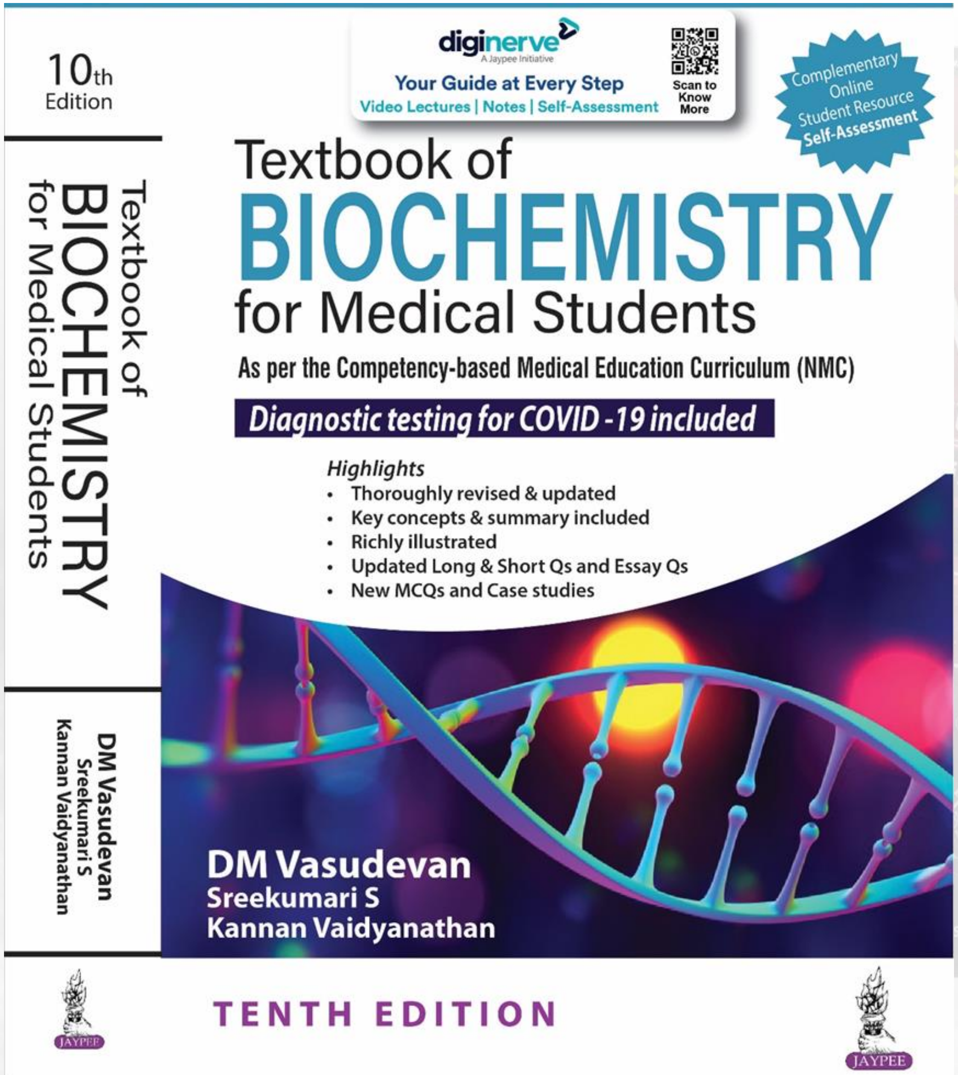


Chapter 20B: Hemoglobin, Functions, Abnormal Hemoglobins

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Normal Hemoglobin

- Normal level of Hemoglobin (Hb) in blood in males is **14-16 g/dl and in females, 13-15 g/dl.**
- Hb is globular in shape.
- The adult Hb (HbA) has 2 alpha chains and 2 beta chains.
- Molecular weight of HbA is **67,000 Daltons**.

- Hb F (Fetal Hb) is made up of 2 alpha and 2 gamma chains.
- Hb A2 has 2 alpha and 2 delta chains.
- Normal adult blood contains 97% HbA, about 2% HbA2 and about 1% HbF.
- Each alpha chain has 141 amino acids.
- The beta, gamma and delta chains have 146 amino acids.
- There are 36 histidine residues in Hb molecule; these are important in buffering action.
- The 58^{th} residue in alpha chain is called **distal histidine,** because it is far away from the iron atom.
- The 87th residue in alpha chain is called **proximal histidine, because it lies near to the iron atom.**

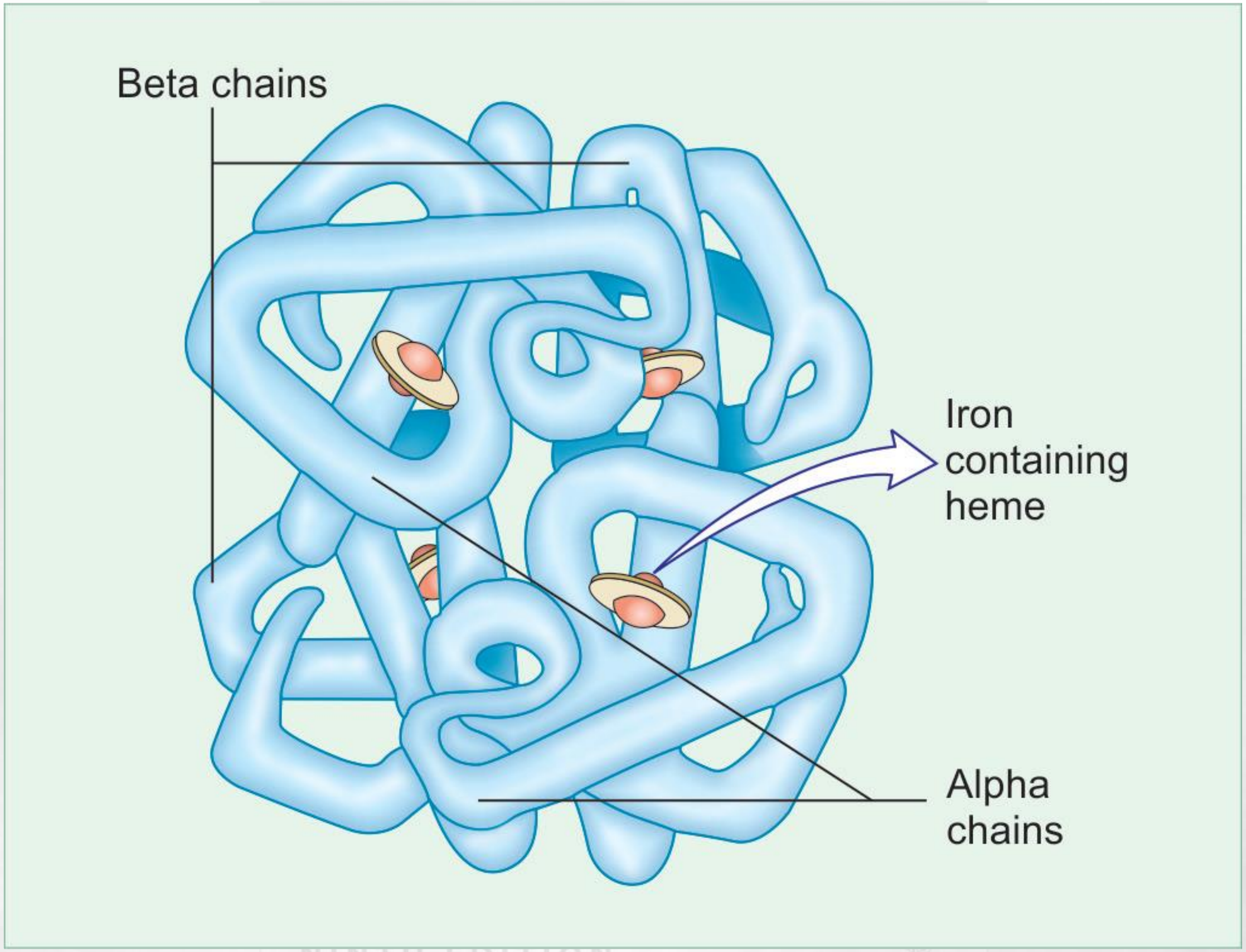
Fetal Hemoglobin (HbF)

- HbF has 2 alpha chains and 2 gamma chains. Gamma chain has 146 amino acids.
- The differences in physicochemical properties when compared with HbA are:
    - a. Increased solubility of deoxy HbF
    - b. Slower electrophoretic mobility for HbF
    - c. Increased resistance of HbF to alkali denaturation
    - d. HbF has decreased interaction with 2,3-BPG.

- The synthesis of HbF starts by 7th week of gestation; it becomes the predominant Hb by 28th week.
- At birth, 80% of Hb is HbF.
- During the first 6 months of life, it decreases to about 5% of total.
- HbF level may remain elevated in children with anemia and beta thalassemia, as a compensatory measure.

Hemoglobin A2

- It is a normal adult Hb; it is about 2% of total Hb.
- It has 2 alpha chains and 2 delta chains.
- The delta chain has sequence homology with beta chain.
- In beta thalassemia, as a compensation, HbA2 is increased.
- The iso-electric pH of HbA2 is 7.4, while HbA has the pI value of 6.85.
- HbA2 is slower moving on electrophoresis.



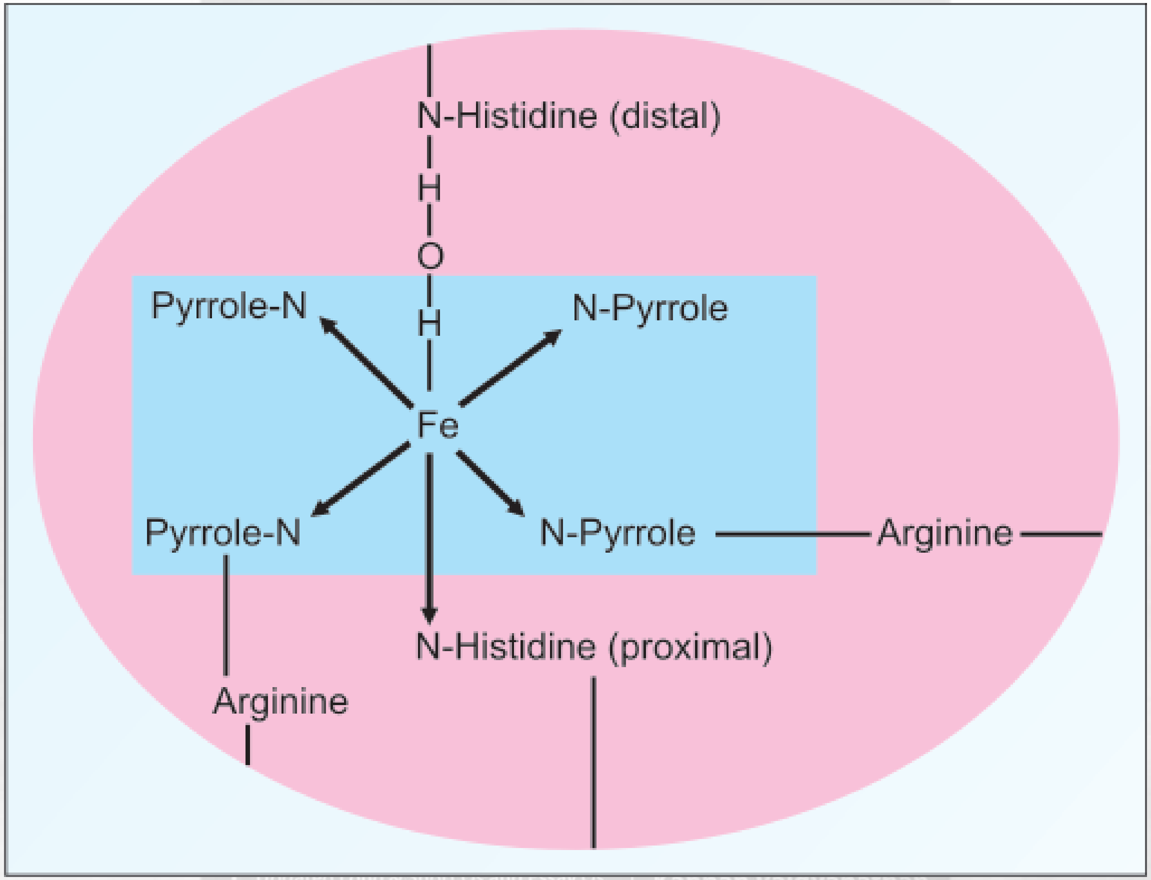
Hemoglobin molecule is made up of two alpha and two beta chains.

Oxygenation and Oxidation

- When hemoglobin carries oxygen, the Hb is **oxygenated.**
- **The iron atom in Hb is still in the** ferrous state.
- **Oxidised Hemoglobin is called** Met-Hb; then iron is in ferric state and the oxygen carrying capacity is lost.

- The alpha and beta subunits are connected by relatively weak non-covalent bonds like van der Waals forces, hydrogen bonds and electrostatic forces.
- There are 4 heme residues per Hb molecule, one for each subunit in Hb.
- The 4 heme groups account for about 4% of the whole mass of Hb.
- The heme is located in a hydrophobic cleft of globin chain.

Linkage of Heme with Globin



Pink circle represents the globin chain. Blue rectangle represents the protoporphyrin ring

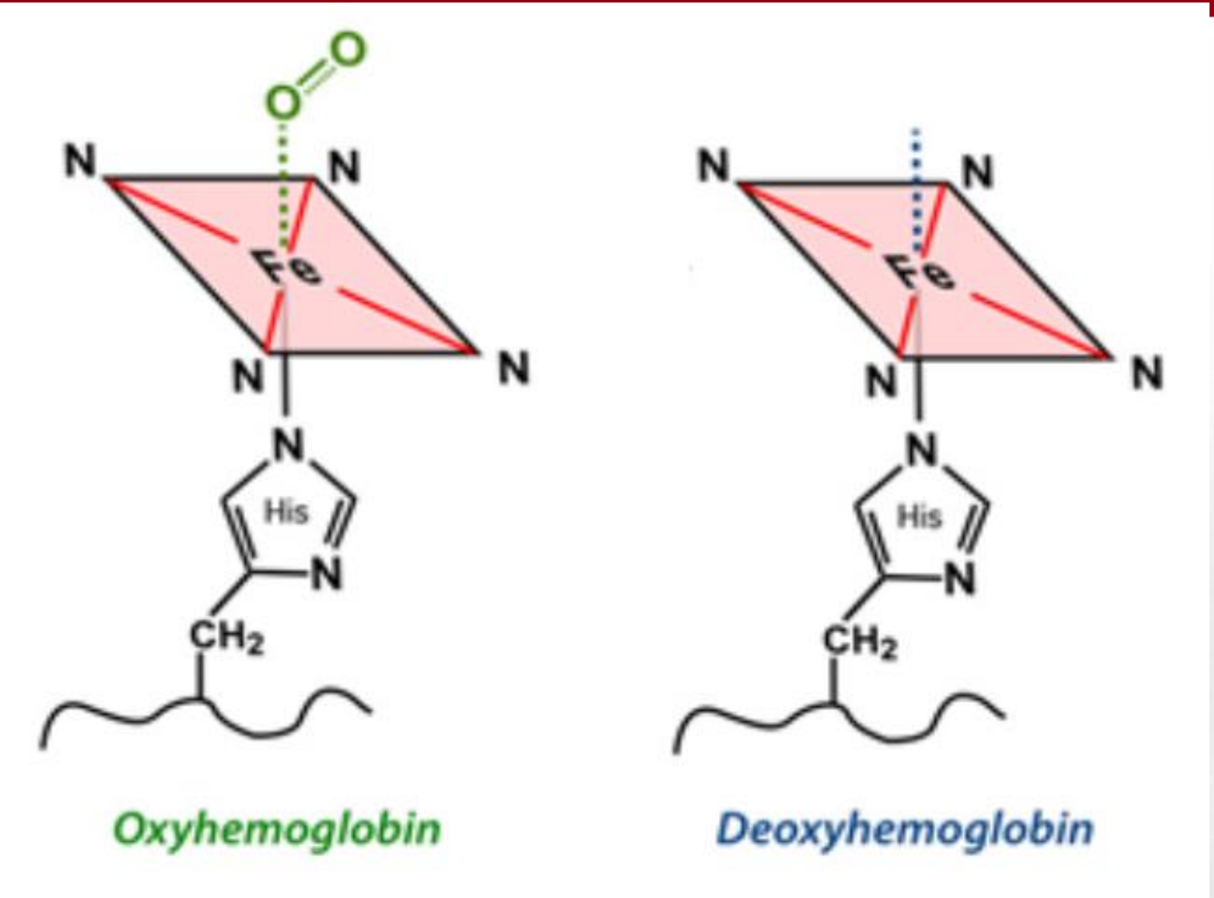
- The iron atom of heme occupies the central position of the porphyrin ring.
- The reduced state is called ferrous (Fe++) and the oxidized state is ferric (Fe+++).
- The ferrous iron has **6 valencies and ferric has 5 valencies.**
- **In** hemoglobin, **iron remains in the ferrous state.**
- The iron is linked to the pyrrole nitrogen by 4 co-ordinate valency bonds and a fifth one to the imidazole nitrogen of the **proximal histidine.**
- In oxy-Hb, the 6th valency of iron binds the O2.
- The oxygen atom directly binds to Fe, and forms a hydrogen bond with an imidazole nitrogen of the distal histidine.

- In deoxy-Hb, a water molecule is present between the iron and distal histidine.
- As the porphyrin molecule is in resonance, central iron atom is linked by coordinate bond.
- The **distal histidine lies on the side of the heme** ring.

Transport of Oxygen by Hemoglobin

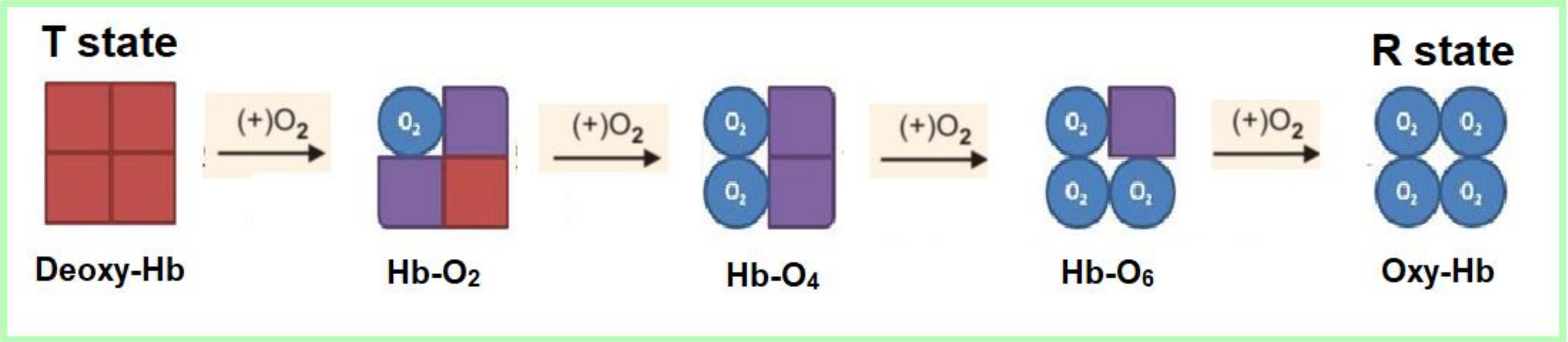
- Hemoglobin is an ideal oxygen carrying pigment because –
  - a. It can transport large quantities of oxygen
  - b. It has great solubility
  - c. It can take up and release oxygen at appropriate partial pressures
  - d. It is a powerful buffer.

Transport of Oxygen by Hemoglobin

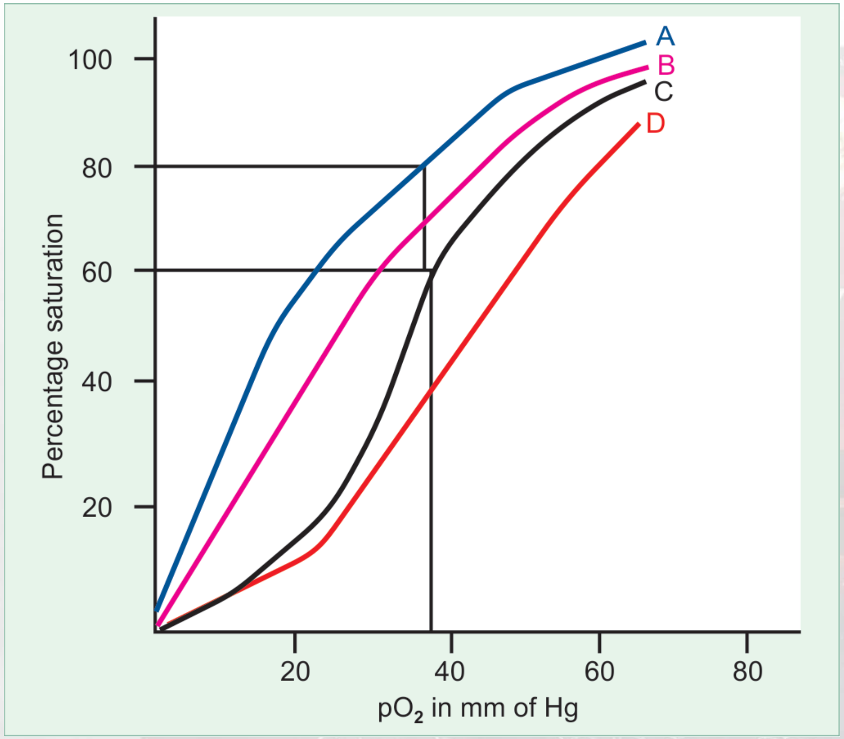


In oxy-Hb, the 6th valency of iron binds the O_2. The oxygen atom directly binds to Fe. In deoxy-Hb, oxygen is removed and a water molecule is present between the iron and distal histidine.

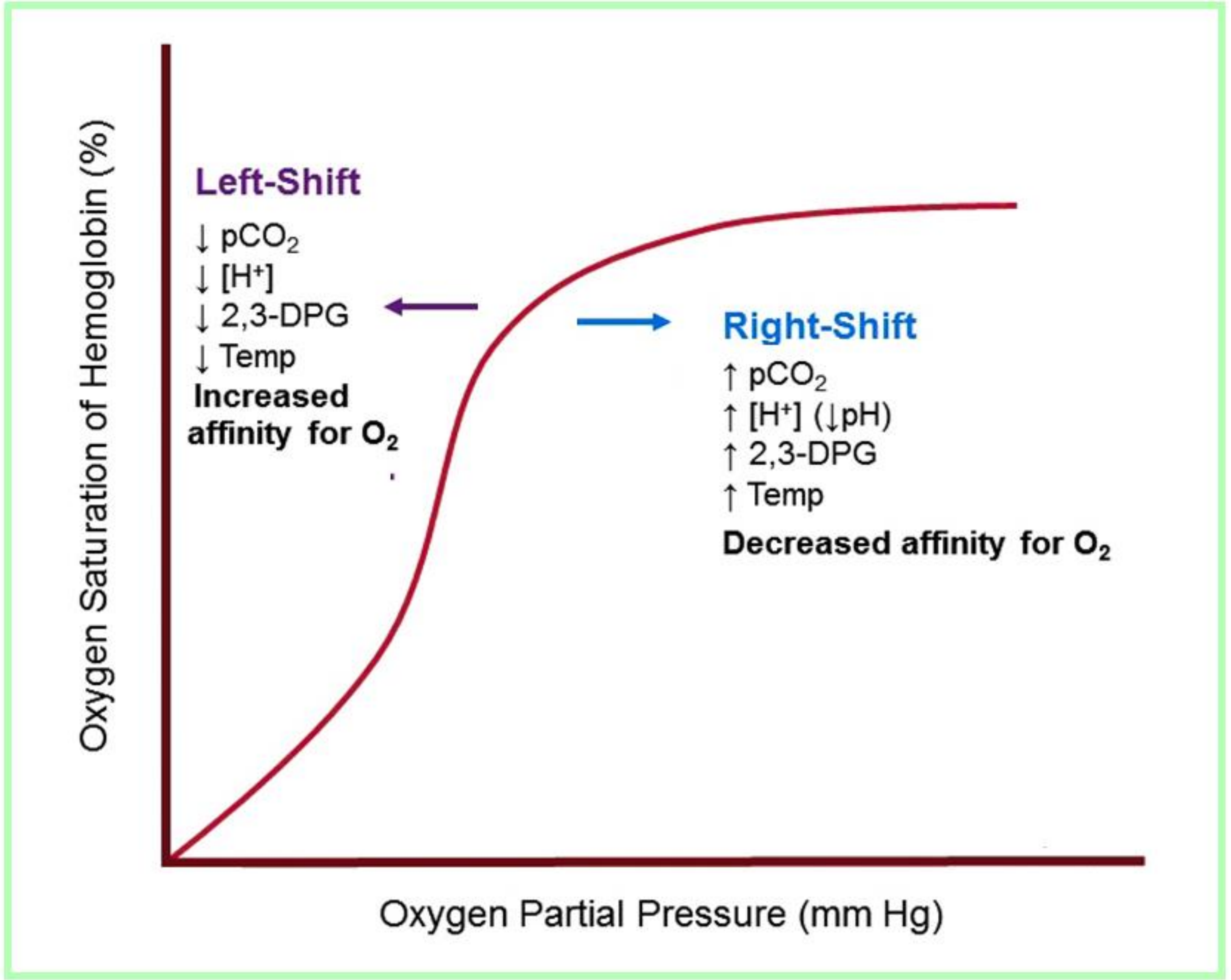
Subunit Interaction in Hemoglobin



Diagrammatic representation of the subunit interaction in hemoglobin. Rectangles represent T state Hb monomers and circles represent R state Hb monomers. As oxygen is added, salt bridges are successively broken. Simultaneously the T (taught) confirmation of deoxy-Hb is changed into R (relaxed) confirmation of oxy-Hb.

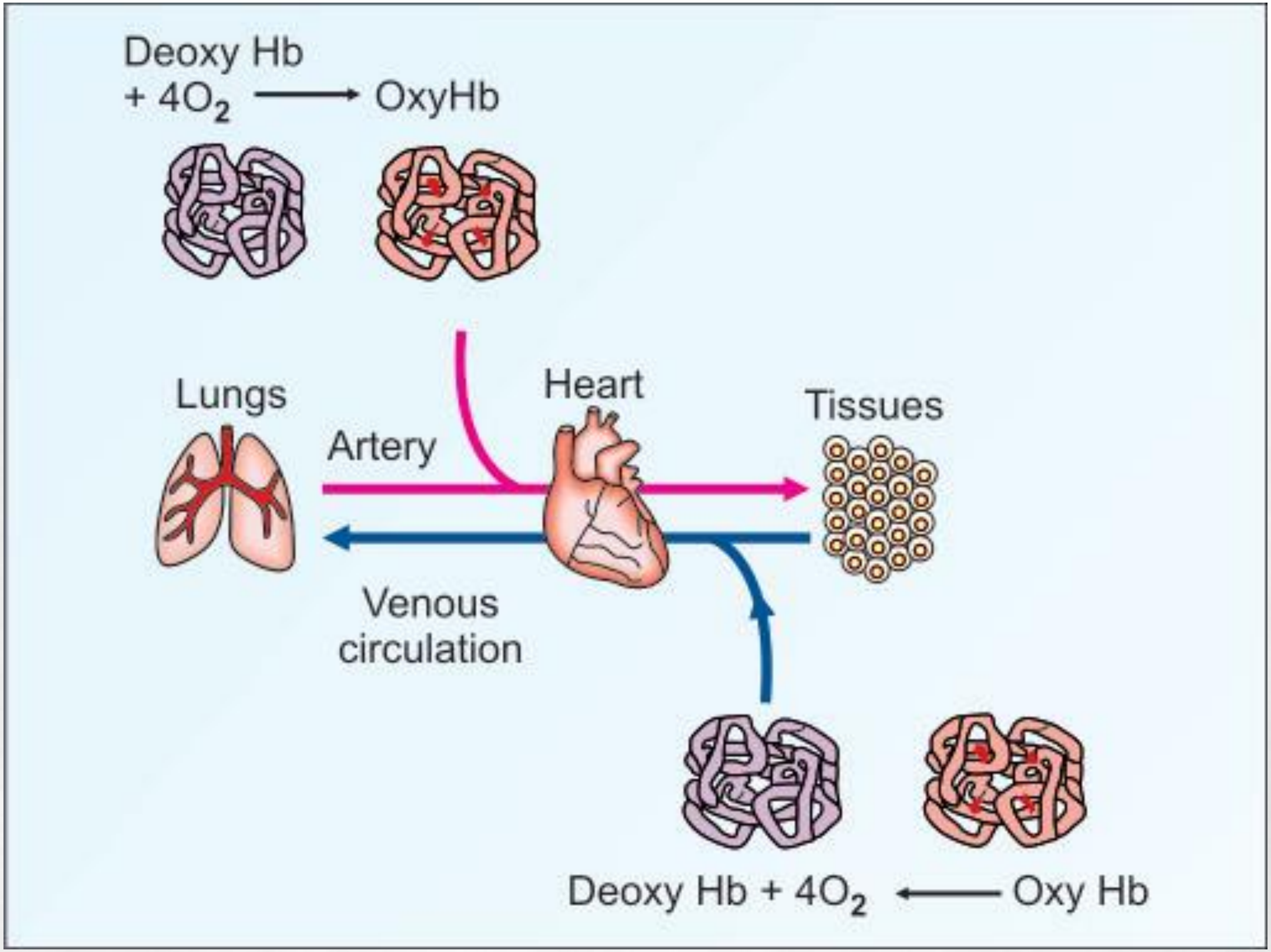


Oxygen dissociation curve (ODC). A = Theoretical curve as per mass action. B = Sigmoid curve, due to heme-heme interaction (Hill effect). C = Further shift to right due to carbon dioxide (Bohr effect) and PG. This curve represents the pattern under normal conditions. D = Further shift to right when temperature is increased to 42oC.



Factors affecting oxygen dissociation curve.

In Tissues, Oxy Hb Releases O2



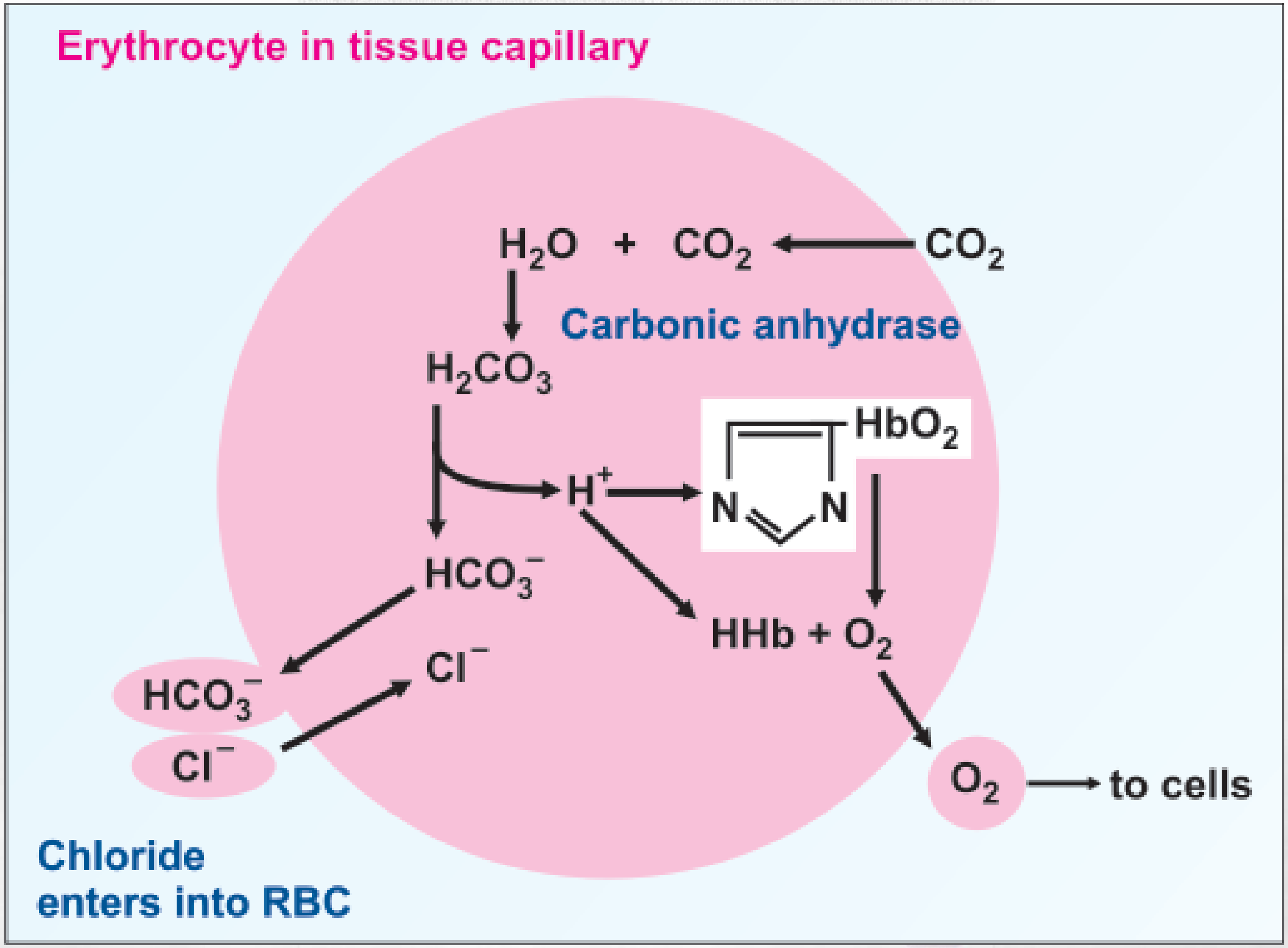
Affinity of Oxygen with Oxygen

Affinity 1 time	Affinity 2 times	Affinity 4 times	Affinity 18 times
$Hb + O_2 \rightarrow HbO_2$	$HbO_2 + O_2 \rightarrow HbO_4$	$HbO_4 + O_2 \rightarrow HbO_6$	$HbO_6 + O_2 \rightarrow HbO_8$

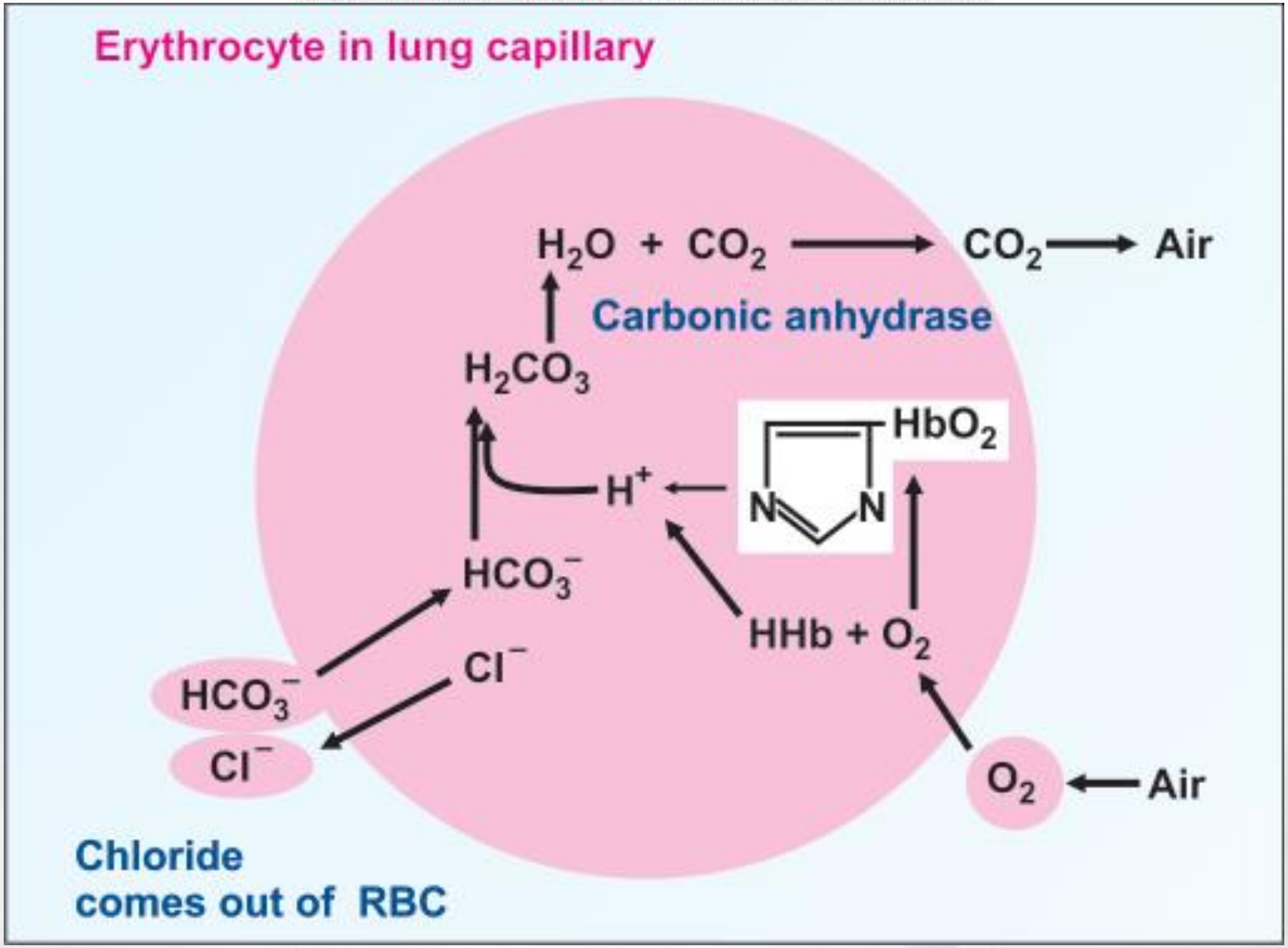
Clinical Applications of Oxygen Dissociation

1. In all hypoxic states the O_2 affinity is decreased with a shift in ODC to the right and an increase in 2,3-BPG inside RBC. The **adaptation** to the high altitude where pCO_2 is low, includes increased pulmonary ventilation, polycythemia, and an increase in 2,3-BPG level with a shift in ODC to the right.
2. In **anemia** where the total concentration of Hb is reduced, increased oxygen unloading alone will ensure proper oxygenation of tissues.
3. in many cases, the 2,3-BPG level varies inversely as the Hb concentration.
4. in chronic pulmonary diseases and cyanotic cardiac disorders also, the 2,3-BPG level is increased, ensuring maximum unloading of O_2 to the tissues.
5. The red cell 2,3-BPG level is decreased in **acidosis** and increased in **alkalosis**. Hence, the expected shift in ODC to the right or left is not observed.
6. **Transfusion** of large volumes of stored blood, which has a low level of 2,3-BPG can lead to sudden hypoxia, since it can cause a left-shifted ODC.

Chloride Shift in Tissues



Chloride Shift, in Lungs



Transport of Carbon Dioxide

1. Dissolved Form

About 10% of CO_2 is transported as dissolved form.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

The hydrogen ions thus generated, are buffered by the buffer systems of plasma.

2. Isohydric Transport of Carbon Dioxide

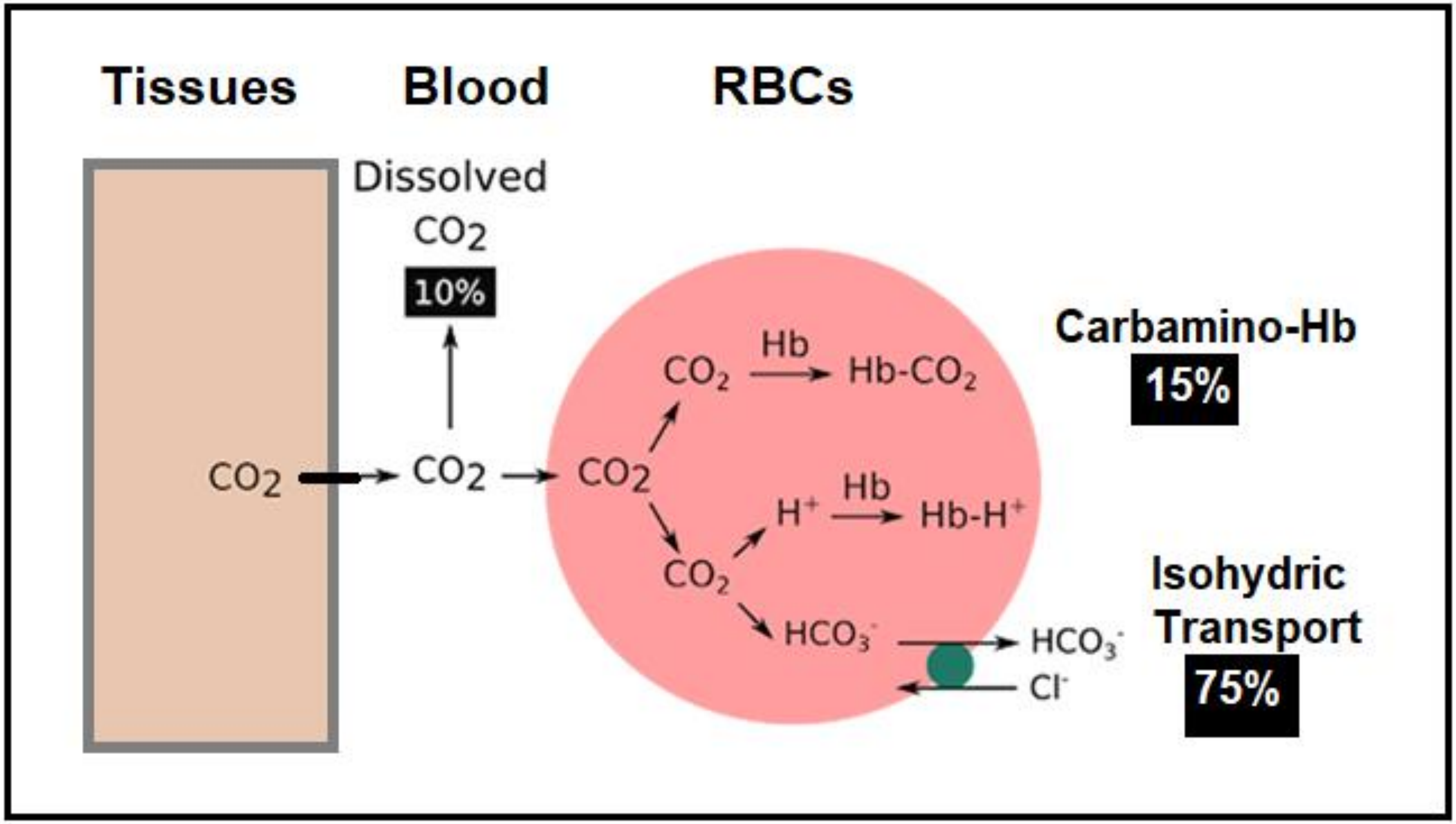
Isohydric transport constitutes about 75% of CO_2. It means that there is a minimum change in pH during the transport. The H+ ions are buffered by the deoxy-Hb and this is called the Haldane effect.

3. Carriage as Carbamino Hemoglobin

The rest 15% of CO_2 is carried as carbamino-hemoglobin, without much change in pH. A fraction of CO_2 that enters into the red cell is bound to Hb as a carbamino complex.

$$R\text{–}NH2 + CO_2 \text{--------}\rightarrow R\text{–}NH\text{–}COOH$$

Transport of Carbon Dioxide



Embryonal, Fetal and Adult Hemoglobins

Name of hemoglobin	Globin chains present	Period of life
Hb Gower I **Hb Gower2** **Hb Portland**	2 Zeta, 2 epsilon 2 alpha, 2 epsilon 2 gamma, 2 delta	Embryonal hemoglobins. Upto the end of first trimester of pregnancy
Fetal Hb or Hb F	2 alpha 2 gamma	Second trimester to early post natal life, upto 2 years
Hb A (adult Hb)	2 alpha ,2 Beta	Immediately after birth to end of life span
Hb A2 (adult)	2 alpha ,2 delta	Adult life

Hemoglobin Derivatives

- Hemoglobin derivatives are formed by the combination of different ligands with the heme part, or change in the oxidation state of iron.
- Oxy-Hb is dark red, deoxy-Hb is purple, met-Hb is dark brown, COHb is cherry red and sulph-Hb is green in color.
- Normally concentration of deoxy-Hb is less than 5% of the total Hb.
- If the level increases **cyanosis** occurs.

Carboxy-Hemoglobin (Carbon Monoxy Hb) (CO-Hb)

- Hemoglobin binds with carbon monoxide (CO) to form carboxy-Hb.
- The affinity of CO to Hb is 200 times more than that of oxygen.
- It is then unsuitable for oxygen transport.
- When one molecule of CO binds to one monomer of the hemoglobin molecule, it increases the affinity of others to O_2; so that the O_2 bound to these monomers are not released.
- This would further decrease the availability of oxygen to the tissues.

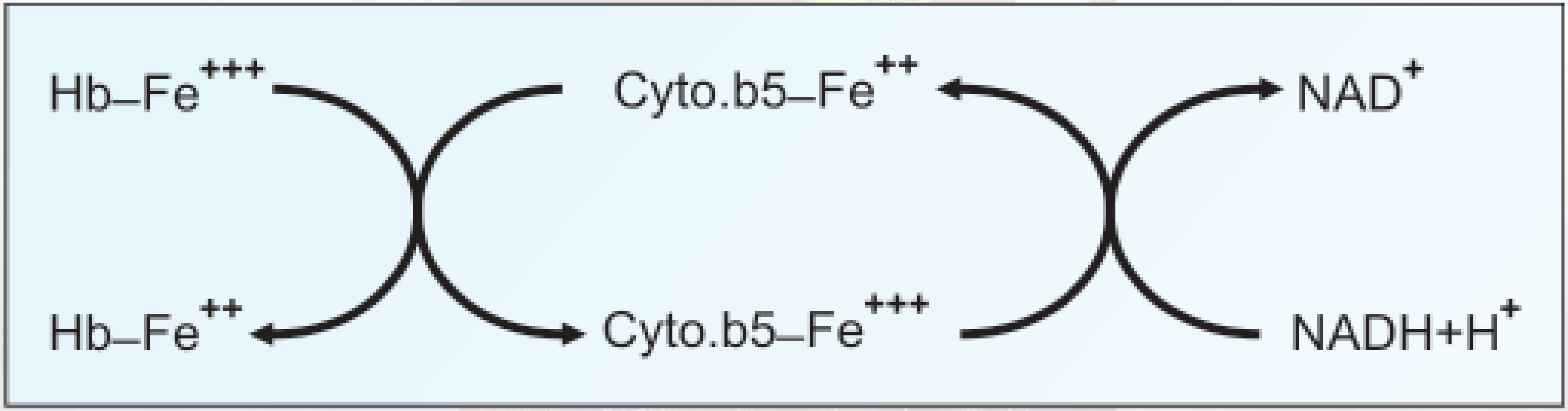
Carbon Monoxide Poisoning

- CO is a colorless, odorless, tasteless gas generated by incomplete combustion.
- CO poisoning is a major occupational hazard for workers in mines.
- Breathing the automobile exhaust in closed space is the commonest cause for CO poisoning.
- The carboxy-Hb level in normal people is 0.16%.
- An average smoker has an additional 4% of CO-Hb.
- One cigarette liberates 10–20 ml carbon monoxide into the lungs.

- Clinical symptoms manifest when carboxy-Hb levels exceed 20%.
- Symptoms are breathlessness, headache, nausea, vomiting, and pain in chest.
- At 40-60% saturation, death can result.
- Administration of O_2 is the treatment.
- In severe cases, oxygen under high pressure (hyperbaric oxygen) is helpful.

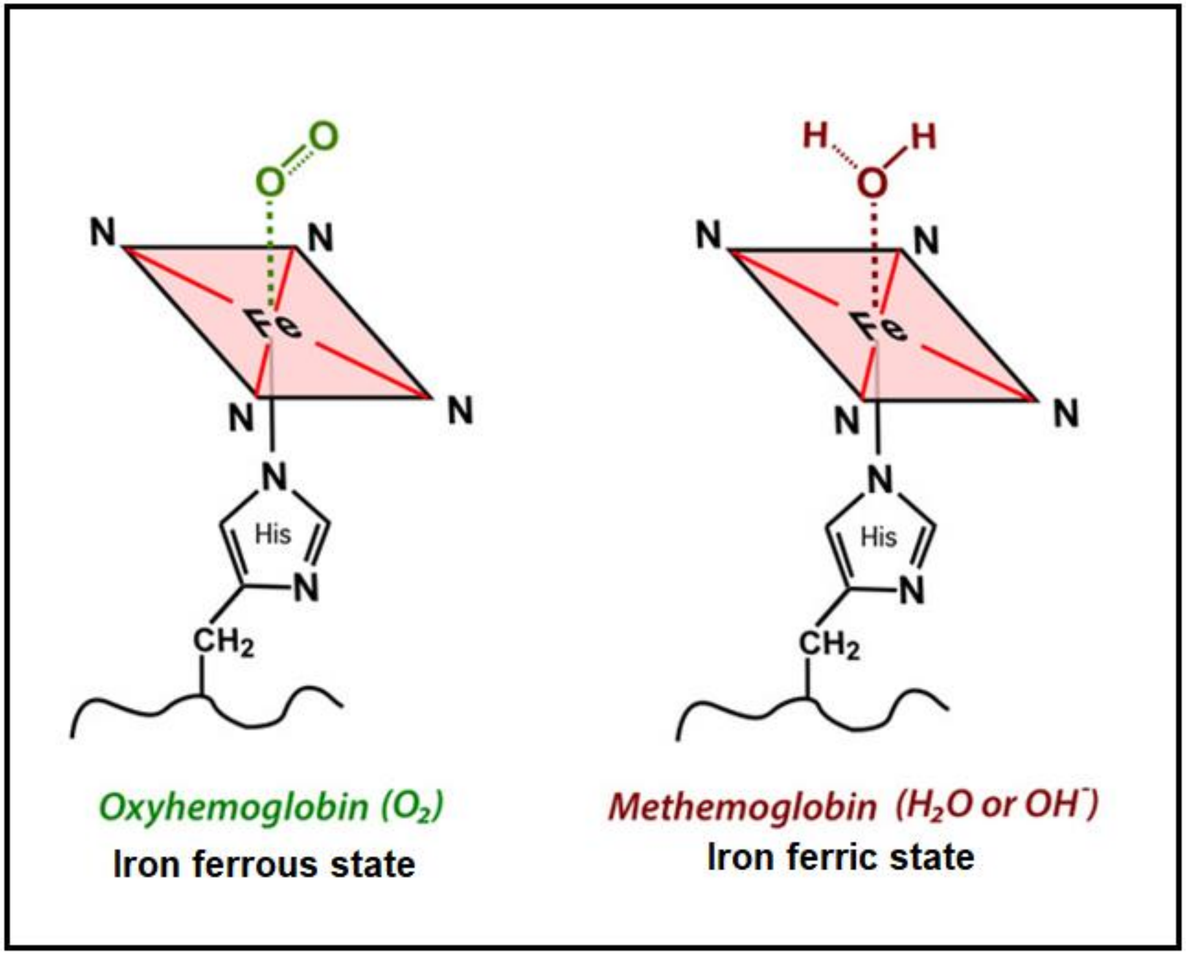
Met-hemoglobin (Met-Hb)

- When the ferrous (Fe++) iron is oxidized to ferric (Fe+++) state, met-Hb is formed.
- Small quantities of met-Hb formed in the RBCs are readily reduced back to the ferrous state by met-Hb reductase enzyme systems.
- About 75% of the reducing activity is due to enzyme system using **NADH and cytochrome b5.**



Methemoglobin Reductase System

Another 20% of the reducing activity is due to **NADPH dependent system.**
Glutathione dependent Met-Hb-reductase accounts for the rest 5% activity.



Difference between OxyHb and MetHb.

Met-hemoglobinemias

- Normal blood has only less than 1% of met-hemoglobin.
- It has markedly decreased capacity for oxygen binding and transport.
- An increase in methemoglobin in blood, (met-hemoglobinemia) is manifested as **cyanosis.**
- **Causes may be congenital** or acquired.

Congenital Met-hemoglobinemia

- Cytochrome b5 reductase deficiency is characterized by cyanosis from birth.
- 10-15% of hemoglobin may exist as met-hemoglobin.
- Oral administration of methylene blue, 100-300 mg/day or ascorbic acid 200-500 mg/day decreases met-Hb level to 5-10% and reverses the cyanosis.

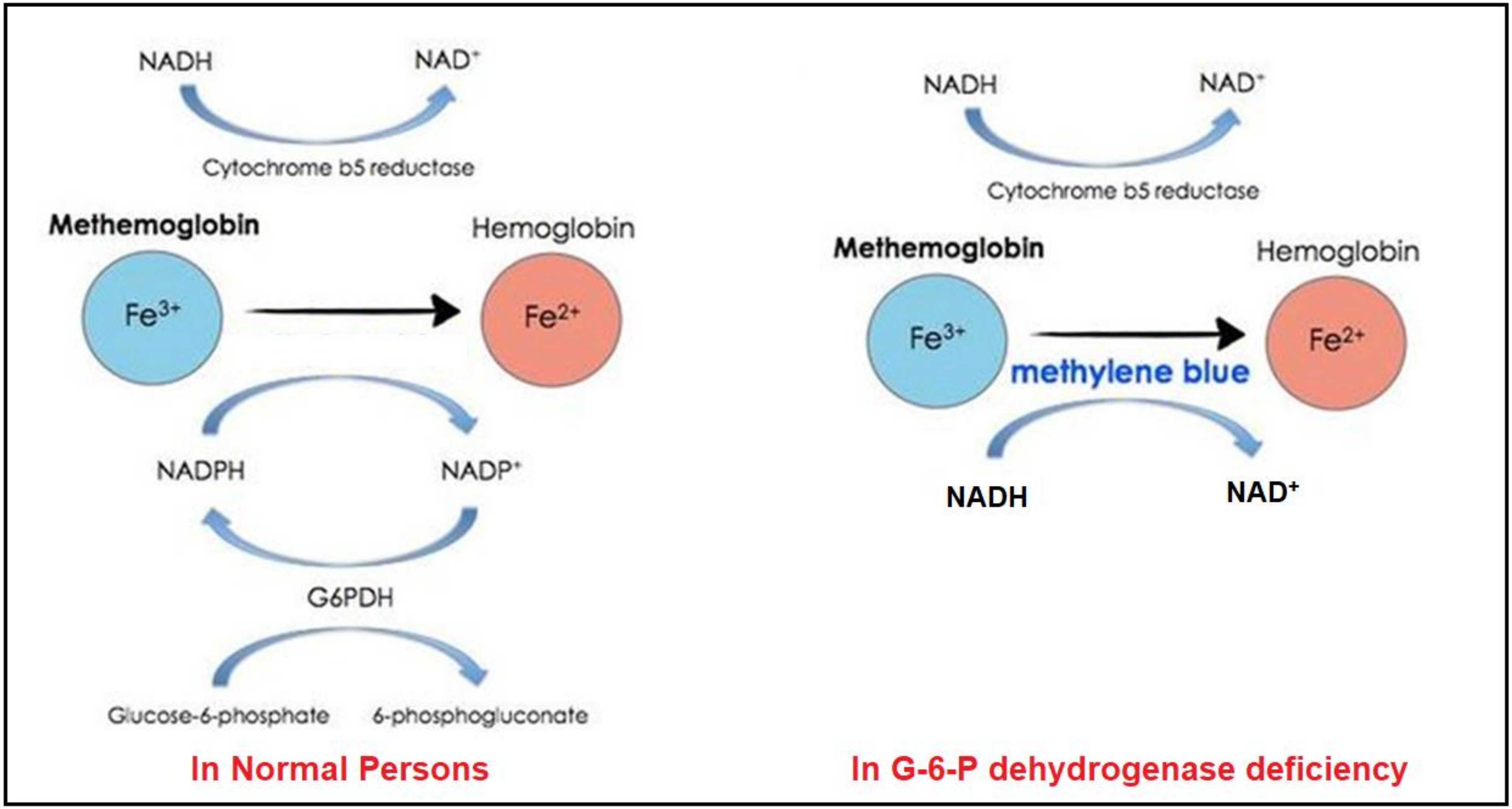
Acquired or Toxic Met-hemoglobinemia

- Met-hemoglobinemia may develop by intake of water containing nitrates or due to absorption of aniline dyes.
- Aniline dye workers have been known to develop met-hemoglobinemia.
- Drugs which produce met-hemoglobinemia are: acetaminophen, phenacetin, sulphanilamide, amyl nitrite, and sodium nitroprusside.

Glucose-6-phosphate Dehydrogenase Deficiency

- In persons with this enzyme deficiency, the condition may be manifested even with small doses of drugs.
- In such persons, NADPH is not available in the RBC.
- In such individuals, disease is manifested easily.
- In such patients, intravenous leukomethylene blue 2 mg/kg is effective, which will substitute for the NADPH.

Glucose-6-phosphate Dehydrogenase Deficiency



Laboratory Analysis

- Ferricyanide can oxidize oxy- or deoxy-Hb to metHb.
- The color changes to dark brown and absorption spectra show a band in the red with its center at 633 nm, while the bands for Oxy-Hb persist.
- Sodium hydrosulfite or dithionite reconverts met-Hb to oxy-Hb.

Hemin Crystals

- When iron is oxidized to Fe+++, it has a net positive charge.
- It can combine with negatively charged chloride, to form hemin or hematin chloride.
- Hemin crystals can be prepared from even very old blood stains in medicolegal cases.
- Blood or eluted blood stains are heated with Nippe's fluid (1% solution of KCI, KBr and Kl in glacial acetic acid) over a glass slide, when dark brown rhombic crystals are seen under the microscope.
- The test is sensitive, but is answered by the heme part of blood of all species.

Sulf-hemoglobinemia

- When hydrogen sulfide acts on oxy-hemoglobin, sulf-hemoglobin is produced.
- It can occur in people taking drugs like sulphonamides, phenacetin, acetanilide, dapsone, etc.
- It cannot be converted back to oxy-hemoglobin.
- It is seen as basophilic stippling of RBC, throughout its lifespan.

Nitric Oxide

- Hemoglobin binds nitric oxide (NO) with high affinity similar to binding of carbon monoxide (CO).
- The NO is delivered at its site of action, i.e. capillary endothelium.
- The positive co-operative effect and effect of H+ also play a role in the binding and delivery of NO.
- Binding of NO by hemoglobin increases its half-life.

- The heme iron preferentially binds NO in the T conformation.
- When hemoglobin acquires the R conformation, the NO is transferred to a cysteinyl SH group on the beta chain and then to the SH group of a small molecule like GSH, when R form reverts to T state.
- The X-S-NO complex is biologically very potent and even under low oxygen tension delivers NO to tissue capillaries.

Hemoglobinopathies and Thalassemias

Abnormalities in the primary sequence of globin chains lead to **hemoglobinopathies,** e.g. hemoglobin S (HbS).

Abnormalities in the rate of synthesis would result in **thalassemias.** In other words, normal globin chains in abnormal concentrations result in thalassemias, e.g. beta thalassemia.

Hemoglobinopathies

- Hundreds of hemoglobin variants
- Alpha chain variants or beta chain variants.
- Gamma and delta chain variants - Rare
- The hemoglobin variants may be classified into 5 major types, based on their clinical manifestations.

Important Hemoglobinopathies

Hemoglobin	Point mutation position	Amino acid substitution	Codon and base substitution
HbS	Beta 6	Glu → Val	GAG→ GUG
HbC	Beta 6	Glu → Lys	GAG → AAG
HbE	Beta 26	Glu → Lys	GAG → AAG
HbD (Punjab)	Beta 121	Glu → Gln	GAG → CAG
HbM	Proximal or distal histidine in α or β chains	His → Tyr	CAC → UAC

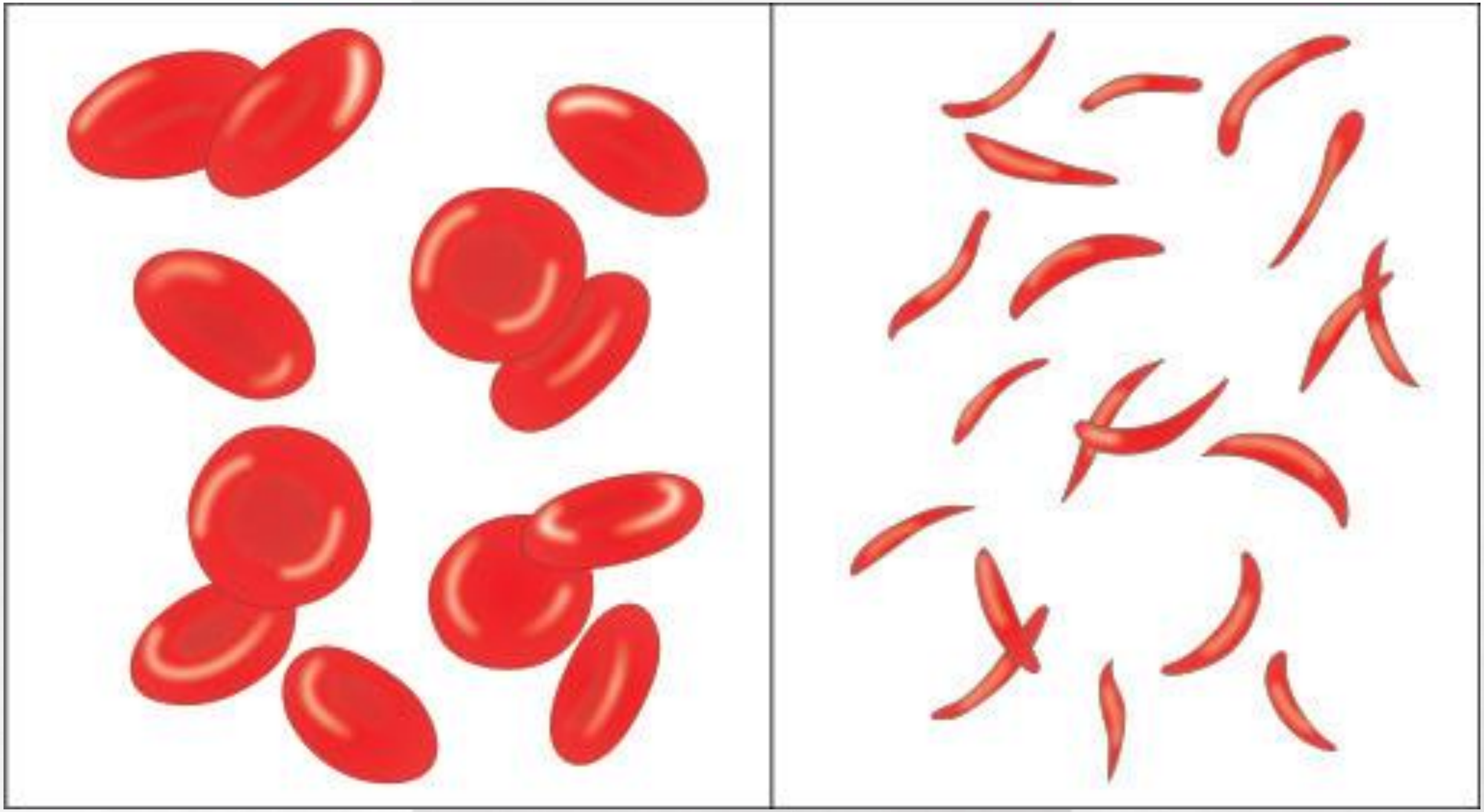
1. Sickle syndromes

- A. Sickle-cell trait (AS)
- B. Sickle-cell disease with SS, SC, SD, SO varieties and S beta thalassemia

2. Unstable hemoglobins
3. Hemoglobins with abnormal O2 affinity
4. Structural variations leading to thalassemia
5. Non symptomatic Hb variants – HbP, Q, N, J

Hemoglobin S (HbS) (Sickle Cell Hemoglobin)

- HbS constitutes the **most common variety worldwide.**
- Hemoglobin with abnormal electrophoretic mobility is responsible for the sickling disease (Pauling, 1949).

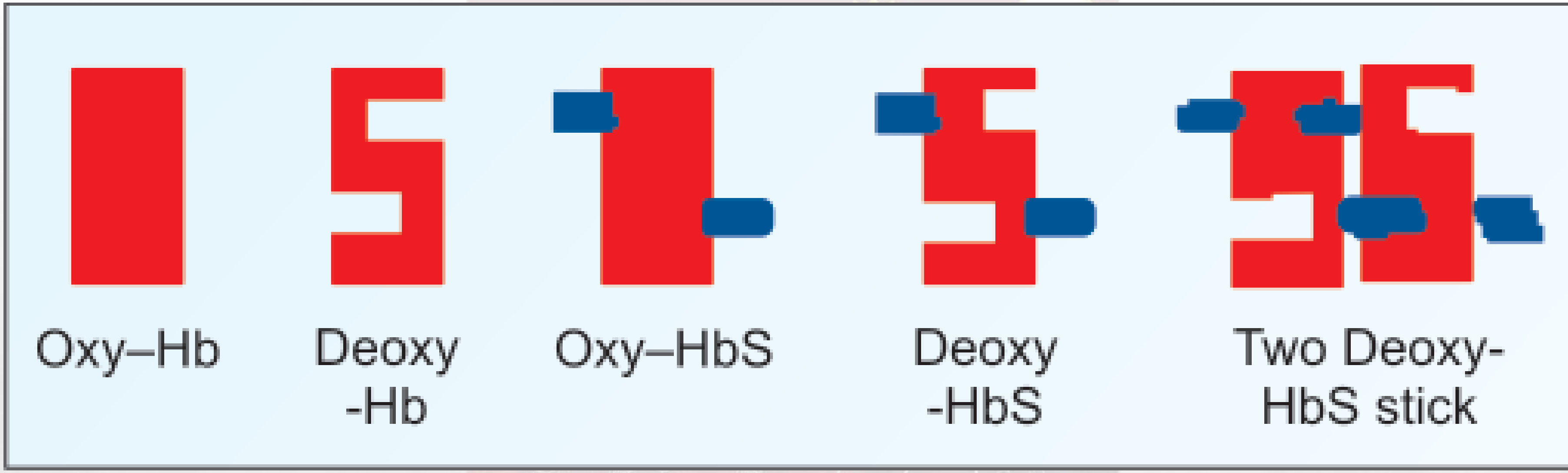


Sickle Cell Disease

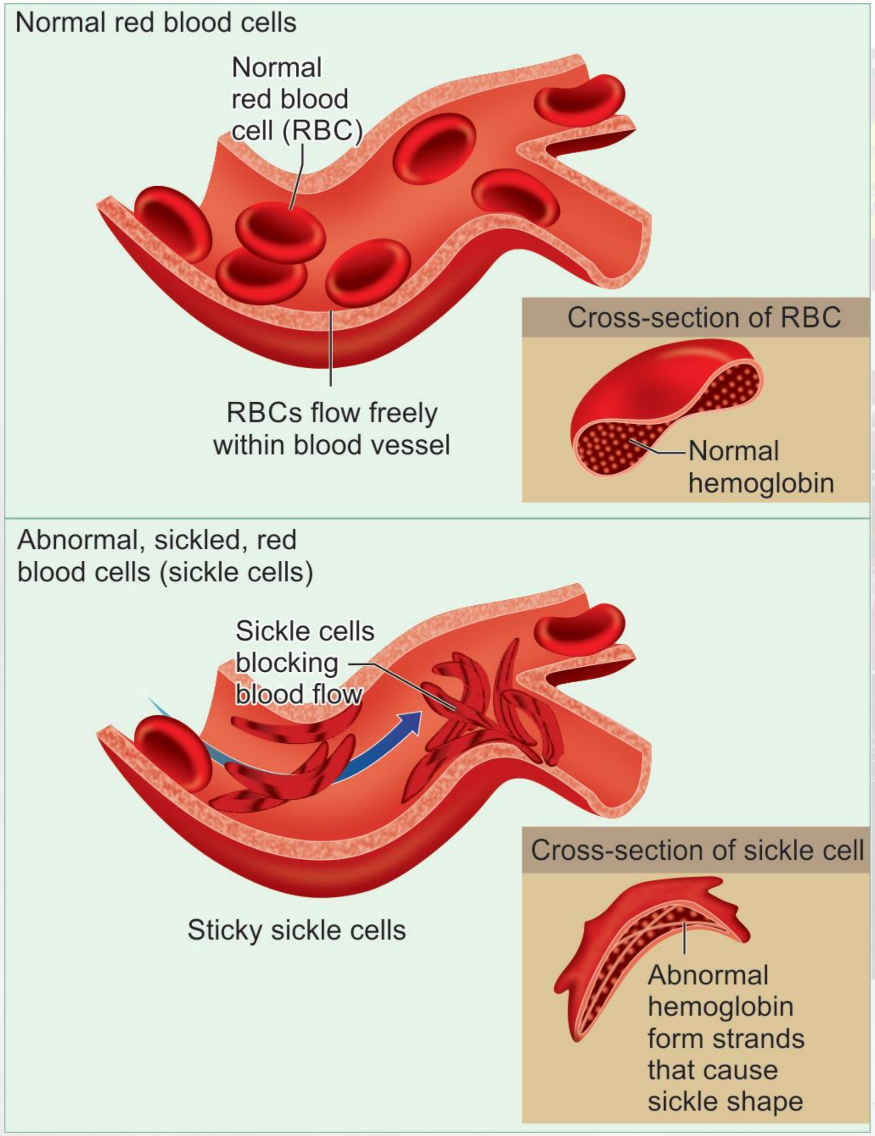
- The glutamic acid in the **6th position of beta** chain of HbA is changed to valine in HbS.
- Leads to polymerization of hemoglobin molecules inside RBCs.
- Causes a distortion of cell into sickle shape.
- The substitution of hydrophilic glutamic acid by hydrophobic valine causes a localized stickiness on the surface of the molecule.

- The deoxygenated HbS depicted with a protrusion on one side and a cavity on the other side, so that many molecules can adhere and polymerize.
- HbA and HbF will prevent sickling, because they do not co-polymerize with HbS.
- HbS can bind and transport oxygen.
- The sickling occurs under deoxygenated state.

Sticky Patches on HbS Molecule



- The sickled cells form small plugs in capillaries.
- Occlusion of major vessels can lead to infarction in organs like spleen.
- Death usually occurs in the second decade of life.
- Heterozygous state is very common in Central and West Africa, East and Central India.
- Tribals all over India show an increased incidence of SS and AS.
- The slave trade has played an important role in spreading the gene from Africa to different parts of America.



Mechanism of vascular occlusion in sickle cell anemia.

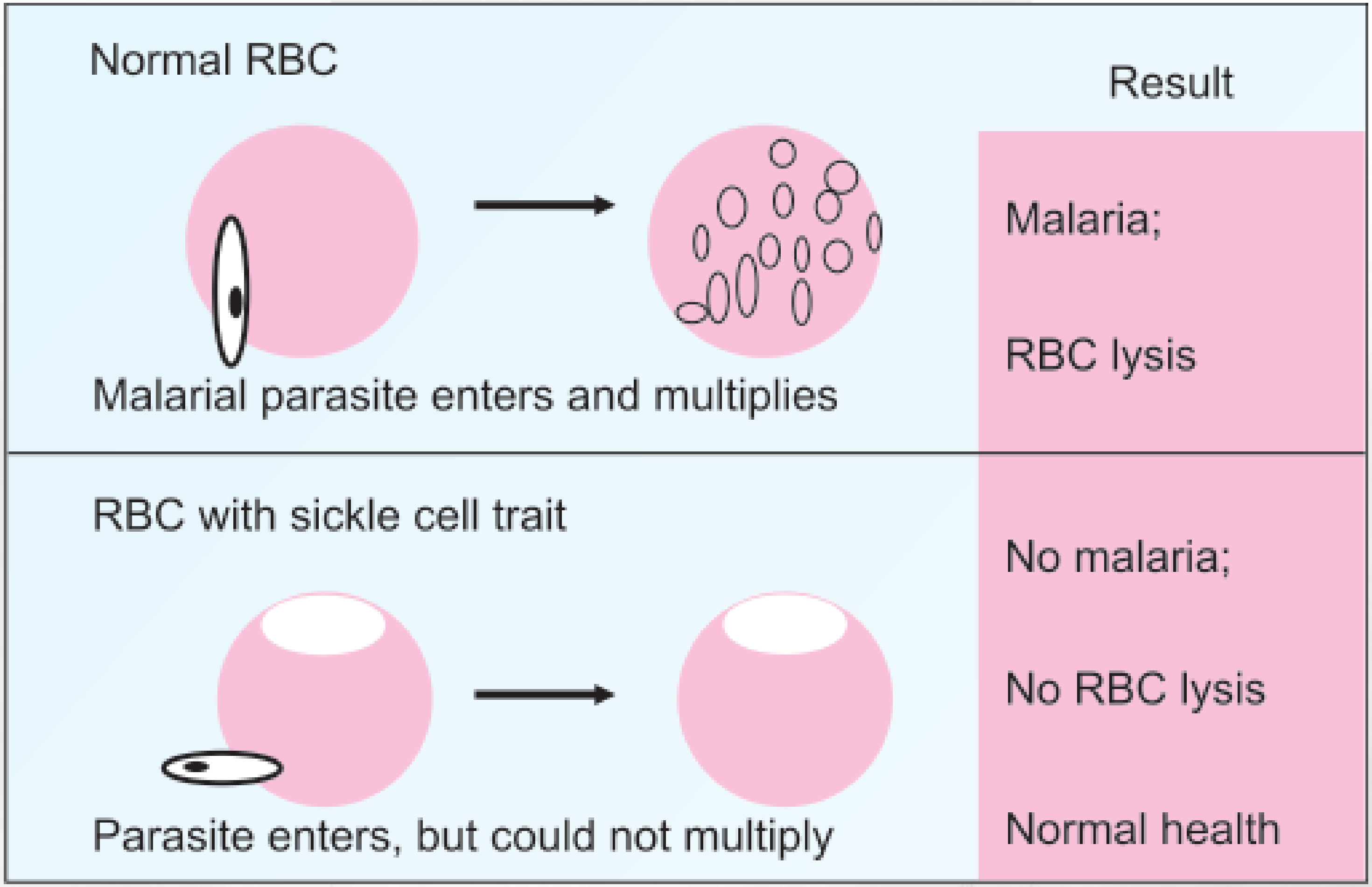
Sickle Cell Trait

- Heterozygotes (AS) - 50% of Hb normal.
- Sickle cell trait as such does not produce clinical symptoms.
- Such persons can have a normal lifespan.
- At higher altitudes, **hypoxia may cause** manifestation of the disease.
- Chronic **lung disorders may also produce hypoxia-induced** sickling in HbS trait.

HbS gives Protection Against Malaria

- The high incidence of the sickle cell gene in population coincides with the area endemic for malaria.
- HbS affords protection against *Plasmodium falciparum infection.*
- Hence the abnormal gene was found to offer a biologic advantage.

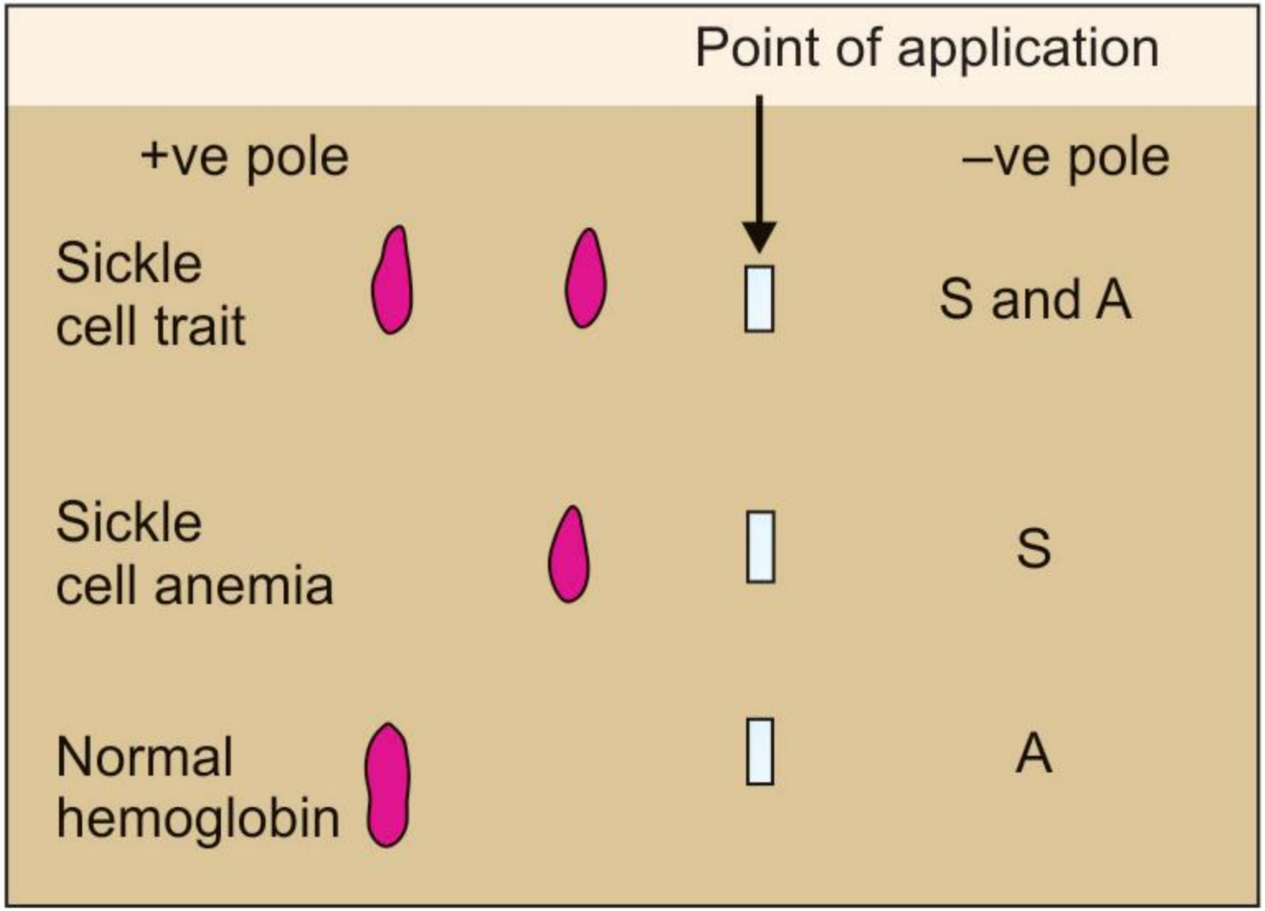
Sickle Cell Trait Protects from Malaria



Electrophoresis

- Electrophoresis at alkaline pH shows a slower moving band than HbA.
- At pH 8.6, carboxyl group of glutamic acid is negatively charged.
- Lack of this charge on HbS makes it less negatively charged, and decreases the electrophoretic mobility towards positive pole.
- At acidic pH, HbS moves faster than HbA. In sickle cell trait, HbA and HbS.

Electrophoresis at pH 8.6



In the electrophoresis, the abnormal HbS can be detected along with normal Hb in persons with HbS trait.

Sickling Test

- A blood smear is prepared.
- A reducing agent such as sodium dithionite is added.
- Blood smear examined under the microscope shows sickled RBCs.

Management of Sickle Cell Disease

- Repeated blood transfusions may be required in severe anemia.
- But this can lead to iron overload and cirrhosis.
- Treatment with anti-sickling agents like urea, cyanate and aspirin, that interfere with polymerization are tried.
- Sodium butyrate will induce HbF production with clinical improvement.

Hemoglobin E

- It is the **second most prevalent** variant.
- It is due to the replacement of beta 26 glutamic acid by Lysine.
- It is primarily seen in orientals of South-East Asia (Thailand, Myanmar, Bangladesh, etc).
- The variant is very prevalent in West Bengal.
- Heterozygotes are completely asymptomatic.
- Similar mobility as of A2 on electrophoresis.

Other Hemoglobin Variants

1. **Hb C-** 6^{th} amino acid beta chain glutamic acid is replaced by lysine.

- Double heterozygotes (HbSC) have moderate disease.
- Homozygotes (CC) have hemolytic anemia.

2. **Hb D** – Beta 121 glutamic acid to glutamine (HbD Punjab). HbSD is severe disease.
3. **Hb M** – Proximal or distal hisitidine substitutions (HbM Boston, HbM Hyde Park).

Molecular differences of HbA, HbS and HbC

HbA

Val	His	Leu	Thr	Pro	**Glu**	Glu	Lys
1	2	3	4	5	6	7	8

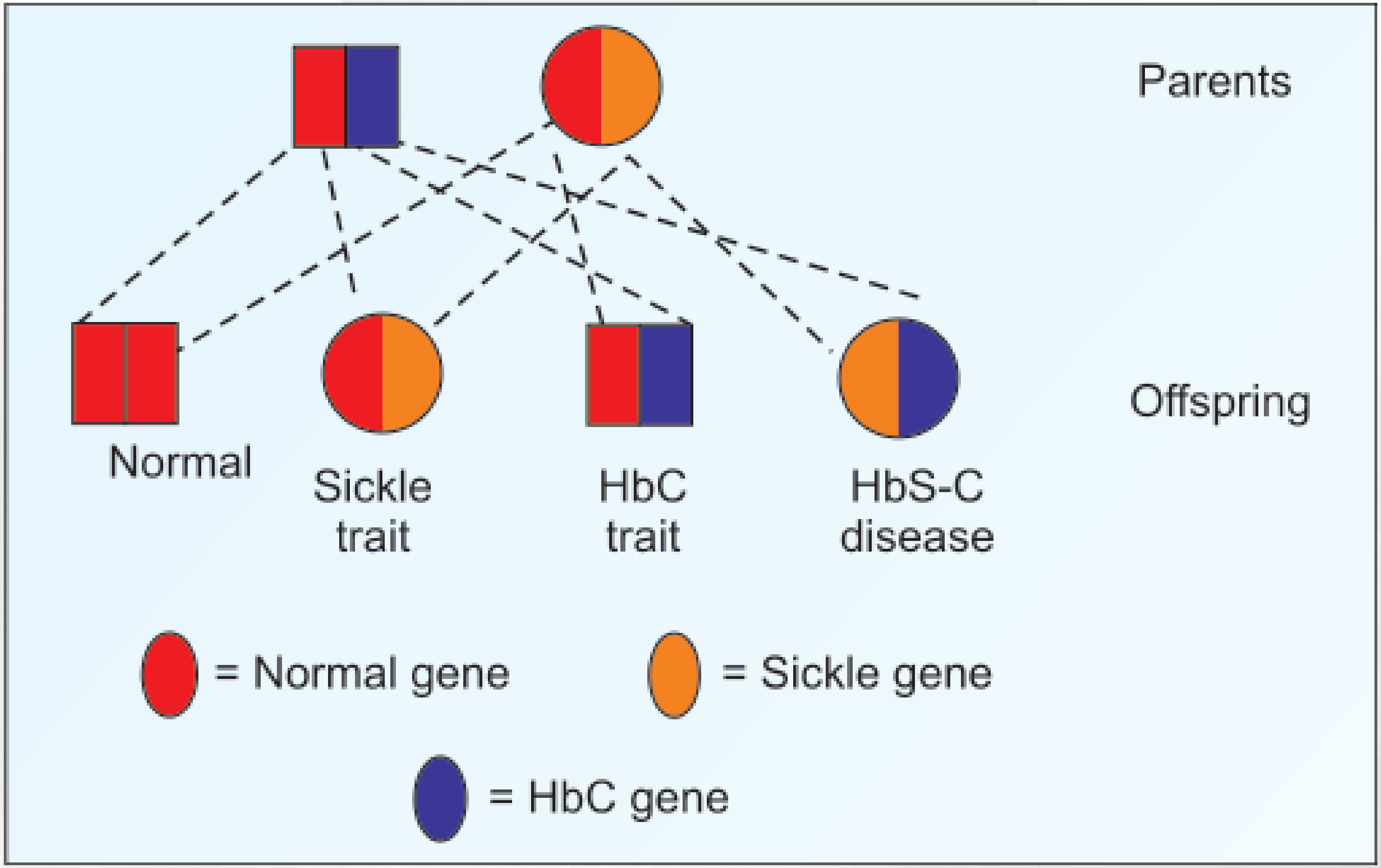
HbS

Val	His	Leu	Thr	Pro	**Val**	Glu	Lys
1	2	3	4	5	6	7	8

HbC

Val	His	Leu	Thr	Pro	**Lys**	Glu	Lys
1	2	3	4	5	6	7	8

Inheritance of HbC Trait and Generation of HbS-C Disease



Thalassemias

- The name is derived from the Greek word, "*thalassa*", *which means "sea"*.
- *Greeks identified* this disease present around Mediterranean sea.
- Thalassemia may be defined as the normal hemoglobins in abnormal proportions.
- The gene function is abnormal, but there is no abnormality in the polypeptide chains.
- Reduction in alpha chain synthesis is called alpha thalassemia, while deficient beta chain synthesis is the beta thalassemia.
- Other types like delta-beta thalassemia, Hb Lepore, hereditary persistence of HbF (HPF) are related conditions.

Beta Thalassemia

- Beta thalassemia is more common than alpha variety.
- Beta type is characterized by a decrease or absence of synthesis of beta chains.
- As a compensation, gamma or delta chain synthesis is increased.

Inheritance

- Beta thalassemias are phenotypically described as beta (+) or beta (o) depending on whether there is beta chain synthesis or not.
- Beta (o) thalassemia may result from base substitutions.
- Beta (+) thalassemias are produced from defects in post transcriptional processing of mRNA.

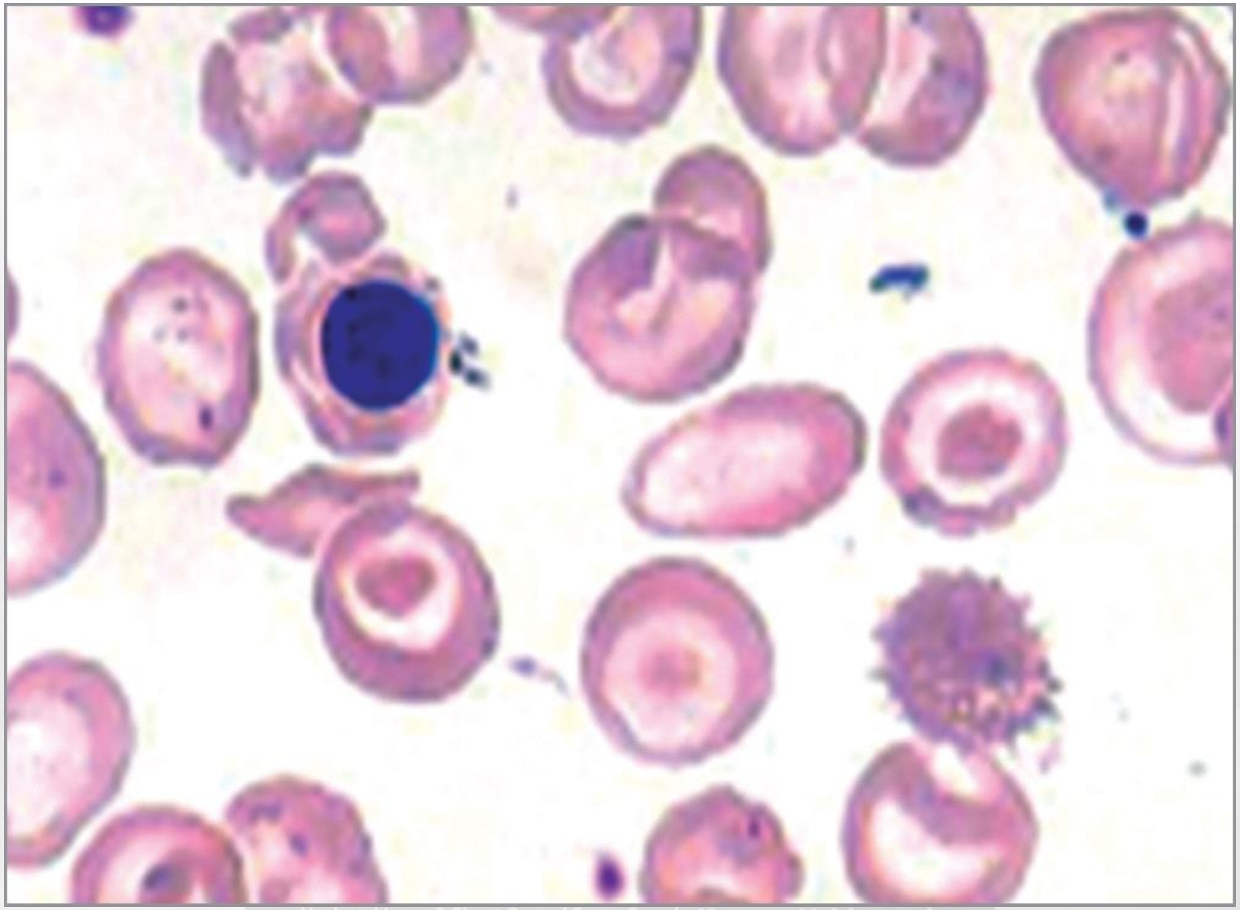
Alpha Thalassemias

- They may result from different types of gene deletions.
- Since there are 2 pairs of alpha genes per cell, a single gene deletion in one chromosome or a pair of genes in the chromosomes does not have much effect on a chain production.
- Alpha thalassemia is rarer because alpha chain deficiency is incompatible with life.

Thalassemia Syndromes

- These syndromes are mainly seen in people of Asian, African and Mediterranean origin.
- All cases of thalassemias are characterized by **deficit of HbA synthesis.**
- Hypochromic microcytic anemia is seen
- In homozygous state, clinical manifestations are severe, and hence called **Thalassemia major.**
- There will be nucleated RBCs in peripheral circulation.
- In heterozygous conditions, the clinical signs and symptoms are minimal; they are called Thalassemia **minor.**
- The synthesis of unaffected chains occurs at the normal rate.

- Since they do not have complementary chains to bind, they form aggregates and precipitate within the cell.
- These precipitates or **inclusion bodies lead** to membrane damage and destruction of red cells.
- The co-existence of HbS and beta thalassemia trait is fairly common.
- Homozygous beta thalassemia is characterised by severe anemia, hypersplenism and hepato splenomegaly.
- The marrow in the skull bones expand producing the "hair-on-end appearance" described in X-ray.



Thalassemia. Nucleated red blood cell, target cells, spherocytosis, and poikilocytosis are seen.

Expansion of marrow of skull bones lead to Hair-on-end appearance in beta Thalassemia

- It is the radiologic pattern seen as calcified spicules perpendicular to bone surface

- Repeated transfusion is the only available treatment.
- This may lead to iron overload.
- Splenectomy may also lessen the anemia.
- Marrow transplantation has been successfully tried in a few cases.

MYOGLOBIN (Mb)

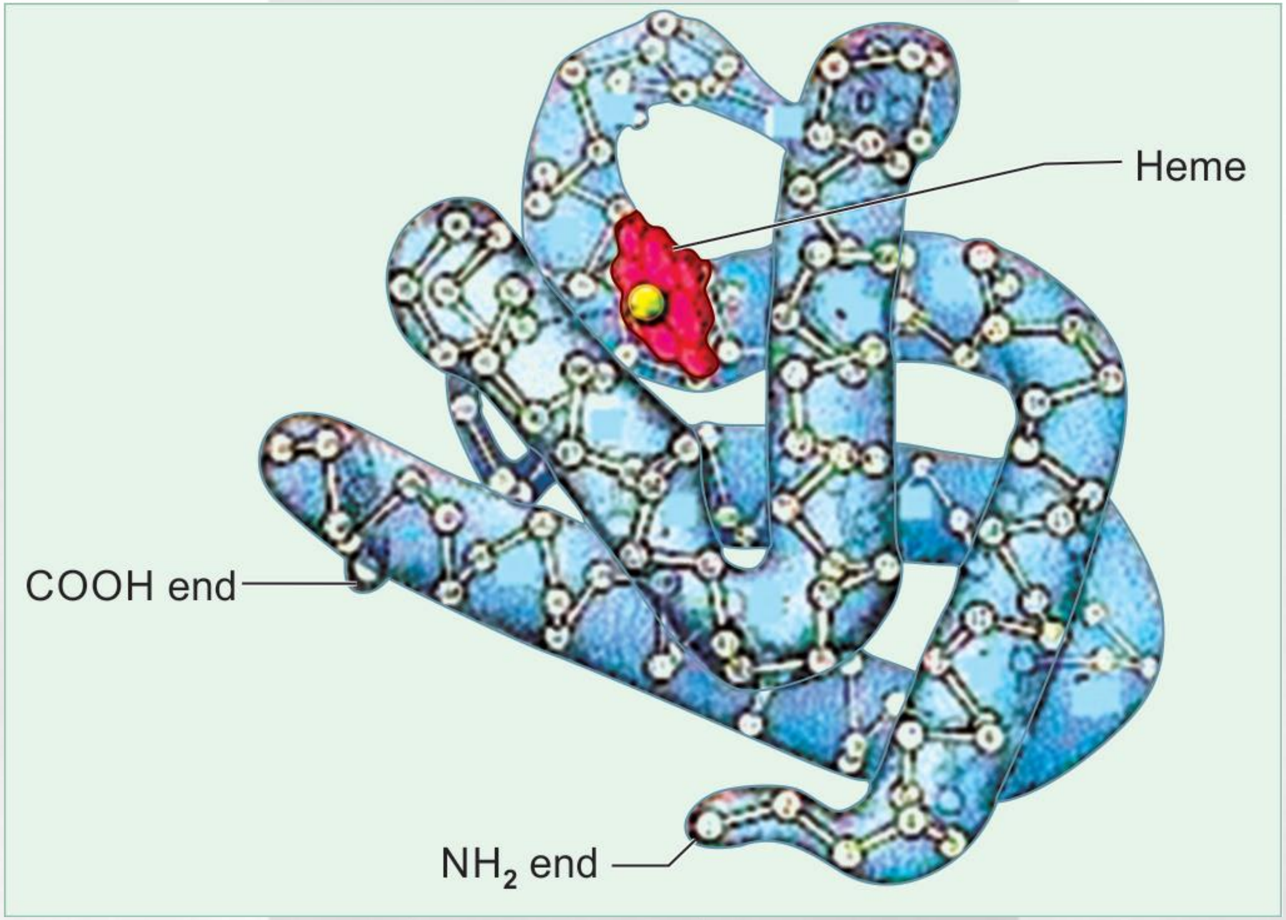
- One molecule of Mb can combine with 1 molecule of oxygen.
- In the muscles, the oxygen is taken up by Mb for the sake of tissue respiration.
- Mb has **higher affinity for oxygen than that** of Hb.
- The pO_2 in tissue is about 30 mm of Hg, when Mb is 90% saturated.
- At this pO_2, Hb saturation will be only 50%.

- In severe physical exercise, pO2 in muscles lowers to 5 mm Hg, when myoglobin releases all the bound oxygen.
- Mb has a high oxygen affinity while Bohr effect, co-operative effect and 2,3-BPG effect are absent.
- Severe crush injury causes release of myoglobin from the damaged muscles.

Clinical Significance of Myoglobin

Severe crush injury causes release of myoglobin from the damaged muscles. Being a small molecular weight protein, Mb is excreted through urine (myoglobinuria). Urine color becomes dark red.

Myoglobin will be released from myocardium during myocardial infarction, and is seen in serum. Serum myoglobin estimation is useful in early detection of myocardial infarction.



Myoglobin Chain.

Causes of Anemias

1**. Hemolysis due to impaired production of RBCs**

a. Defect in heme synthesis: Nutritional deficiency of iron, copper, pyridoxal phosphate, folic acid, vitamin B12 or vitamin C. Lead will inhibit heme synthesis.

b. Defect in regulators: Erythropoietin synthesis is reduced in chronic renal failure.

c. Defect in stem cells: Aplastic anemia due to drugs (e.g. Chloramphenicol), infections or malignant infiltrations.

2. Hemolysis due to intracorpuscular defect

a. Hemoglobinopathies such as HbS, HbC:

b. Thalassemias—major and minor

c. Abnormal shape: Spherocytosis and elliptocytosis.

d. Enzyme deficiencies: Deficiency of glucose-6- phosphate dehydrogenase.

Causes of Anemias, Continued

3. Hemolysis due to extracorpuscular causes

   a. Infections: Malarial parasites

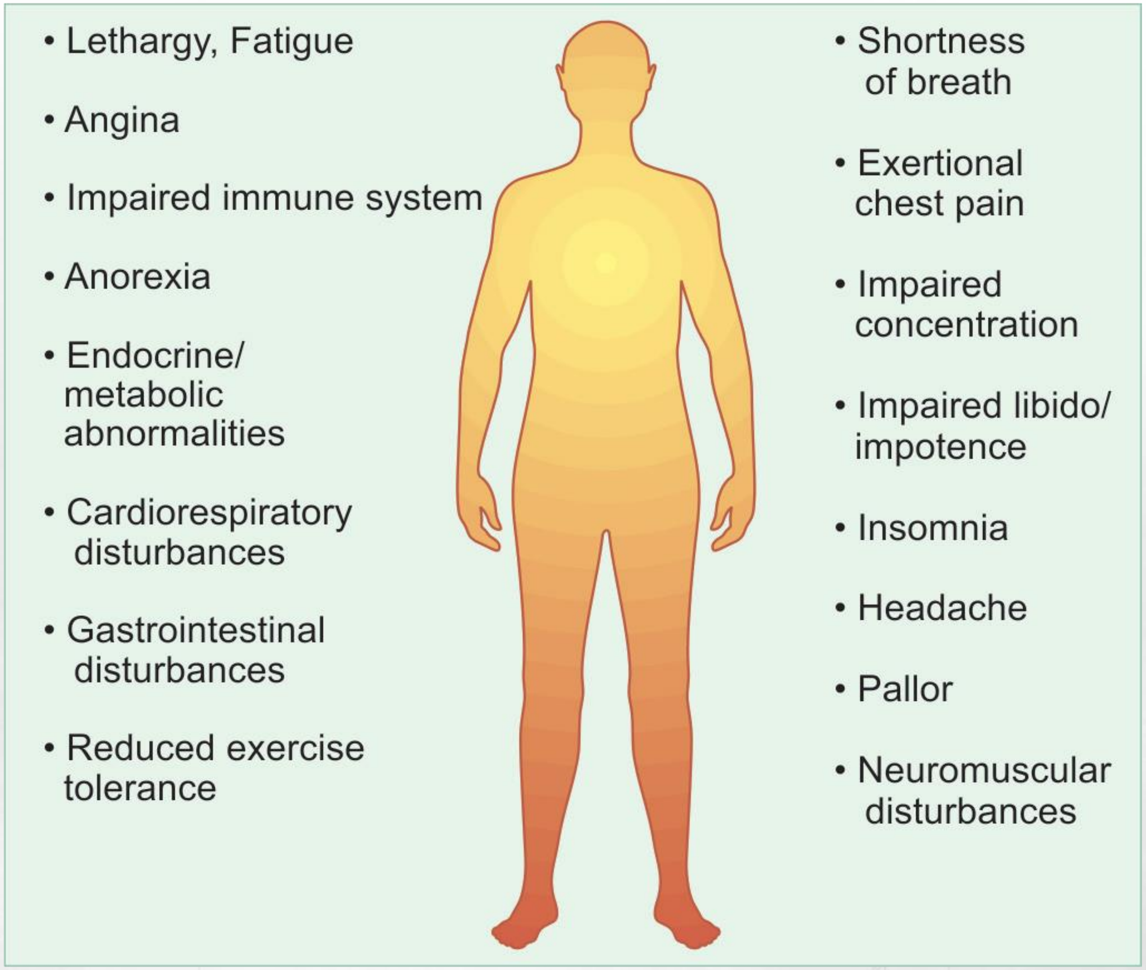
   b. Autoimmune hemolysis: Antibodies are seen against RBC membrane components.

   c. Isoimmune hemolysis: Rh incompatibility.

   d. Hemolysis due to drug sensitisation: Many drugs (e.g. alpha-methyl dopa, quinine) may fix on RBC membrane, and produce antibodies against the altered membrane.

4. Hemorrhage

Hematuria, hematemesis, hemoptysis, peptic ulcer metrorrhagia and hemorrhoides are the usual causes for hemorrhage. Hemophilia (absence of AHG) and thrombocytopenia are other major causes for bleeding tendencies.



Common symptoms of anemia.