University of Papua New Guinea
School of Medicine and Health Sciences
Division of Basic Medical Sciences
Discipline of Biochemistry and Molecular Biology

PBL SEMINAR

HEMOGLOBIN, O_2-TRANSPORT and CYANOSIS – An Overview

What are the major O_2-binding proteins in humans?
- Hemoglobin (Hb) and Myoglobin (Mb)

What are the major differences between Hb and Mb?

- **Hemoglobin (Hb):**
  - Made up of Four-subunits
  - Found in Red Blood Cells (RBC)
  - Facilitates O_2 transport in blood from Lungs to Tissues
  - Facilitates CO_2 transport in blood from Tissues to Lungs
  - Cooperative binding with Oxygen

- **Myoglobin (Mb):**
  - Made up of One-subunit
  - Found in Red Muscles (e.g., Cardiac muscle)
  - Stores O_2 in tissues
  - Non-cooperative binding with Oxygen

What is the general structure of Hb in adult?
- Major type of Hb in adult (HbA) consists of 4 subunits (2α and 2β)
- Each subunit is made up of a polypeptide chain and a Heme prosthetic group,
- Heme prosthetic group is located within a hydrophobic cleft of the folded polypeptide chain
- Functional unit of HbA has a quaternary structure consisting of 4-subunits packed tightly and held together by multiple non-covalent interactions
- **Heme** prosthetic group in HbA is made up of a Protoporphyrin IX ring structure with an Iron atom in the Ferrous (Fe^{2+}) oxidation state that forms Six Coordinate bonds
- Fe^{2+} ion forms **Four Coordinate** bonds with the Four Pyrrole rings of Protoporphyrin
- Fifth and Sixth coordinate bonds are perpendicular to the plane of the Protoporphyrin ring
- Fifth coordinate bond is with a Histidine residue called the **Proximal Histidine** (**His F8**) in the Globin protein
- Sixth coordinate bond is close to another Histidine residue, **Distal Histidine (His E 7)**
- Sixth position of the coordinate bond is either unoccupied (**Reduced Hb**) or occupied by O_2 (**Hb O_2**) or by other compounds (such as CO, HCN)

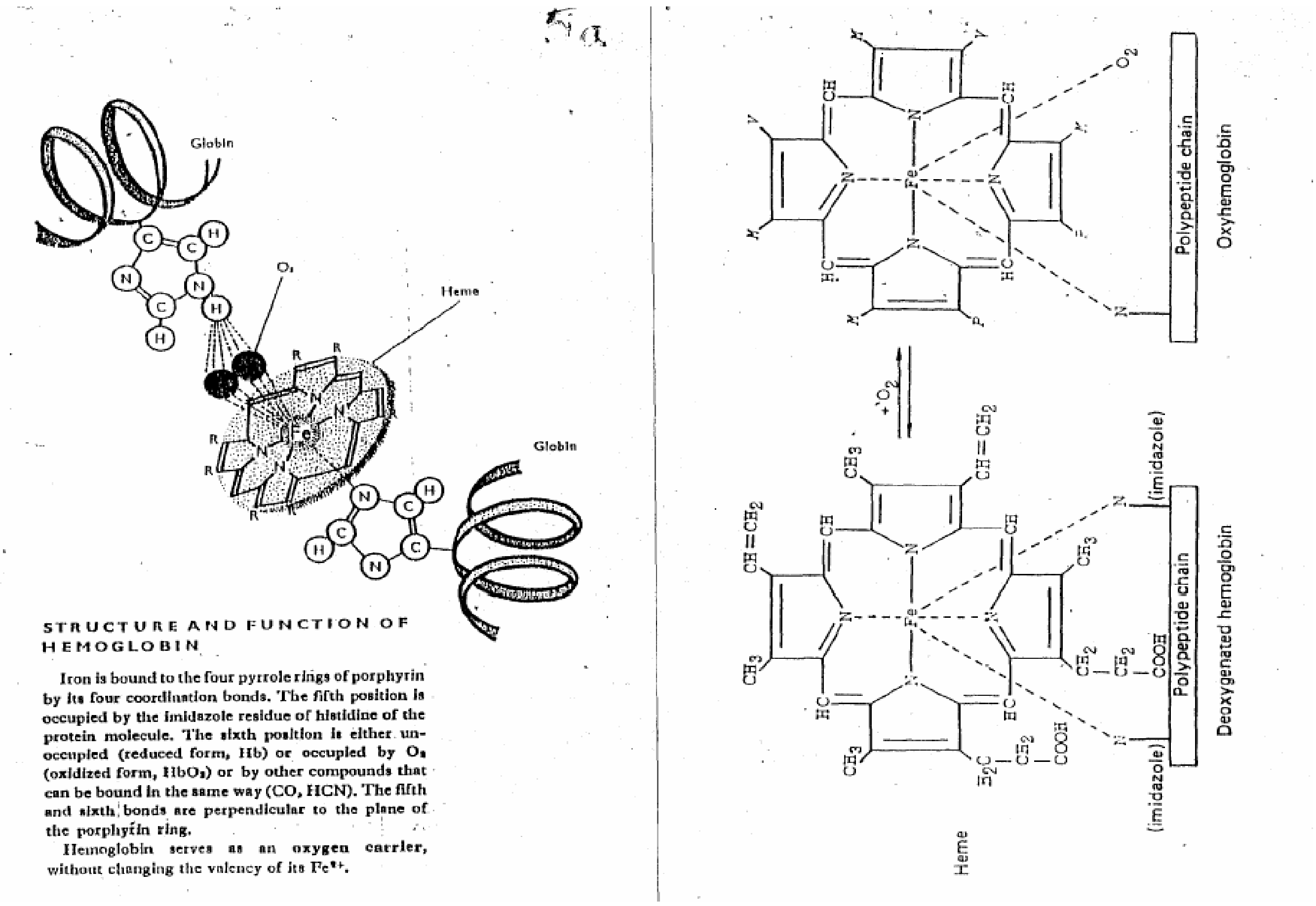
(**Fig. 1**: Schematic diagram of **Heme and Hb**)

What does cooperative binding means in regard to Hb?
- Hb is an allosteric protein
- Two forms of Hb are:

- o Taut (T, deoxygenated): Reduced Hb
- o Relaxed (R, oxygenated): Hb O_2

- Hb interacts with O_2 via **Cooperative binding**
- Binding of O_2 at one Heme group increases the Oxygen affinity of the remaining Heme groups in the same Hb molecule
- Allosteric nature of Hb is indicated thus:
- As O_2 binds, Hb takes on a Relaxed configuration, thus subsequent binding of O_2 is facilitated as each O_2-binging site is exposed
- Myoglobin consist of one subunit, thus O_2 binding is **non-cooperative**

FIG. 1:

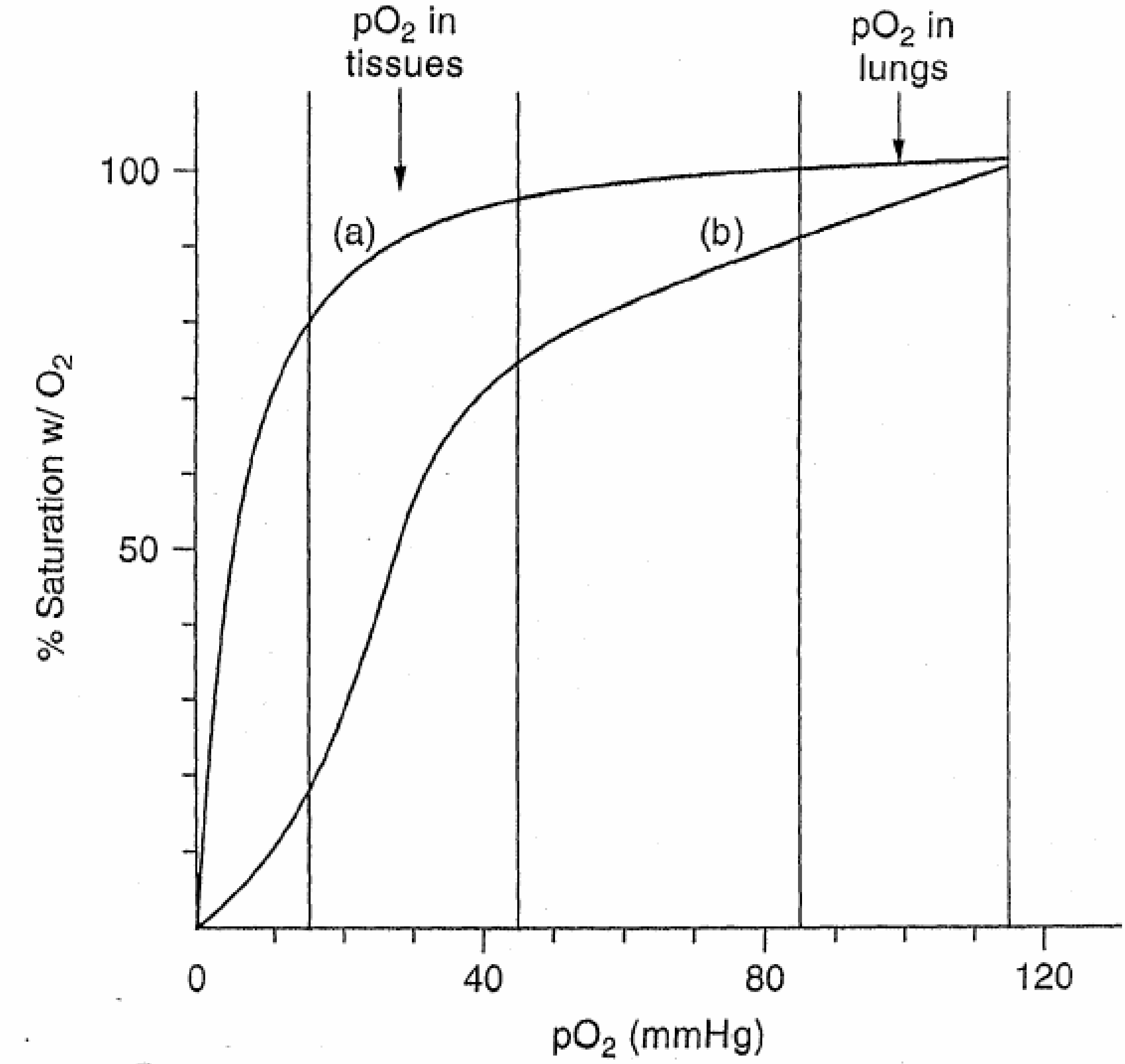


What is the significance of the Oxygen dissociation curve for Hb and Mb?

- **Fig 2** shows schematic diagram of O_2 dissociation curves for Hb and Mb
- Oxygen dissociation curve for Hb is Sigmoid shape, reflecting cooperative binding
  - o Sigmoid shape allows Hb to carry and deliver O_2 efficiently from sites of high O_2 to sites of low O_2
- Oxygen dissociation curve for Mb is Rectangular Hyperbolic shape, reflecting non-cooperative binding
- O_2-dissociation curve for Mb is to the left of the Hb curve
- From the O_2-dissociation curves it can be seen that for any particular O_2 pressure the degree of saturation of Mb is higher than that for Hb
- **Indicating that Mb has a higher affinity for O_2 than does Hb**

- Significance of this is that in the blood capillaries in the muscle, for example, Hb will release its O_2 to Mb for storage

FIG. 2:



Myoglobin and Hemoglobin Oxygen Dissociation Curves. (a) Myoglobin; (b) Hemoglobin.

What is the Bohr Effect?

- Effect of concentration of H^+ ions on the affinity of Hb for Oxygen
- As concentrations of H^+ ions increases, affinity of Hb for O_2 decreases, causing release of O_2 to the tissue

$$\mathbf{HbO_2 + H^+ \Leftarrow\!=\!=\!=\!=\!=\!\Rightarrow HHb + O_2}$$

- Binding of O_2 to hemoglobin is affected by the concentration of H^+ ions and CO_2 in the surrounding tissue

- Actively metabolizing tissue, such as muscle, concentrations of H^+ ions and CO_2 are relatively high
- H^+ ions tend to displace O_2 from HbO_2 to form DeoxyHb
- Increase in CO_2 also causes increase in H^+ ions due to the action of the enzyme **Carbonic Anhydrase**, which catalyzes the reaction:

$$\mathbf{CO_2 + H_2O \Leftarrow\!\!==\!\!\Rightarrow H_2CO_3 \Leftarrow\!\!==\!\!\Rightarrow HCO_3^- + H^+}$$

How is O_2 transported in blood?

- Hemoglobin binds and transport O_2 as Oxyhemoglobin (OxyHb)
    - A fully loaded Hb molecule carries $4O_2$
- Total O_2 content in blood is the sum of dissolved O_2 and OxyHb
- Total O_2 capacity of blood is about 20.0ml of O_2 per 100ml of blood
- Normally, about 97 – 98% of O_2 is transported as OxyHb from Lungs to tissues
- Usually about 0.33ml of O_2 is physically dissolved in 100ml of blood
- At the lungs the Partial Pressure of O_2 (PO_2) is highest (PO_2 = 100mm Hg)
- At the tissue PO_2 is about 40 mm Hg

How is CO_2 transported in blood?

At the Tissues:

- CO_2 produced during metabolism diffuses freely down concentration gradient across cell membrane into ECF and RBC
    - Concentration gradient for CO_2 in this direction is because metabolism in RBC is Anaerobic, thus no CO_2 is produced in RBC
- RBC is the principle site for formation of H^+ and HCO_3^- ions in blood by action of Carbonic Anhydrase:

$$\mathbf{CO_2 + H_2O \Leftarrow\!\!====\!\!\Rightarrow H_2CO_3 \Leftarrow\!\!====\!\!\Rightarrow H^+ + HCO_3^-}$$

- H^+ ions are mainly buffered inside RBC by Deoxygenated Hb (HHb) formed when OxyHb gives up O_2 in the tissues:

$$\mathbf{HbO_2 + H^+ \Leftarrow\!\!====\!\!\Rightarrow HHb + O_2}$$

- HCO_3^- ions pass from RBC down their concentration gradient into plasma, in exchange for Chloride ions (Cl^-) so as to maintain electrical neutrality (**Chloride Shift**)

At the Lungs:

- Reverse of these processes occur at the lungs
    - PCO_2 in blood of Pulmonary capillaries is higher than PCO_2 in Alveoli
    - Low level of PCO_2 in Alveoli is maintained by ventilation
- **Fig. 3:** Schematic diagram of O_2 and CO_2 exchange at Tissue and Lungs

How does H^+ ion affect the affinity of Hb for O_2?

- H^+ ion concentration (pH) affects the affinity of hemoglobin for O_2
- **Low pH** (i.e., increased acidity or high H^+ ions) **decreases the affinity of Hb for O_2** and therefore, **Shifts the O_2-dissociation curve to the Right** (**Fig. 4)**
    - Enhancing the release of O_2 by HbO_2 at the tissues
- **High pH (low acidity or low H^+ ions) increases the affinity of Hb for O_2** and therefore, **Shifts the O_2-dissociation curve to the Left** (**Fig. 4**)
- Influence of pH on O_2 binding is Physiologically significant, because:
    - Decrease in pH is often associated with increased O_2 demand – as can occur in increased metabolic rate that results in production of either CO_2 and/or Lactic acid

How does 2,3-BPG affect the affinity of Hb for O_2?

- **2,3-Bisphosphoglycerate** (**2,3-BPG**) is a highly Anionic organic phosphate molecule present in RBC along with the Hemoglobin
- **2,3-BPG promotes the release of O_2 from HbO_2 by lowering the affinity of Hemoglobin for O_2**
- One molecule of 2,3-BPG binds in the small cavity located between the β-chains in DeoxyHb (**Fig. 5**)
- On binding it stabilizes the quaternary structure of DeoxyHb
- For O_2 to overcome this and bind to Hb, a higher concentration of O_2 is required
- O_2 tension in the lungs is sufficiently high under most conditions to saturate Hb almost completely, even when 2,3-BPG levels are high
- Binding of 2,3-BPG to HbO_2 does not occur because the cavity is too small and therefore cannot accommodate 2,3-BPG

What is the significance of 2,3-BPG binging to Hb?

- Significance of a High 2,3-BPG concentration is that the efficiency of O_2 delivery to tissues is increased
- High levels of 2,3-BPG enhance formation of DeoxyHb at Low Partial Pressure of O_2, thus HbO_2 delivers more of its O_2 to the tissues
    - Resulting in a substantial increase in the amount of O_2 delivered because the venous blood returning to the heart of a normal individual (at rest) is at least 60% saturated with O_2
        - Much of this can dissociate in the peripheral tissues if the 2,3-BPG concentrations rises
- High 2,3-BPG shifts the O_2-dissociation curve to the Right
- Low 2,3-BPG shifts the O_2-dissociation curve to the Left (**Fig. 4**)
- Tissue Hypoxia increases 2,3-BPG in RBC
    - Examples: In conditions such as Anemia, Cardiopulmonary Insufficiency, High Altitude, etc

How does Temperature affect the affinity of Hb for O_2?

- Temperature has a significant effect on the affinity of Hb for O_2
- At **temperatures below normal** (Hypothermia) the **affinity of Hb for O_2 increases**, resulting in tighter binding of O_2 to Hb
    - **O_2-dissociation curve is Shifted to the Left** (**Fig. 4**)

- At **higher temperatures the affinity is lower**, and therefore binding is weaker, thus Shifting the O_2-dissociation curve to the **Right** (**Fig. 4**)
- Effect of Higher temperature is similar to that of High levels of 2,3-BPG, in that both enhance the unloading of O_2 at the tissue

Take Note:
- At lower temperatures:
    - Decreased utilization of O_2 by the body
    - Increased solubility of O_2 in plasma,
    - Increased solubility of CO_2 (which acidifies the blood),
- Compensate for the diminished ability of Hb to release O_2
- H^+, CO_2 and 2,3-Bisphosphoglycerate are all Allosteric effectors (**Why?**)
- Because:
- These molecules act at different sites on Hb and their effects are additive
- They favor the conformation of HHb and therefore promote the release of O_2 from HbO_2

What parameters can shift O_2- dissociation curve of Hb to the Right?
- Remember the Acronym: **CADET Right (See diagram below)**
- Increased:
    - **C**O_2
    - **A**cidity (low pH)
    - 2,3-**D**PG (2,3-Diphosphoglycerate or 2,3-Bis-phosphoglycerate)
    - **E**xercise
    - **T**emperature

CYANOSIS

What is Cyanosis?
- Condition that causes Skin, Lips, Mucous Membrane and/or Fingernails to appear bluish in color or (in severe cases) purple-magenta
- Higher than normal HHb in small superficial blood vessels
- Higher than normal Met-Hb in blood

When can cyanosis be observed?
- Cyanosis may be observed when:
    - O_2 saturation of blood is below 80%
    - Mean capillary concentration of HHb in blood is greater than 50g/L
        - Bluish color characteristic of Cyanosis is due to the presence of more than 50g/L of HHb in Capillary Blood;
        - In “Healthy” Individuals (Hb = 150g/L) Cyanosis occurs when more than One-third of their total Hb is Deoxygenated
    - Amount of Met-Hb in circulation is greater than 10% of total Hb

What are some of the causes of Cyanosis?
- Several causes of Cyanosis:
    - Some basic mechanisms that can cause Cyanosis are:

- O_2 saturation of Arterial blood is lower than normal or
- Circulation may be slowed causing more extraction of O_2 per gram of Hb, thus increasing the concentration of HHb in capillaries

- A variety of diseases and factors may cause Cyanosis:
  - Lack of O_2 (such as in suffocation or Cyanotic Heart disease),
  - Congenital Heart disease, Pulmonary disease,
  - Terminal event as in Cardiopulmonary Arrest
  - Abnormal Hb (such as Met-hemoglobinemia)
  - Toxins (such as Cyanide, Carbon Monoxide)
  - Exposure to Cold Air or Cold Water,
  - High Altitude, Shock, Breath holding,
  - Asthma, Seizures, Drug Overdoses (Narcotics, Sedatives), etc.

Fig 3A
SCHEMATIC DIAGRAM OF TRANSPORT OF O_2 AND CO_2 IN BLOOD

(A) REACTIONS AT THE LUNGS: RBC (Venous blood) from Tissue to Lungs

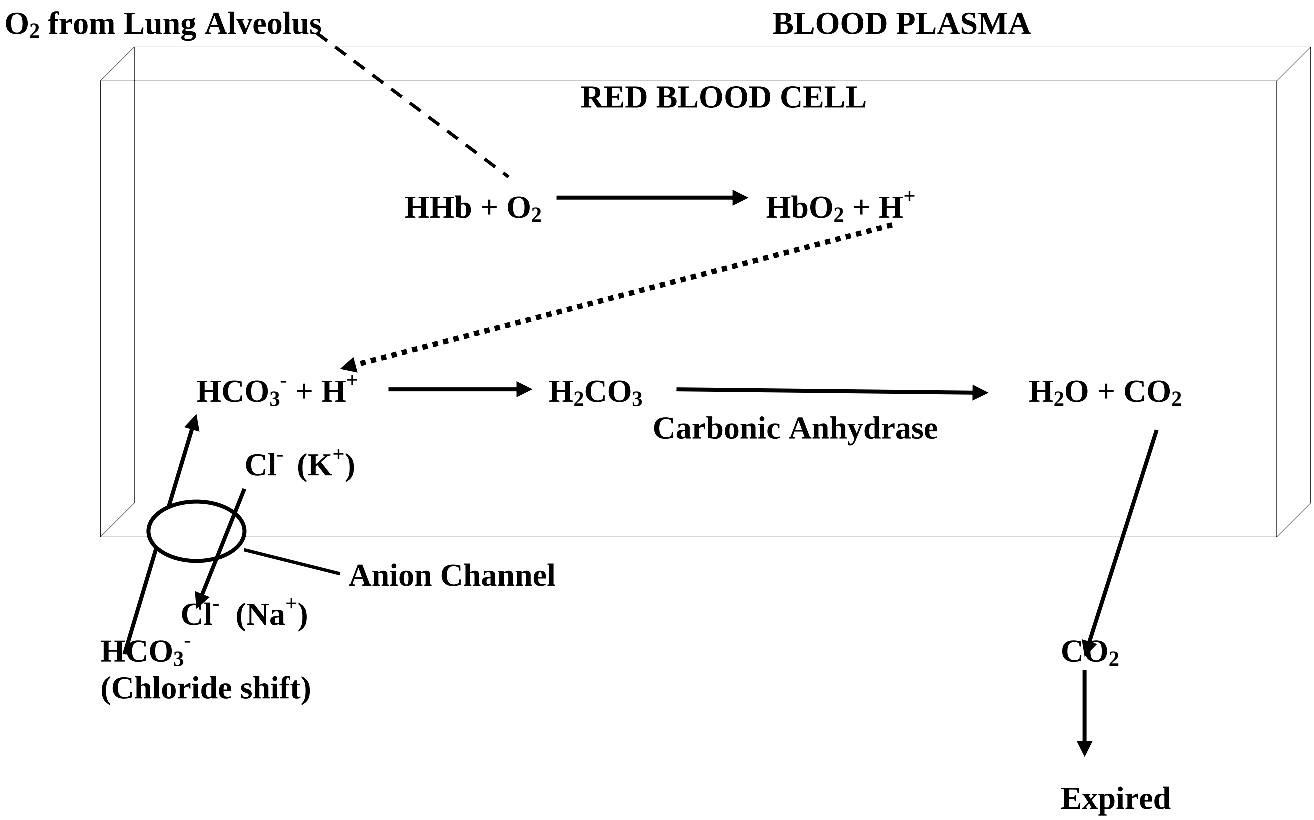
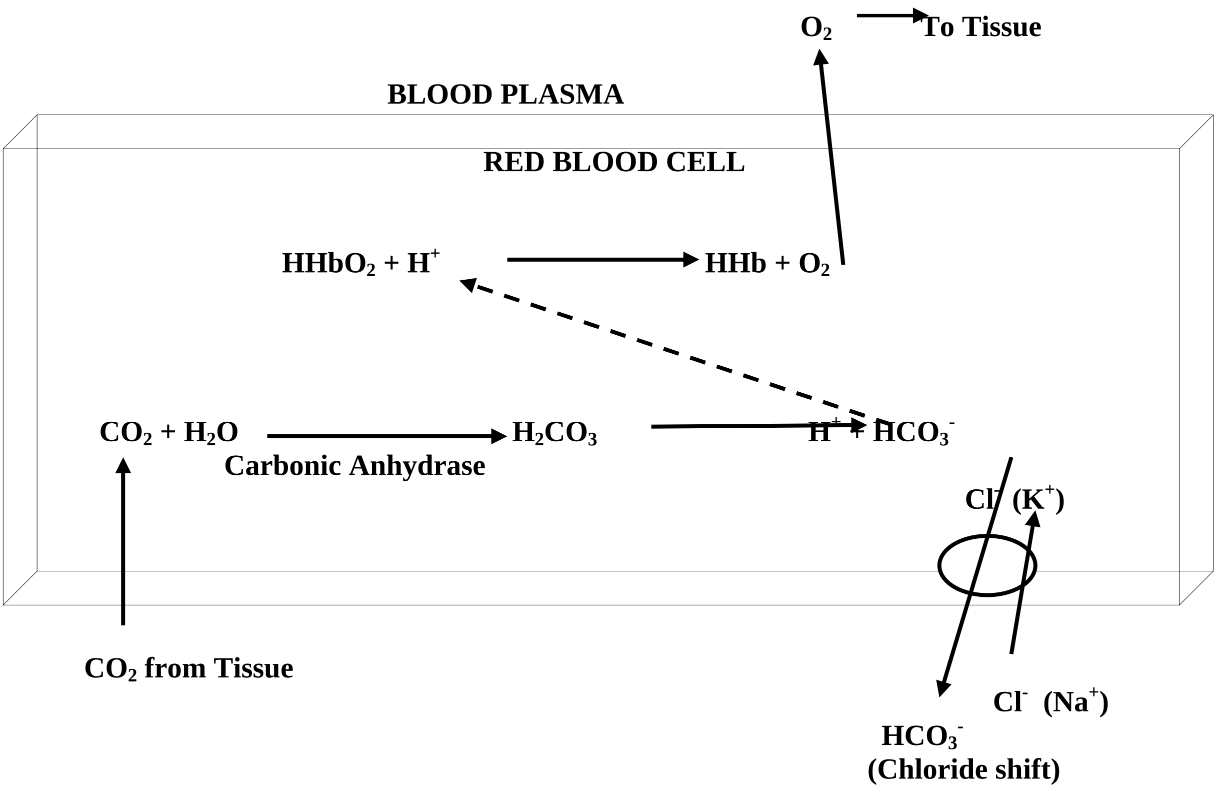


Fig 3B

(B) REACTIONS AT THE TISSUE: RBC (Arterial blood) from Lungs to Tissue



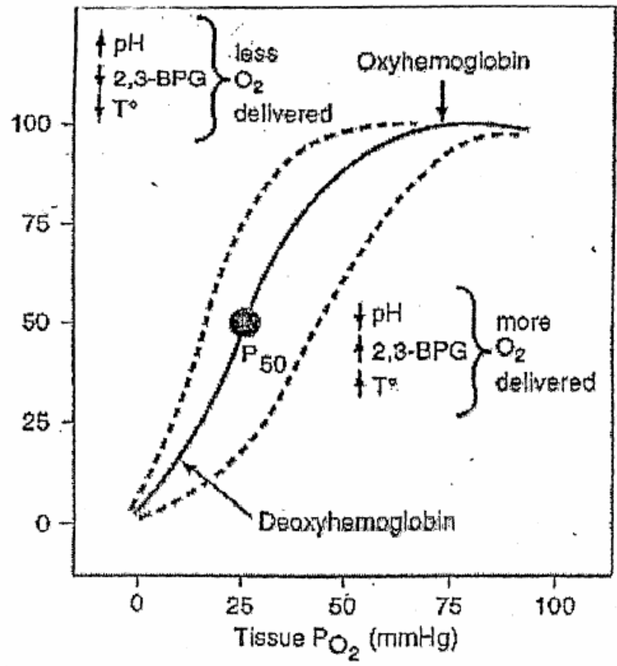


Figure 4

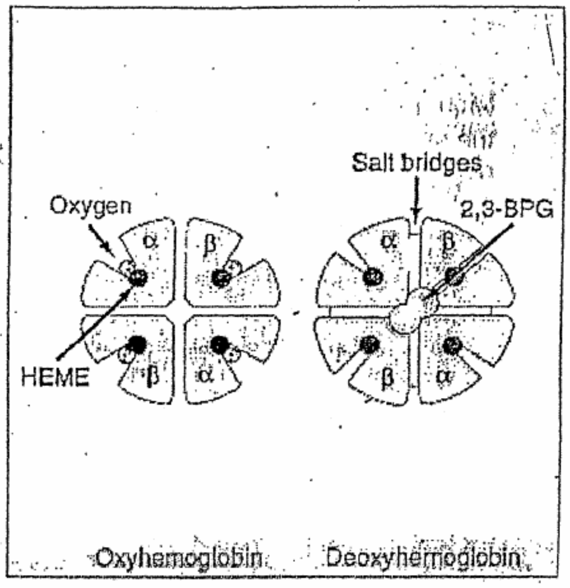


Figure 5

Printed from: *Principles of internal medicine.* Fauci AS *et al.* 14th ed. 1998.

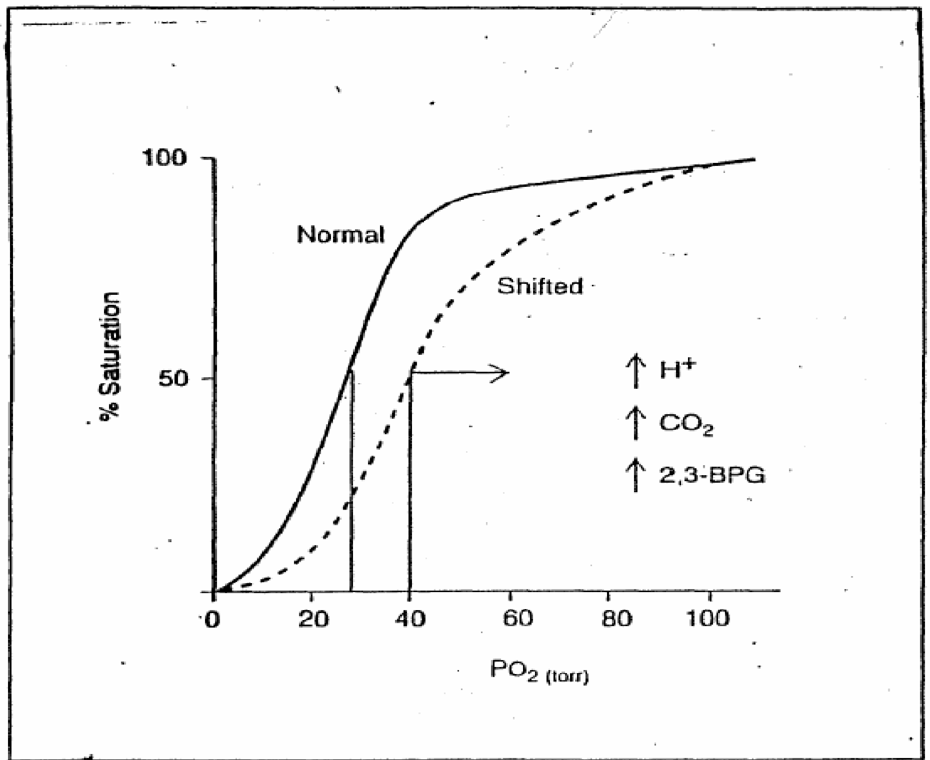


Fig 6 Rightward shift of the hemoglobin oxygen-binding curve in the presence of an increased concentration of protons (decreased pH), CO_2, or 2,3-bisphosphoglycerate (2,3-*BPG*).

CADET: Right