CHEMISTRY OF HEMOGLOBIN

- Describe structure of hemoglobin and structure function relationships
- Describe the transport of oxygen and carbondioxide by hemoglobin
- Explain the functional relevance of physiological factors in gas exchange and role of hemoglobin in buffering
- Enumerate the derivatives of hemoglobin normally found in blood and indicate their clinical relevance
- Describe the genesis of hemoglobinopathies and thalassemias and their molecular pathology
- Mention the clinical features of sickle cell anemia, thalassemias and other common hemoglobin variants
- Explain the pathophysiology and laboratory diagnosis of these molecular diseases
- Enumerate the functions of myoglobin
- Classify the different types of anemias, causes and salient features of nutritional and hemolytic anemias

- **Haemoglobin** is the Red blood pigment of erythrocytes
- Carries O_2 from lungs to tissues &CO_2 from tissues to lungs

Structure of Hemoglobin

- It is **globular , tetrameric protein**.
- It is a **conjugated protein**
- Hemoglobin → **Heme –prosthetic** group(nonprotein part)

  &

  globin-**apoprotein** part

Structure of Heme

- The characteristic red colour of Hb and blood is due to **heme**
- **Heme** is present as prosthetic group in Hb as well as other compounds like **cytochromes, catalase, tryptophan pyrrolase**
- Heme is a **metalloporphyrin** & made up of **protoporphyrin IX (III)** with **iron** at its centre

- Protoporphyrin IX is a **cyclic tetrapyrrole** to which **4 methyl, 2 vinyl and 2 propionate** groups are attached .
- Each pyrrole ring is a **5 –membered ring** structure containing **N atom**.
- The 4 pyrroles are linked in a planar ring by four **methylene bridges**

- The **ferrous iron** can form **6 coordination** bonds.
- The ferrous iron is attached to **4 N atoms** of porphyrin ring.
- At the **fifth** coordination position, it is attached to **N of proximal Histidine residue of globin**
- The **sixth coordination position** is occupied by **oxygen molecule**

- 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.**

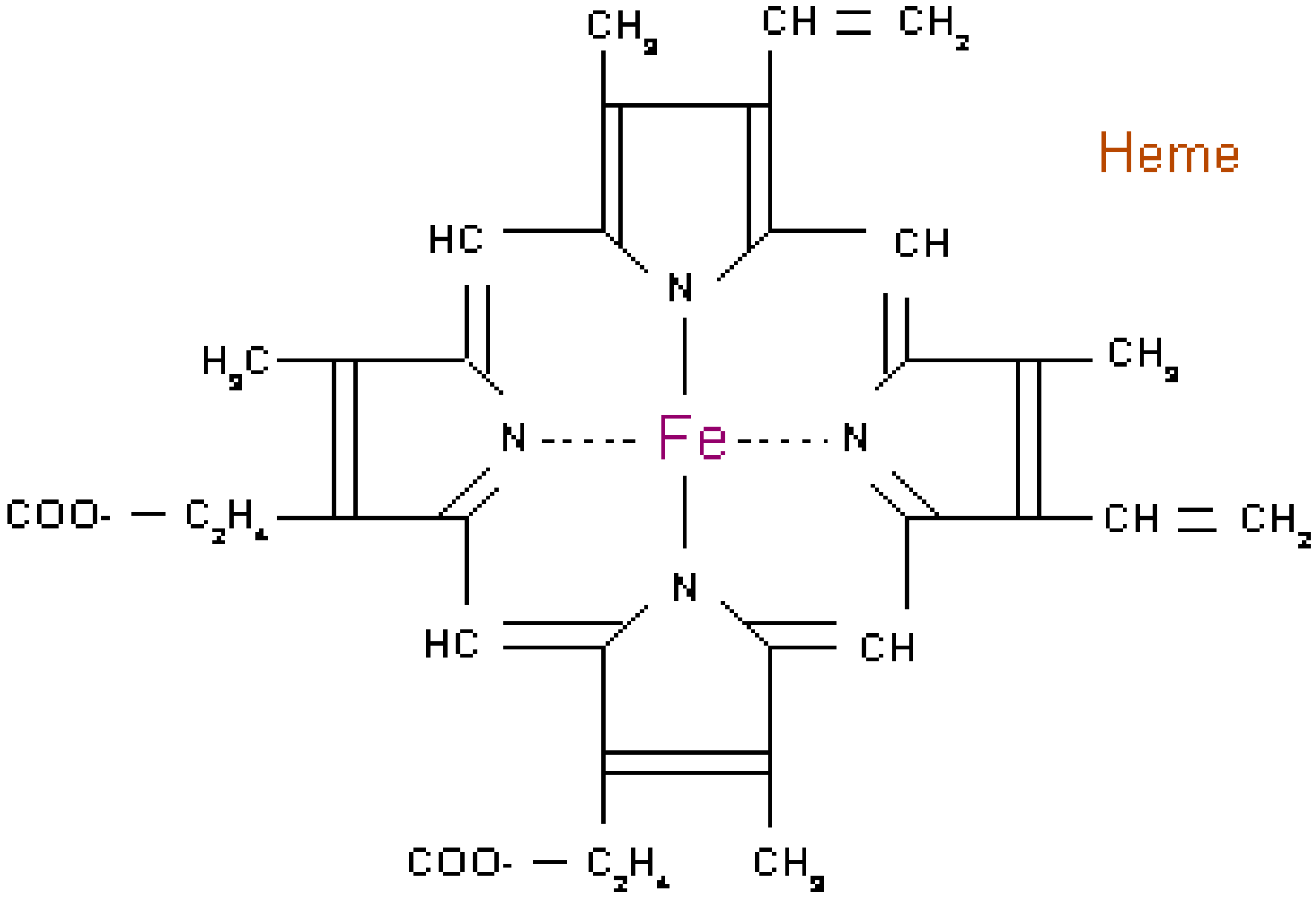
- Each alpha chain has **141 amino acids**.

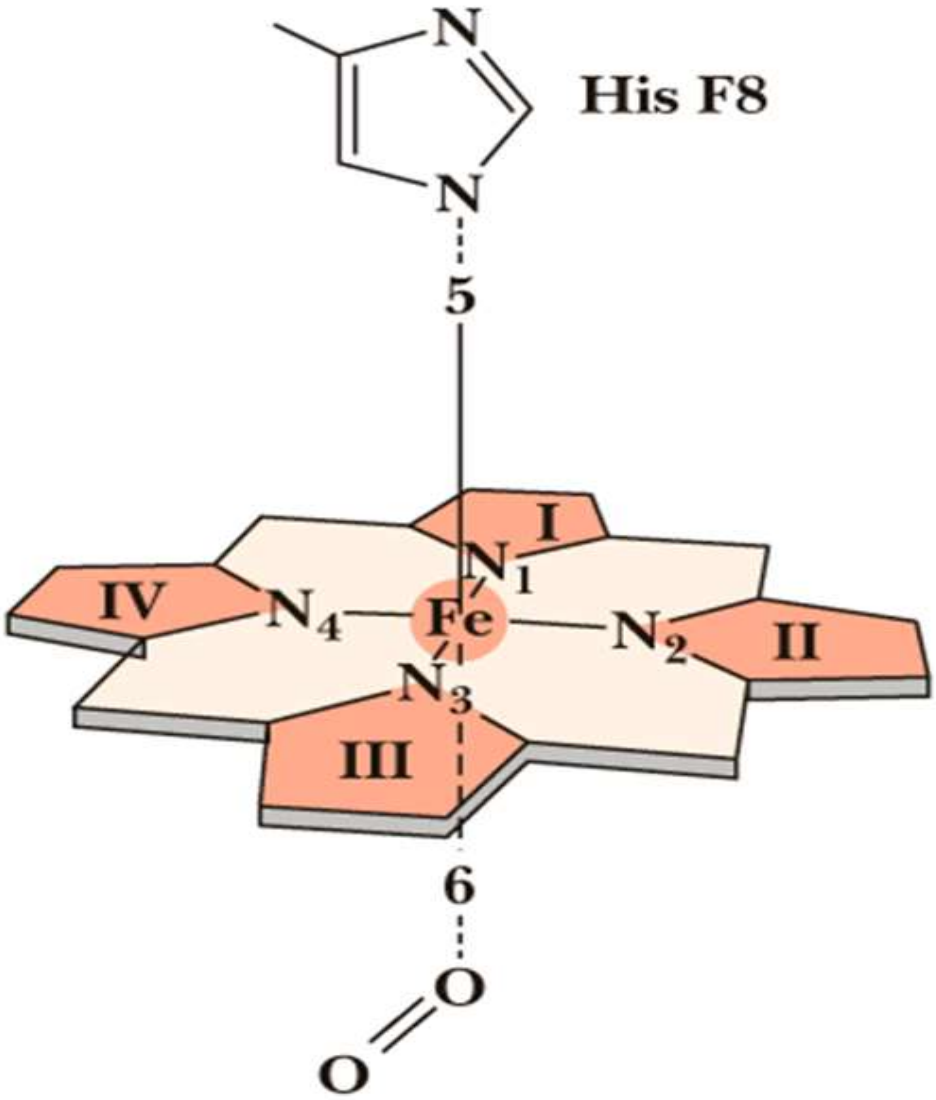
- The beta, gamma and delta chains have **146 amino acids.**

- There are **36 histidine** residues in Hb molecule; these are important in **buffering action**.

- The **58th 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.**


Heme
CH3
CH = CH2
HC
CH
N
H3C
CH3
N
Fe
N
COO- — C2H4
CH = CH2
N
HC
CH
COO- — C2H4
CH3



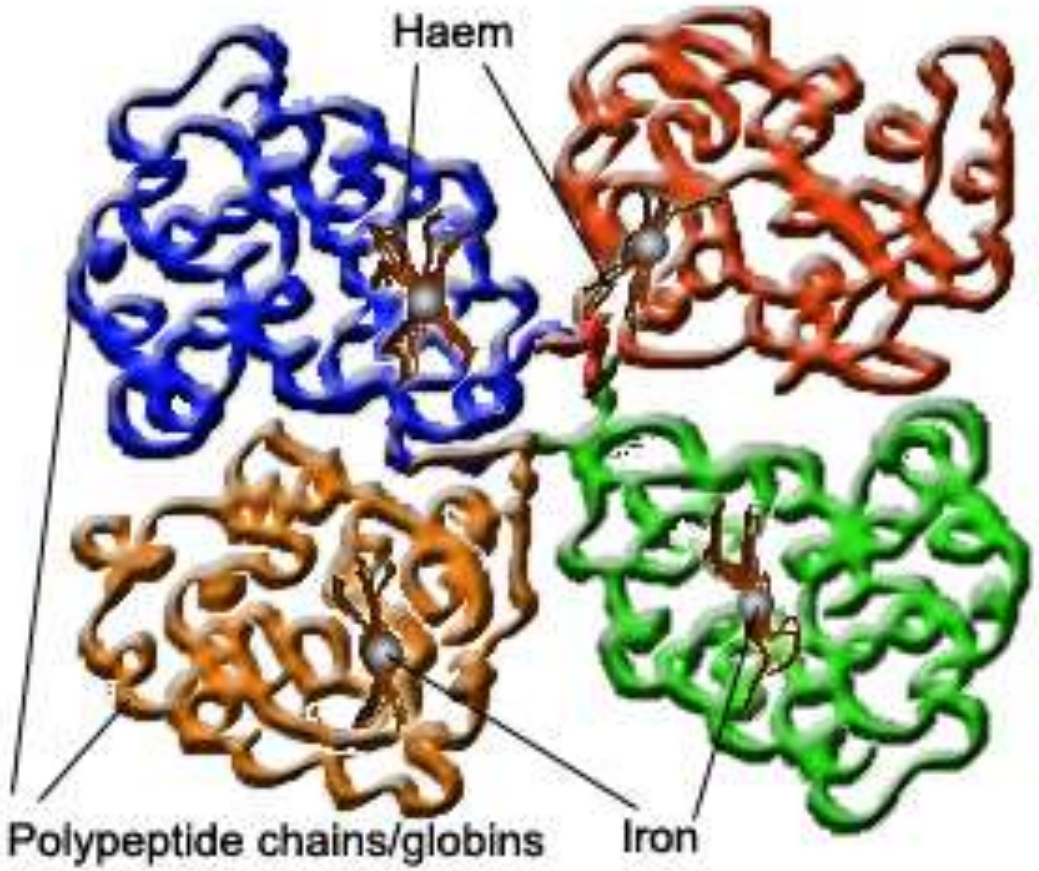
The heme plane

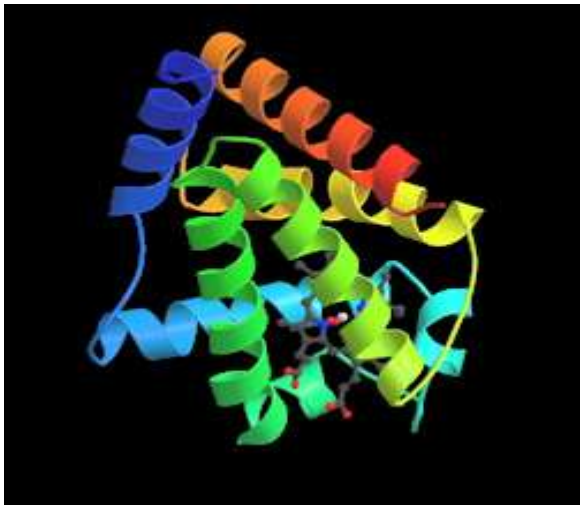
B. **Structure of Globin Chain**

- **Globin** is composed of **4** polypeptide chains
- **Hb A1** -2 α chains &2 β chains
- α chain -141 amino acids
- β chain -146 amino acids
- Total **574 amino acids**
- **Hb A2** – 2 α chains & 2 δ chains
- **Hb F** -2 α chains & 2 γ chains

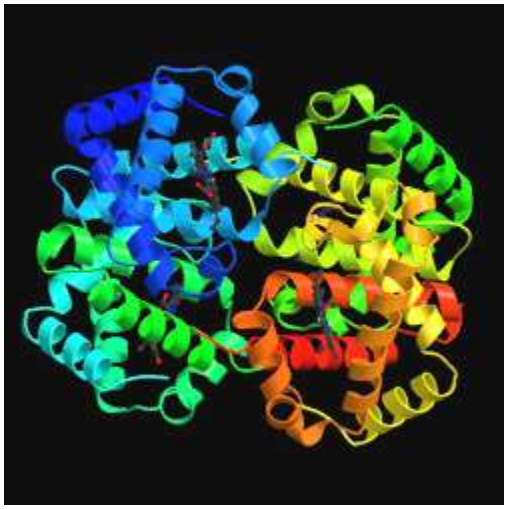
- The 4 polypeptide chains are held together by **non-covalent interactions**
- Each polypeptide chain of globin is attached to **one heme group**

One haemoglobin molecule consists of 4 globin polypeptide chains,4 heme groups and 4 oxygen molecules

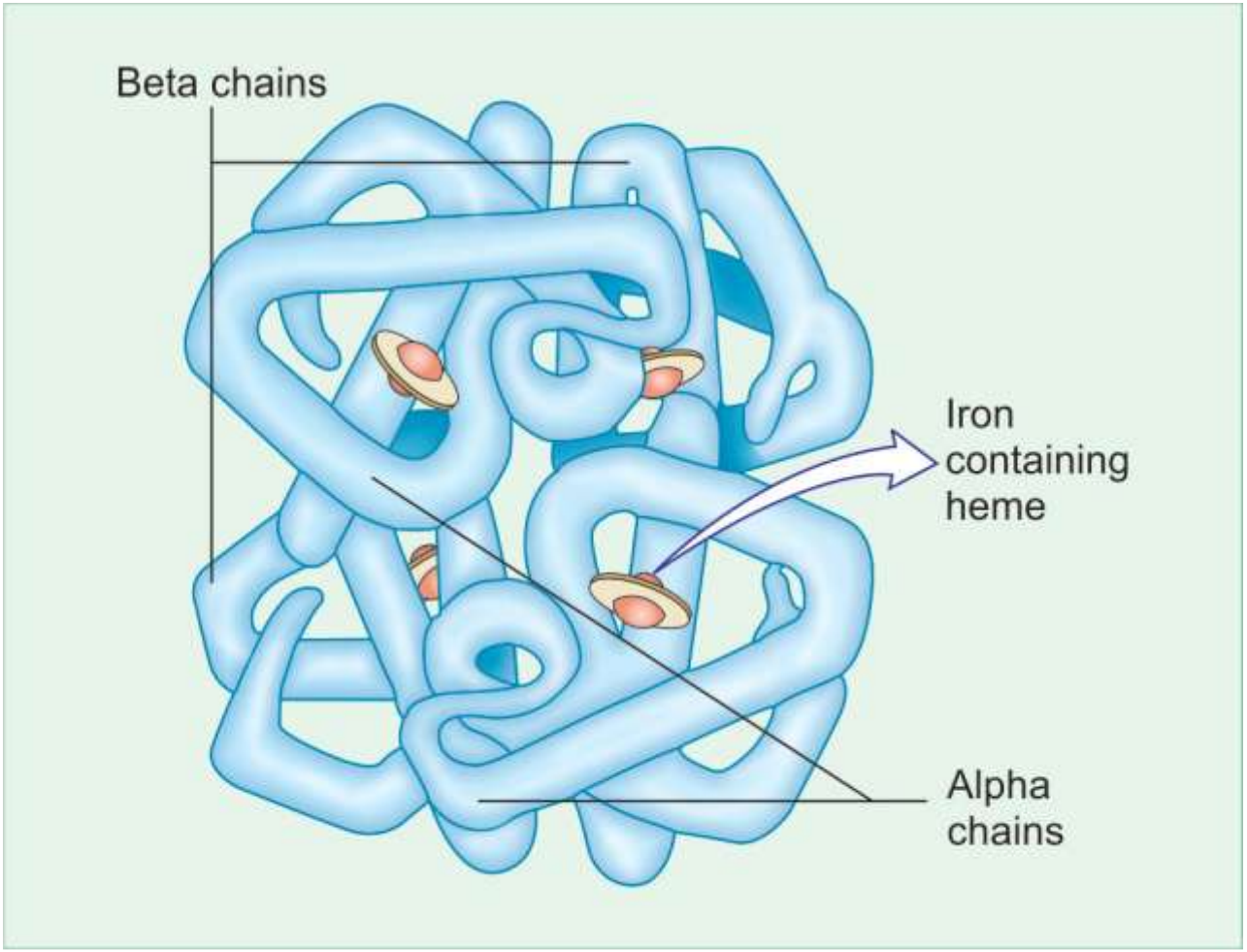




Haemoglobin monomer



Haemoglobin tetramer



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

Normal variants of Hb

1) **Hb A** (Adult hemoglobin)

2) **Hb A2** (Adult hemoglobin)

3) **Hb F** (Fetal hemoglobin)

4) **Hb A1c** (Glycated hemoglobin)

Normal Adult human hemoglobin

- The α-chains in these hemoglobins are identical.

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%

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 (HbA)**
- 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.

Glycosylated hemoglobin (HbA1c)

- Bound form of hemoglobin to glucose is having **clinical importance**.

- HbA1c level **increases in diabetes mellitus**.

- Monitoring levels of HbA1c in DM patients provides valuable information about the **dynamic changes occurring in blood glucose level prior three months**.

FUNCTIONS OF HEMOGLOBIN

1) **Hb transports oxygen from lungs to tissues.**

2) It also helps **to transport CO2 and protons from tissues to lungs.**

3) It **acts as buffer** during **acid base balance of blood ,maintaining normal pH of blood.**

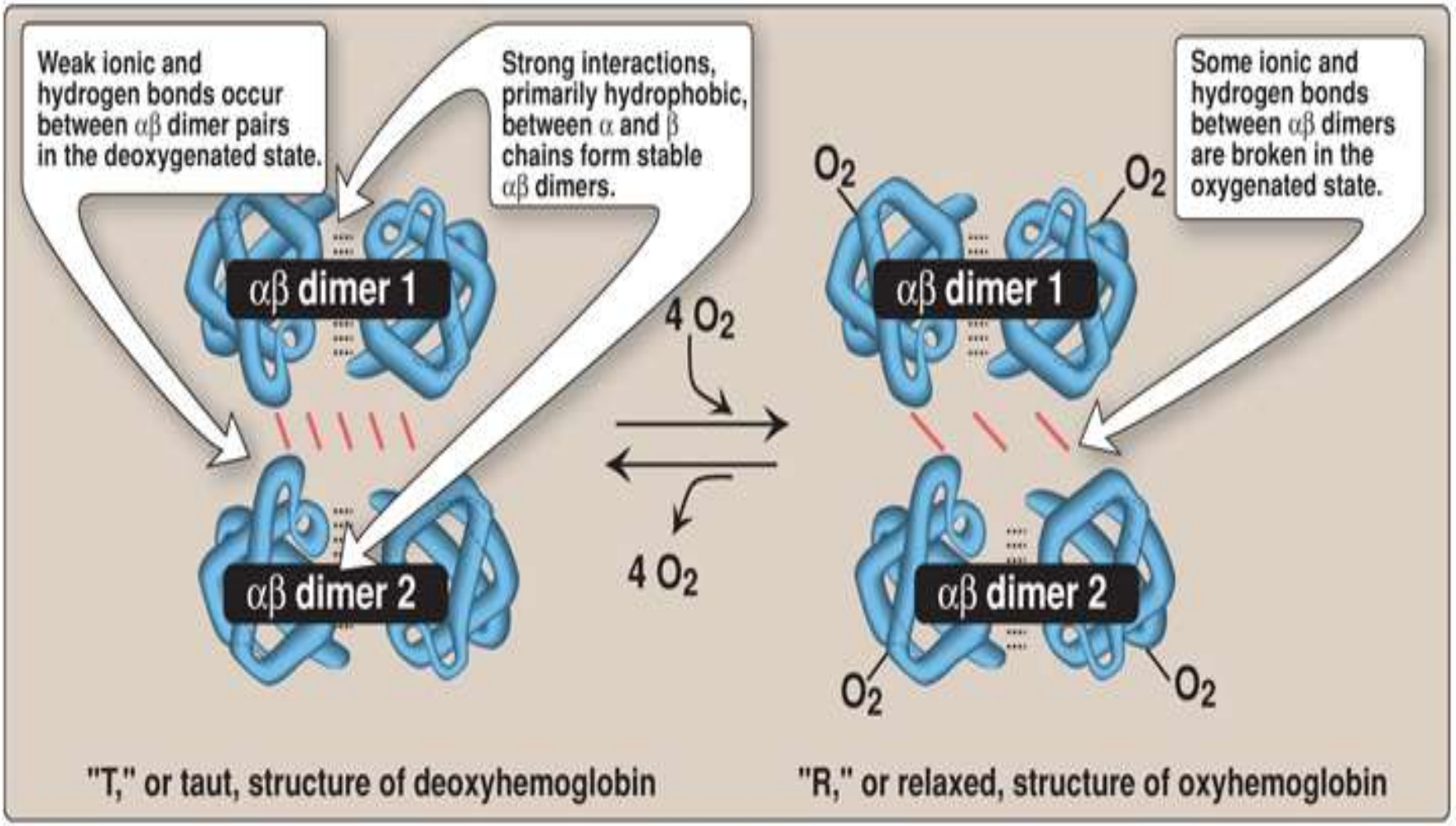
Transport of CO_2 by Hb

- Transported as **carbaminohaemoglobin**
- When **pCO2 ↑ the H+ ion conc. ↑ i.e. pH ↓**
- **In tissue**, pCO2 is high and pH is low due to metabolic acids. The affinity of hemoglobin to O2 ↓ which shifts ODC to right. So more O2 is released to tissue.
- **In lungs** , opposite reaction is found. Where pCO2 is low pH is high and pO2 is significantly elevated . More O2 binds to Hb and ODC is shifted to the left.

Hb as buffer

- Important in **regulation of pH** of blood
- **Chloride shift**:
- **When CO_2 is taken up, ↑ HCO_3^-** diffuse out of cell into plasma and chloride ions Chloride from plasma enter in cell to establish electrical neutrality.
- So RBCs from **venous side are slightly buldged** with higher Chloride conc.
- When blood reaches lungs the reverse reaction takes place.(reversal of chloride shift)

Different forms of Hemoglobin



- When hemoglobin is bound to O2, it is called **oxyhemoglobin**. This is the **relaxed (R)** state where some of the hydrogen and ionic bonds are destabilised.
- **R state has a higher affinity for O2**.
- The form with a vacant O2 binding site is called **deoxyhemoglobin** and corresponds to the taut or tense (**T) state**.
- **In the absence of O2, T state is more stable; has low affinity for oxygen**
- when O2 binds, R state is more stable and hemoglobin make a conformational change from T to the R state.
- The structural change involves **readjustment of interactions between subunits.**

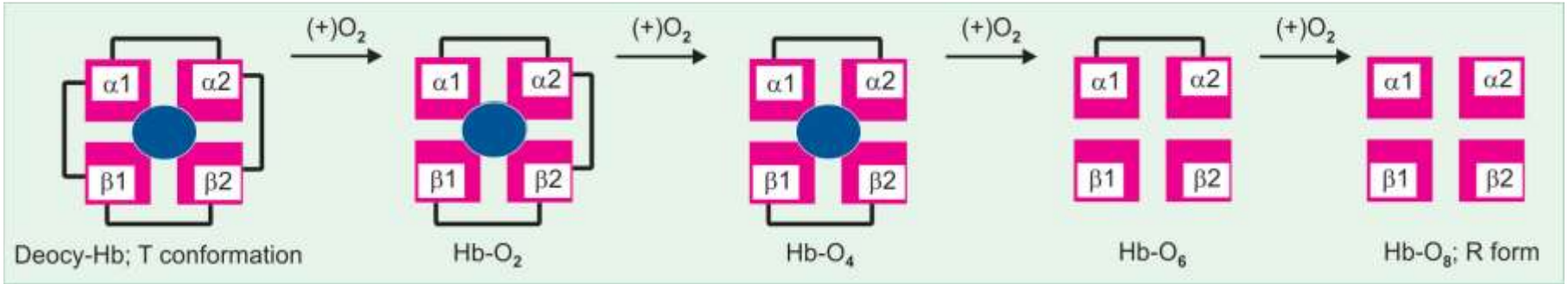
- When hemoglobin carries oxygen, the Hb is **oxygenated.**
- **The iron atom in Hb is still in the** ferrous state.
- **Oxidised Hemoglobin is called** Met-Hb; then iron is in ferric state and the oxygen carrying capacity is lost.

- 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.

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**.

Hemoglobin



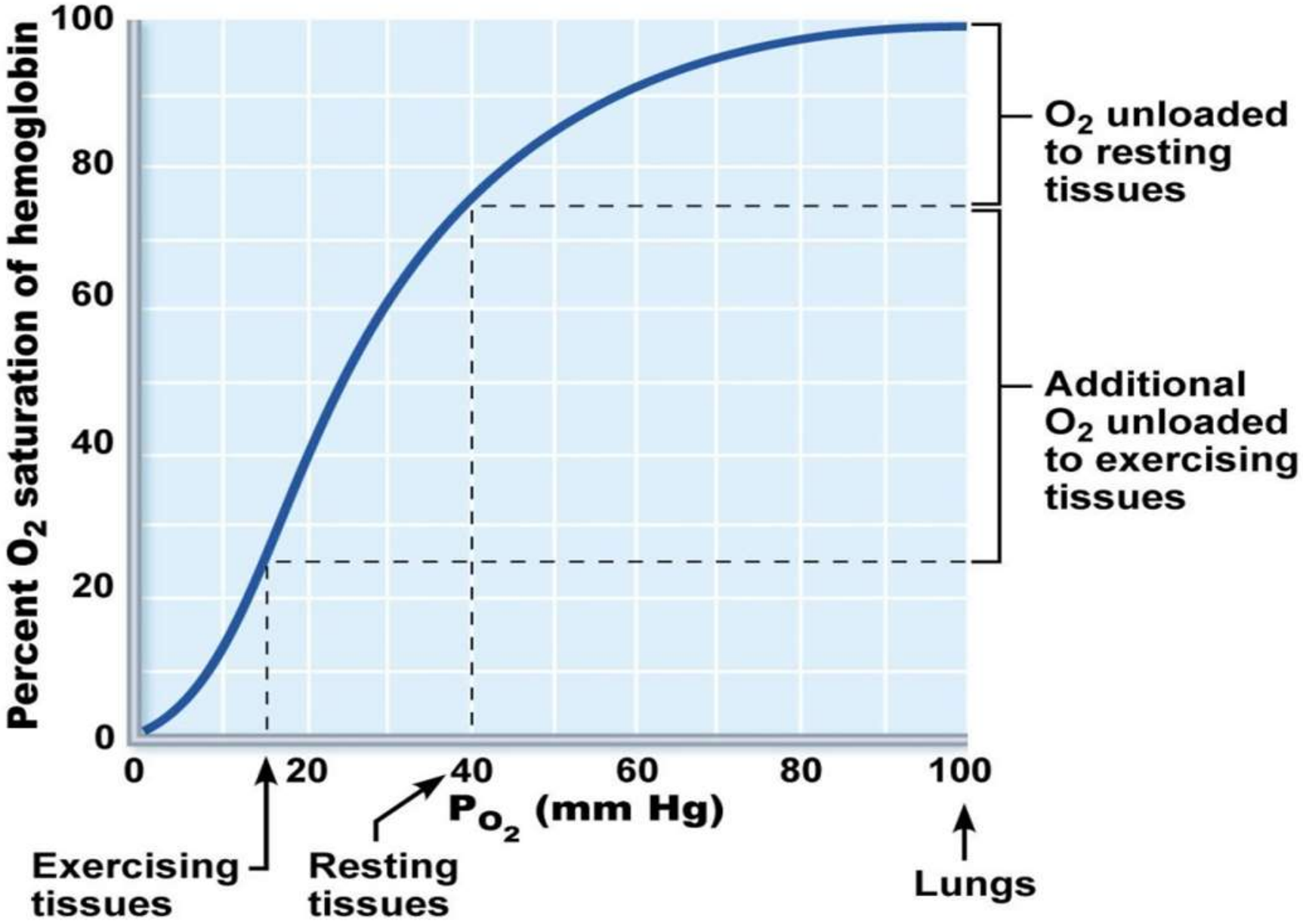
Pink rectangles represent hemoglobin (Hb) monomers. Black connection lines represent salt bridges. As oxygen is added, salt bridges are successively broken and finally 2,3-bisphosphoglycerate (BPG) is expelled. Simultaneously the T (taught) confirmation of deoxy-Hb is changed into R (relaxed) confirmation of oxy-Hb. Blue circle represents 2,3- BPG.

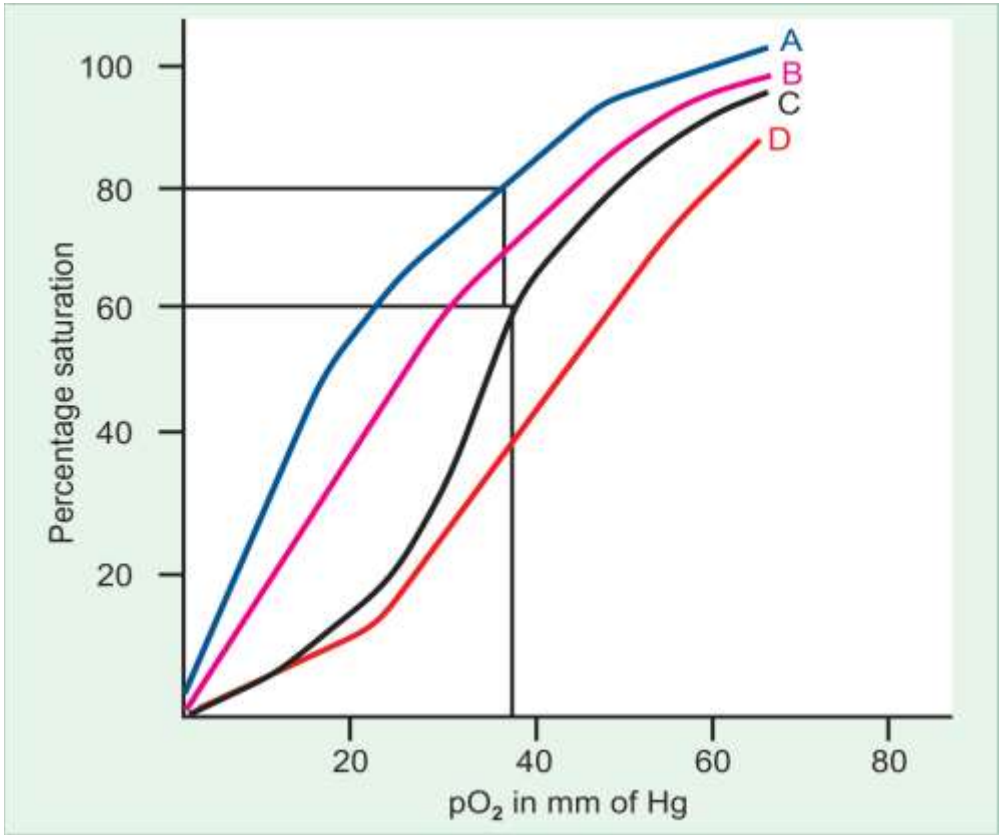
Oxygen transport by Hb

- Oxygen combines with heme portion in the blood
- Combination of Hb & oxygen is dependant on partial pressure of oxygen (pO_2)
- There is **progressive increase in percent of Hb** that is bound with oxygen as pO_2 increases.
- This is called **percent saturation of Hb**.

Oxygen Dissociation Curve

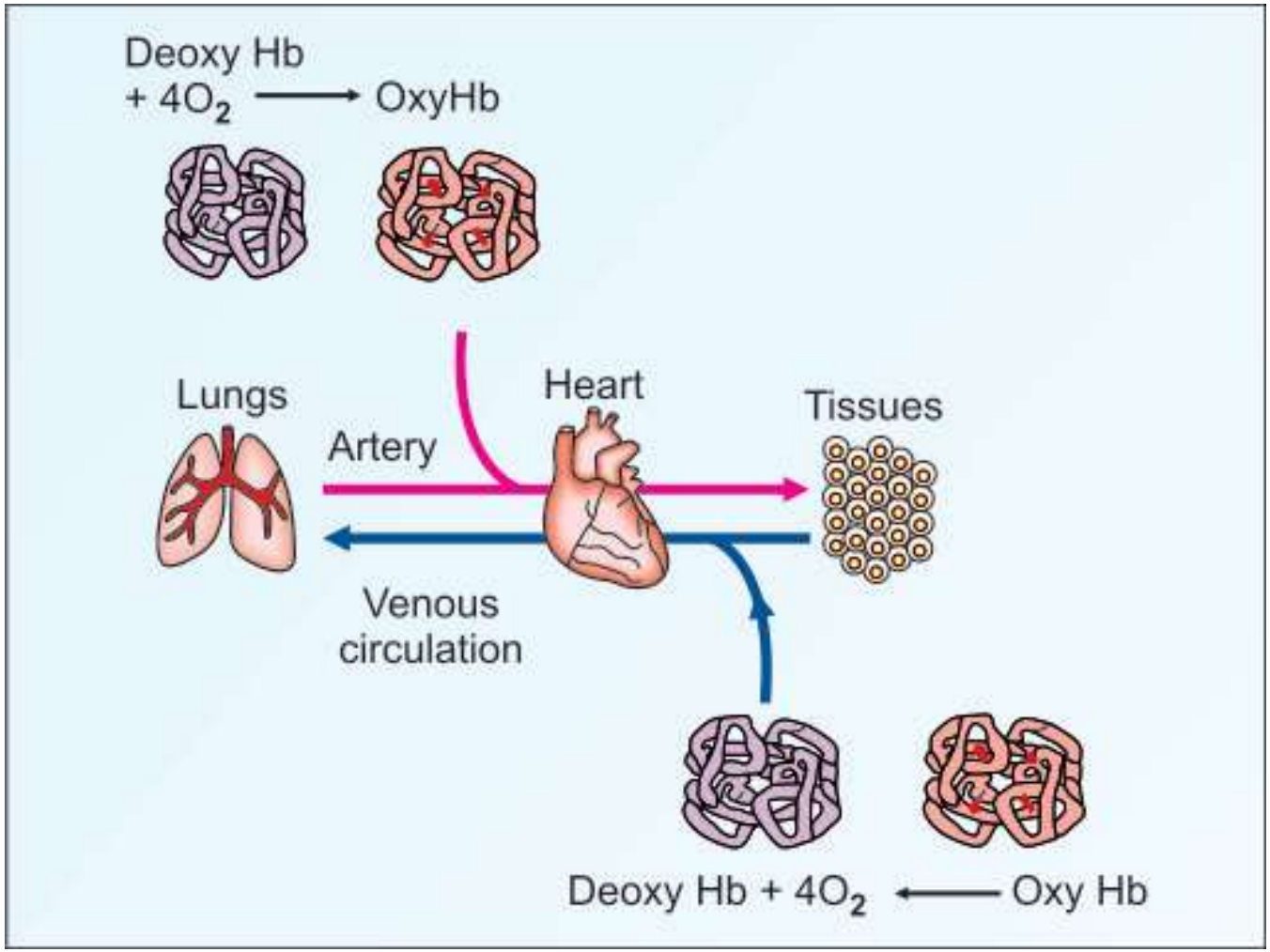
- The binding ability of hemoglobin with O_2 at **different pO_2**
- It is **sigmoid or 'S'** shaped curve
- There is **cooperative binding** of oxygen to Hb



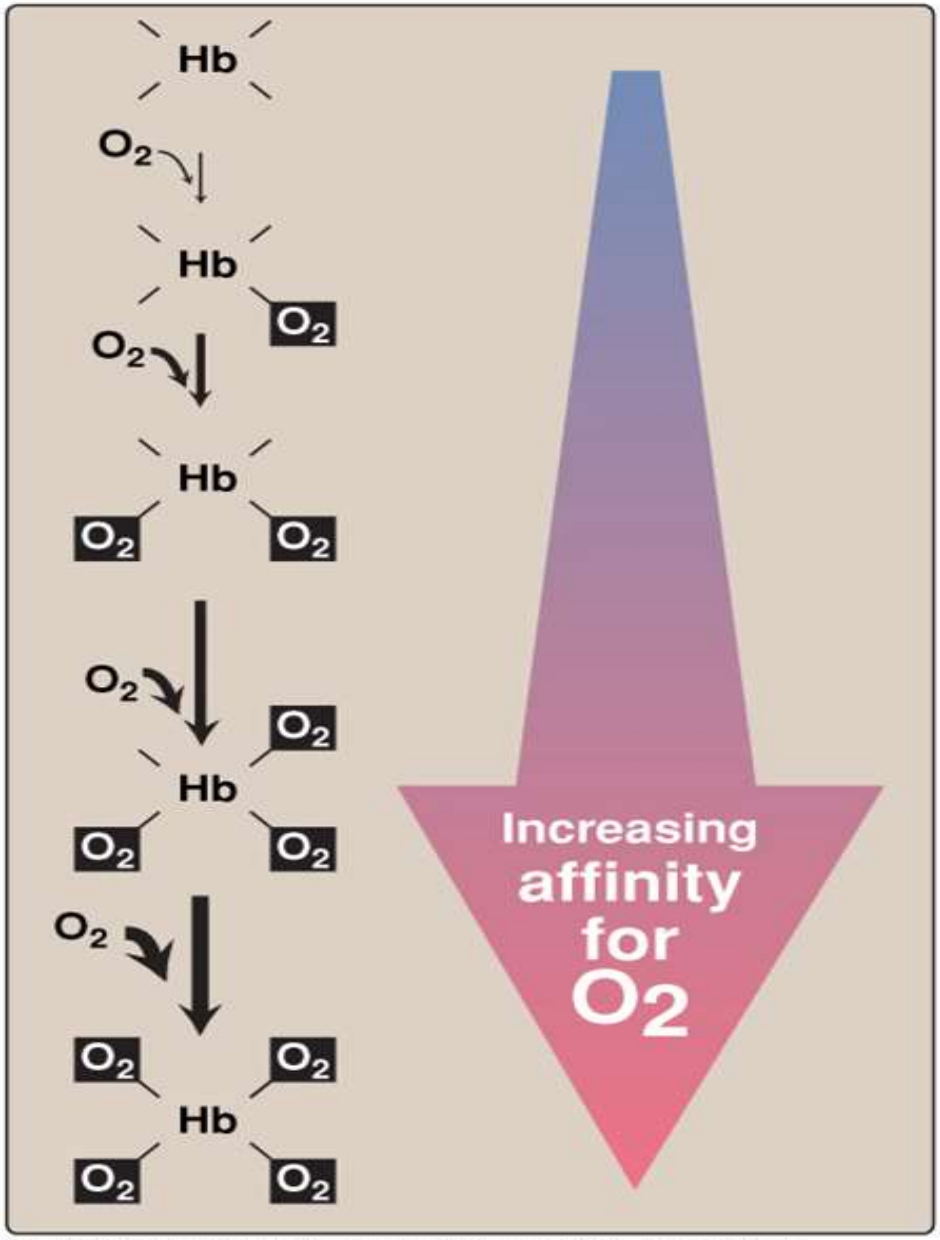


Oxygen dissociation curve (ODC). A = Theoretical curve as per mass action. B = Sigmoid curve, due to heme-heme interaction (Hill effect). C = Further shift to right due to carbon dioxide (Bohr effect) and PG. This curve represents the pattern under normal conditions. D = Further shift to right when temperature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

Tissues, Oxy Hb Releases O2



- **Co-operativity** means affinity of Hb for binding the **first molecule of O_2** is relatively low ,but subsequent oxygen molecules are bound with a higher affinity
- Binding of one molecule facilitate the **second molecule binding**

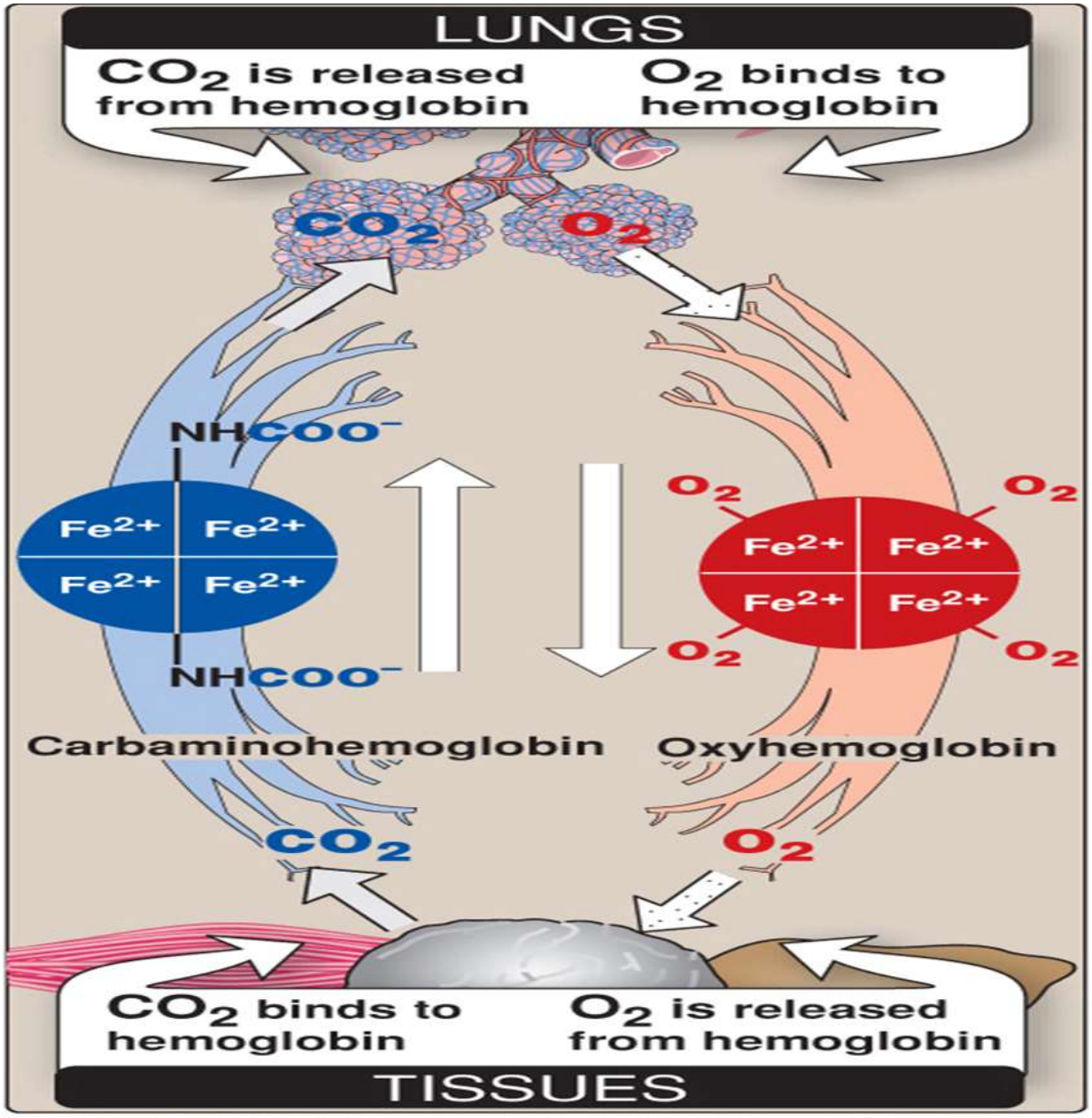


- Cooperative binding of oxygen by the four subunits of hemoglobin means that the **binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule**

- In the lung, the concentration of oxygen is high and hemoglobin becomes saturated (or “loaded”) with oxygen.
- In the peripheral tissues, oxyhemoglobin releases (or“unloads”) much of its oxygen for use in the tissues

Affinity of Oxygen with Oxygen

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



Bohr Effect

- **The change in oxygen affinity with pH** is known as the **Bohr effect**.
- Hemoglobin oxygen affinity is reduced as the **acidity increases**.
- Since **the tissues** are relatively rich in carbon dioxide, the pH is lower than in arterial blood; therefore, **the Bohr effect facilitates transfer of oxygen.**
- The **Bohr effect is a manifestation** of the acid-base equilibrium of hemoglobin.

- The regulation of O_2-binding to hemoglobin by H+ and CO2 is called the Bohr effect
- Both H^+ and CO_2 are negative effectors of O2-binding.
- CO2 reduces O2 affinity
- Metabolically active tissues need more O2; they generate more
- CO2 and H+ which causes hemoglobin to release its O2.

Factors shifting ODC to right

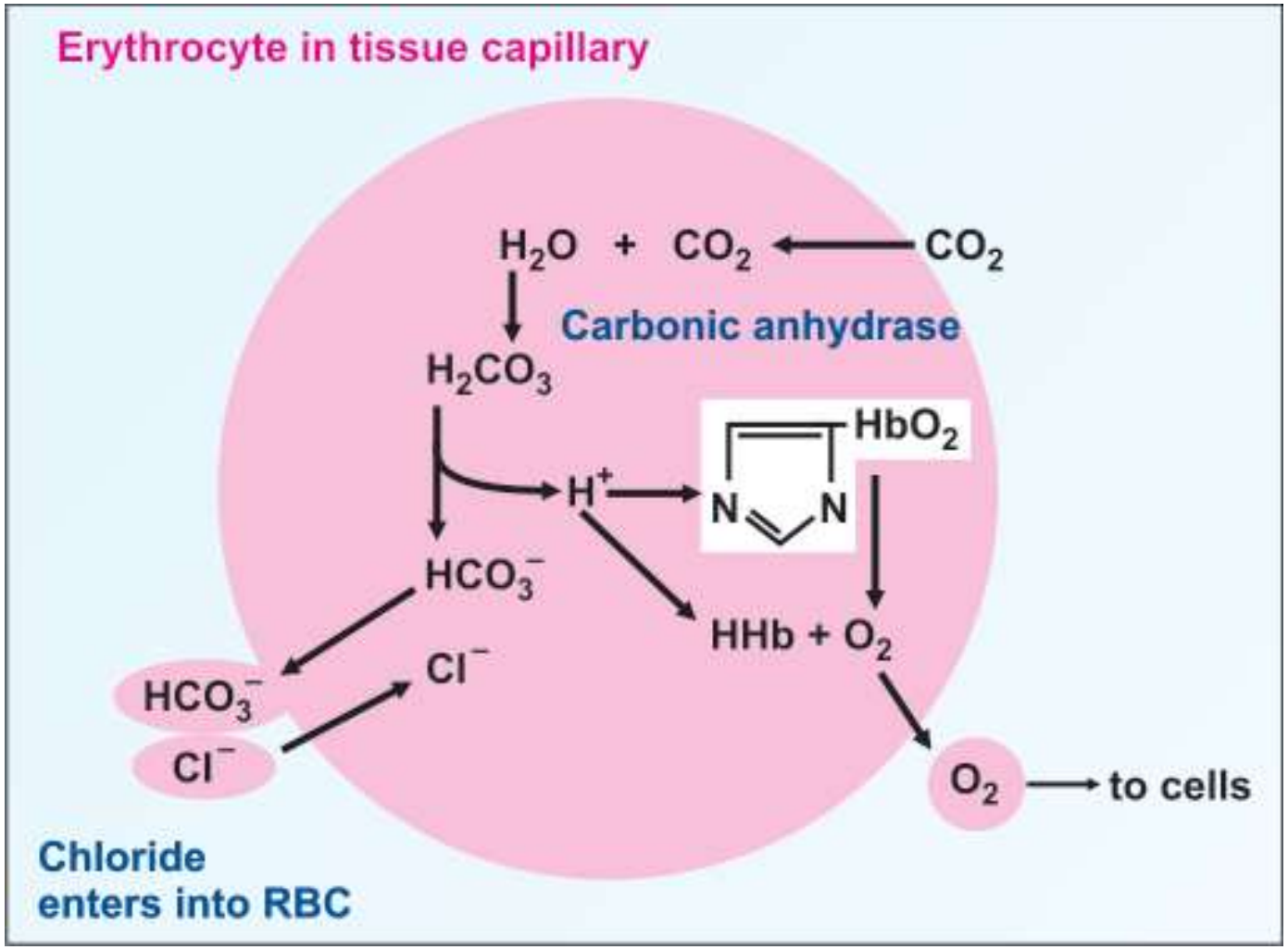
- 1) Increased hydrogen ion conc.(blood pH)
- 2) Increased CO_2 tension (Bohr effect)
- 3) Increased temperature
- 4) Increased erythrocyte concentration of 2,3-BPG

- **Clinical importance of 2,3 –Bisphosphoglcerate**

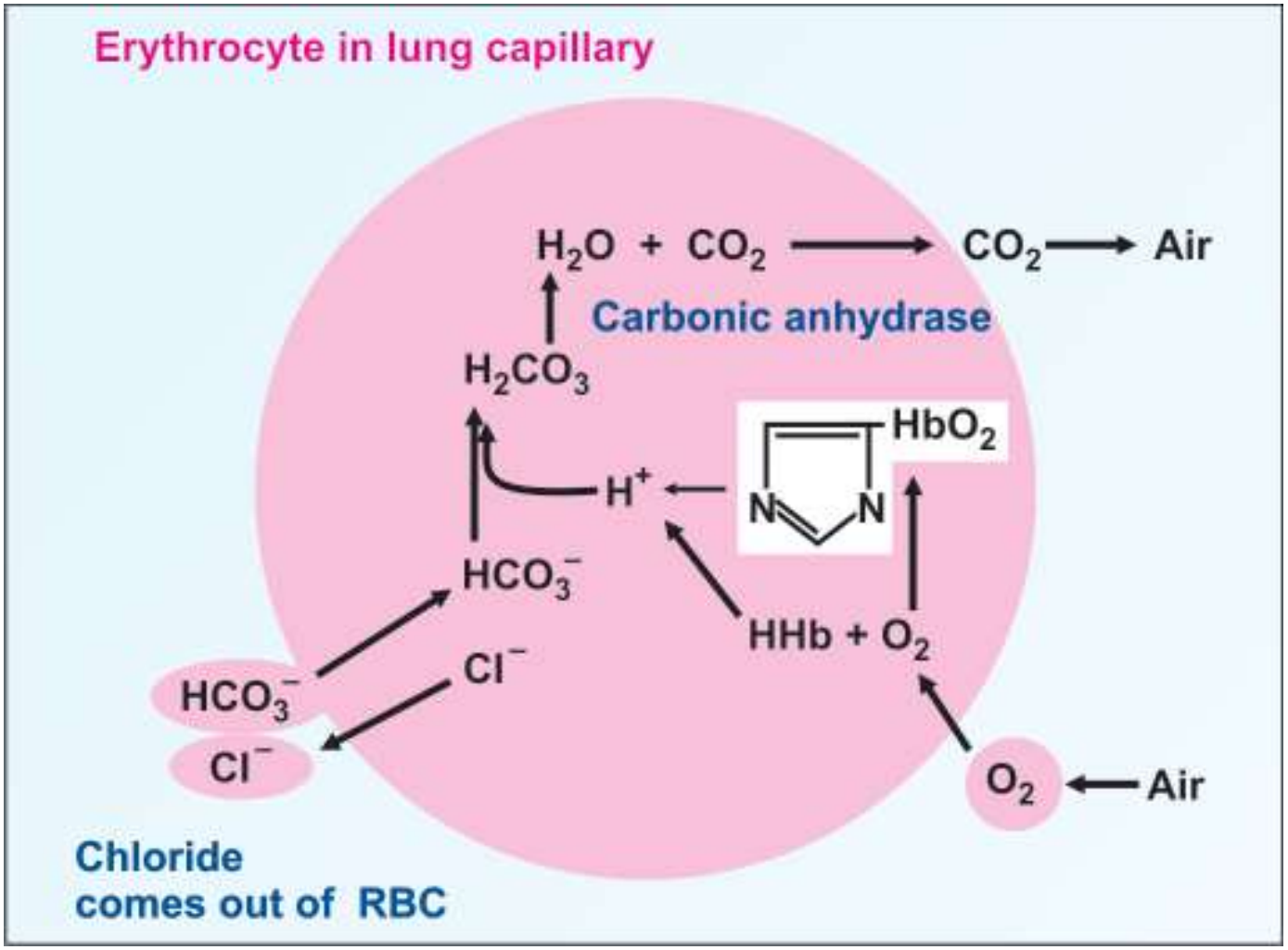
- The concentration of 2,3 –BPG increases in response to chronic hypoxia
- That helps in greater unloading of oxygen
- Levels of 2,3 –BPG decrease in stored blood

- **Chloride shift**:
- When CO_2 is taken up, ↑ HCO_3^- diffuse out of cell into plasma and chloride ions from plasma enter in cell **to establish electrical neutrality**.
- So RBCs from **venous side** are **slightly buldged** with **higher Chloride conc**.
- When blood reaches lungs the reverse reaction takes place.(**reversal of chloride shift)**

Chloride Shift in Tissues



Chloride Shift, in Lungs



Clinical Applications

- In **all hypoxic states**, the O2 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 pO2 is low, includes increased pulmonary ventilation, polycythemia and increase in 2,3-BPG level with a shift in ODC to 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 2,3-BPG level varies inversely as the hemoglobin concentration.
4. In chronic **pulmonary diseases and cyanotic cardiac** diseases also the 2,3-BPG level is increased ensuring maximum unloading of O2 to 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.

Hemoglobin derivatives

- 1) Oxyhemoglobin
- 2) Reduced hemoglobin
- 3) Carboxy hemoglobin
- 4) Methemoglobin
- 5) Cyanmethemoglobin
- 6) Carbaminohemoglobin
- 7) Sulphemoglobin

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**.

- 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 O2** is the treatment.
- **In severe cases**, oxygen under high pressure (**hyperbaric oxygen**) is helpful.

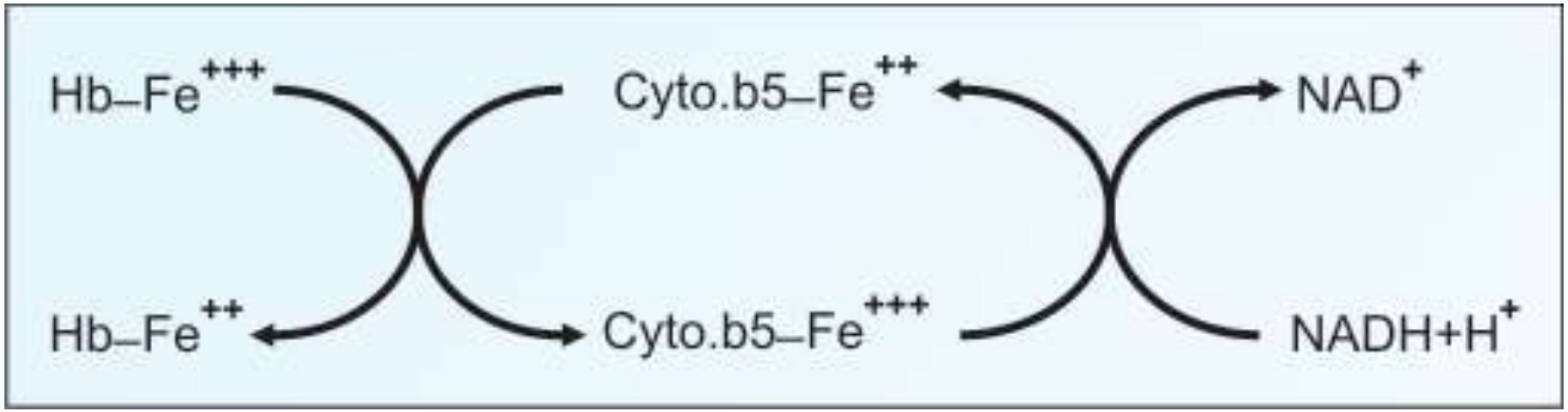
- The absorption bands of carboxy-Hb is similar to that of oxy-Hb.
- But on adding a reducing agent like sodium dithionite, it fails to convert carboxy-Hb to deoxy-Hb; whereas oxy-Hb is converted.

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.

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**.

- **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.

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**.

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**.

Hemoglobinopathies and Thalassemias

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

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.

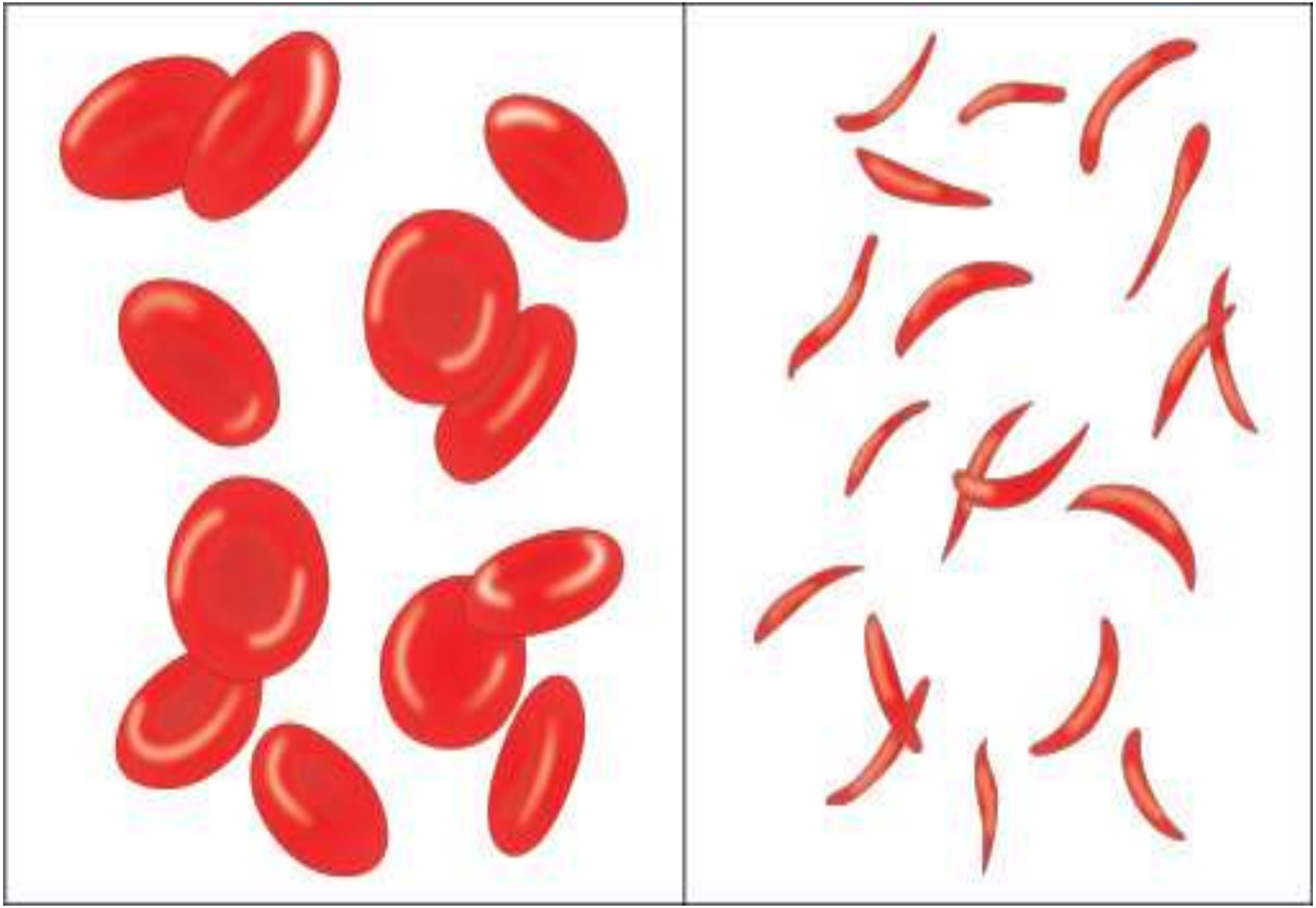
Hemoglobinopathies

- **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.

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)

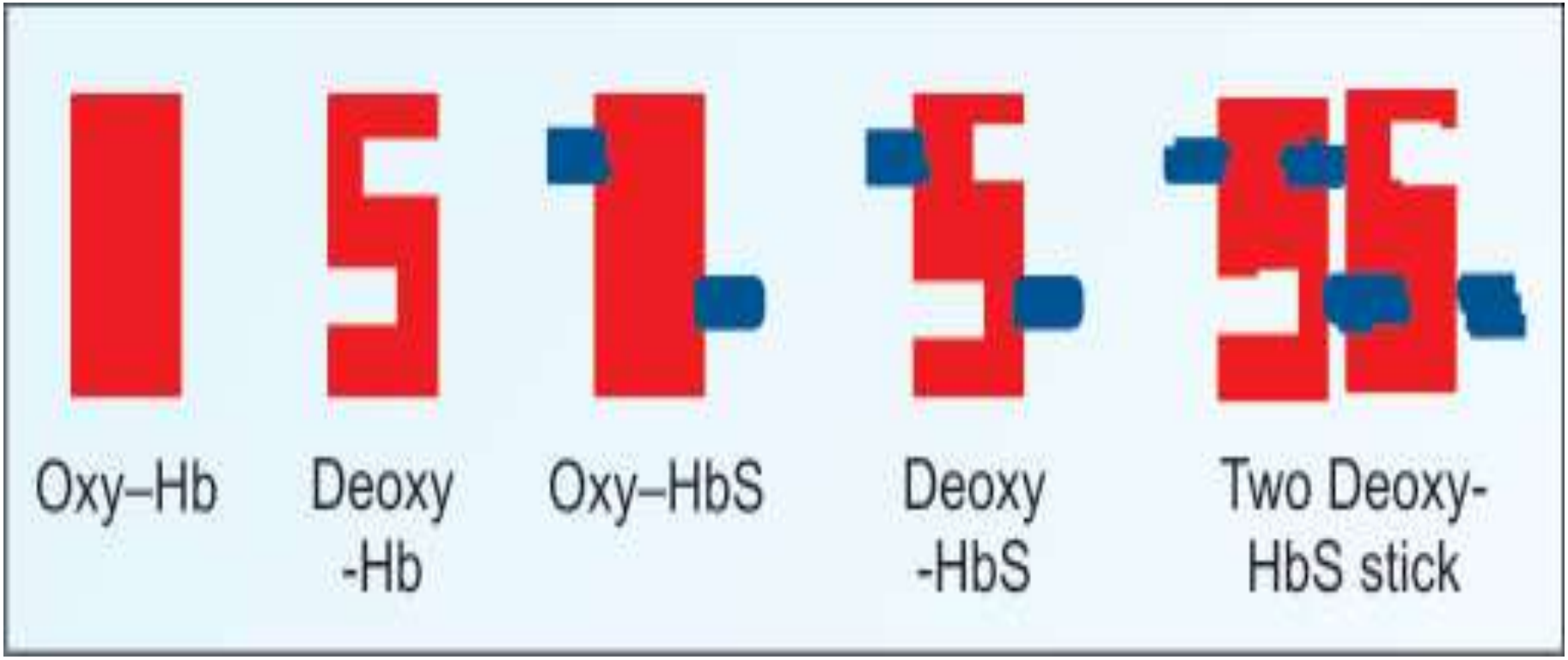
- HbS constitutes the **most common variety worldwide.**
- Hemoglobin with **abnormal electrophoretic mobility** is responsible for the **sickling disease** (Pauling, 1949).



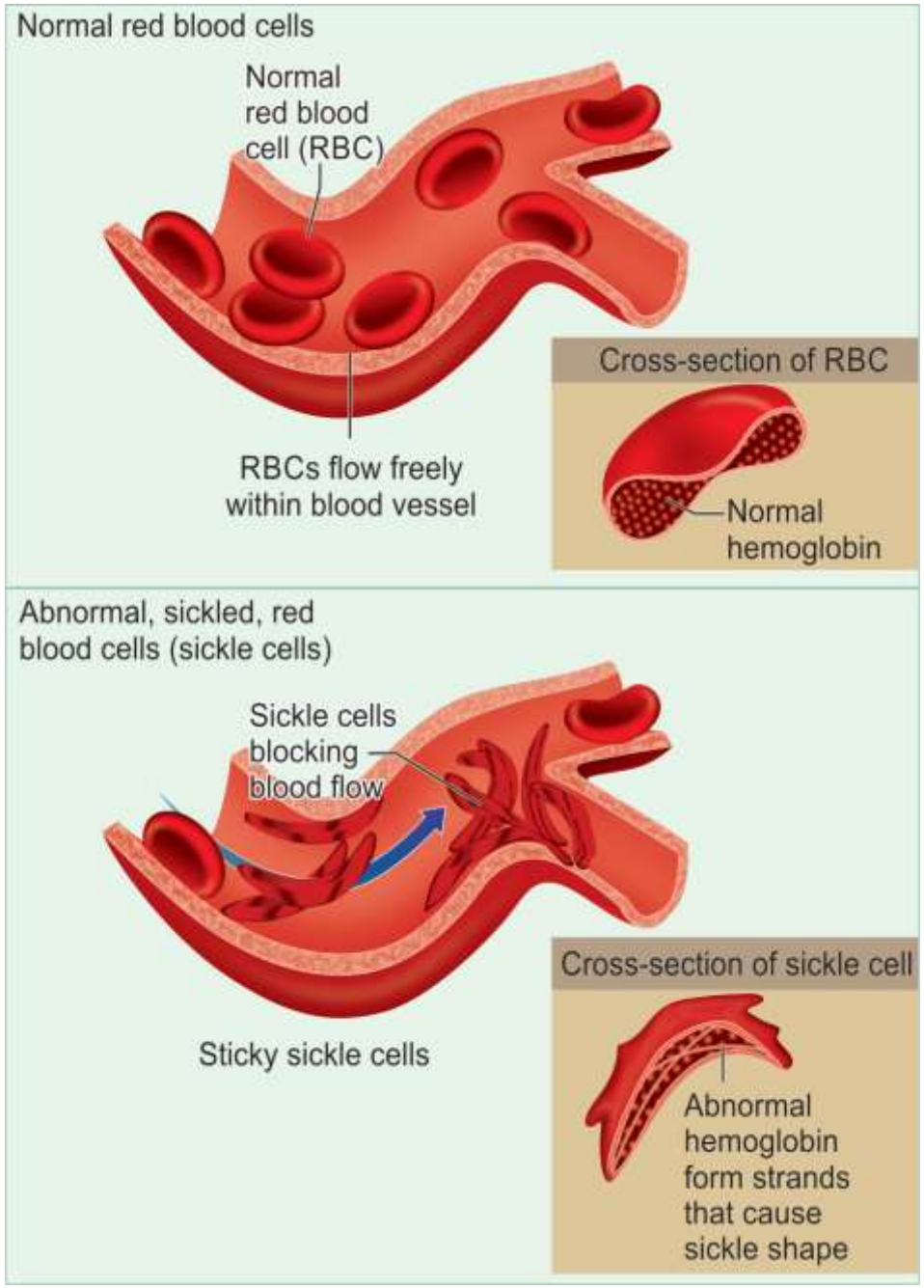
- The **glutamic acid** in the **6th position of beta** chain of HbA is changed to **valine** in **HbS**.
- Leads to **polymerization of hemoglobin molecules inside RBCs.**
- Causes a **distortion of cell into sickle shape**.
- The substitution of **hydrophilic glutamic acid** by **hydrophobic valine**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

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.

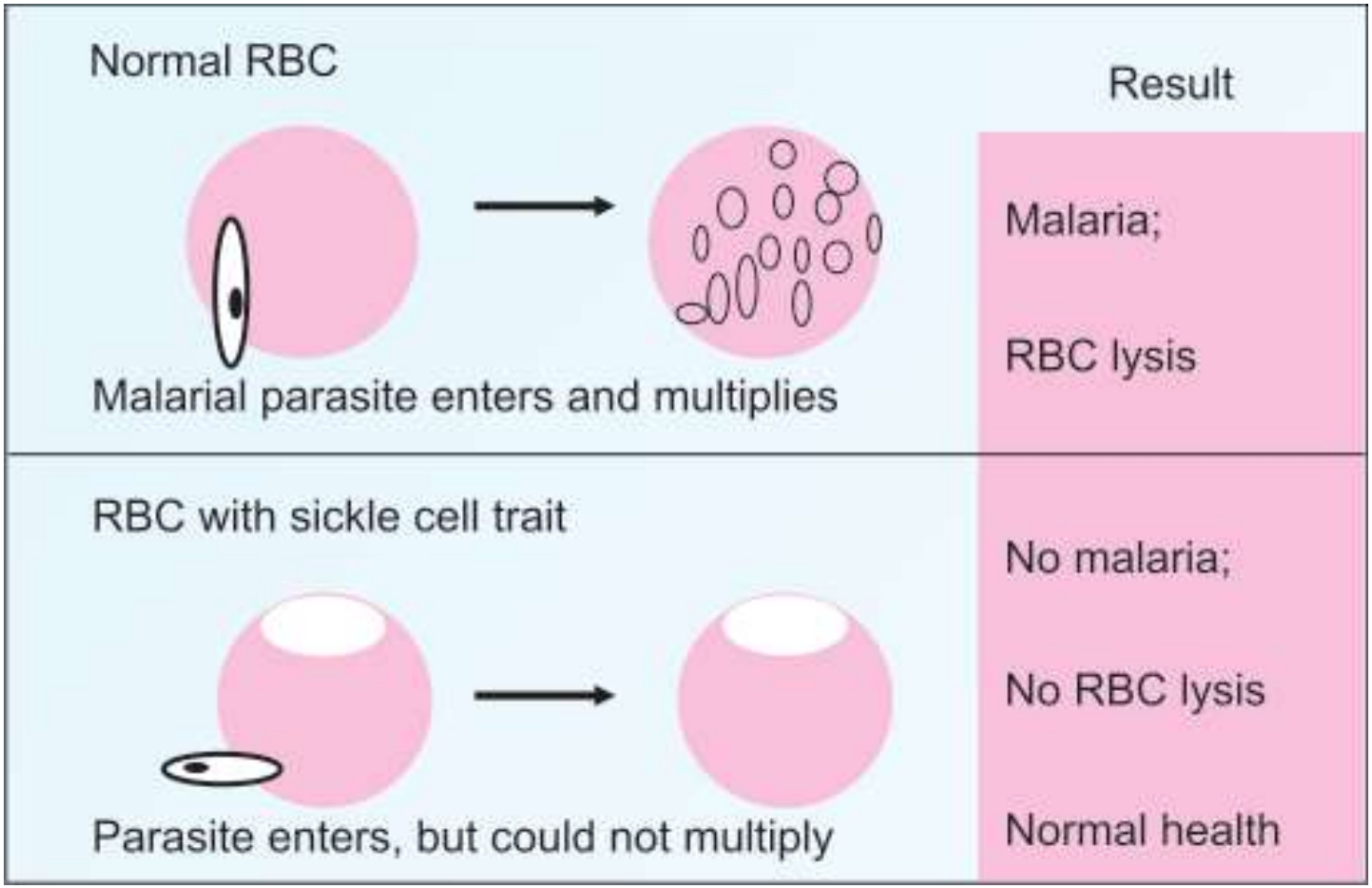
Sickle Cell Trait

- **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**.

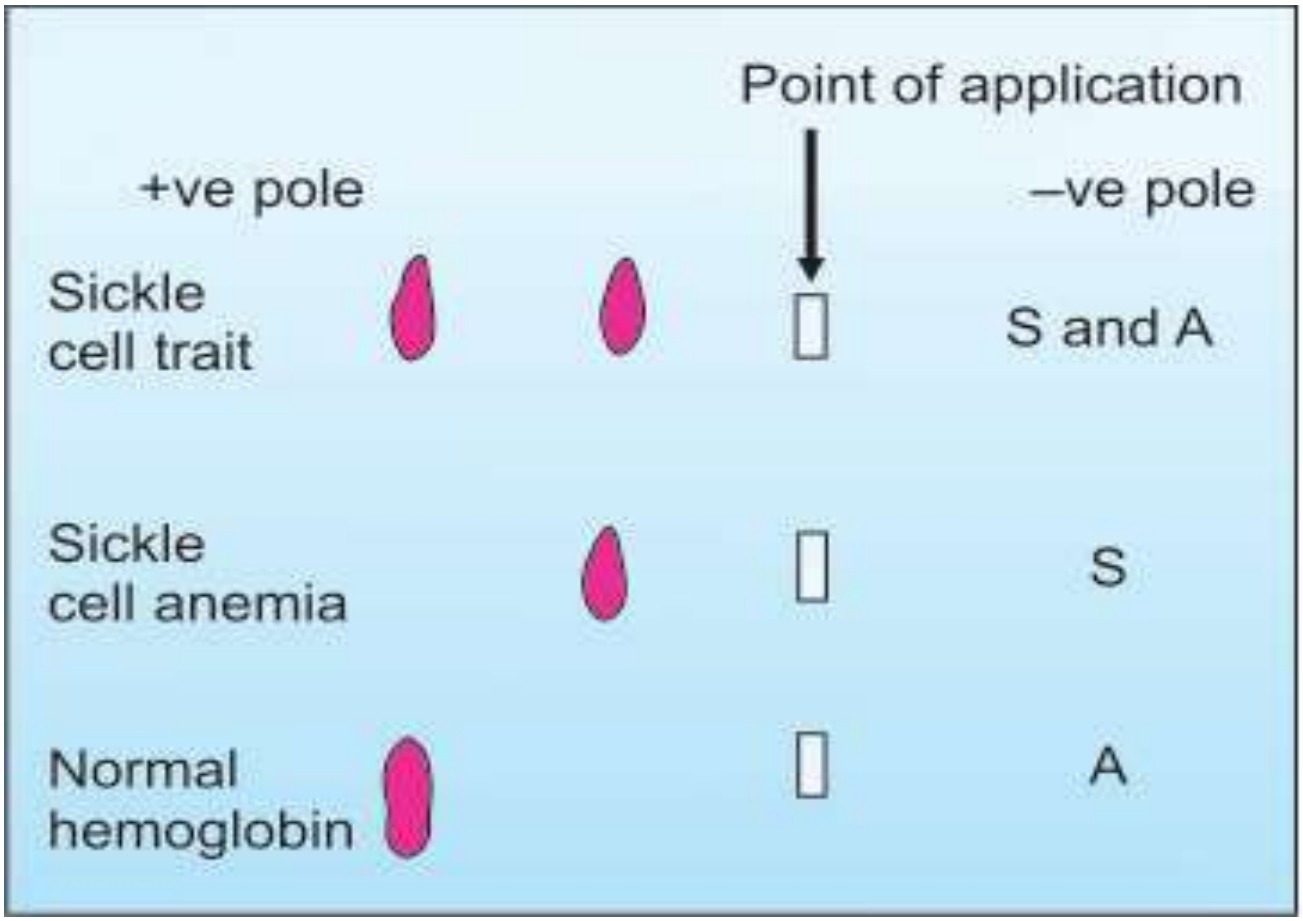
Sickle Cell Trait Protects from Malaria



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



Linus Pauling
NP 1954
1901-1994

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 Hemoglobins

1. **Hb C-** 6th amino acid beta chain **glutamic acid** is replaced by **lysine**.
- Double heterozygotes (HbSC) have moderate disease.
- Homozygotes (CC) have **hemolytic anemia**.

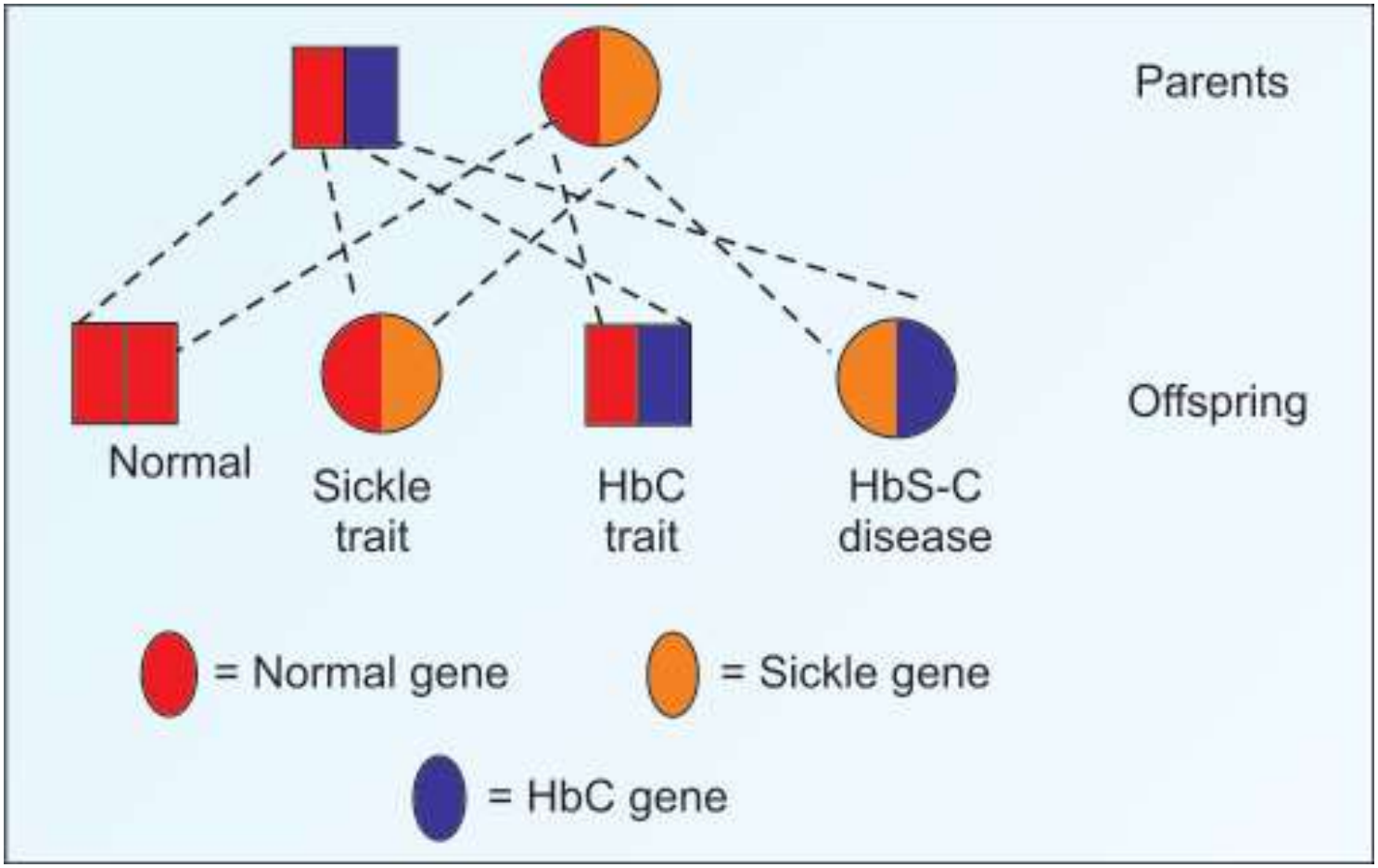
2. **Hb D** – Beta 121 **glutamic acid** to **glutamine (HbD Punjab). HbSD is severe disease**.
3. **Hb M** – Proximal or distal hisitidine substitutions (**HbM Boston, HbM Hyde Park**).

Molecular differences of HbA, HbS and HbC

Important Hemoglobinopathies

Hemo-globin	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

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

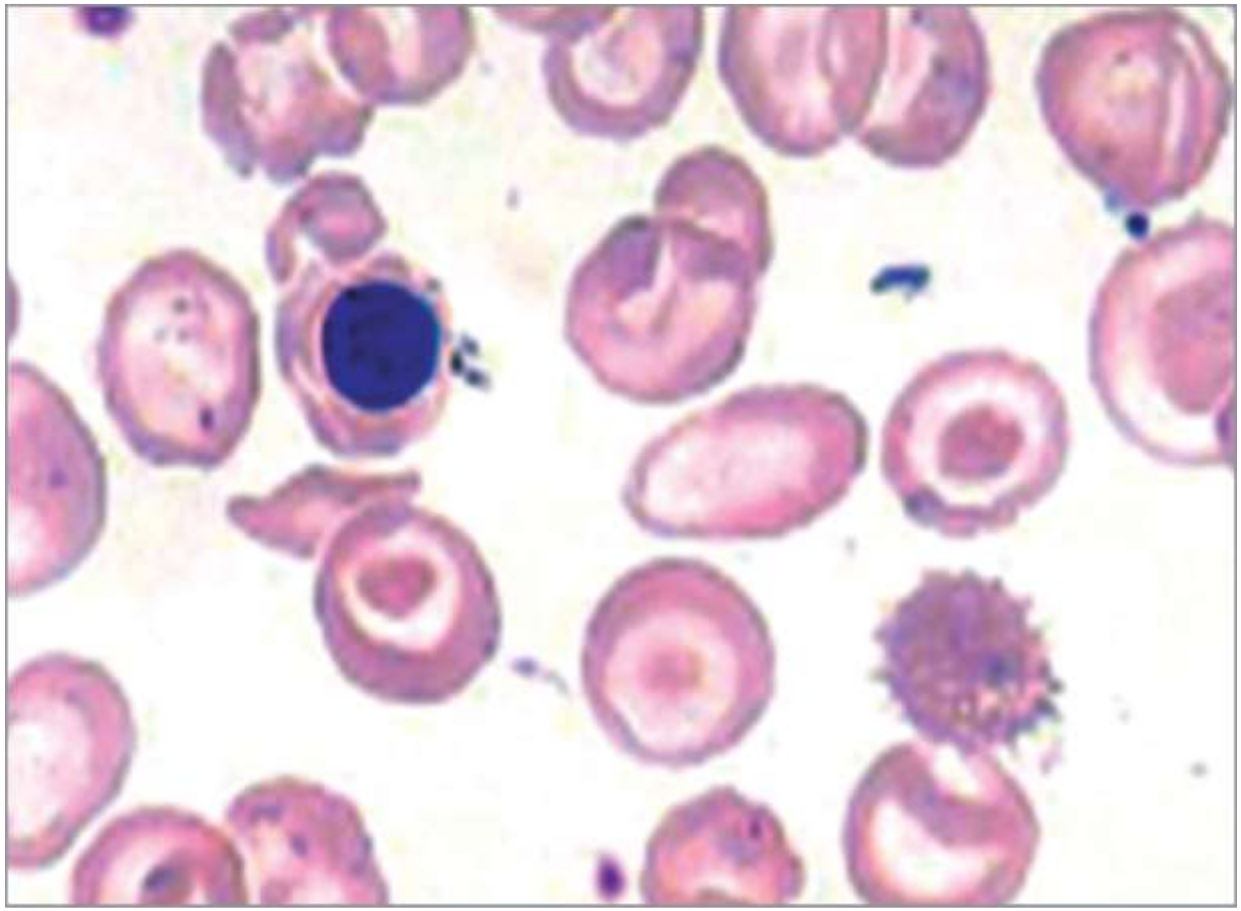
- Beta thalassemias are phenotypically described as **beta (+) or beta (o)** depending on whether there is beta chain synthesis or not.
- Beta (o) thalassemia may result from **base substitutions.**
- Beta (+) thalassemias are produced from **defects in posttranscriptional processing of mRNA**.

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

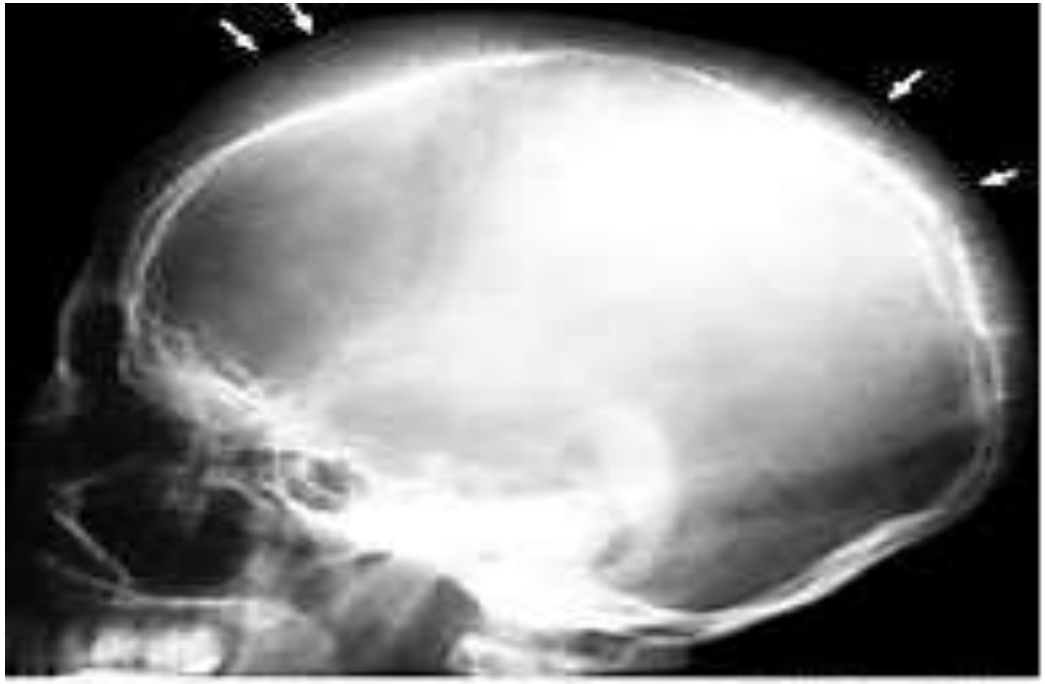
- These syndromes are mainly seen in people of **Asian, African and Mediterranean origin**.
- All cases of thalassemias are characterized by **deficit of HbA synthesis.**
- Hypochromic microcytic anemia is seen.
- In homozygous state, clinical manifestations are severe, and hence called **Thalassemia major.**
- There will be **nucleated RBCs** in peripheral circulation.



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 pO2 in tissue is about 30 mm of Hg, when Mb is 90% saturated.**
- At this pO2, Hb saturation will be only 50%.

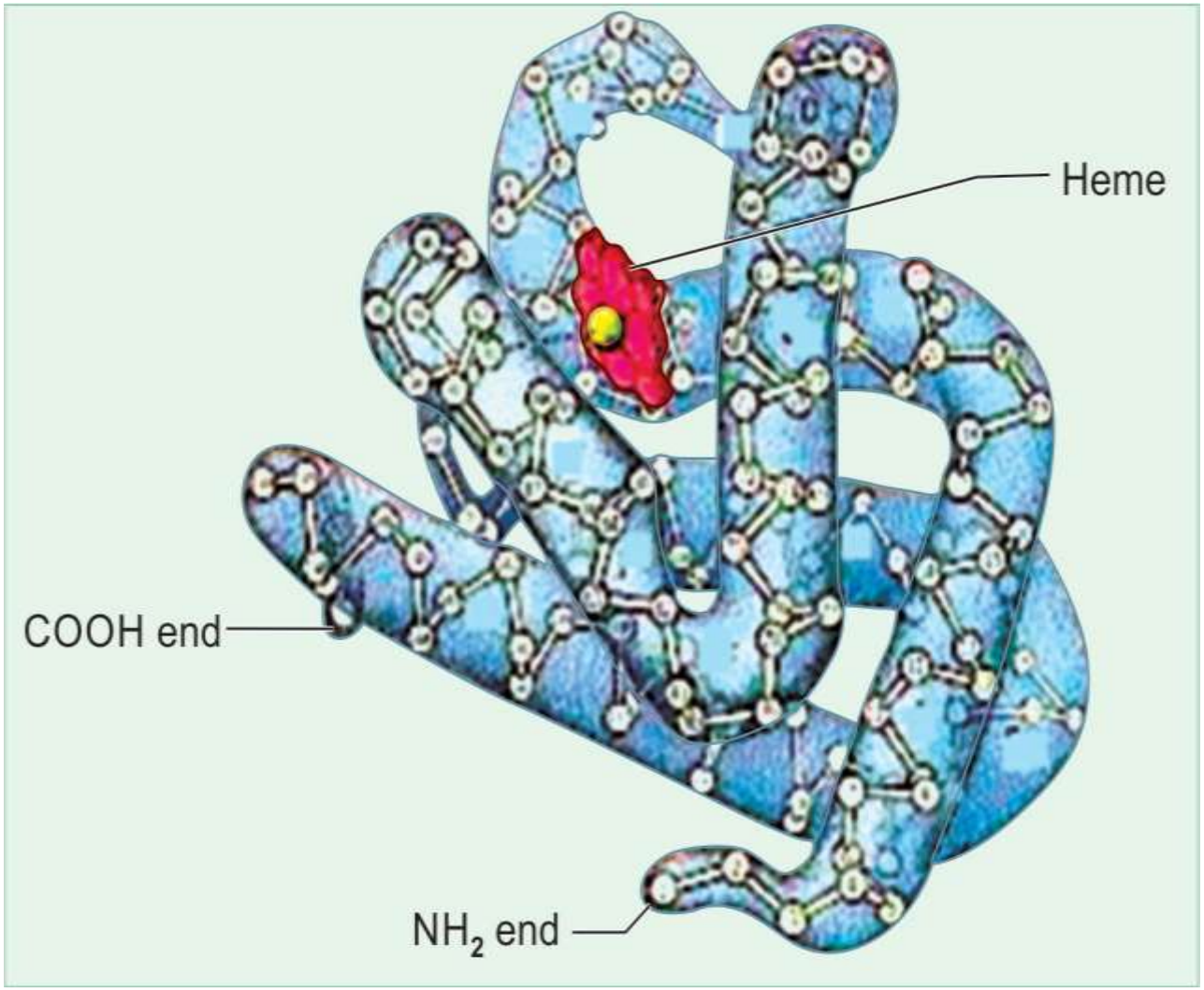
- **In severe physical exercise**, pO2 in muscles lowers to 5 mm Hg, when **myoglobin releases all the bound oxygen.**
- **Mb has a high oxygen affinity while Bohr effect, co-operative effect and 2,3-BPG effect are absent.**
- Severe crush injury causes **release of myoglobin from the damaged muscles.**

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 and thrombocytopenia** are other major causes for bleeding tendencies.


Thank
You