HEMOGLOBIN: STRUCTURE AND FUNCTION

Dr Bela Goyal

Case 1

- A 11-year-old male child was admitted with a history of having been found unconscious in the bathroom by the elder brother of the patient.

- There was no evidence of associated tonic-clonic movements, tongue bite, frothing from mouth, vomiting, bladder, and bowel incontinence or trauma. There was no history of fever, seizures, headache, vomiting, and substance abuse prior to this episode.

- At the time of admission, patient was unresponsive. Pupils were normal size and well reacting to light, and plantar reflex was extensor bilaterally and systemic examination was normal.
- Patient was intubated, mechanically ventilated, and was started on antiedema measures and other supportive treatment

- **MRI**: bilateral gyral swelling of the frontal and parietal lobes.
- Patient improved and was extubated on the fourth day of admission and was discharged in satisfactory condition after seven days without any neurological sequelae.
- Ref: Sharma S, Gupta R, Paul BS, et al: Accidental carbon monoxide poisoning in our homes. Indian J Crit Care Med 2009;13:169-170.

Case 2

- A 25-year-old male was found lying unconscious and brought out after breaking open the bathroom door after one hour.
- Similar Examination and MRI like Case 1
- He had residual hypoxic damage in the form of persistent vegetative state and was discharged to a domiciliary care on request.

Discussion

- **Gas geysers** have emerged as a cost-effective efficient method for heating water at homes. Gas geysers run on LPG, the combustion of which leads to generation of CO, hydrocarbons, and nitrogen oxides.
- CO toxicity occurs by **competitive binding of CO to the hemoglobin** heme groups **shifting the oxygen-hemoglobin dissociation curve** to the left.
- The **predilection for the globus pallidus** may relate to hypotensive effect of CO poisoning in the watershed territory of the arterial supply.

Treatment: ??

Hints:

Competitive Inhibitor

Affects oxygen dissociation curve

Learning objectives

- Structure of Hemoglobin
- Binding of oxygen to Hemoglobin
- Binding of CO to hemoglobin
- Importance of Oxygen

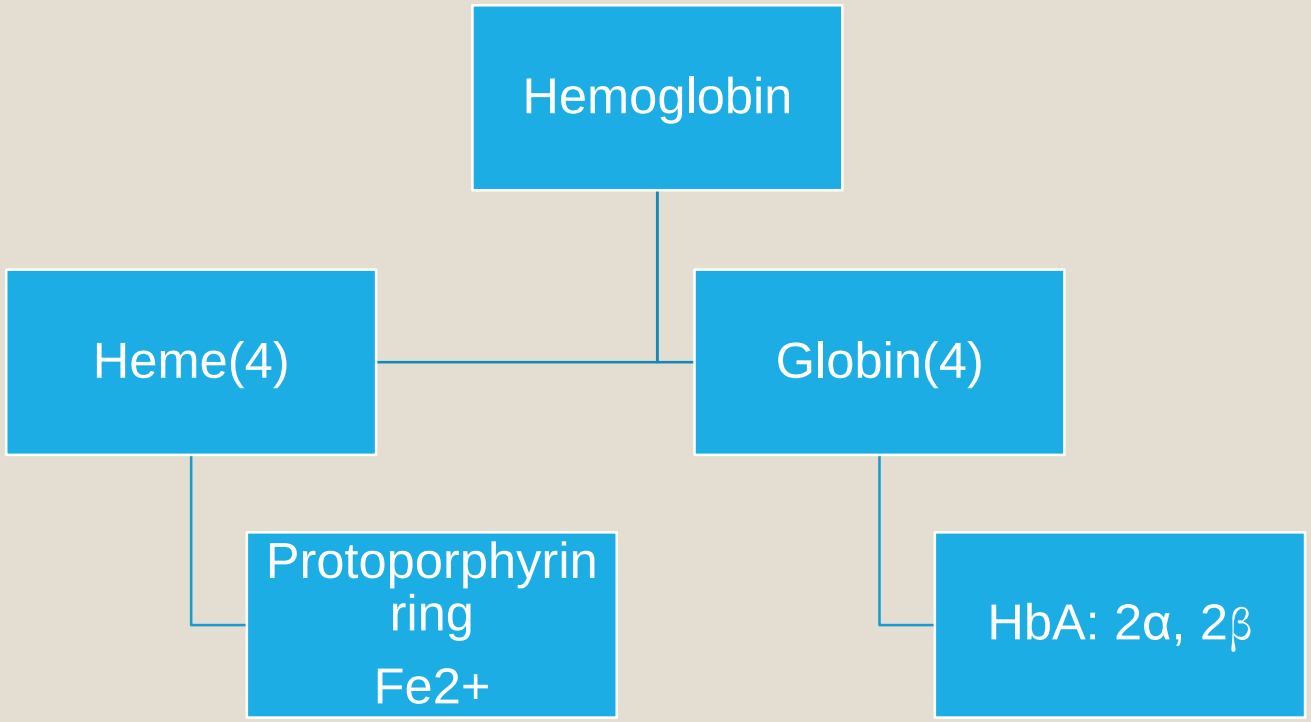
Why these many symptoms by inhaling a gas?

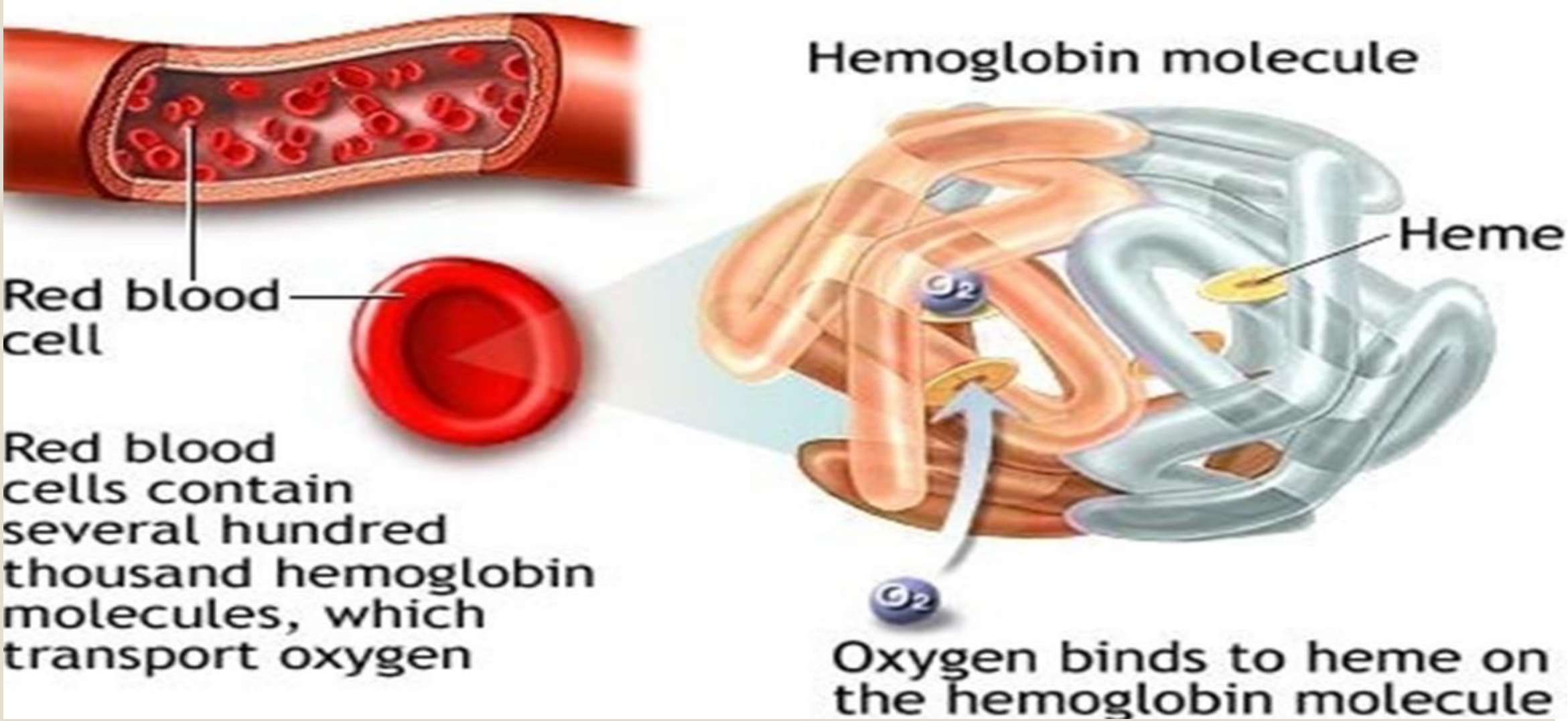
- ANOXIA
- Oxygen needs to be transported from lungs to tissues efficiently to protect against anoxic episodes
- What is the mechanism for this efficient transport?
- What is the biomolecule responsible and its characteristics that supports this function?

Hemoglobin(Hb)

- Quaternary structure
- Hemoglobin

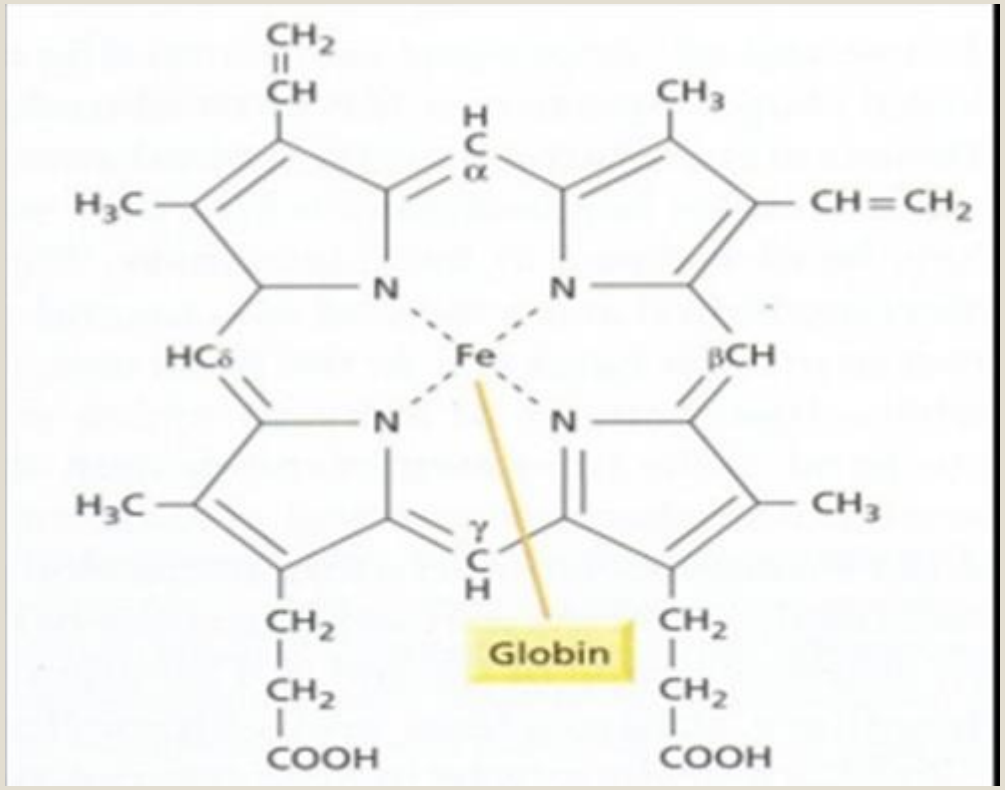
- Heme : Prosthetic group
- Globin: Protein part




Hemoglobin molecule
Heme
Red blood cell
Red blood cells contain several hundred thousand hemoglobin molecules, which transport oxygen
O_2
O_2
Oxygen binds to heme on the hemoglobin molecule

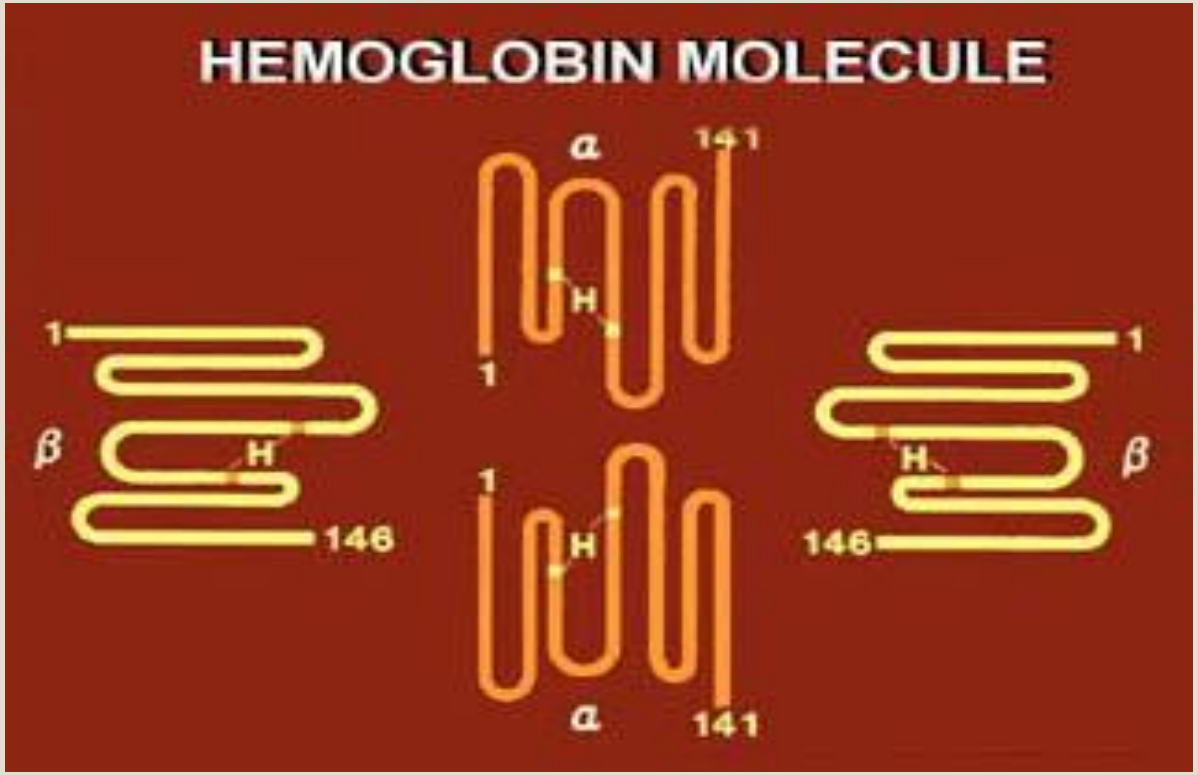
Structure of Heme

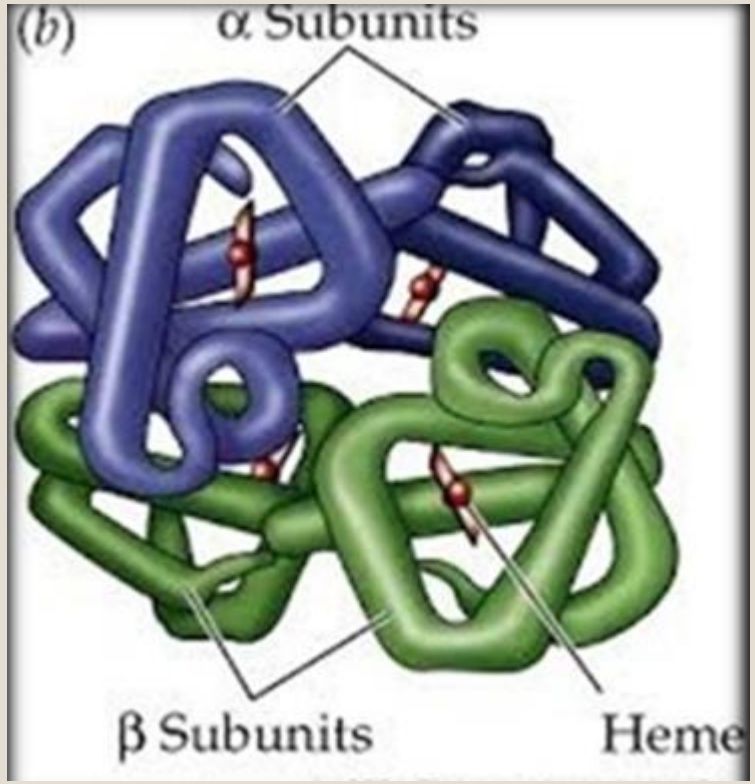
- Iron containing pigment **heme** is attached to protein **globin**
- **Heme** is iron porphyrin complex called **Iron protoporphyrin IX**
- **Conjugated double bonds**
  → **Red color to Heme.**
- Functional form Iron in Heme is-
  - **Ferrous form(Fe^{++})**
  - **Reduced state**



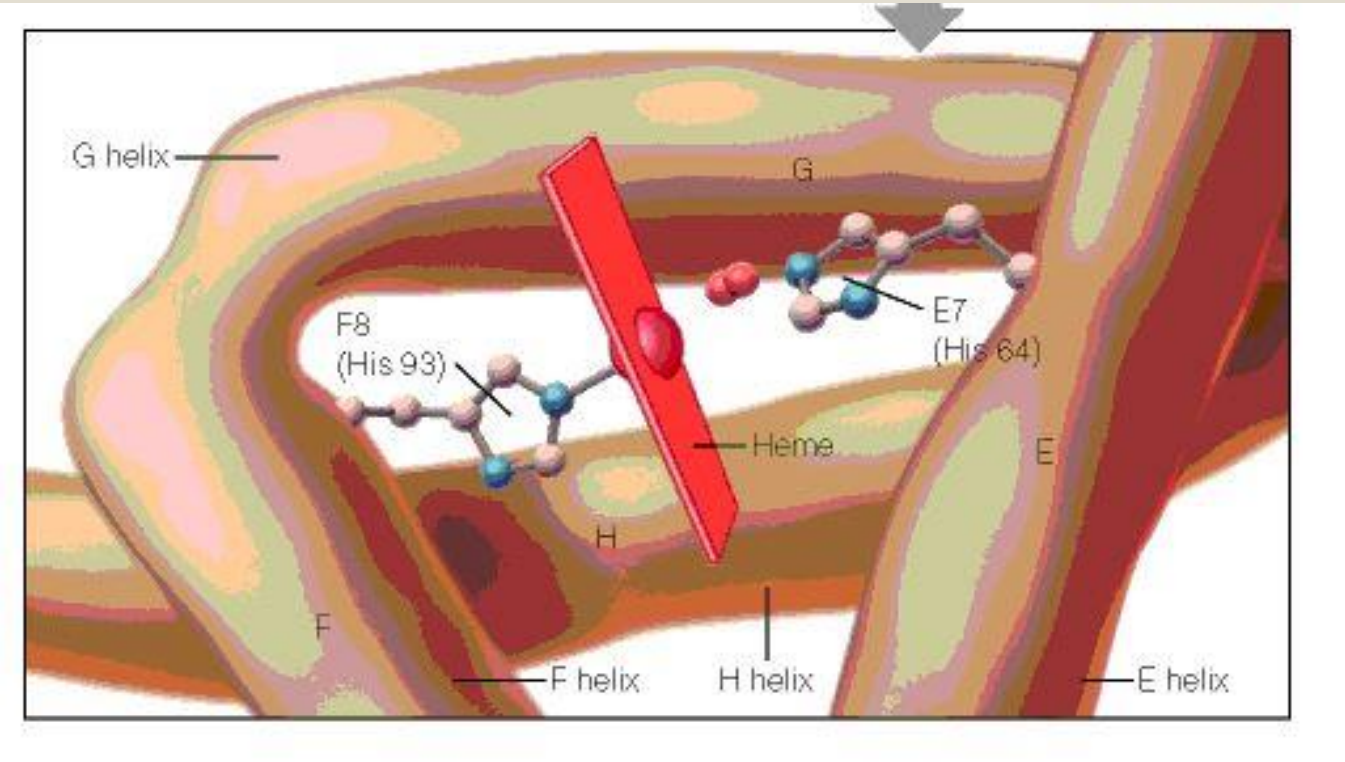
Structure of Globin

Adult Hemoglobin has 4 Polypeptide chains : **2α and 2β**





Heme Pocket

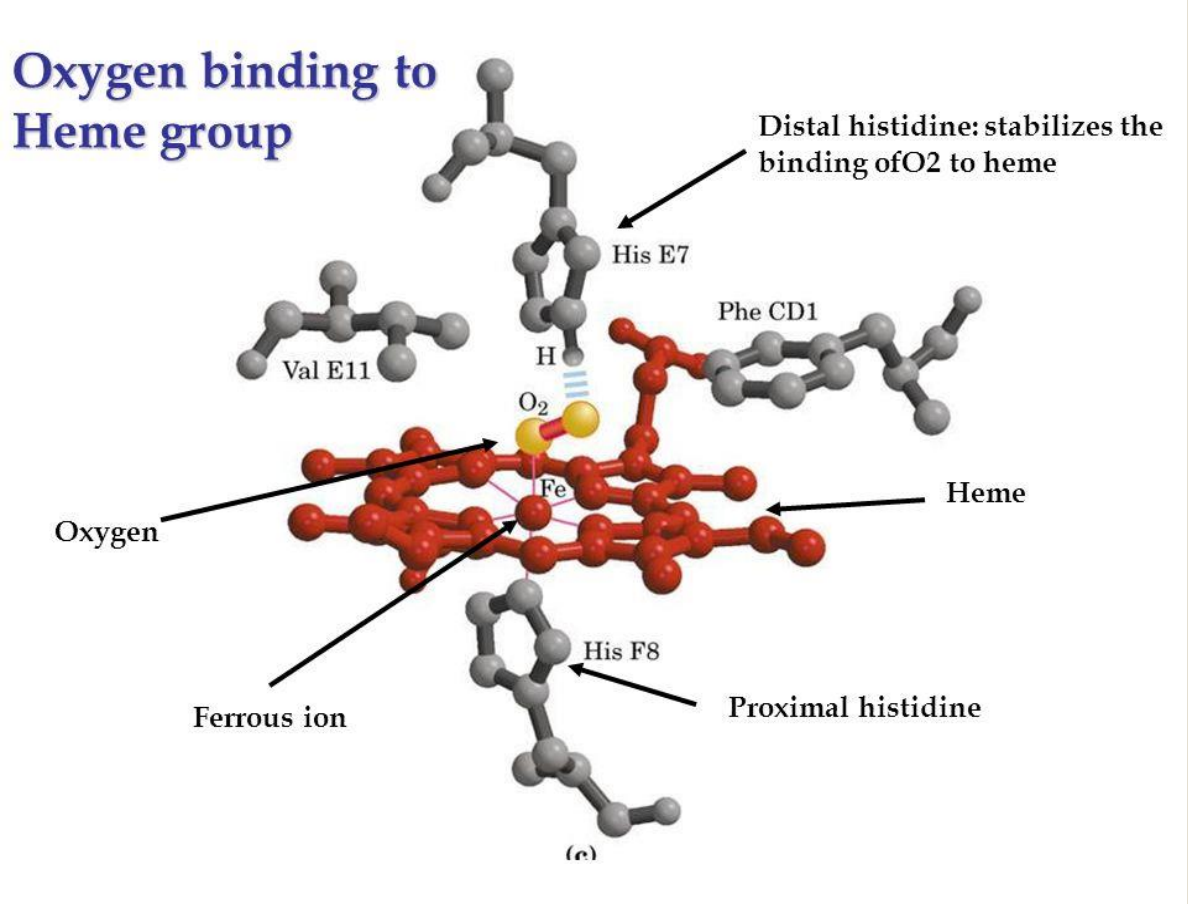


- Heme Pocket is a **crevice/ hollow hydrophobic area**
- Formed in the **interior of Globin subunits**
- Heme group is **tucked between E and F helices** of Globin subunit.

Amino acids in Globin chain are **identified by Helix name** and **Position of a.a in that helix**.

E7 His (Distal His), **F8 His (Proximal His)**

Oxygen binding of Hemoglobin



- Iron has 4 covalent bonds with pyrrole rings, Fifth coordination bond with proximal histidine(F8), Sixth coordination bond with divalent oxygen
- Divalent oxygen is linked to Fe++ of Heme and imidazole group of Distal histidine(E7)

Where did CO bind in Case 1 and 2??

- Hint :
- **Competitive binding**

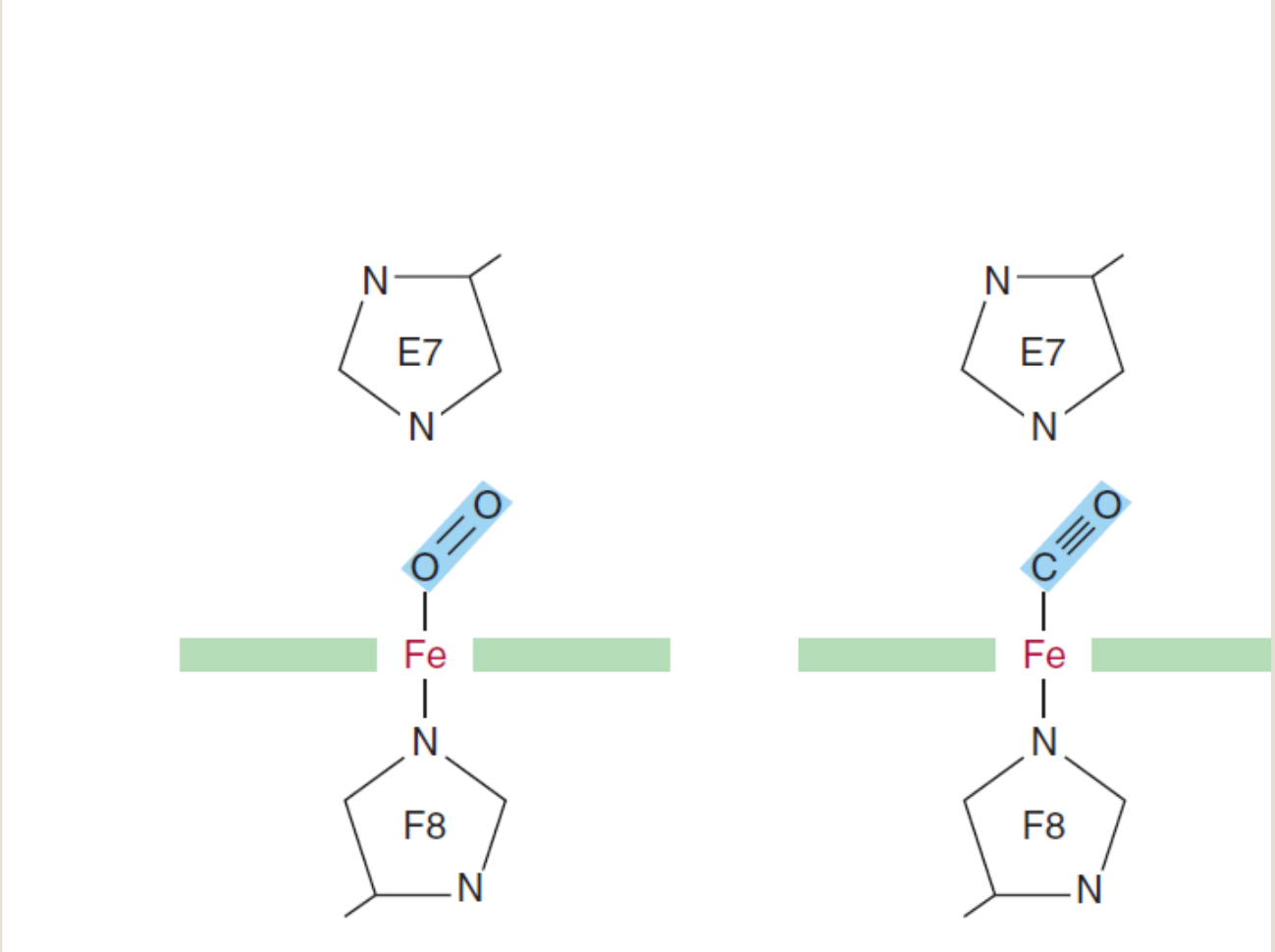
- **Affects Mitochondrial ETC**

(Respiratory Chain/

Cellular respiration)

- To **generate ATP**

(Oxidative Phosphorylation)



Treatment for CO Poisoning

- **Treatment** includes immediate removal of the victim from the exposure and administration of high-flow or 100% oxygen by a non-rebreather reservoir oxygen mask

- **Prevention**: The geyser should not be switched on after locking the door from inside, ventilation should be kept open
- Gas geyser unit should be placed outside the bathroom with a hose of hot water going inside.

References

1. Victor W. Rodwell, David A. Bender, Kathleen M. Botham, Peter J. Kennelly, P. Anthony Weil. Harper's Illustrated Biochemistry, 30th Edition

- 2. Denise R. Ferrier; Lippincott Illustrated Reviews Biochemistry, 7th Edition

- 3. Kasper, Fauci, Hauser et al., Harrison's Principles of Internal Medicine, 19th Edition

Lancet. 1954 Jan 16;266(6803):119-24.

Defective gas-transport function of stored red blood-cells.

VALTIS DJ.

PMID: 13118742

Arch Biochem Biophys. 1967 Jul;121(1):96-102.

Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes.

Chanutin A, Curnish RR.

PMID: 6035074

Why 2,3 BPG reduces in stored blood?

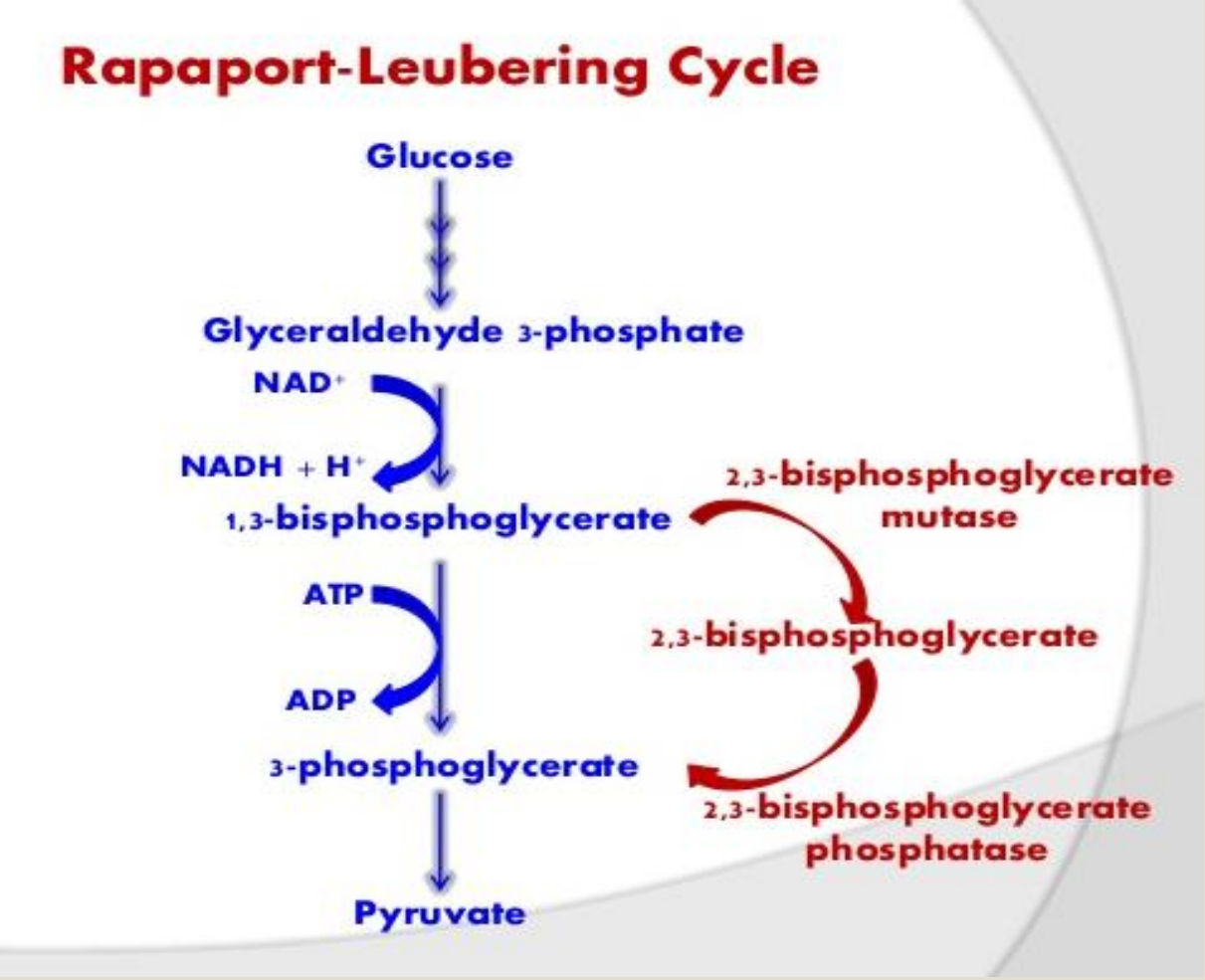
- During storage of RBCs, large amounts of lactate are formed and blood pH drops rapidly.

- When the pH falls below 7.2, the bisphosphoglycerate phosphatase will be activated and the normally high concentration of 2,3-DPG is rapidly depleted

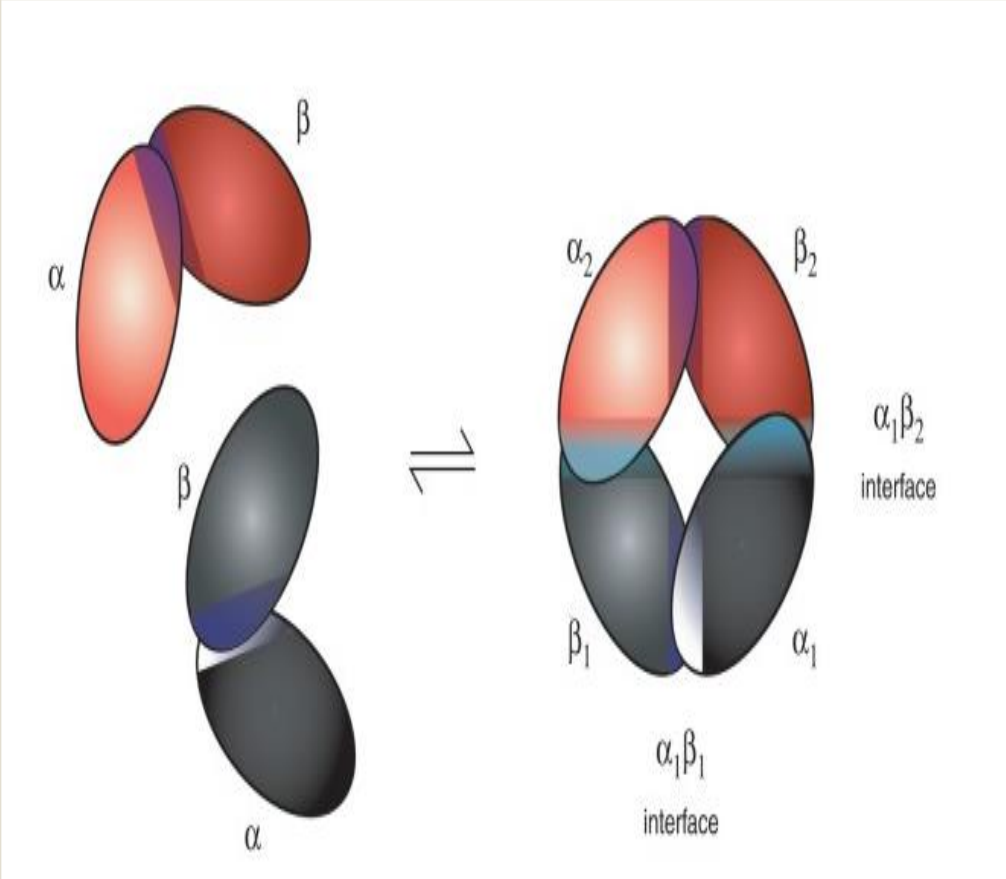
Duhm J, Gehrlach E. Metabolism and function of 2,3- diphosphoglycreate in red blood cells. Greenwalt TJ, Jamieson GA, editors. The human red cell in vitro. New York: Grune & Stratton; 1974. p. 111-48.

Where was 2,3 BPG in Glycolysis?

- Was it 1,3 BPG or 2,3 BPG?



What do 2,3 BPG has to do with Oxygenation and Oxygen transport??

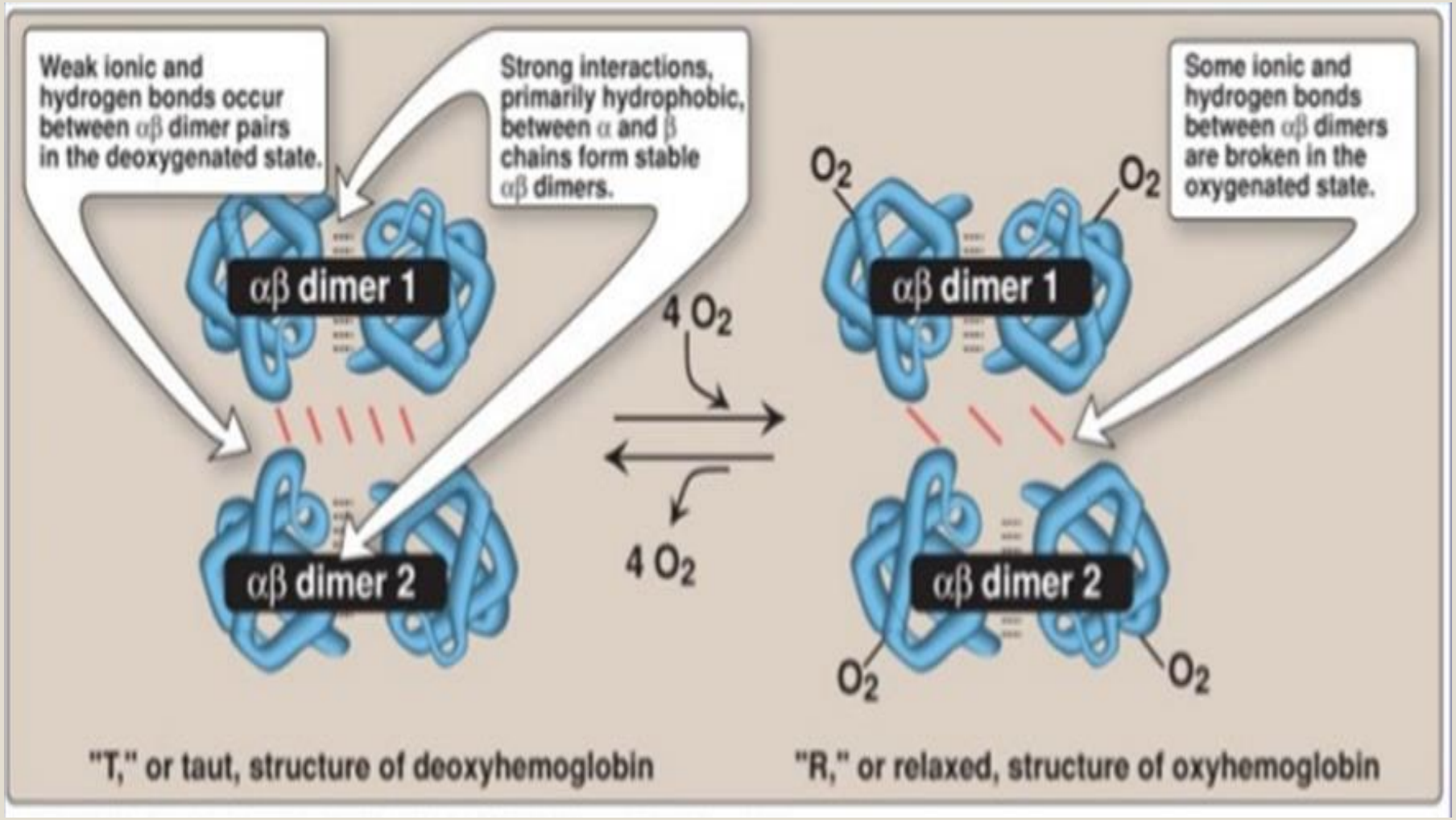


- In Hb 4 polypeptide chains are visualized as **two identical dimers, (αβ)1 and (αβ)2 held by**

Ionic bonds and **Hydrophobic interactions**,

- **Two dimers are linked** to each other by **weak polar bonds.**

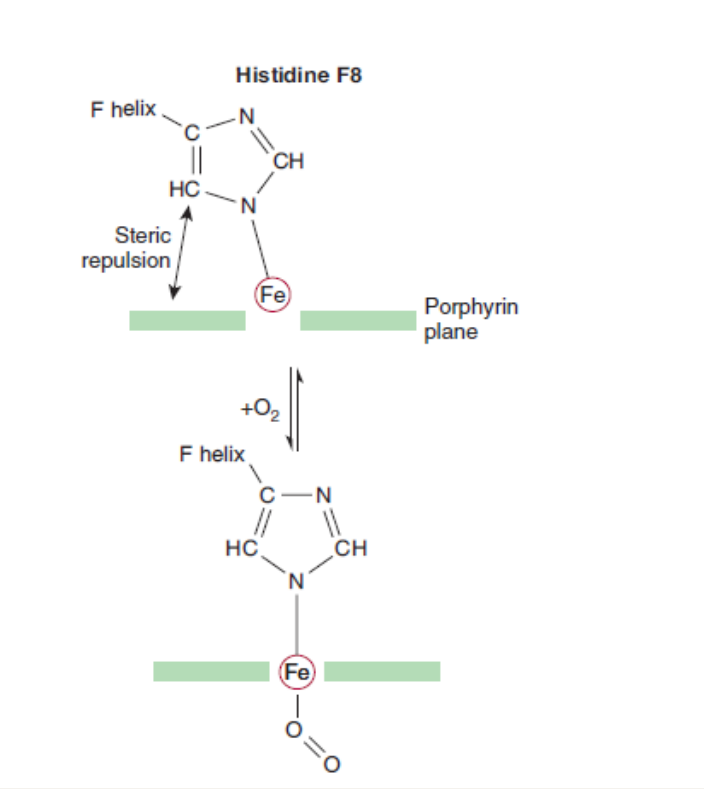
Taut and Relaxed forms of Hb

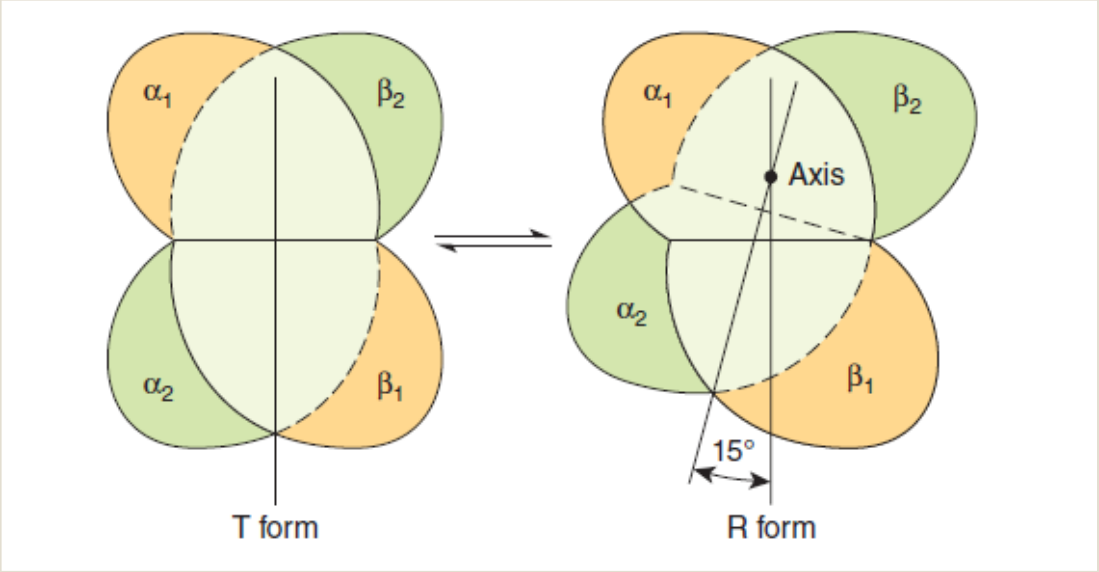


T Form of Hb	R Form Of Hb
Deoxy Hb is in T form binds with CO2,H^+ and 2,3BPG	**Oxy Hb is in R form binds only with Oxygen**
T form has **salt bridges** linked in between the dimer subunits	**Salt bridges are broken** in between the dimer subunits during oxygenation of Hb.
More constrained form	**Less constrained form**
T form has **low affinity for Oxygen**	R form has **higher affinity for Oxygen**
T form of Hb **predominates in low pO2**	R form of Hb **predominates at high pO2**

How does these bonds break on Oxygenation?

Changes in Hb on oxygenation



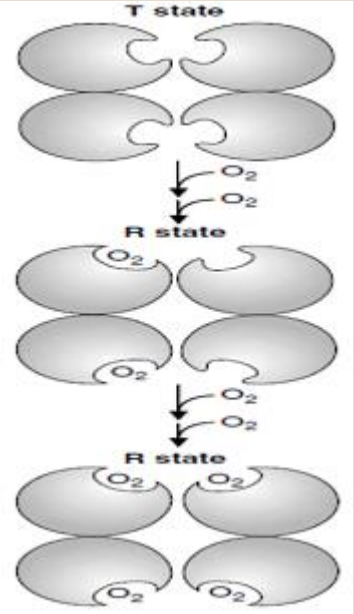


Fe moves into plane of of heme ($0.3A^0$ to $0.1A^0$)

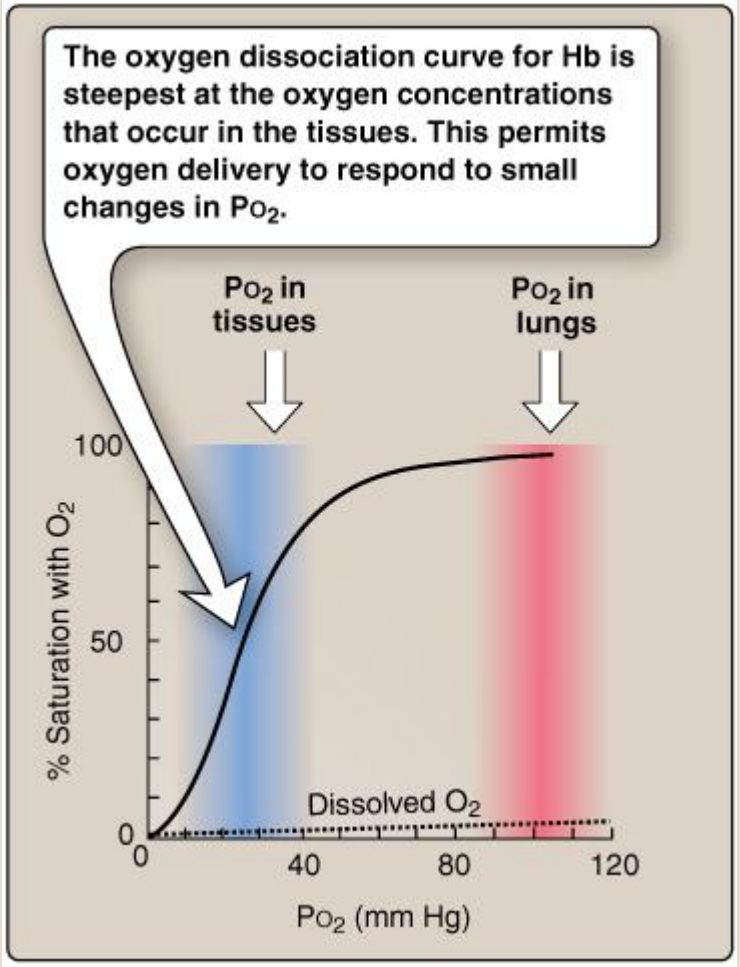
α2β2 moves 15^0 relative to α1β1

Features of oxygenation of Hb

- Oxygen links to **Ferrous** form of Iron, of Heme (Reversible, non-enzymatic, no oxidation of Ferrous)
- **One Hb molecule with 4 Heme can bind to four O2 molecules**
- **Shows co-operative binding like allosteric regulatio**
- **Follows Hills equation**
- **Due to tetrametric structure of Hb**
- **Makes it efficient in oxygen transport**



How cooperative binding mechanism makes Hb efficient in oxygen transport

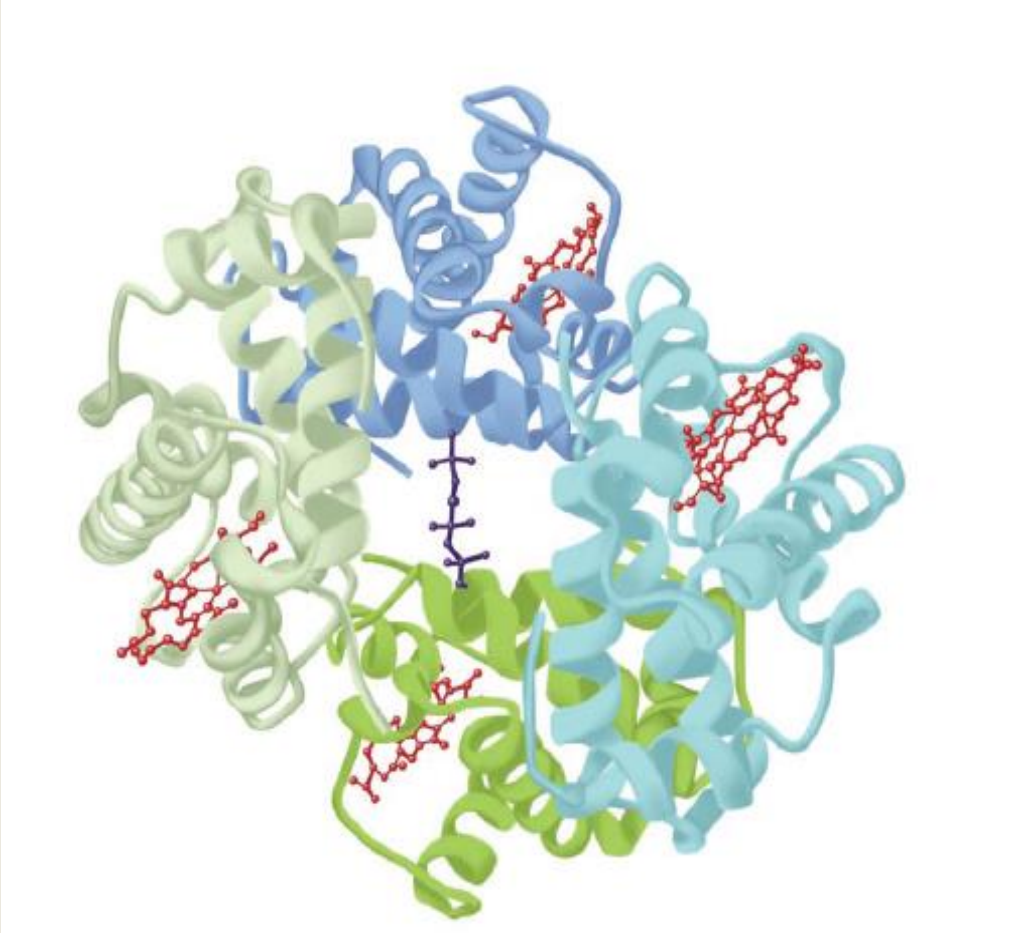


Allows 100% saturation at pO2 much lower than what it actually requires

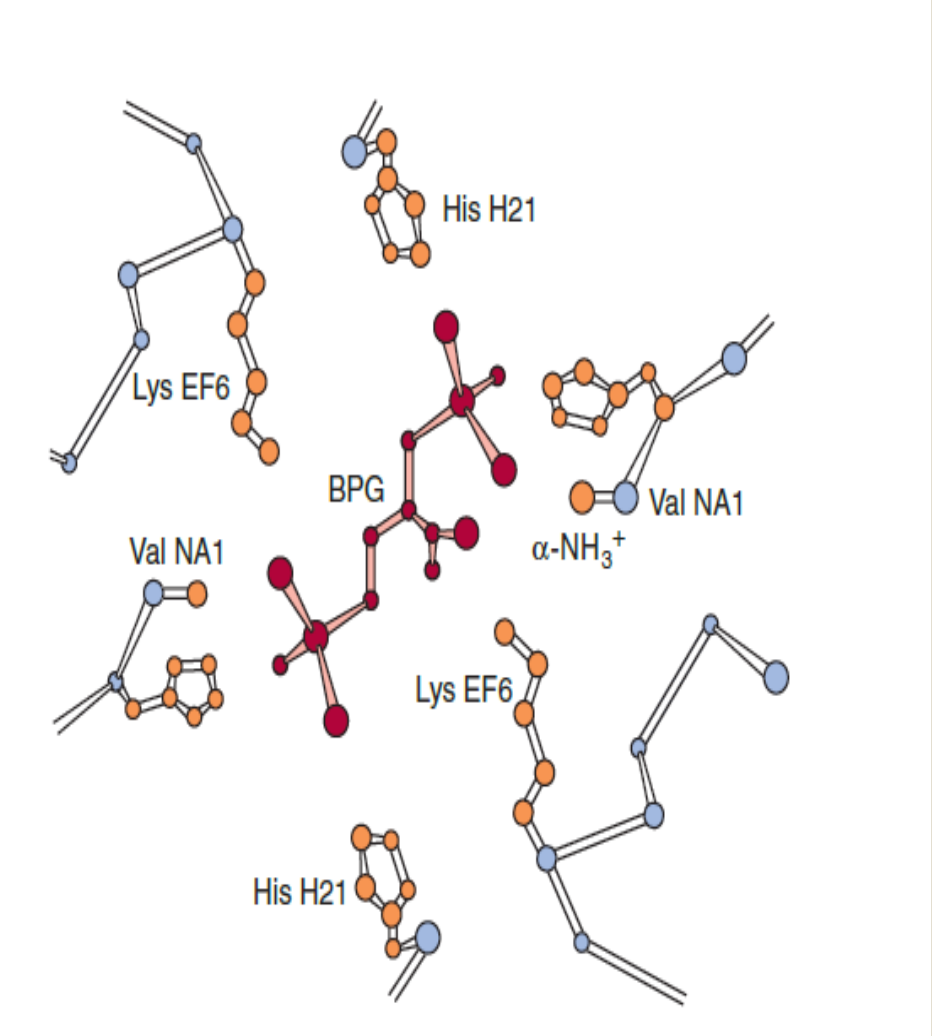
Hemoglobin picks up the **largest possible load** of **Oxygen** in the lungs, and delivers the Oxygen **where and when** needed.

pO_2 (torr)	Per cent saturation of Hb
100 (in alveoli)	96
40 (in resting muscles)	64
20 (in working muscles)	20
10 (in vigorously exercising muscles)	10

Role of 2,3 BPG in release of oxygen



- One molecule of 2,3-BPG binds to a pocket, formed by the **two β-globin** chains, in the center of the deoxyhemoglobin tetramer



- This pocket contains several positively charged amino acids(lysine, valine) that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG.
- Stabilizes T form of deoxyHb

Consequence of Transfusing stored blood

- Hemoglobin deficient in 2,3-BPG thus acts as an **oxygen "trap"** rather than as an oxygen transport system.

- Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6–24 hours.

- However, severely ill patients may be compromised if transfused with large quantities of such 2,3-BPG–"stripped" blood

Transfusion. 2006 Sep;46(9):1543-52.

Storage of red blood cells with improved maintenance of 2,3-bisphosphoglycerate.

Högman CF[1], Löf H, Meryman HT.

Author information

Erratum in
Transfusion. 2007 Jan;47(1):176.

Abstract

BACKGROUND: During storage, red blood cells (RBCs) rapidly lose 2,3-bisphosphoglycerate (2,3-DPG) leading to an increase in the affinity for O(2) and a temporary impairment of O(2) transport. Recent clinical evaluations indicate that the quality of transfused RBCs may be more important for patient survival than previously recognized.

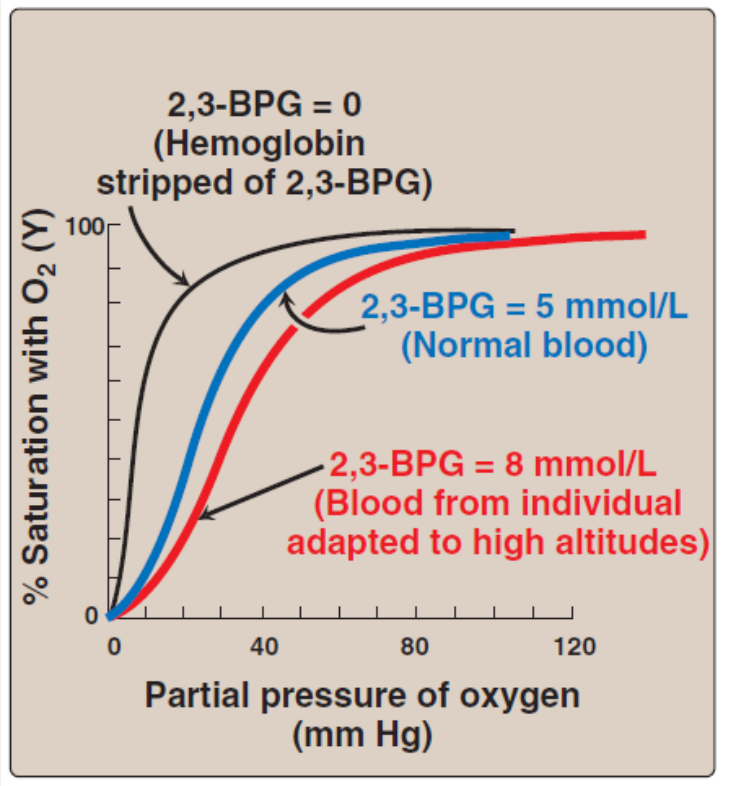
STUDY DESIGN AND METHODS: Glucose-free additive solutions (ASs) were prepared with sodium citrate, sodium gluconate, adenine, mannitol, and phosphates at high pH, a solution that can be heat-sterilized. CP2D was used as an anticoagulant. Additional CP2D was added to the AS to supply glucose. RBCs were stored at 4 degrees C and assayed periodically for intracellular pH (pHi), extracellular pH, glucose, lactate, phosphate, ATP, 2,3-DPG, hemolysis, and morphology.

RESULTS: Storage in 175 mL of the chloride-free, hypotonic medium at a hematocrit (Hct) level of 59 to 60 percent resulted in an elevated pHi and the maintenance of 2,3-DPG at or above the initial value for 2 weeks without loss of ATP. The addition of 400 mL of storage solution

The maximum storage time for red cells has been doubled (21 to 42 days, with median time of 15 days) by changes in H+, phosphate and hexose sugar concentration

Response of 2,3-BPG levels to chronic hypoxia or anemia

- Allosteric effect of 2,3 BPG on oxygen affinity
- 2,3 BPG concentration increases in RBC in hypoxia/ anemia
- **HbF: Low affinity for 2,3 BPG**



References

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- **Thank You!**