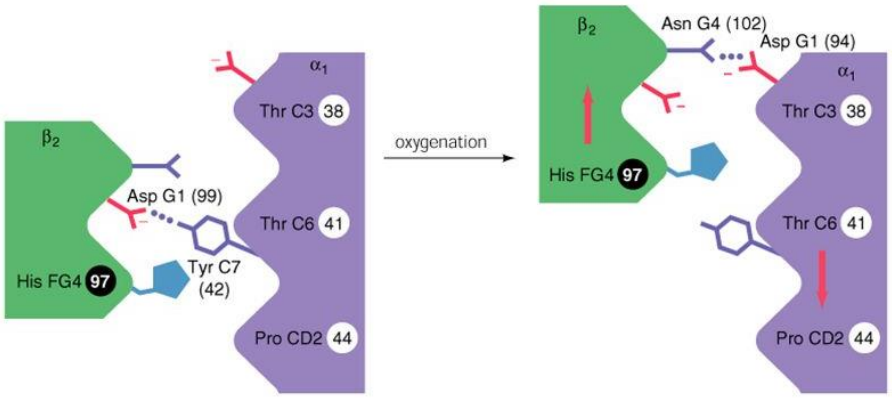
7.Mutations that alter the equilibrium of the T-state and R-state:

Hb Cowtown: Is a mutant with increased oxygen affinity. It results from the substitution of His146 (last AA in the beta globin that is responsible for the Bohr Effect) to Leucine which, in turn, produces more hemoglobin in the R state

Elimination of hydrogen bonds between the chains can also alter the quaternary structure and thus the T/R equilibrium.

Ex. I) Hb Kansas: stabilization of the T state (Asn to Thr).

II) Hb Yakima: stabilization of the R state (Asp to His or His) Note: The reason for the name is the names of the cities in which these mutations were found.



(a) T Form (deoxy) (b) R Form (oxy)