Biochemistry - HLS

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Hemoglobinopathies

Prof. Mamoun Ahram Hematopoietic-lymphatic system

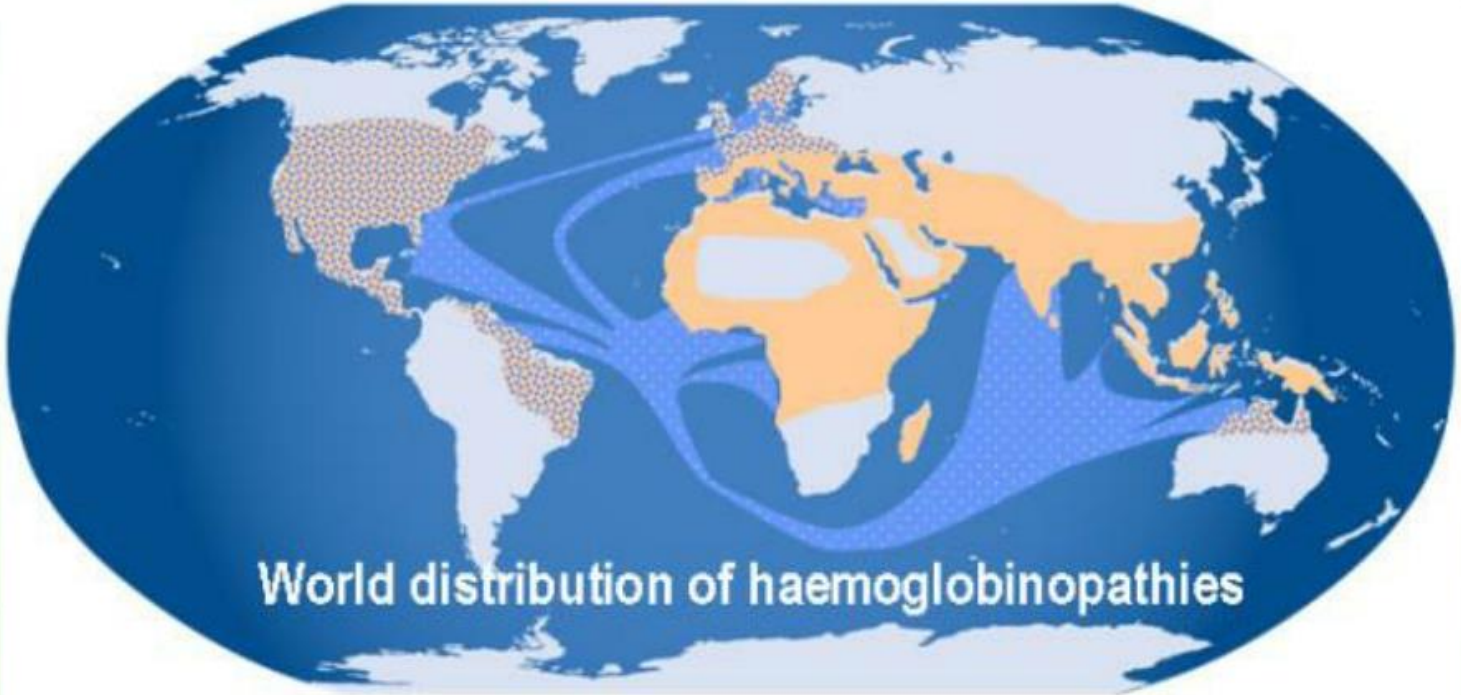
Resources

- This lecture
- Mark's Basic Medical Biochemistry, Ch. 44

What are hemoglobinopathies?

- Hemoglobinopathies: Disorders of human hemoglobin **(deficiency in the oxygen transport system) .**
- The most common genetic disease group in the world –so they are really serious diseases- (5% of people are carriers) with substantial morbidity (about 300,000 born each year).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.

Notice the distribution of hemoglobinopathies around the world that concentrated in the old world – including Jordan-



Hereditary hemoglobins disorders

Types of abnormalities:

- Qualitative abnormalities: mutations resulting in structural variants in hemoglobin that makes it defective in O2 transport, (each amino acid has a function in a hemoglobin molecule , and there is a good chance that one of them would be mutated , and this results in a defective hemoglobin)
  - Over 800 variants have been identified.
- Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias) [the hemoglobin is fine in structure].
  Jordan has high prevelance of thalassemias.
- Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin.
  - NO switching from gamma to beta globin-

Qualitative abnormalities

Classification of molecular mutations

Basically We are talking about single point mutations that can take anywhere in the hemoglobin molecule .

- Mutations in surface residues
  - Usually asymptomtic (e.g. HbE); an exception is HbS >>sickle cell anemia[it is a single point mutation that really causes a dramatic effect on the function of hemoglobin molecule.]

- Mutations in internal residues (remember that Hb is a globular protein with a hydrophilic AAs outside and hydrophobic AAs inside)
  - Often producing unstable hemoglobin, Heinz bodies and causing hemolytic anemia (e.g. Hb Hammersmith)

- Mutations stabilizing methemoglobin – a hemoglobin with iron in the ferric state-
  - Stabilizing heme-Fe $^{+3}$; resulting in cyanosis – cannot bind to oxygen-

- Mutations at α1-β2 contacts

That will cause an alteration in the equilibrium in T and R hemoglobin

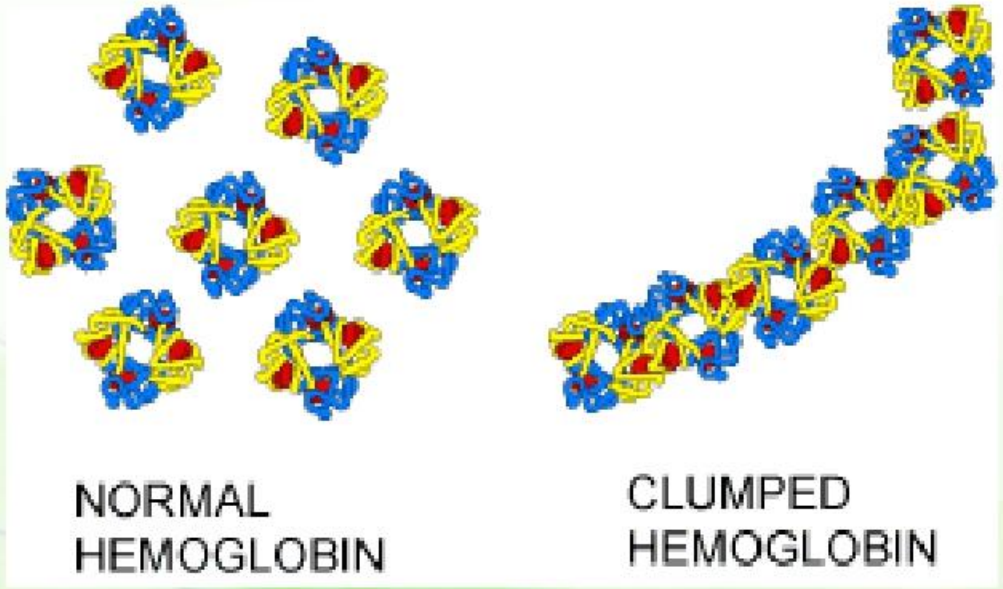
  - Altered oxygen affinity (mainly higher; a condition known as polycythemia)

Sickle cell hemoglobin (HbS)

It is caused by a single point mutation in one nuclide in DNA

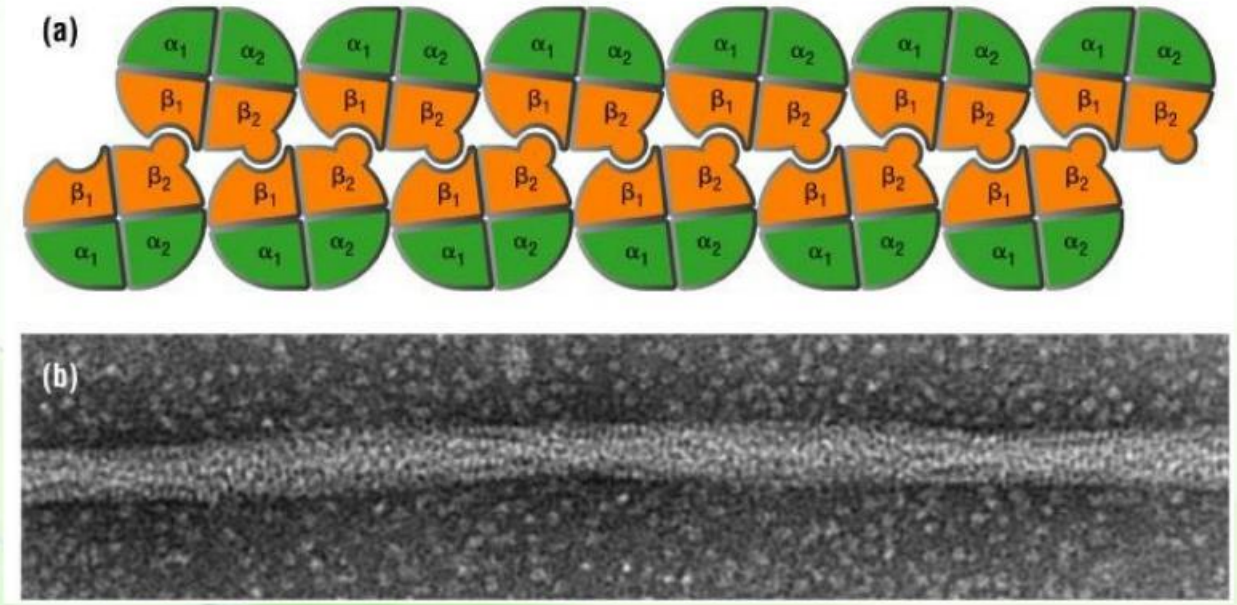
- It is caused by a change of amino acids in the 6th position of β globin (negatively charged Glu to non-polar Val).
  - The hemoglobin is designated α2βs2 - there are 2 beta chains that sickled- or HbS.
  - The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues.
  - This aggregation leads to deformation of the red blood cell because we have millions of these hemoglobin molecules and they all aggregate together as chains .
  - It can also cause hemolytic anemia (life span of RBCs is reduced from 120 days to <20 days).

We have the hemoglobin proteins as aggregates, forming a chain of proteins instead of having hemoglobin proteins as individual molecules.



Cellular effect on system

- Repeated cycles of oxygenation and deoxygenation (that would cause clumping and clustering of the hemoglobin molecules , so blood flow is restricted) lead to Irreversible sickling.
- Cells cannot squeeze though capillaries in a single file and therefore block blood flow causing local hypoxia (low oxygen levels in tissue).
- Long-term recurrent clogging of the capillary beds leads to damage to the internal organs, in particular the kidneys, heart and lungs.



So, why they cluster together?

How does the fiber form?

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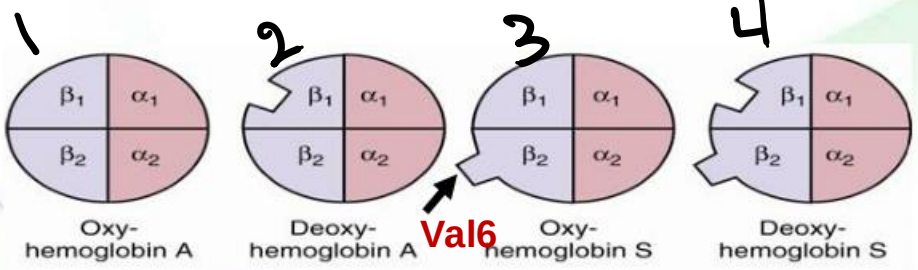
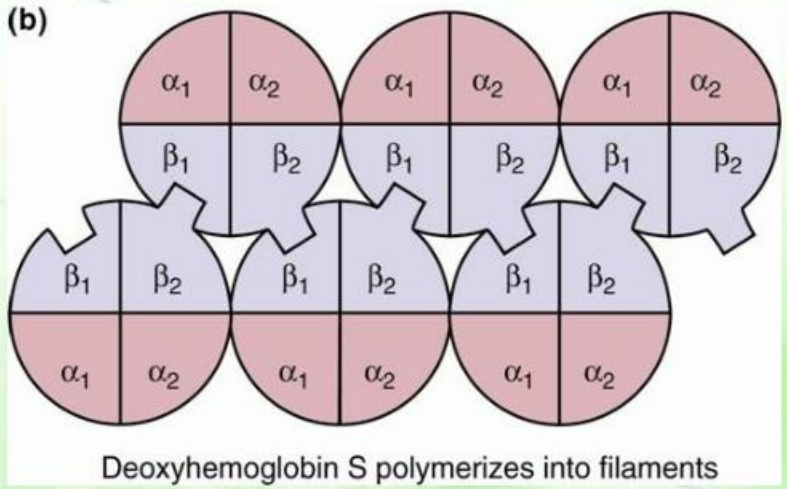
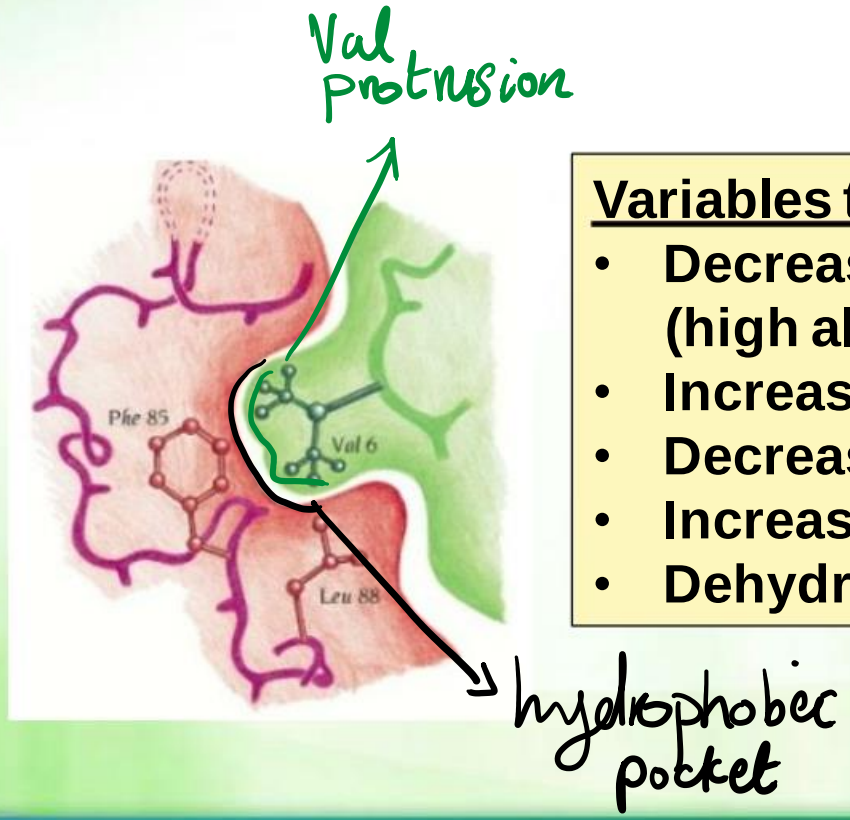
- Fiber formation only occurs in the deoxy or T-state.
- The mutated valine of β2 chain is protruded and inserts itself into a hydrophobic pocket on the surface of β1 chain.







Variables that increase sickling

- **Decreased oxygen pressure (high altitudes)**
- **Increased pCO_2**
- **Decreased pH**
- **Increased 2,3-BPG**
- **Dehydration (why?)**

Explanation of the previous slide

- we have here in **(1)** a hemoglobin molecule that's oxygenated, if it's deoxygenated as **(2)** it will cause a formation of a hydrophobic pocket within the beta 1 chain.
- In a sickled hemoglobin we have the formation of a Valine protrusion (hydrophobic) that includes a Valine molecule - the sickled hemoglobin here is **(3)** -
- So, when it becomes deoxygenated, the protrusion still exists + we have the hydrophobic pocket = so what happens is this protrusion of one molecule would fit the pocket of another molecule and that would cause clumping of the hemoglobin molecules

- remember that sickling increases with repeated oxygenation-deoxygenation cycles
So, WHAT does increase the sickling?
Producing more T form, like:
1. Decreased O2 pressure
2. Increased CO2 pressure
3. Decreased PH
4. Increased 2,3 BPG
5. Dehydration

Sickle cell trait

This is a case when one allele is normal (HbA producing normal B chain) and the other one is mutated (so you have the formation of HbS)>>[combination between HbA and HbS]

- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but their cells sickle when subjected to low oxygen.

- Advantage: selective advantage from plasmodium falciparum that causes malaria – they become resistant to infection by malaria -. Why?

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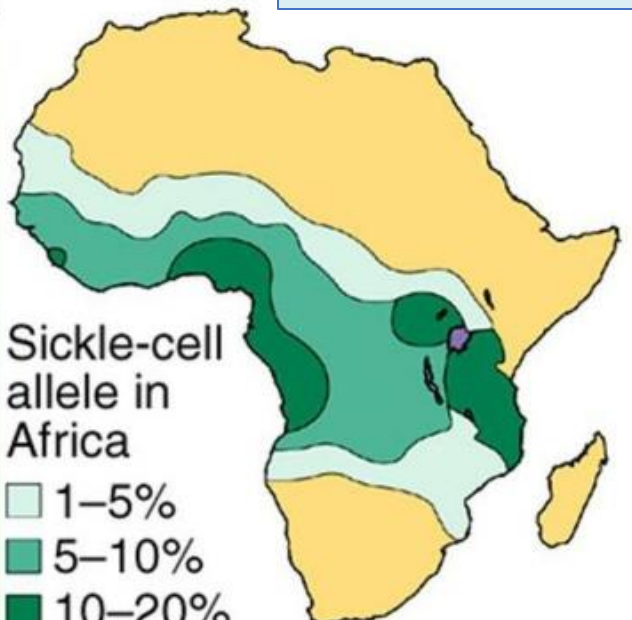
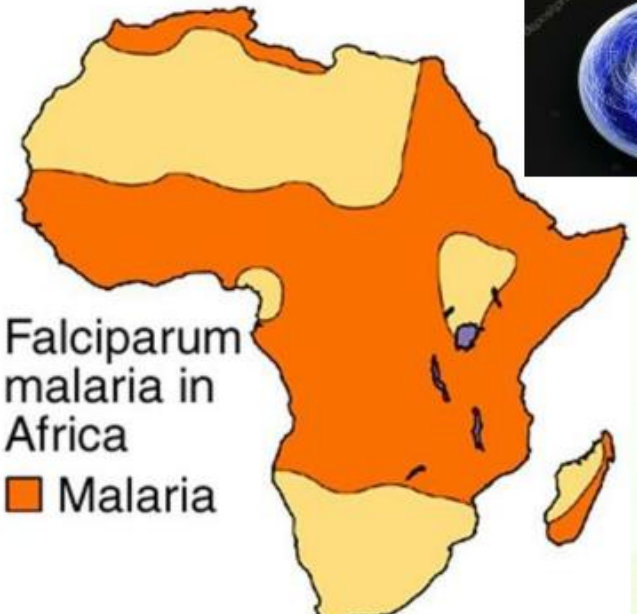
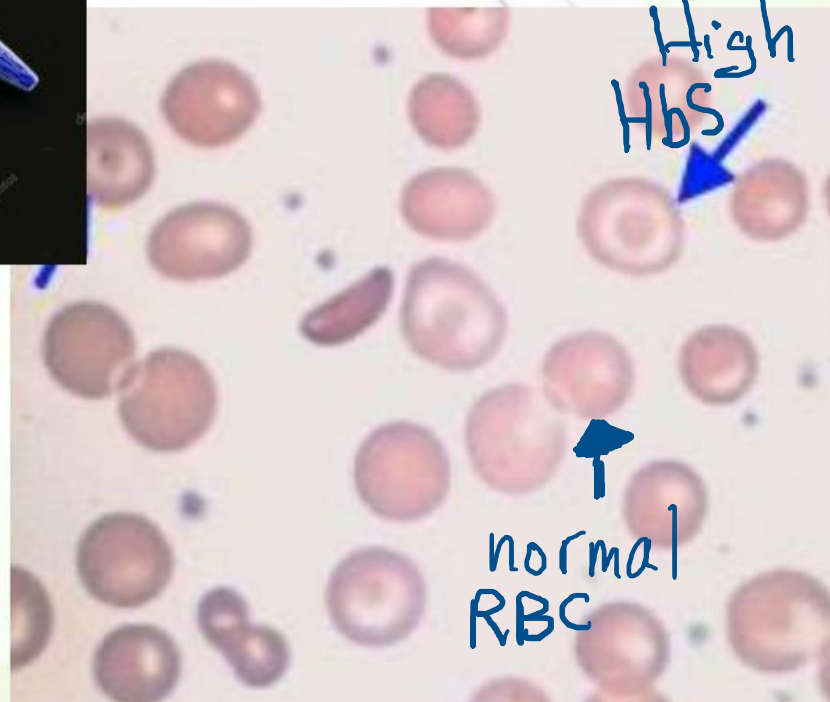






About the previous slide

Why??

One reason is that sickled RBCs have low half life(40 days or less) and the reproductive duration of malaria is 60 days and malaria infects RBCs so this prevents malaria from completing its growth inside cells .

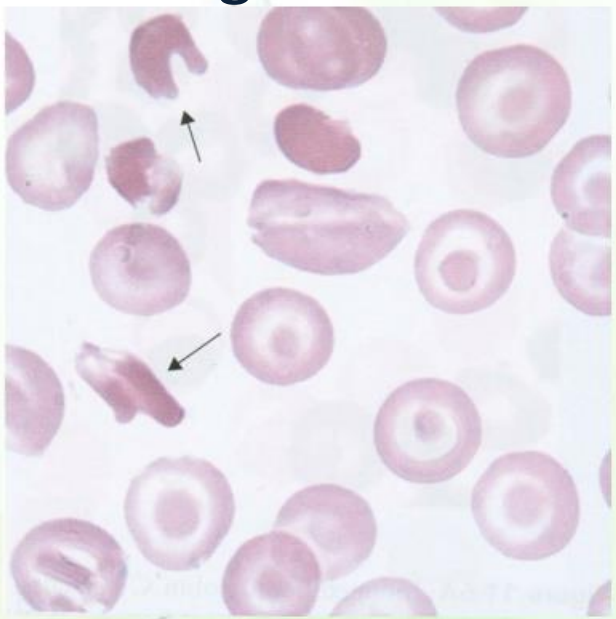
About the map

If you look at the map (on the left) which shows the distribution of sickle cell allele in Africa , you can see that it actually matches the distribution of Malaria in these countries. (On the right)> so there is an advantage for theses individuals

Hemoglobin C (HbC)

- (HbC) is also due to a change at the 6th position of β globin replacing the glutamate with lysine - **positively charged** - (designated as βc).
- This hemoglobin is less soluble than HbA so it crystallizes in RBCs reducing their deformability in capillaries –decrease the ability of RBCs to change their shape as the squeeze in capillaries-
- HbC also leads to water loss from cells leading to higher hemoglobin concentration.
- This problem causes only a minor hemolytic disorder.

(Insignificant effect on function contrary to HbS)



If you look at the blood sample you will see the abnormality in the morphology of RBCs in individuals having HbC

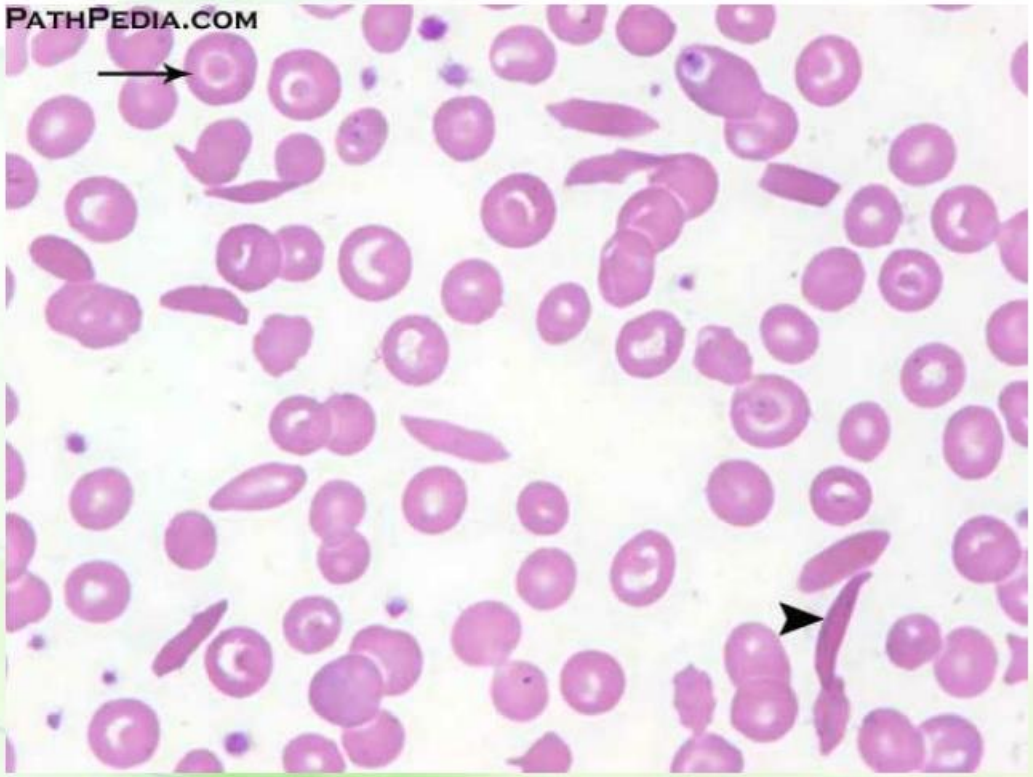
You have finished 1/3 of the slides, GO ON :)

HbSC disease

1st allele : Glu to Val
2nd allele : Glu to Lys

- Individuals with both βc and βs mutations have HbSC disease, a mild hemolytic disorder which may have no clinical consequences, but it is clinically variable.

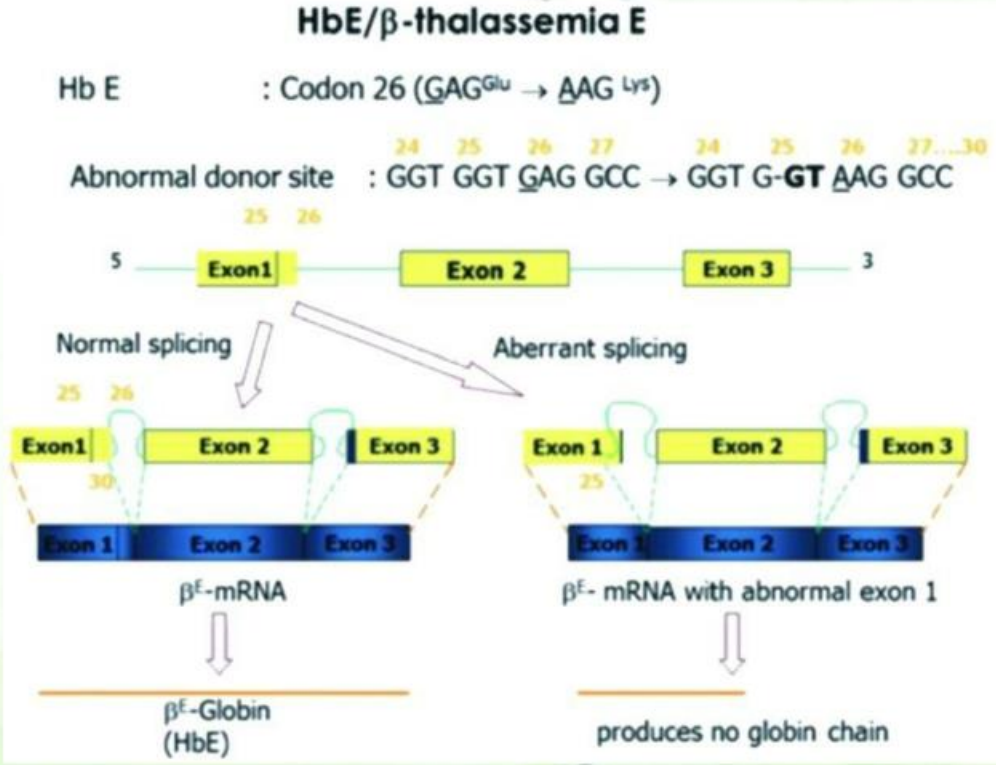
They produce defective hemoglobin molecule just like normal people or patients with sickle cell trait –mild clinical abnormalities-



Hemoglobin E

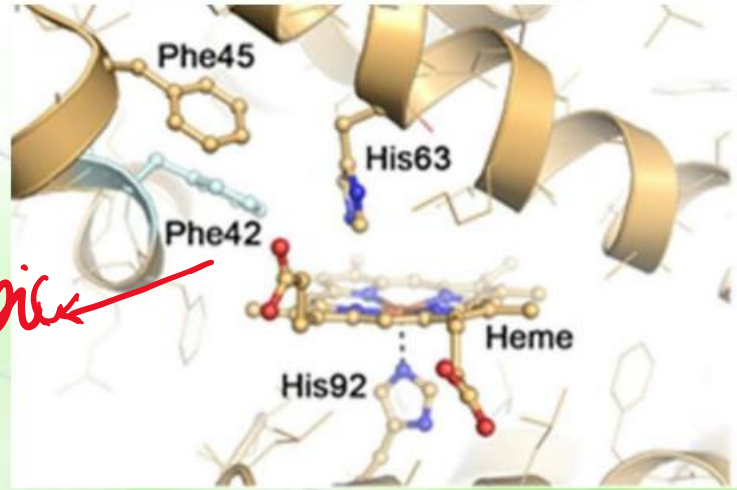
- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- It is caused by a point mutation in beta chain in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein(it's not stable as the normal protein , so it would tend to be degraded)

- Individuals with this mutation make only around 60% of the normal - low- amount of β-globin protein.and Hb has deficient ability to carry O2.

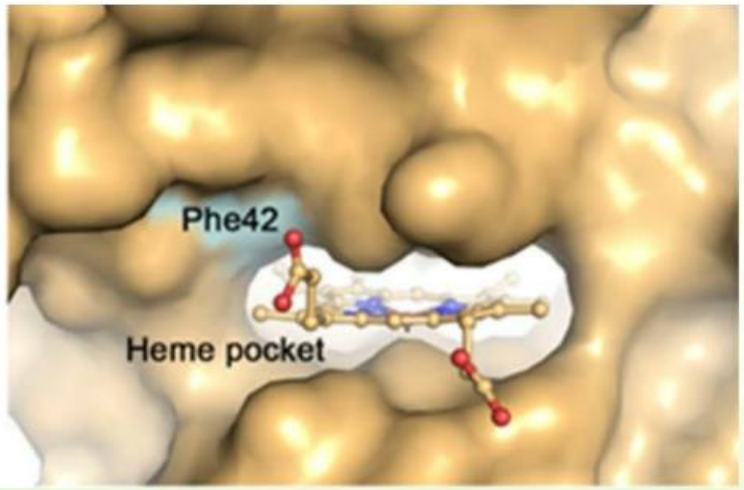


Hb Hammersmith

- 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 phenylalanine(which exists in the heme pocket) with a serine(polar) within the β globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and oxygen binding affinity causing cyanosis.
  That will cause destabilization of the interaction between heme and hemoglobin molecule



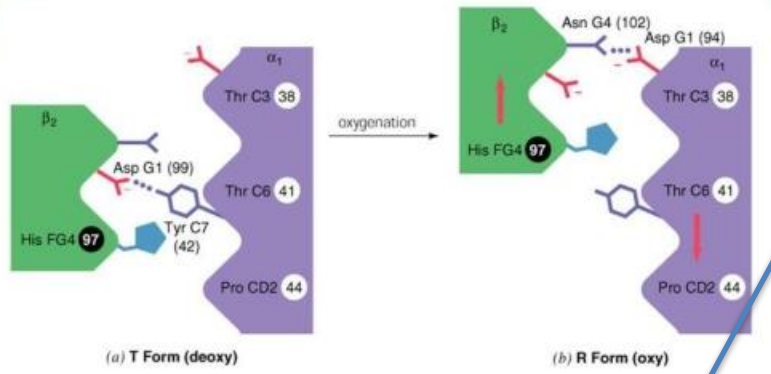
hydrophobic



Mutations at α1-β2 contacts

- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increased affinity)
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
  - Hb Kansas: stabilization of the T state (Asn G4 (102) to thr); Decreased cooperativity.
  - Hb Yakima: stabilization of the R state (Asp G1 (99) to His **or Asn**).

You would have in this case destabilization of the T sate

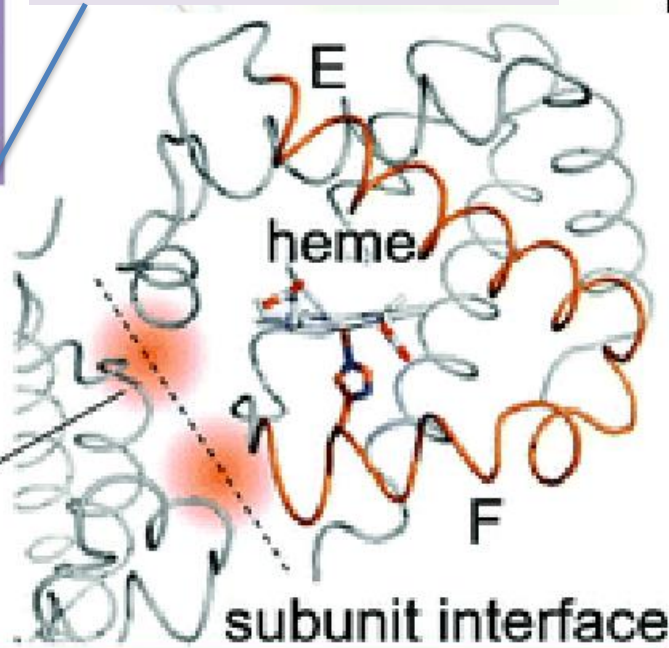


There are different mutations of the same amino acid

Or His

β99 Asp→Asn (R-fixed)

β102 Asn→Thr (T-fixed)



Explanation of the previous slide

We talked about non-covalent interactions that take place between the polypeptide chains and how they stabilize the T or R states, one of them is **His 146 –beta chain- with Lys 40 – alpha chain-**

-Also, **His 146** can also interacts non covalently **with aspartate 94 –in the same chain(beta) which called intra-molecular interaction** and this stabilizes the T state

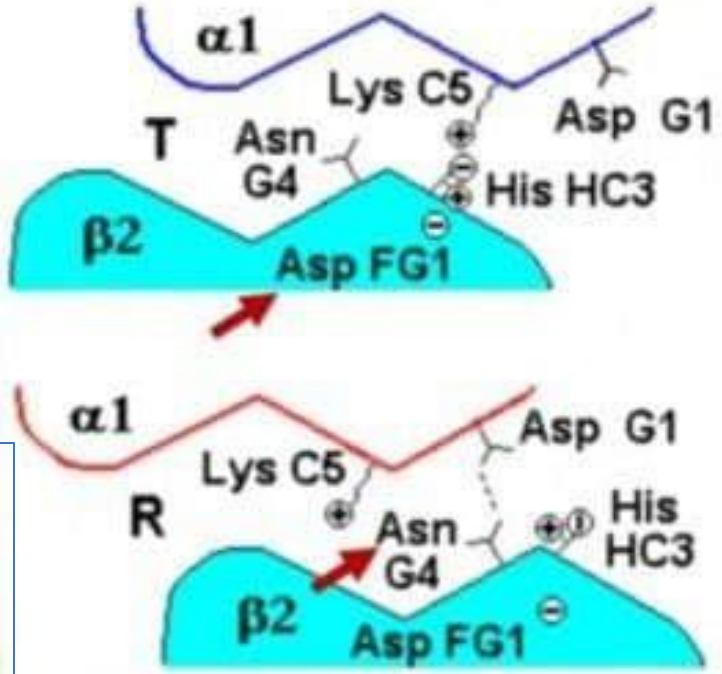
SO WHAT?

If His 146 is mutated to leucine for example that would actually stabilize the R state –high affinity - because there is no more interaction and the T state is destabilized and this variant is known as Hb CowTown .

There are many single mutations of hemoglobin and these are given the names of the towns where these mutations were identified such as Hb Kansas and Hb Yakima

Stabilization of T/R state

- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increase affinity).
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
  - Hb Kansas: stabilization of the T state (Asn to thr).
  - Hb Yakima: stabilization of the R state (Asp to His).



NOTE: this slide is not in the original file of slidess on e learning or in live lecture but I found it in the 2021 recorded lecture and it is almost a review so check the next slide for the explanation :)

Explanation of the previous slide

The mutations change the equilibrium between the 2states of the hemoglobin molecule.

Remember,

In the T state we have interactions between His - in beta- with Asp - in beta- , and His - in beta- with Lys - in alpha-, that stabilize the T state.

Once we have oxygen formation hemoglobin changes to the R state and what happens is sliding of the alpha chain and loss of interaction between His with Asp and His with Lys and Instead of that we have formation of interaction between Asn-in beta- with Asp - in alpha-

So, when there is a mutation and Asn changes to Thr, so the reaction between Asn and Asp that stabilizes R state will not happen which would stabilize and fix the hemoglobin molecule in the T state (Hb Kanses)

And when Asp –beta chain- changes to His we won't have the interaction that stabilizes the T state, so the equilibrium changes the formation of R state and the molecule will stay in the R state (Hb Yakima)

The doctor said that he may ask a question about this idea but with different amino acids mentioned in the lectures so check the modified slides and make sure that you memorized them.

Altered Oxygen Transport

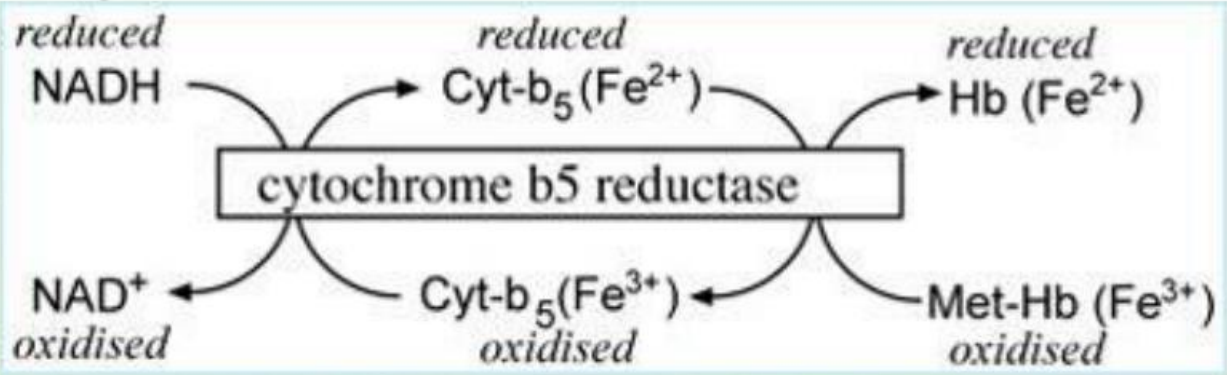
These are examples about qualitative hemoglobinopathies and remember that there are 700 variants, so we can talk about every single amino acid and there are different names for different hemoglobin variants

Methemoglobin (HbM)

Methemoglobin is a Hemoglobin molecule that is bound to iron in the ferric state rather than the ferrous state So it is not able to bind oxygen

Check the next slide

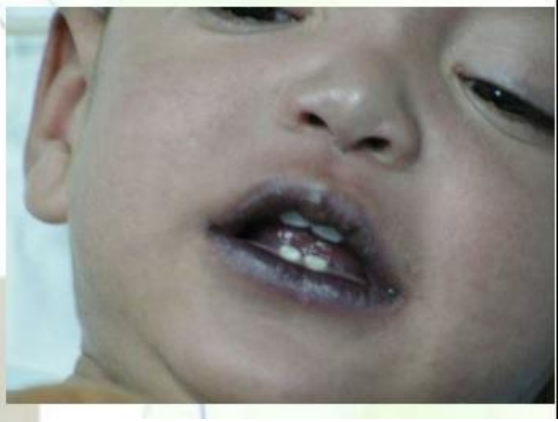
- Oxyhemoglobin can undergo reversible oxygenation because its heme iron is in the reduced (ferrous, Fe^{+2}) state.
- During oxygen release from heme, Fe^{+2} is oxidized to Fe^{+3}, forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.
- If not, a condition known as methemoglobinemia develops.



Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase

Normal Blood

Chocolate Brown coloured Blood



Chocolate lips because of cyanosis

About the previous slide

Normal people have HbM but in very low quantities because of :

1.The hydrophobic pocket surrounding the heme molecule prevents the iron oxidation from the ferrous to ferric state.

2. Individuals have methemoglobin reductase so it immediately reduces HbM into Hb with iron in the ferrous state, and this requires cytochrome molecule within the enzyme itself and NADH.

So, we oxidize NADH and transfer the electrons to iron.

- this process takes place in RBCs
- The source of NADH is glycolysis

Why HbM?

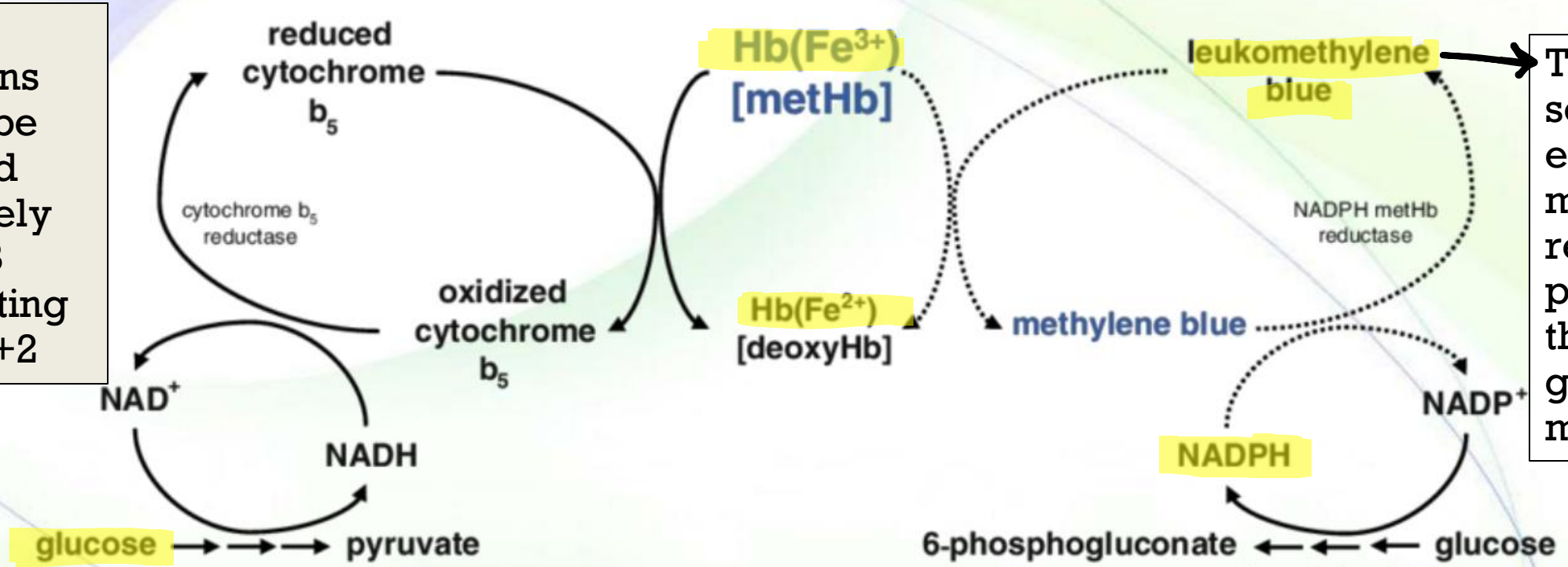
- Some mutant globins (α and β) bond heme in such a way as to resist the reductase.
  - Hb Boston: distal histidine – that stabilizes the oxygen binding to a heme molecule- is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen. It also attracts H2O into the pocket.
  - HbM Iwate: -it was discovered in Japan- proximal histidine (that binds to iron in the 5th coordinate) is replaced by a tyrosine.
- A deficiency of the reductase enzyme.
- Certain drugs or drinking water containing nitrates.

All of these causes increase the probability of oxidizing Iron from Fe+2 to Fe+3

Treatment (methylene blue)

Alternative pathway

These electrons would be donated ultimately to Fe+3 converting it to Fe+2



The second source of electrons for methemoglobin resulting in the production of the normal globin molecule

NADH from glycolysis is the main source of electrons

Pentose phosphate pathway

In pentose phosphate pathway we have production of NADPH

Methylene blue can be used in treating patients with methemoglobinemia

NADPH is the source of electrons the would be used to reduce methylene blue into leukomethylene blue

Quantitative abnormalities (thalassemias)

Thalassemias

- Thalassemias: the most common human single-gene disorder specially in the old world including the middle east .
- They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio –in normal RBCs we have an equal amount of alpha and beta chains-

The Alpha-Thalassemias

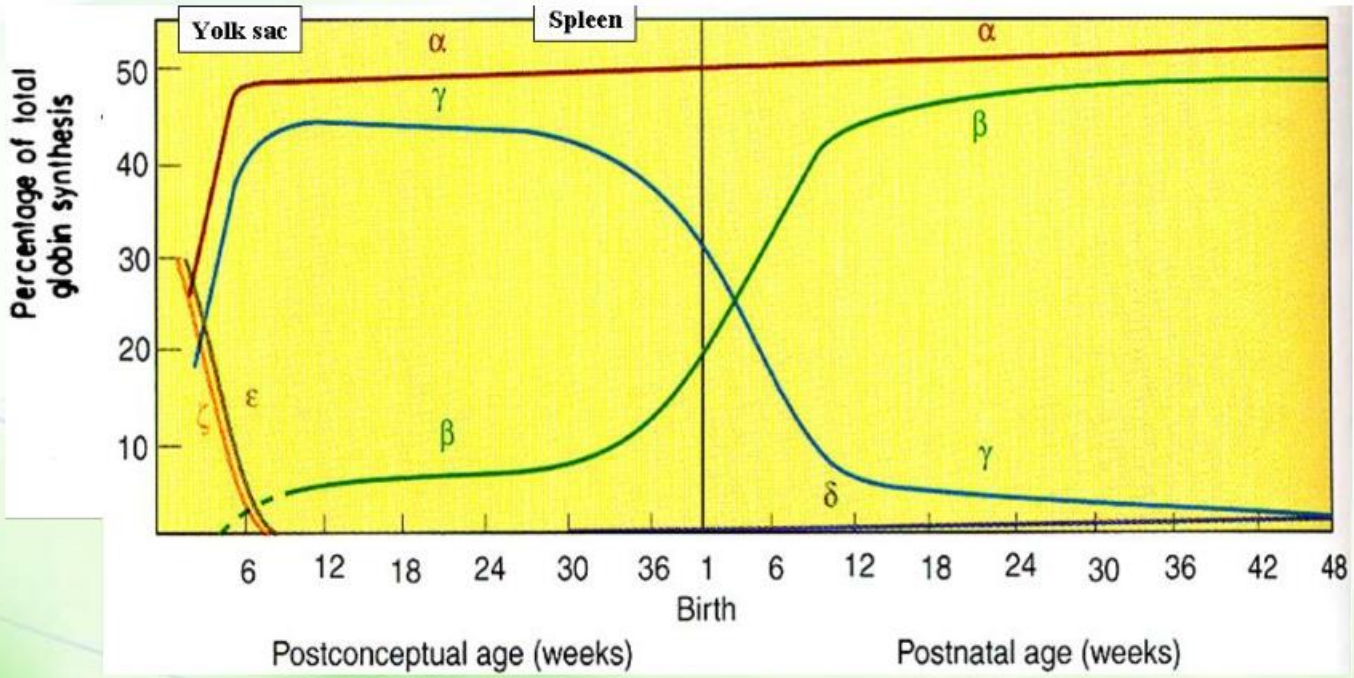
- Alpha-thalassemia: underproduction of the α-globin chains

–we have more beta than alpha- .

- HbA (α2β2), HbF (α2γ2), and HbA2 (α2δ2) are all affected in α-thalassemia.

Remember that,

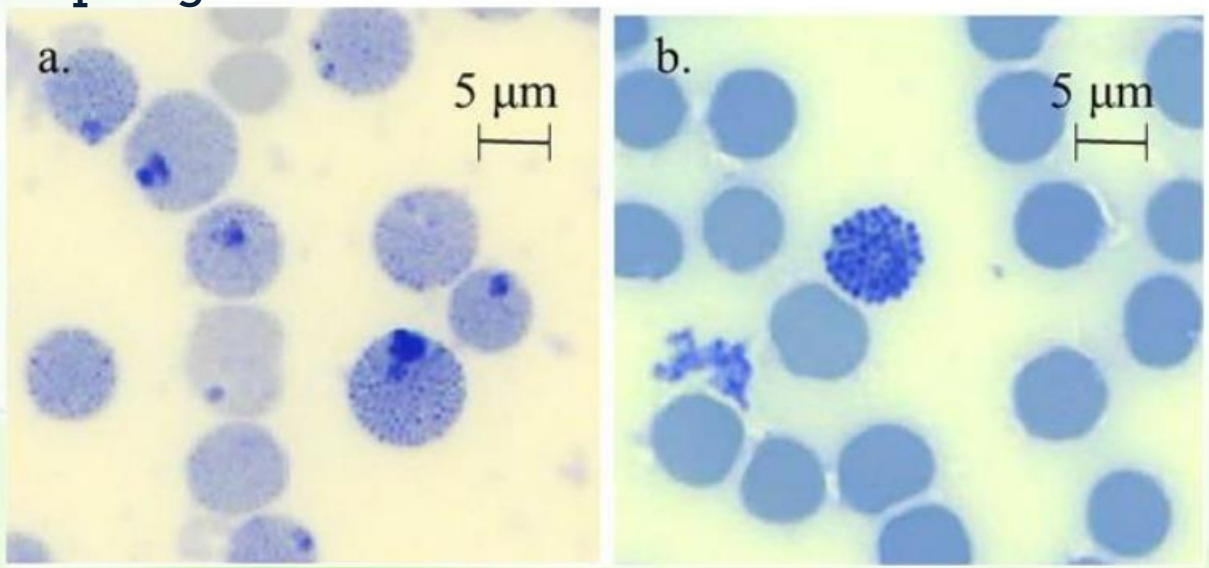
- **Alpha chain is produced at the end of embryonic stage and continues throughout the life .**
- **Beta chain : starts to be produced slowly in the early fetal stage but there is a big jump right before birth and it continues throughout the live having equal quantities of alpha and beta chains**



HbH

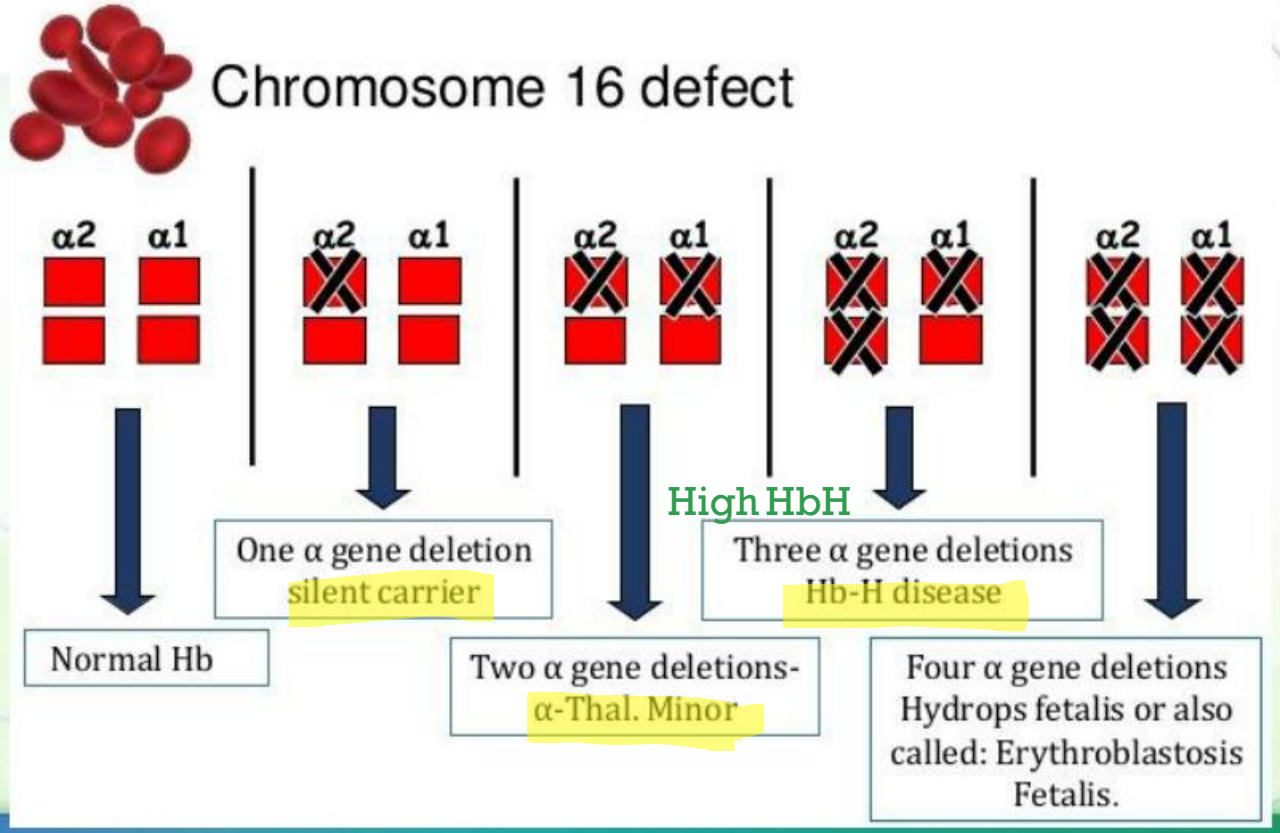
- With reduction of α chain production, and β-chain production is established, homotetramers of β (β4 or HbH) are formed.
- The HbH tetramers have a markedly reduced oxygen carrying capacity.
- Main type of mutation is deletion (rarely point mutations)

Remember that we have 4 alpha genes –2 on each chromosome-



Variable severity

- With the α-thalassemias, the level of α-globin production can range from none to very nearly normal levels.
- This is due in part to the fact that each individual has 4 genes.

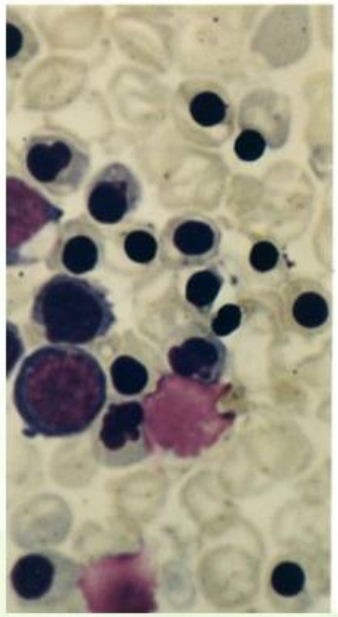


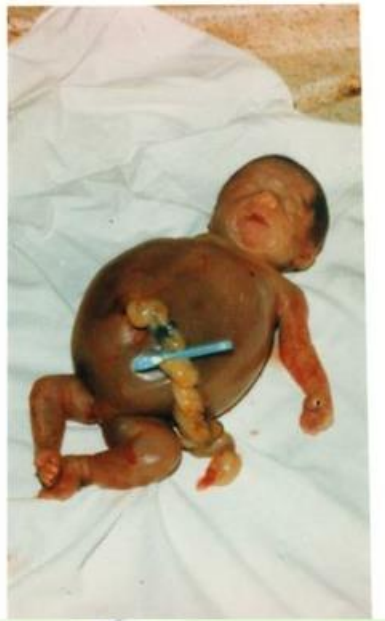
That will result in variable severity

2/3 of the lec is DONE:)

Hydrops fetalis

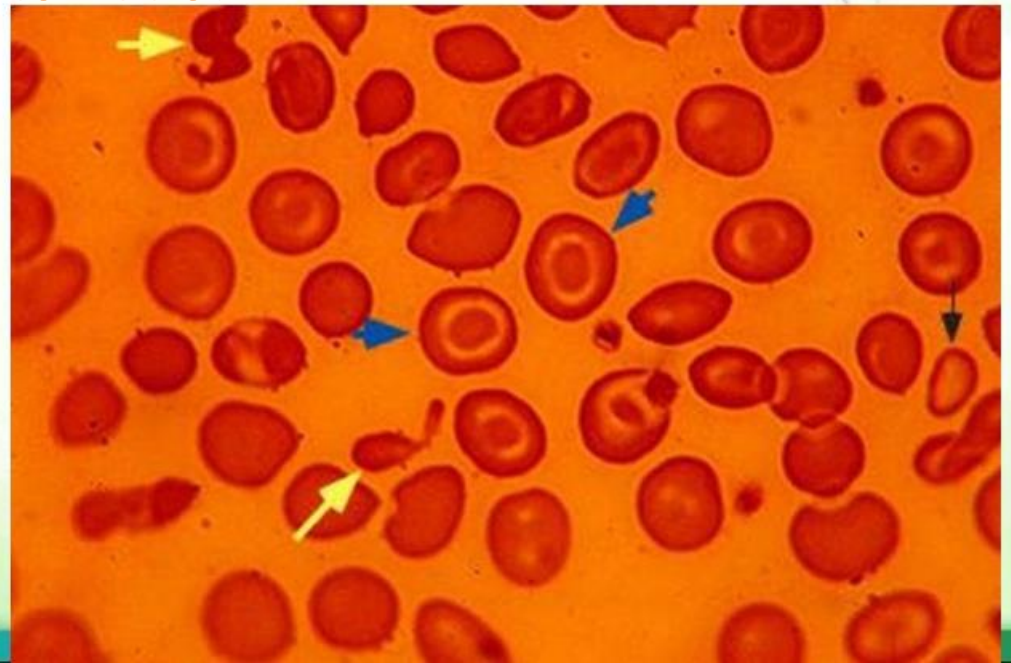
- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ-chains.
- γ4 or Hb Bart: a homotetramer of γ.
- Hb Bart has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues.
- This situation is called hydrops fetalis.
- Stillbirth or death shortly after birth occurs.





Hemoglobin H disease

- 3 of 4 genes deleted one only is active.
- Mild to moderate hemolytic anemia in adults.
- A high level of β4 tetramer is present (high HbH).
- Clinically, it is known as hemoglobin H disease.
- The disease is not fatal but it is symptomatic.



Minor α-thalassemia and silent carrier

- α-Thalassemia trait -minor-: If 2 of the 4 genes are inactivated.
  - The individuals are generally asymptomatic.

- Silent carrier: 1 of 4 genes deleted.
  - Individuals are completely asymptomatic.

Summary of α-thalassemias

For memorizing

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

Deletion of alpha alleles On one chromosome
Or one on each chromosome

[a]Number of normal alpha globin genes

The beta-thalassemias

Normal people have 2 alleles one on each chromosome

- β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers.
- Main type of mutation is point mutations, mutations within the coding region or the promoter, translation initiation codon, splicing positions, or poly-adenylation termination signal.
- The α-globin homotetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.

β-thalassemia major

There are different types depending on the severity of mutation

- A complete lack of HbA is denoted as β0-thalassemia or β-thalassemia major.(no production of beta globin at all)
- Afflicted 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, particularly the heart, liver and pancreas and , finally, death in the teens to early twenties.

β -Thalassemia minor

There is some production of beta globin

- Individuals heterozygous for β-thalassemia is termed β- thalassemia minor.
- Afflicted individuals carry one normal β-globin gene and a mutated gene

or two that produce low amount of beta globin .

- Thalassemia minor individuals are generally asymptomatic.

Classification and types of β-thalassemia

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) No producing of beta globin at all	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

β^0: complete lack of β chain
β^+: some expression of β chain
β: normal expression of β chain
β^E: HbE

Hereditary persistence of fetal hemoglobin

(HPFH) These individuals have an abnormality in not transitioning from gamma to beta during development so they produce HbF throughout their life and they do not know that they have abnormality, so everything is functional and okay.

- Persons with HPFH continue to make HbF as adults.
- Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality.
- Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster.
- There is no deletion of the fetal globin genes

HbF has higher affinity to oxygen but this doesn't give an advantage .

- Think: treatment for β-thalassemia!!!!

There is an idea of switching on the fetal globin gene (gamma globin) in case of beta globin gene is not functional

GENE REGULATION

Switching from fetal to adult hemoglobin

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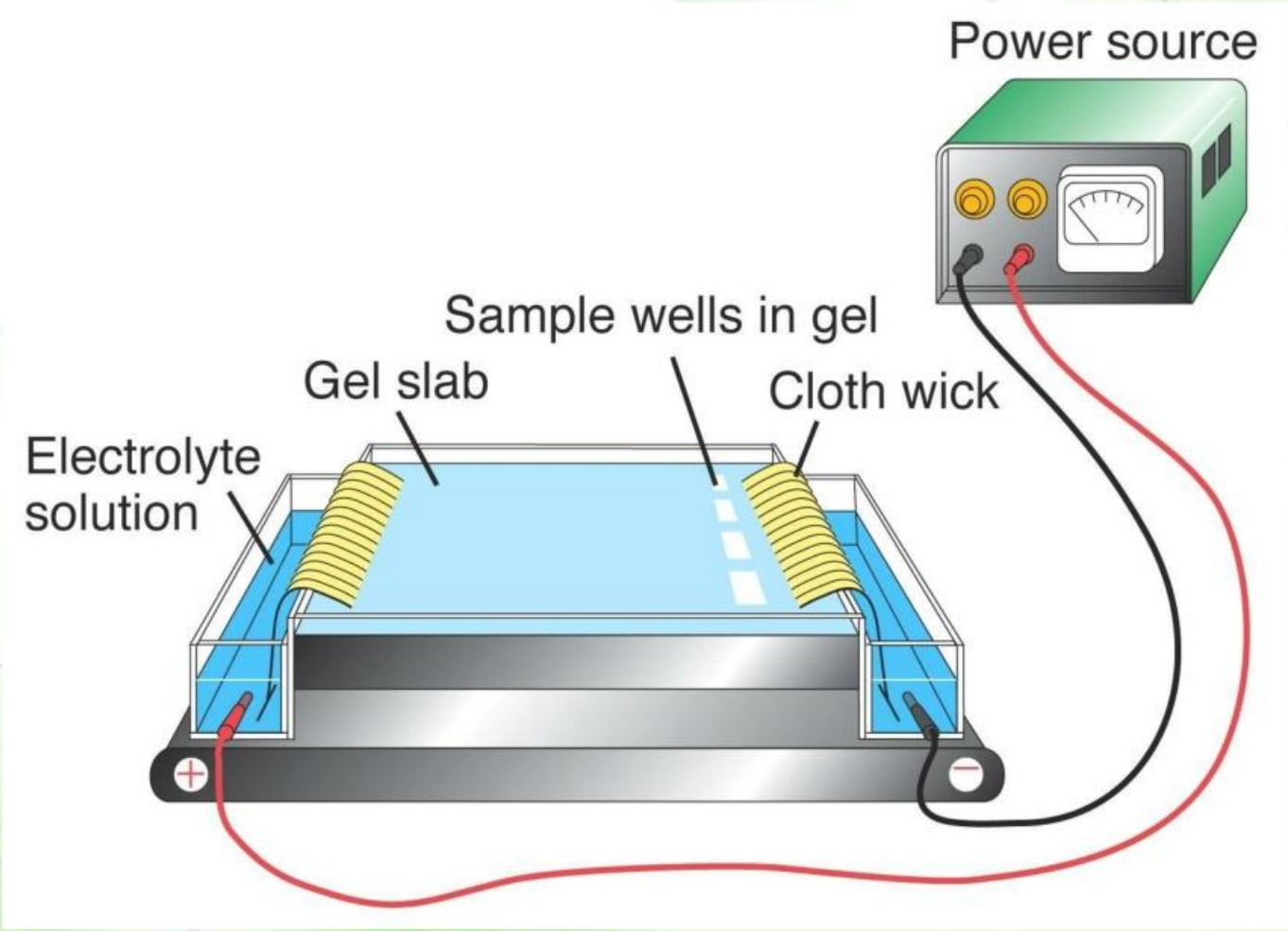
The switch from fetal to adult hemoglobin relies on repression or silencing of the upstream γ-globin gene, but identification of the transcriptional repressors that bind to the sites at which a cluster of naturally occurring variants associated with HPFH (hereditary persistence of fetal hemoglobin) are found has been elusive. A new study provides mechanistic evidence for the direct binding of BCL11A and ZBTB7A, two previously identified γ-globin gene repressors.

Hemoglobin Electrophoresis

For diagnosing hemoglobinopathies

Molecules such as proteins can be separated according to its **charge** or **size**



Mutation and migration

This technique can be used for hemoglobin molecules especially those that have single point mutations because we have a change of one amino acid to another and this will be particularly true if the amino acid that changed or added would change the net charge of the molecule

Ex.
Changing Glu to a Val or to a Lys would change the overall charge of the whole molecule
As a result, hemoglobin molecules would travel differently, so this depends not on size of the Hb molecule rather it depends on the charge of the molecule.

- Amino acid substitution in abnormal Hbs results in an overall change in the charge of the molecule.
- Therefore, Hb migration in a voltage gradient is altered.
- Electrophoresis of hemoglobin proteins from individuals is an effective diagnostic tool in determining if an individual has a defective hemoglobin and the relative ratios of the patient's hemoglobin pattern.

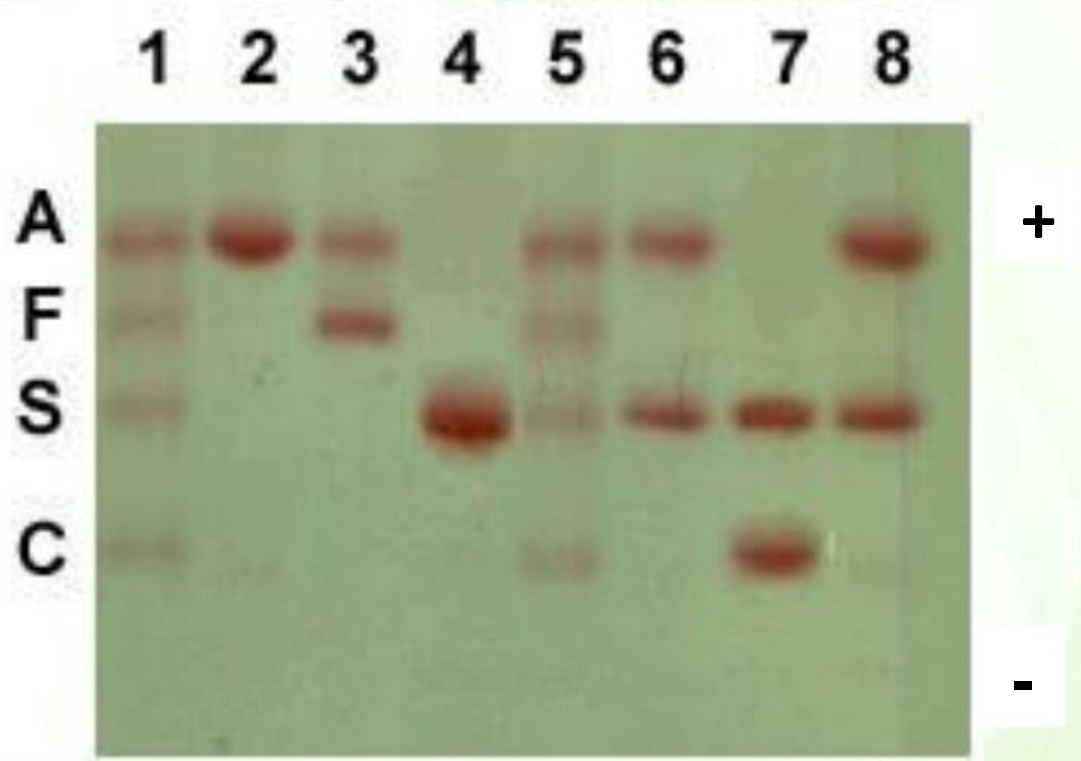
Examples

- In Sickle Cell hemoglobin, replacement of a negatively-charged glu in the standard HbA by a neutral val in HbS results in a protein with a slightly reduced negative charge.
- In homozygous individuals, the HbA tetramer electrophoreses as a single band, and the HbS tetramer as another single band.
- Hemoglobin from a heterozygous individual (with both alleles) appears as two bands.
- Since HbC contains a lysine instead of the normal glutamate, HbC will travel even faster to the cathode.

Results

Check the next slide

- Lanes 1 and 5 are hemoglobin Standards **(a sample that contains all forms of hemoglobin)**
- Lane 2 is a normal adult
- Lane 3 is a normal neonate
- Lane 4 is a homozygous HbS individual
- Lanes 6 and 8 are heterozygous sickle individuals
- Lane 7 is a SC disease individual



Band 1 and 5 : we can see the migration pattern is different among the different hemoglobin molecules, for example HbC is traveling close to the cathode (negative charge end), HbS is migrating a little bit slower than HbC and so on..

Why we see this difference in migration patterns ?

Because in HbC we have a mutation of Glu to Lys so we have a loss of one negative charge and an addition of one positive charge , that's why it travels closer to the cathode.

In HbS the we have a loss of a negative charge(Glu to Val which is with no charge) so it will travel away from the anode and closer to the cathode.

HbF as a whole molecule is more positively charged than HbA.

Band 2: This individual has only HbA and this is an adult individual. And that's good, there is no problem.

Band 3: This individual has both HbA and HbF with more HbF than HbA, so this is a neonate most probably .

Band 4: This individual has only HbS so this is someone with sickled cell anemia

Band 6: This individual has both HbA and HbS so this someone with sickled cell trait

Band 7: This is someone with HbSC

Band 8: This is someone with sickled cell trait