Protein Function. Ch-5-

Dr. Rula Abdul-Ghani

Ligand: molecule bound reversibly to a protein.

Binding site: the site to which the ligand binds, complementary to ligand in size, shape , charge, hydrophobic/philic character.

- Interaction is specific: selective binding to one/few ligands among thousands.
- Interaction coupled to conformational changes →tighter binding.
- Structural adaptation= induced fit = Proteins undergo a conformational change upon ligand binding.
- In a multisubunit protein the binding of a ligand to one subunit affects ligand binding to other subunits.

Myoglobin:

Simple oxygen-binding protein in all mammals primarily in muscle tissue.

Structure of myoglobin:

- Single polypeptide (153a.a)

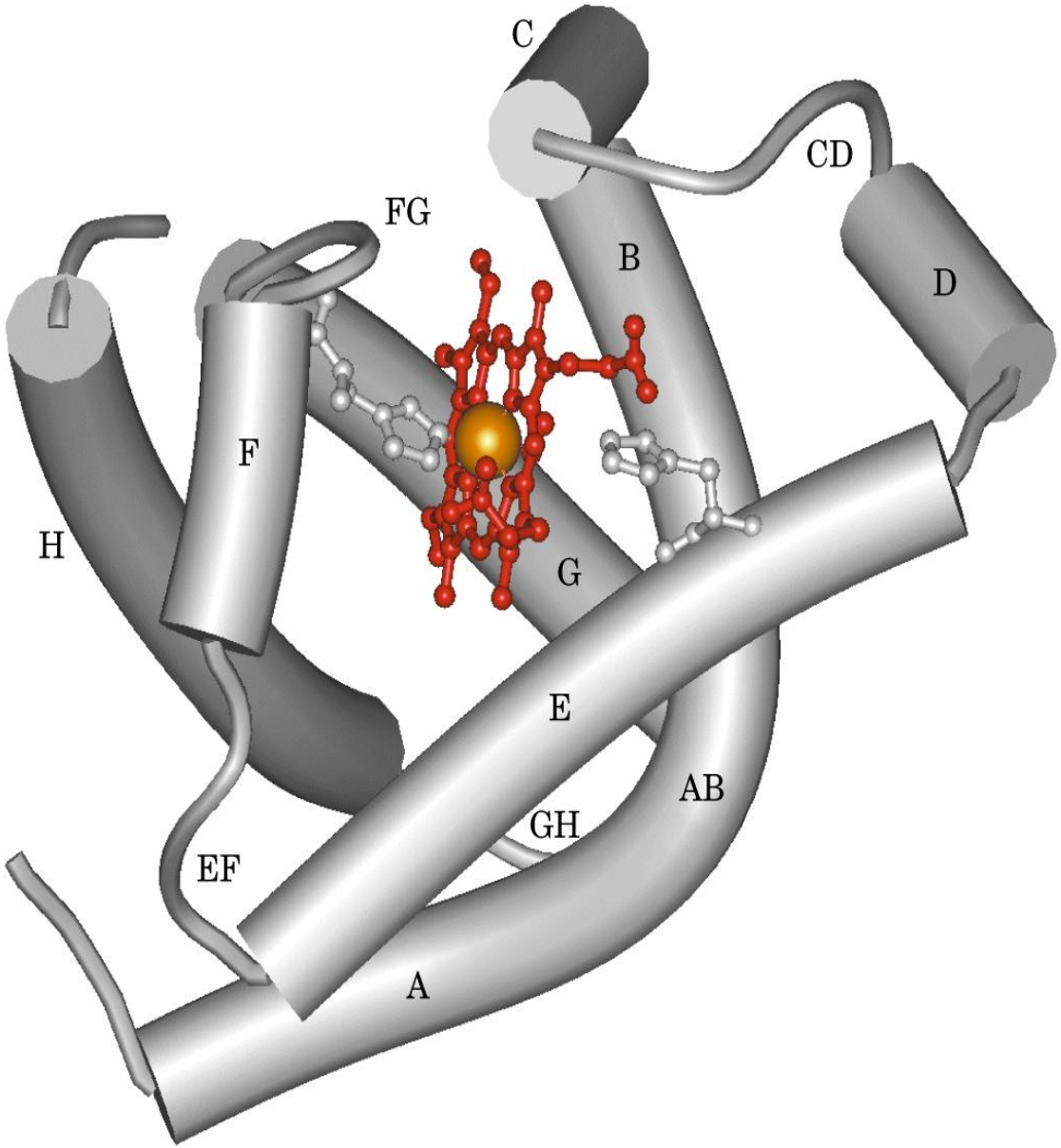
- 8 cylinders A-H

-Nonhelical residues AB,CD …

segments that interconnect

-The heme pocket made up

largely from E and F helices.



Myoglobin's function depends on protein's ability not only to bind oxygen but also to release it.

Reversible binding of a protein P to a ligand L :

$P + L \leftrightarrow PL$

$$Ka = \frac{[PL]}{[P][L]}$$

Ka= affinity of L to its P .

Ka= association constant = M^{-1}

Kd = dissociation constant= 1/Ka = M

Binding equilibrium. (increase in L but ligand binding sites limited)

θ (Theta) = binding sites occupied/ total binding sites

$$\theta = \frac{[PL]}{[PL]+[P]}$$

The more tightly a protein binds a ligand → the lower [ligand] required for the binding sites to be occupied →lower Kd.

table 7–1

Some Protein Dissociation Constants

Protein	Ligand	K_d (M)*
Avidin (egg white)†	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)‡	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)§	Ca^{2+}	3×10^{-6}
		2×10^{-5}

*A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in solution salt concentration, pH, or other variables.

†Interaction of avidin with the enzymatic cofactor biotin is among the strongest noncovalent biochemical interactions known.

‡This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

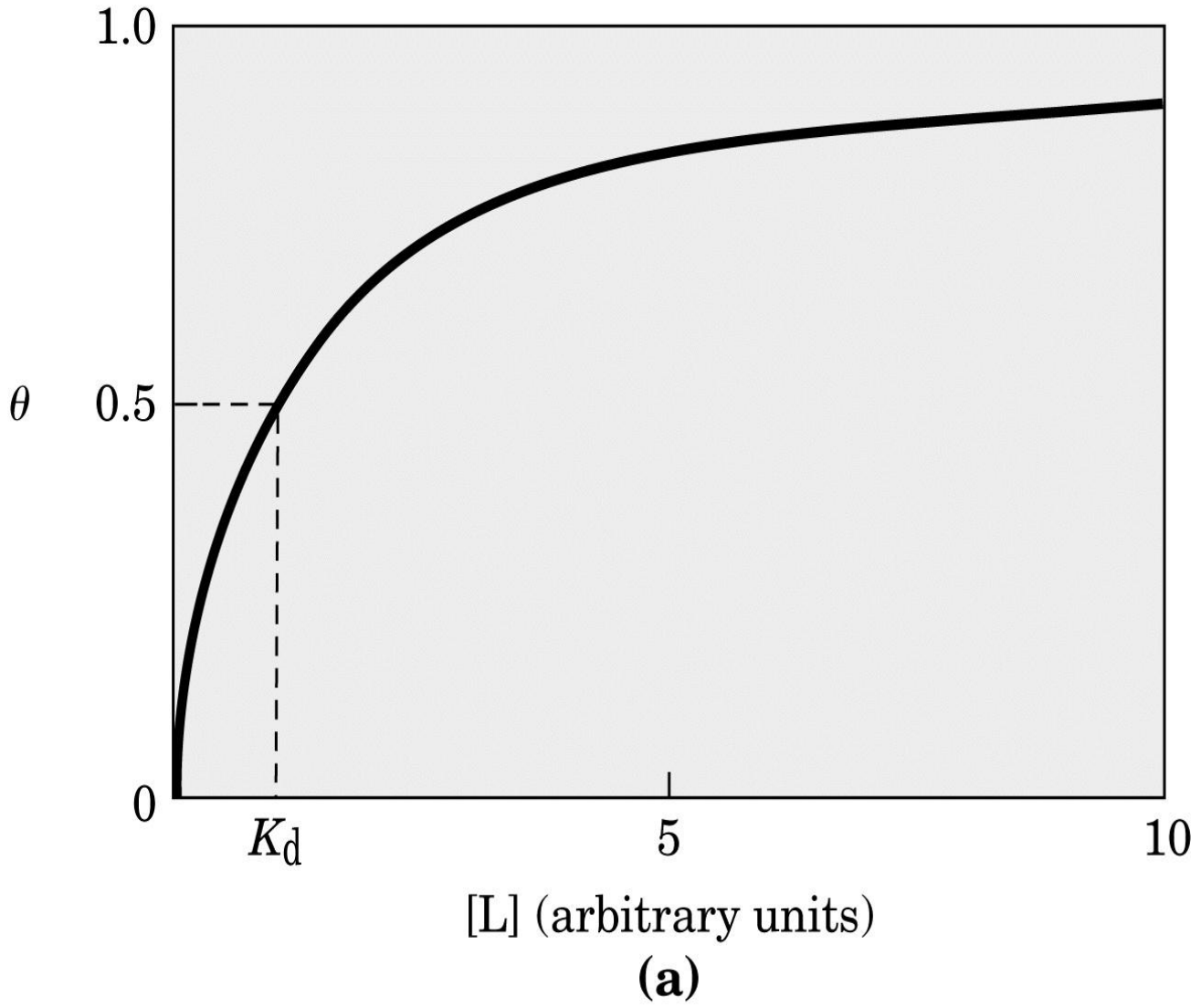
§Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Graphical representation of ligand binding:

The fraction of ligand binding sites occupied, θ plotted against [free ligand]

Kd = 1/Ka =

[L] at which half the available ligand binding sites are occupied.

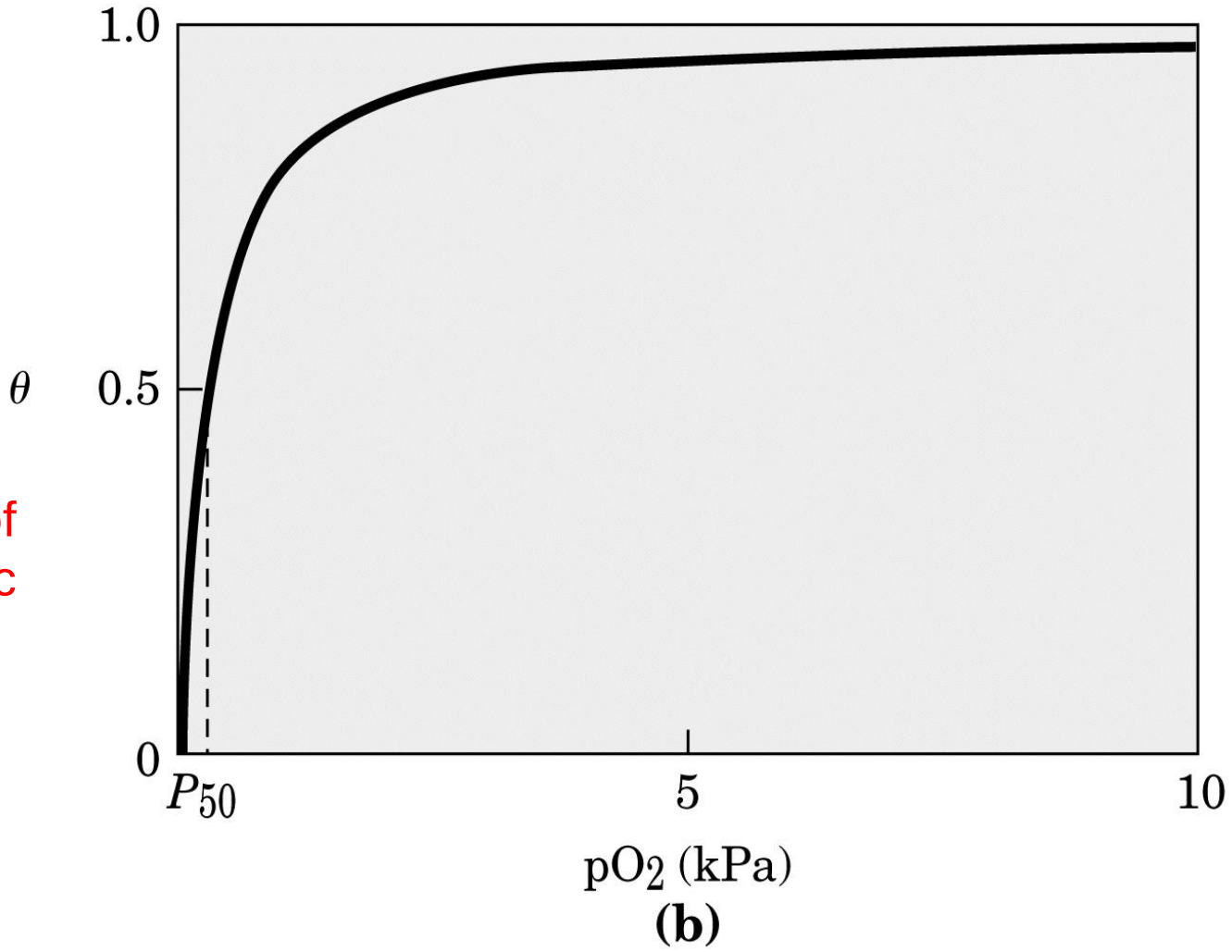


A curve describing O_2 binding to myoglobin:

O_2 is a gas, conc. described by partial pressure of O_2 expressed in kilopascals (Kpa).

O_2 binds tightly to myoglobin with P50 of only 0.26KPa.

O2 dissociation curve of myoglobin → hyperbolic

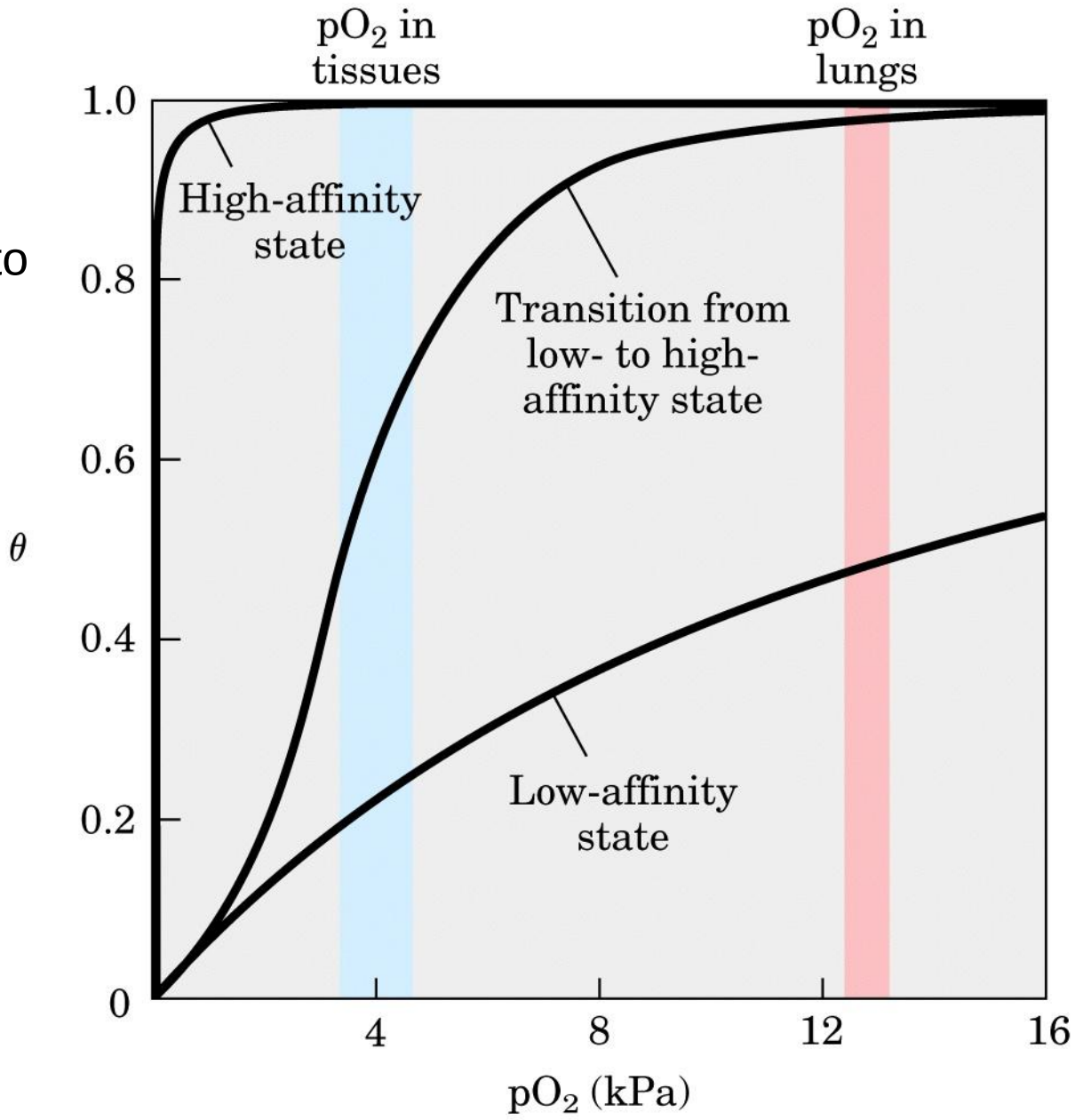


Hemoglobin binding O_2=A sigmoid / cooperative binding curve:

a curve reflects transition from low to high affinity.

Cooperative binding renders Hemoglobin more sensitive to small differences in O_2 conc. bw tissues & lungs allowing hemoglobin to bind O_2 in lungs (high pO_2) & release it in tissue (low pO_2).

O_2 dissociation curve of hemoglobin → sigmoidal



Allosteric protein: binding of a ligand to one site affects the binding properties of another site on the same protein.

allos greek = other.

stereos = solid / shape.

Allosteric protein = having “other shapes” / conformation induced by binding of ligand= modulator (inhibitor or activator).

Conformational changes →more/less active.

Homotropic= normal ligand and modulator are identical.

Heterotropic= modulator other molecule than normal ligand.

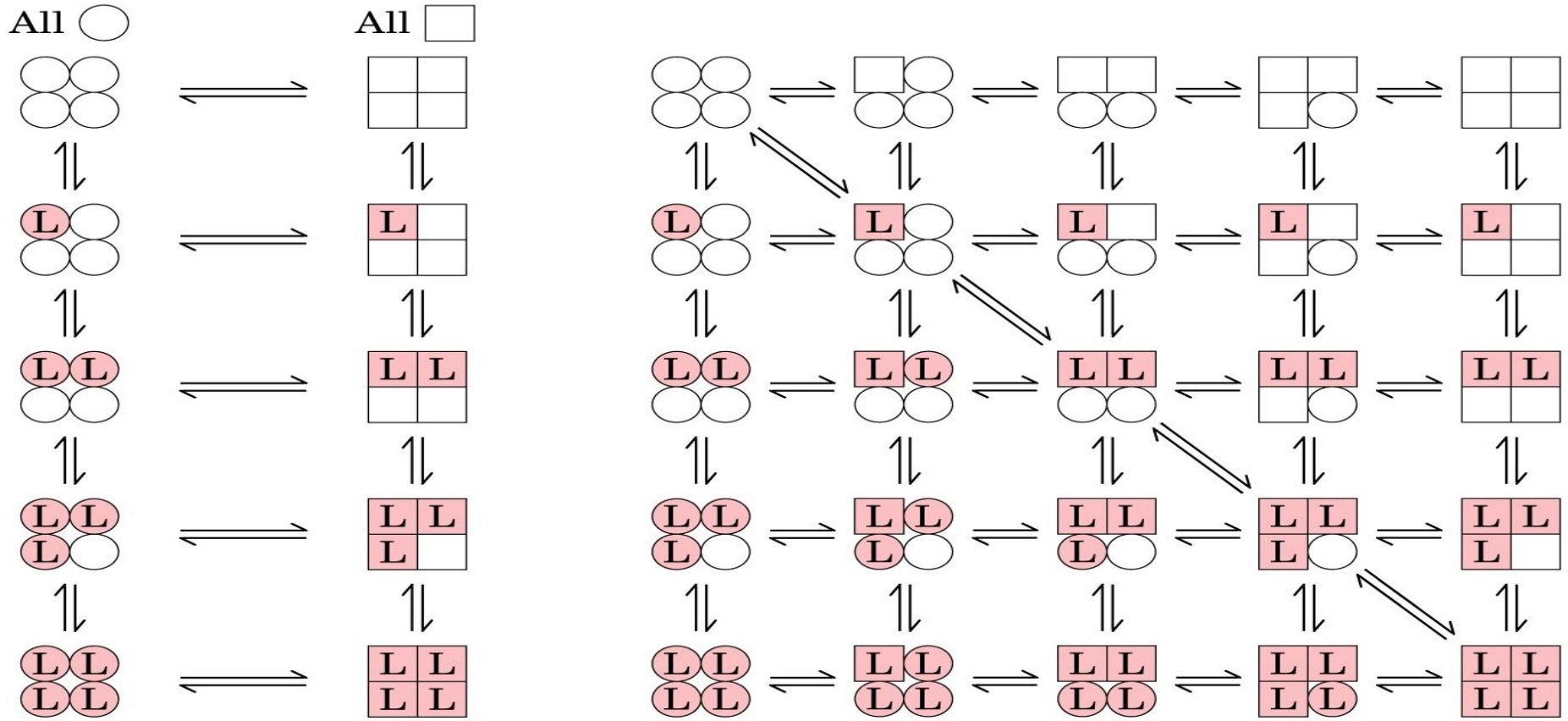
One protein have several modulators homotropic/heterotropic.

O_2 binding to hemoglobin : O_2 ligand + homotropic modulator.

Two models for the interconversions of inactive and active forms of cooperative ligand binding proteins to multisubunit proteins.

a) Concerted model “all or none”: all subunits in the same conformation

b) Sequential model: each individual subunit can be either ○(inactive) or □ (active) →large # of conformations is possible.



- Hemoglobin carries end products of respiration H^+ and CO_2 that stabilize T state.

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$$

carbonic anhydrase (abundant in RBC)

CO_2 insoluble in blood → formation of bubbles in blood and tissues if not converted to bicarbonate.

CO_2 Hydration to bicarbonate → $[H^+]$ → decrease in pH .

Affinity of hemoglobin to O_2 ↓ as pH ↓ in peripheral tissue.

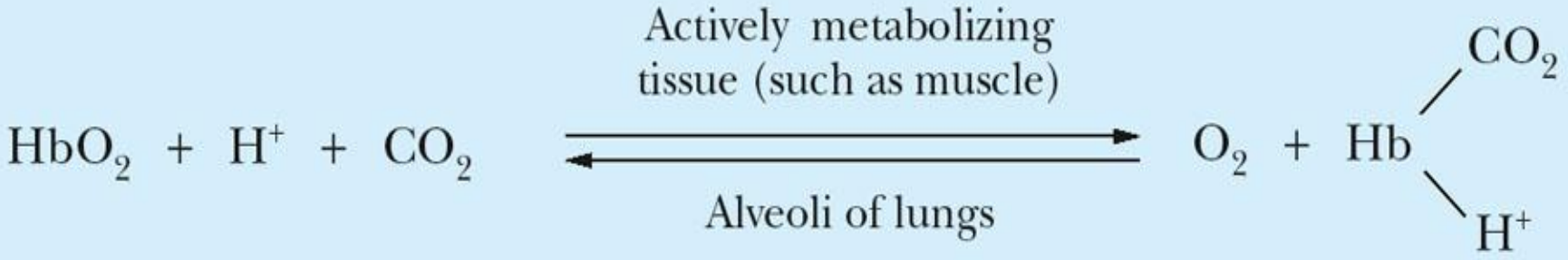
In lungs capillaries CO_2 is excreted.

Bohr effect = effect of pH and $[CO_2]$ on O_2 binding to hemoglobin

$Hb + O_2 \leftrightarrow HbO_2$ (oxygen binds to iron in Heme).

$HHb + O_2 \leftrightarrow HbO_2 + H+$ (proton binds to any of the a.a).

His^{149} (His HC3) of B subunit when protonated ion pair- Asp^{94} → stabilize T state (tensed =inactive) in deoxyhemoglobin.



The general features of the Bohr effect:

In actively metabolizing tissue, hemoglobin releases oxygen and binds both CO2 and H+. In the lungs, hemoglobin releases both CO2 and H+ and binds oxygen.

Table 4.1

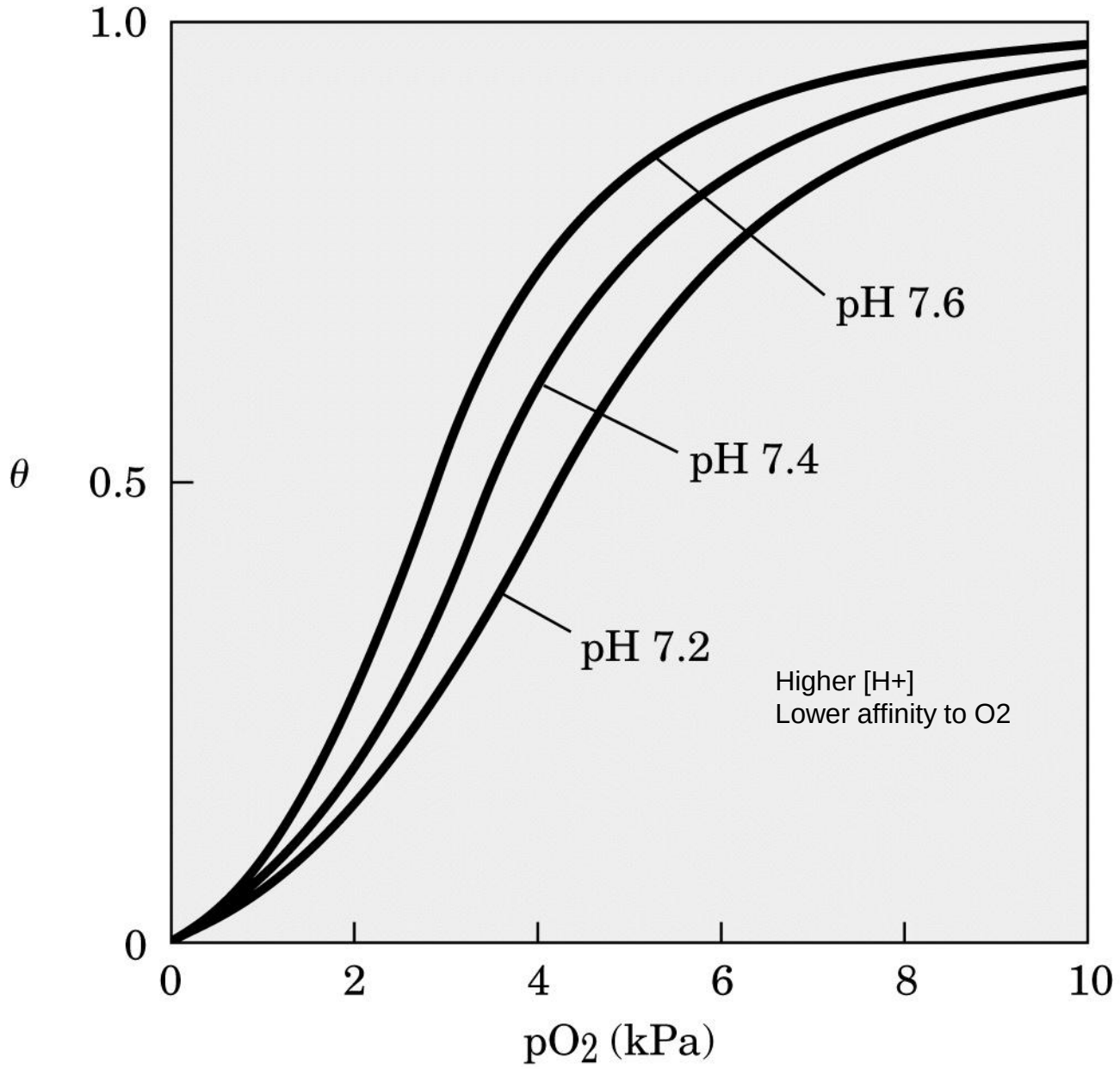
A Summary of the Bohr Effect

Lungs	Actively Metabolizing Muscle
Higher pH than actively metabolizing tissue	Lower pH due to production of H^+
Hemoglobin binds O_2	Hemoglobin releases O_2
Hemoglobin releases H^+	Hemoglobin binds H^+

Effect of pH on O_2 binding to hemoglobin.

pH of blood in lungs = 7.6

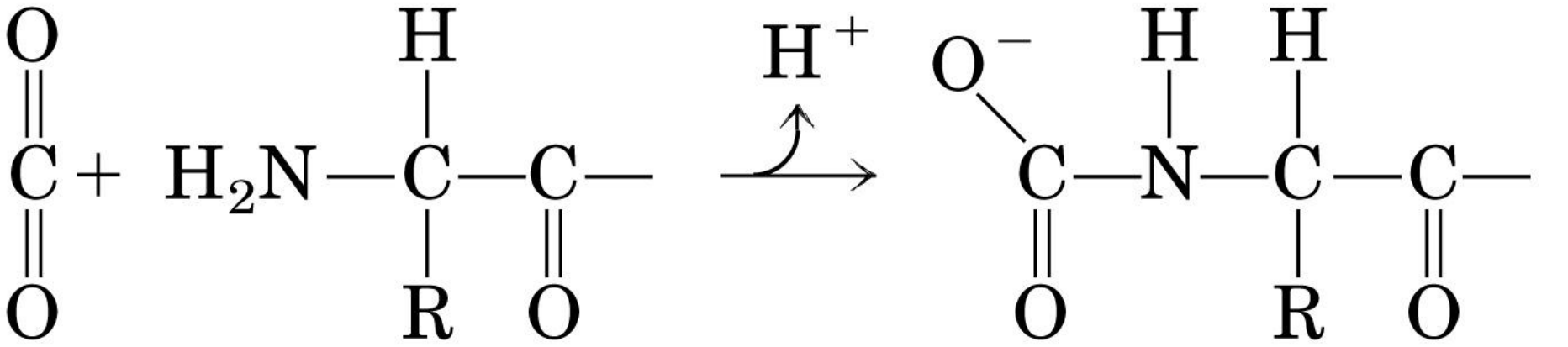
In tissues 7.2



CO_2 binds hemoglobin inversely to O_2

Binds as a carbamate group to the N-terminal end of each globin chain → carbaminohemoglobin.

H^+ produced contribute to Bohr effect.



Oxygen binding to hemoglobin is modulated by 2,3-bisphosphoglycerate which binds to and stabilize T state/ inactive.

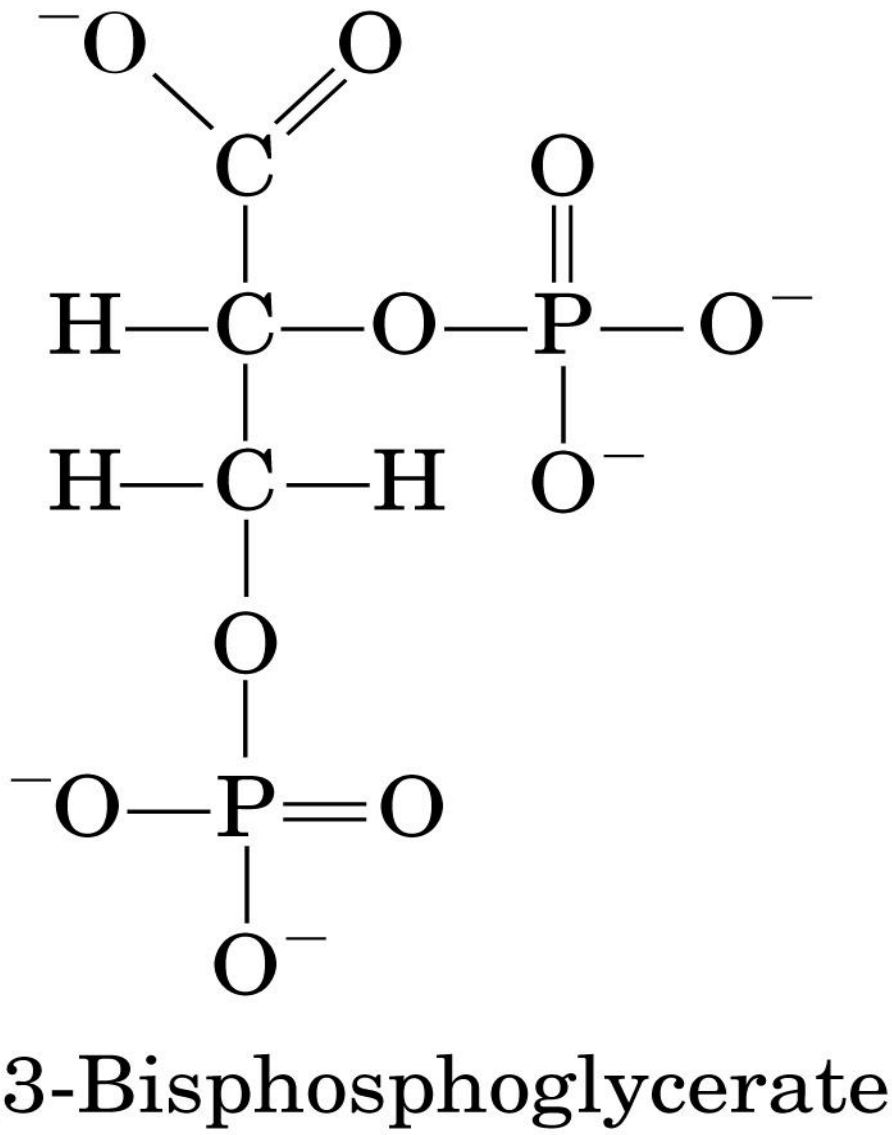
Heterotropic allosteric effect:

Reduce affinity of Hb to O_2.

- Physiological adaptation to the lower pO_2 in high altitudes.

[BPG] ↑ , affinity to O_2 ↓ .

- O_2 delivered to tissues >> 40%.



2,3-Bisphosphoglycerate

Effect of BPG on hemoglobin binding to O2 :

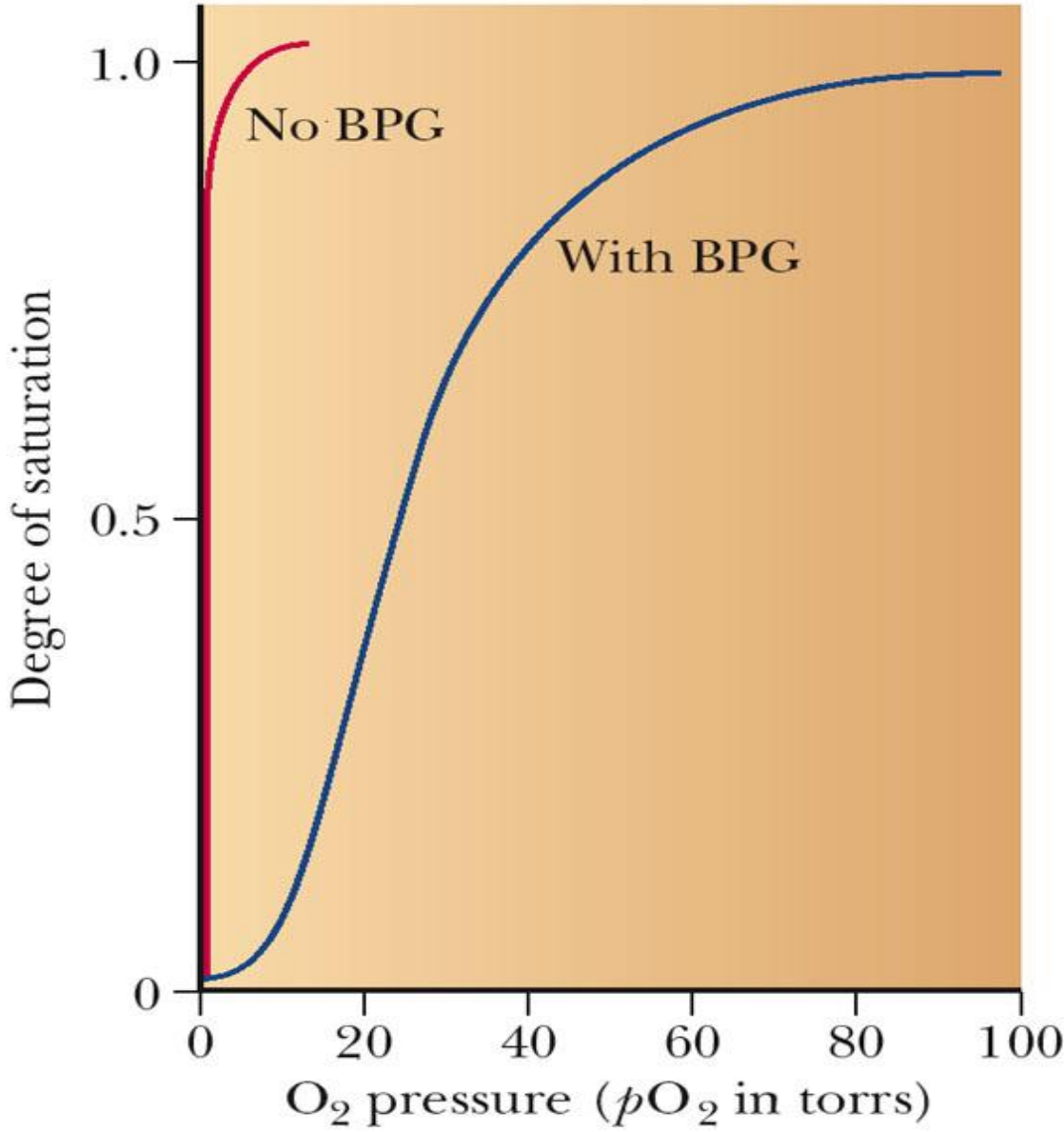
BPG conc in normal human blood= 5nm at sea level, 8nm at high altitudes.

Hemoglobin binds O_2 tightly when BPG is entirely absent,

At sea level hemoglobin is nearly saturated with O_2 in lungs.

But only 60% in other tissues.

At high altitudes O_2 delivery declines by one fourth.

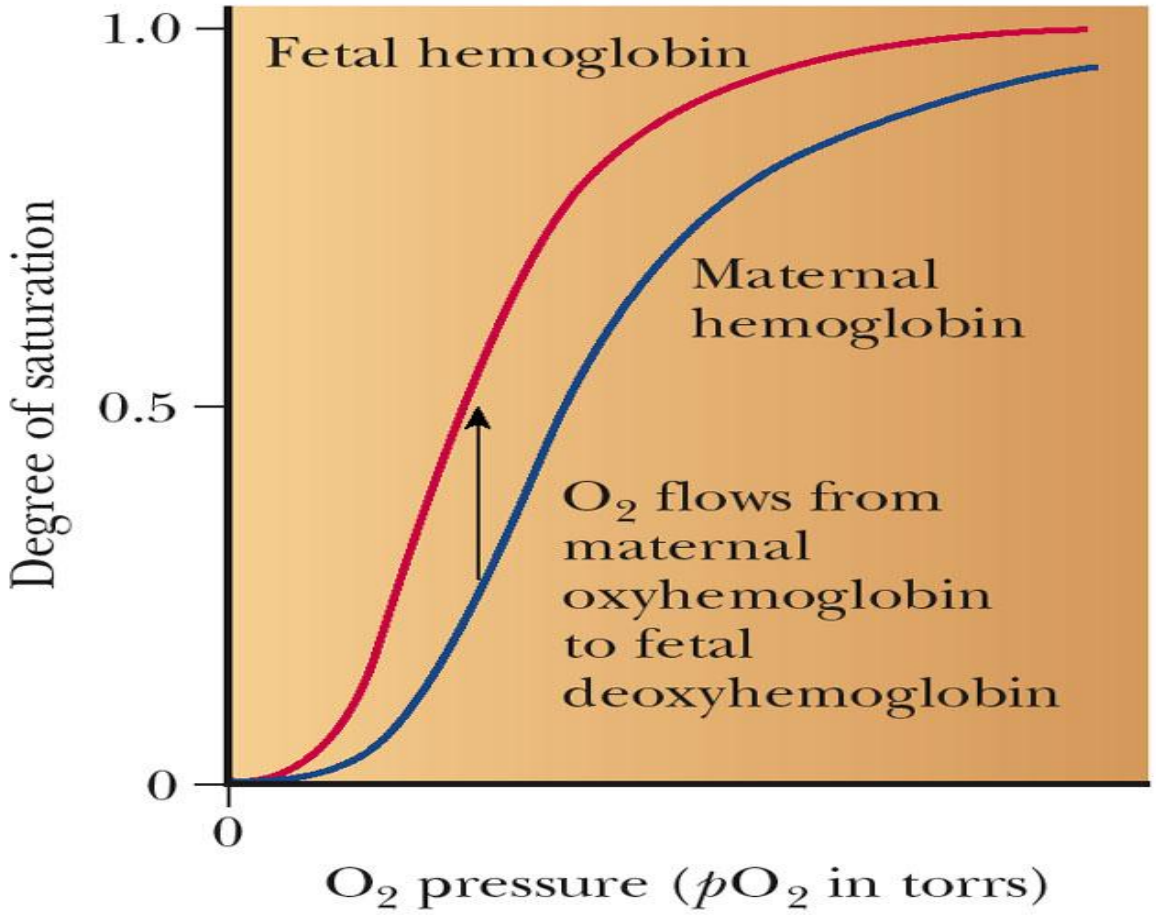


Regulation of O_2 binding to hemoglobin by BPG important in fetal development:

Fetus has $\alpha_2\gamma_2$ hemoglobin.

This tetramer has ↓ affinity to BPG than adult hemoglobin → ↑ affinity to O_2.

Fetus can extract O_2 from maternal blood.



Carbon Monoxide: A Stealthy Killer
(continued from previous page)

It may seem surprising that the loss of half of one's hemoglobin to COHb can prove fatal—we know that people with any of several anemic conditions manage to function reasonably well with half the usual complement of active hemoglobin. However, the binding of CO to hemoglobin does more than remove protein from the pool available to bind oxygen. It also affects the affinity of the remaining hemoglobin subunits for oxygen. As CO binds to one or two subunits of a hemoglobin tetramer, the affinity for O_2 is increased substantially in the remaining subunits (Fig. 2). Thus, a hemoglobin tetramer with two bound CO molecules can efficiently bind O_2 in the lungs—but it releases very little of it in the tissues. Oxygen deprivation in the tissues rapidly becomes severe. To add to the problem, the effects of CO are not limited to interference with hemoglobin function. CO binds to other heme proteins and a variety of metalloproteins. The effects of these interactions are not yet well understood, but they may be responsible for some of the longer-term effects of acute but nonfatal CO poisoning.

When CO poisoning is suspected, rapid evacuation of the person away from the CO source is essential, but this does not always result in rapid recovery. When an individual is moved from the CO-polluted site to a normal, outdoor atmosphere, O_2 begins to replace the CO in hemoglobin—but the COHb levels drop only slowly. The half-time is 2 to 6.5 hours, depending on individual and environmental factors. If 100% oxygen is administered with a mask, the rate of exchange can be increased about fourfold; the half-time for O_2–CO exchange can be

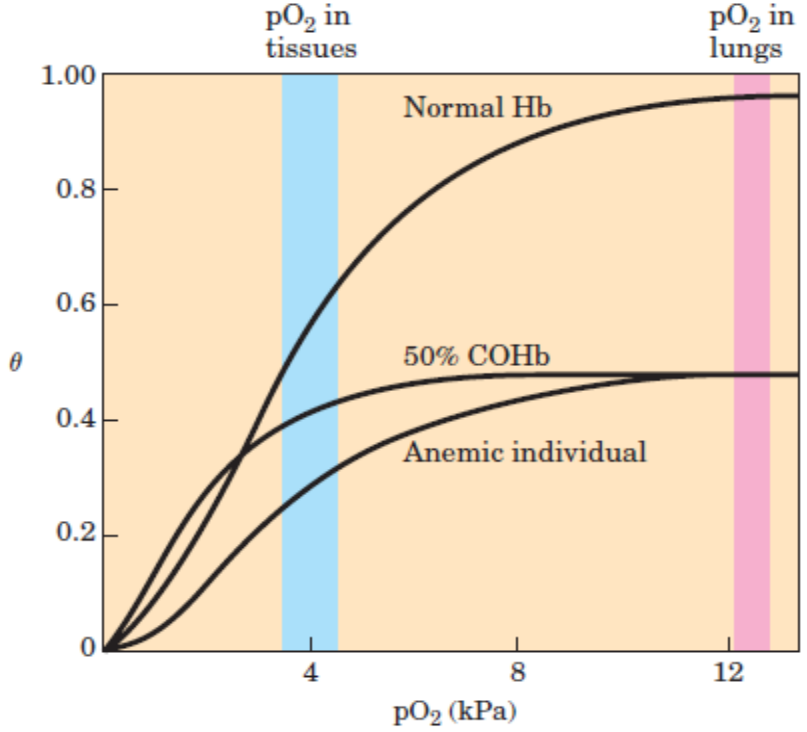


FIGURE 2 Several oxygen-binding curves: for normal hemoglobin, hemoglobin from an anemic individual with only 50% of her hemoglobin functional, and hemoglobin from an individual with 50% of his hemoglobin subunits complexed with CO. The pO_2 in human lungs and tissues is indicated.

of 3 atm (303 kPa) is supplied. Thus, rapid treatment by a properly equipped medical team is critical.

Carbon monoxide detectors in all homes are highly recommended. This is a simple and inexpensive measure to avoid possible tragedy. After completing the re-