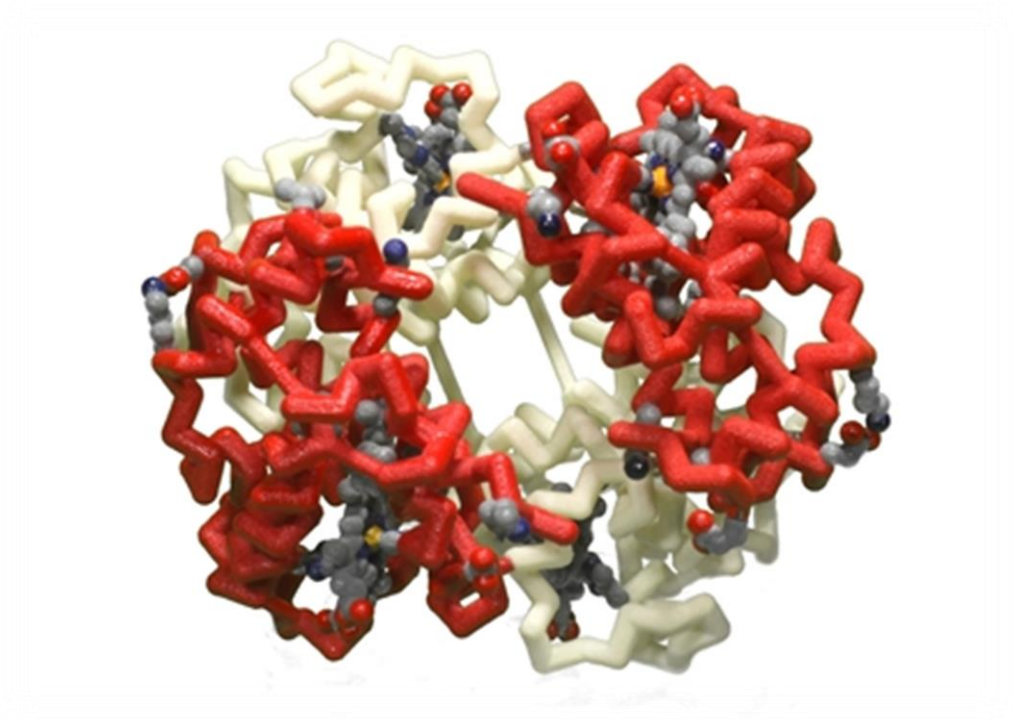

HEMOGLOBIN FUNCTION

Hematopoietic System and Disorders (MED202)

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Lecture outline

- Hb structure
  - Hb derivatives
  - T-state and R-state of Hb
- Functions of Hb
  - Transport of O_2 in the blood
  - O_2-Hb dissociation curve
  - Bohr effect
  - Physiological factors that shift the Oxygen-Hb dissociation curve
  - Transport of CO_2 in the blood
  - CO_2-Hb dissociation curve
  - Haldane effect
- Buffering function of Hb

Hemoglobin

- **Hemoprotein**
- Found only in the cytoplasm of RBCs (erythrocytes)
- 90% of the dry erythrocyte weight
- Responsible for gas transport between lungs and tissues

Hemoglobin Structure

- Globular protein composed of

  4 polypeptide chains: Tetrameric protein

Globular:

  – water soluble, forms colloids in water

- Each polypeptide chain is called **globin**.
- Quaternary structure is important in gaining 3-dimensional shape

Hemoglobin Structure

- Adult Hb (HbA): 2 α- ve 2β-**globin** chains

  $(\alpha\beta)_1 + (\alpha\beta)_2$

  The 2 polypeptide chains within each dimer are held tightly together by strong hydrophbic interactions. There are ionic and hydrogen bonds between αβ dimers

- Each globin chain contains Heme group with Fe^{2+} (ferrous ion) atom at its center.

Structure of Heme

- Ring-shaped molecule
- Holding an iron atom
- 4 pyrroles: small pentagon-shaped molecules made from 4 carbons and 1 nitrogen
- Metalloporphyrin

Properties of the iron in the Heme

- It is the part of the Hb that binds oxygen

Coordination number= 6

6 bonds:

4 bonds: Pyrrol rings (A,B,C,D)

1 bond: Protein (His in globin chain)

1 bond: Oxygen

Types of Hemoglobin

Adult Hb (Hb A) = **2 α** and **2 β** chains

- **HbA_1** It is the most common form of Hb in children older than 7 months and adults
- **HbA_2** (**2 α** and **2 δ**) It is the least common form in adults. It constitutes 2-3% of the total HbA

Fetal Hb (Hb F) = **2 α** and **2 γ** chains

- Higher oxygen affinity compared to HbA
- In the first few months after birth, HbF is replaced by HbA.

Embryonic Hb (Hb E) = **2 ζ** and **2 ε** chains

Hb S – Amino acid substitution at 6^{th} position of β-globin chain

Glu → Val

Characteristic abnormal Hb for sickle cell anemia

Hemoglobin Structure

- Normal Hb levels
  - Men: 14-18 g/dL
  - Women: 12-16 g/dL
  - Newborn: 25 g/dL 3 months: 20 g/dL 1 year old: 17 g/dL
- If there is insufficient Hb production, the percentage of Hb in the RBCs may fall significantly below this value
- In this case, the RBC volume may decrease as the amount of Hb filling the cell is reduced.

Hemoglobin derivatives

- ✓ **Oxyhemoglobin** (HbO_2): O_2 bound Hb (i.e. Hb saturated with O_2)

- ✓ **Deoxyhemoglobin** (deoxyHb): Hb without bound O_2
- ✓ **Carbaminohemoglobin** ($HbCO_2$): CO_2 bound Hb. CO_2 non-covalently binds to globin chain. Approximately 23% of CO2 in the blood is carried as $HbCO_2$.
- ✓ **Methemoglobin** (metHb): Hb containing Fe^{3+} instead of Fe^{2+} in the heme group
- ✓ **Carboxyhemoglobin** (HbCO): In stead of O_2, CO is bound to Fe^{2+} in the Heme group. The affinity of CO to Fe^{2+} is about 200 times higher compared to O_2
- ✓ **Glycohemoglobin** (HbA1c): HbA1c rate of patients with diabetes mellitus is higher than 7%. Glucose bound Hb. It is a measure of long-term glycemia control.

Transport of Oxygen in the Blood

Method	Percent
• Dissolved in the plasma	1.5%
• Bound to Hemoglobin	98.5%

Binding of O_2 to Hemoglobin

•Hb is found in 2 different states: **T-state** and **R-state**.

T-state (T = Taut/Tense): It has a low O_2 affinity

Subunits of Hb are held together by electrostatic interactions.

Binding of the first O_2 to one of the subunits of Hb in T-state leads to conformational change in the Hb molecule that weakens the interaction between other subunits → R-state

R-state (R=relaxed): It has a high O_2 affinity

T-state: Deoxy form of Hb

- ✓ 2 αβ dimers interact via ionic and hydrogen bonds preventing movement of polypeptide chains
- ✓ Favored at low oxygen concentration

R-state: Relaxed form

- ✓ Rupture of some ionic + hydroge bonds between αβ dimers due to binding of oxygen. So polypeptide chains have more freedom of movement.
- ✓ Binding energy with oxygen stabilizes R-state
- ✓ Becomes predominant as oxygen concentration increases

- ✓ Increase in pO_2 causes a shift from T-state to R-state
- ✓ Conformational change in the surface-to-surface interaction between the adjacent subunits. This in turn **induces cooperative binding**
- ✓ When the Hb is oxygenated, one of the αβ dimers rotates 15° with respect to the second αβ dimer. This shift leads to the cooperative nature of Hb.

Oxygen-Hemoglobin binding/dissociation curve

- ✓ Binding of O_2 to Hb has **cooperative** nature
- ✓ Typical sigmoidal (S-shaped) binding/dissociation curve
- ✓ Requires binding of one molecule affects binding of other molecules: Allostery
- ✓ **Binging of the first O_2 to Hb makes it easier to bind the others**
- ✓ It is weakly bound to O_2 at low pO_2 pressures and strongly at high pO_2 pressures.

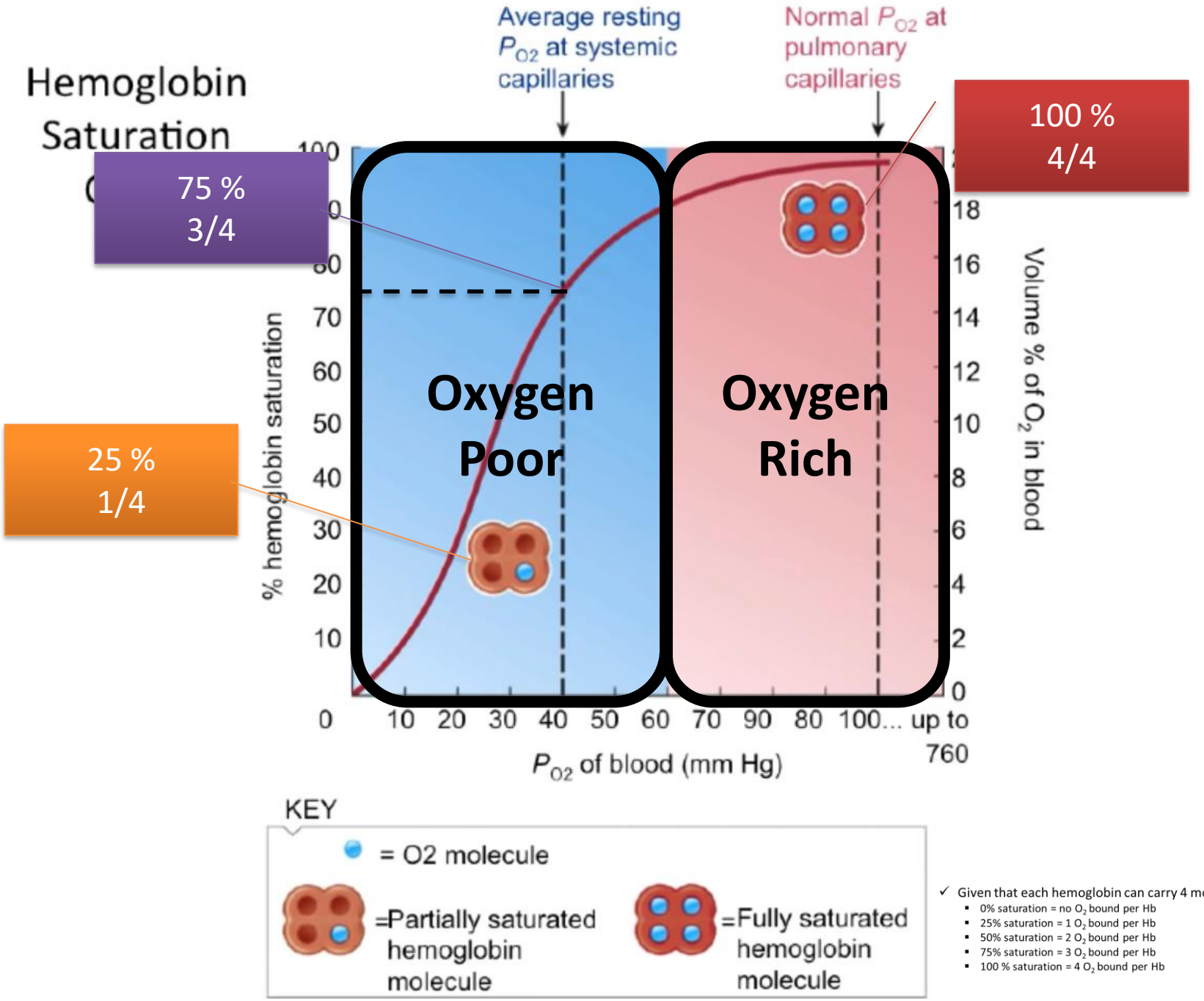
- Hb **binds** oxygen in order to carry it from the lungs to the systemic circulation
- Hb also **unbinds** oxygen in order to deliver it from the systemic circulation to the tissues
- LOADING and OFFLOADING of oxygen from Hb is due to pO_2 gradients at each site
- Oxygen should be loaded onto Hb at the lungs, and offloaded at the tissues

Partial pressures determine Hb loading vs. offloading at each site

- Oxygen binds onto Hb in the pulmonary capillaries
  - pO_2 at alveoli = 100 mmHg
- Oxygen unbinds from Hb at the tissues
  - pO_2 at tissues = 40 mmHg

Regulation of Hemoglobin Saturation

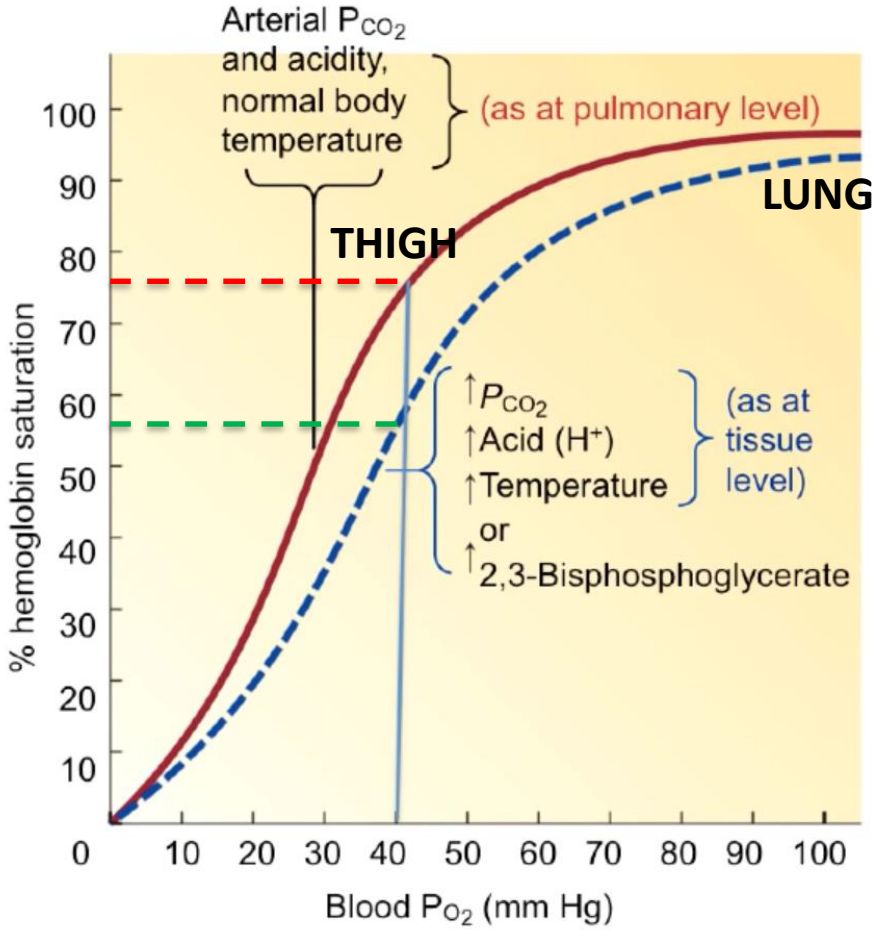
- ✓ % hemoglobin saturation refers to the average amount of oxygen carried on each hemoglobin
- ✓ This is represented on a hemoglobin saturation curve graph, where hemoglobin is more saturated as pO_2 increases, and hemoglobin unloads oxygen as pO_2 decreases
- ✓ Given that each hemoglobin can carry 4 molecules of O_2
  - 0% saturation = no O_2 bound per Hb
  - 25% saturation = 1 O_2 bound per Hb
  - 50% saturation = 2 O_2 bound per Hb
  - 75% saturation = 3 O_2 bound per Hb
  - 100 % saturation = 4 O_2 bound per Hb



- ✓ Given that each hemoglobin can carry 4 molecules of O_2
  - 0% saturation = no O_2 bound per Hb
  - 25% saturation = 1 O_2 bound per Hb
  - 50% saturation = 2 O_2 bound per Hb
  - 75% saturation = 3 O_2 bound per Hb
  - 100 % saturation = 4 O_2 bound per Hb

Hemoglobin saturation is affected by cues in the tissues that signal more O_2 delivery is needed, decreasing saturation and increasing O_2 OFFLOADING:

- Increased temperature
- Increased CO_2 concentration ($\uparrow pCO_2$)
- Increased H^+ levels ($\downarrow$pH)
- Increased 2,3 BPG



Normal: at pO_2 of 40 mmHg, % Hb saturation is 75 %

High activity: at Po_2 of 40 mmHg, % Hb saturation is 55%, indicating more oxgen delivery to tissues

Overall effect **shifts** the Hemoglobin Stauration Curve to the **RIGHT** such that the same amount of Po_2 will lead to more oxygen unloading (i.e. lower %Hb saturation; lower affinity for oxygen)

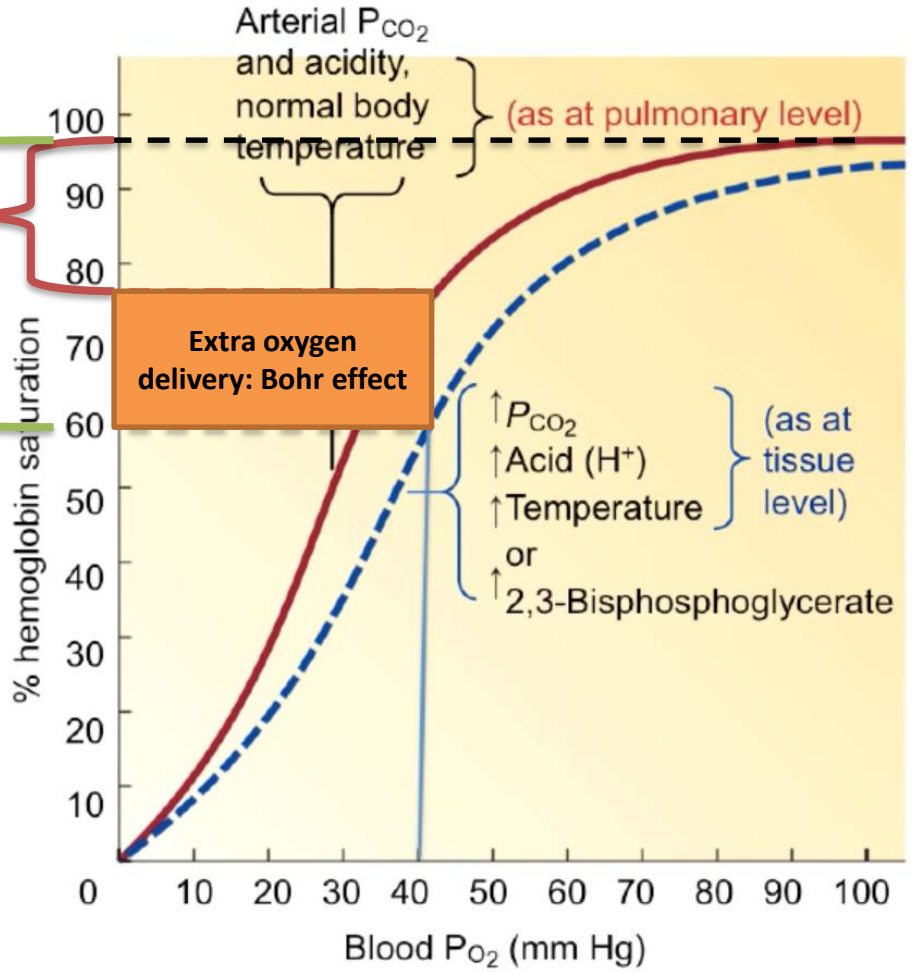
Bohr effect

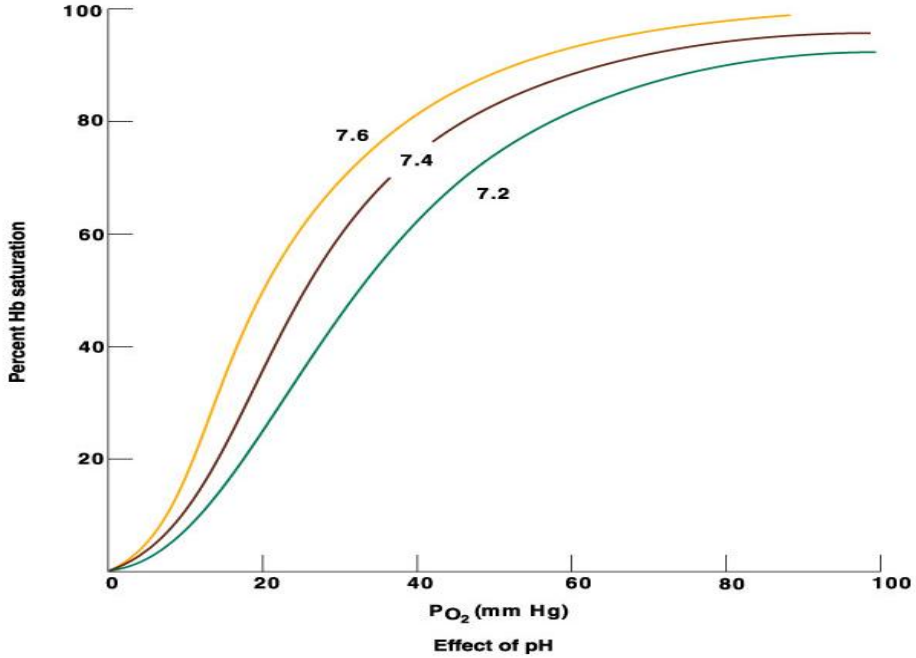
CO_2 / H^+ are affecting the affinity of the hemoglobin for O_2

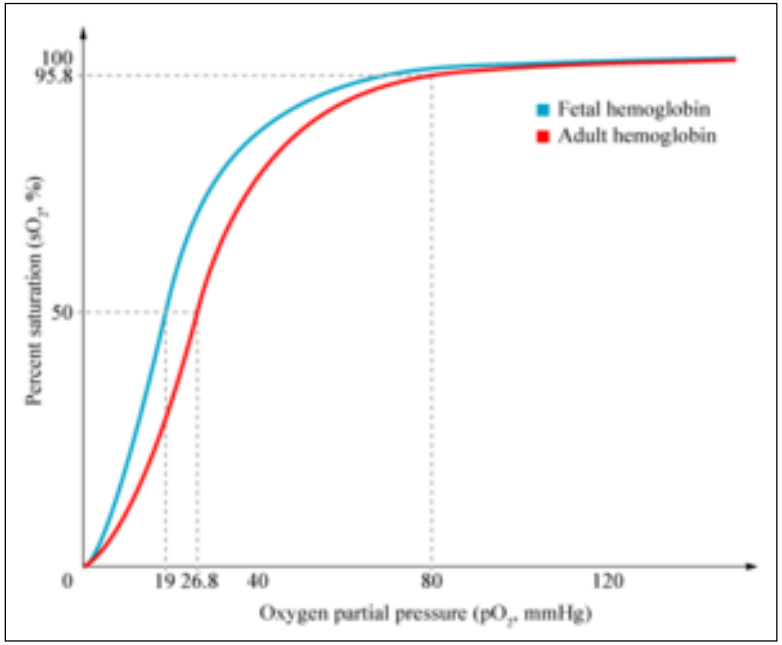
Oxygen delivery

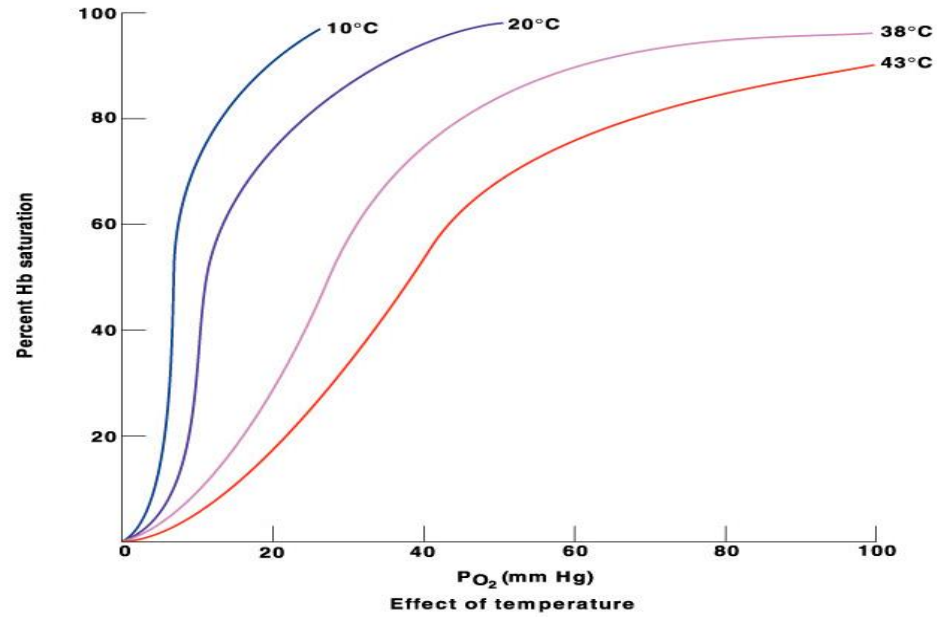
Oxygen delivery

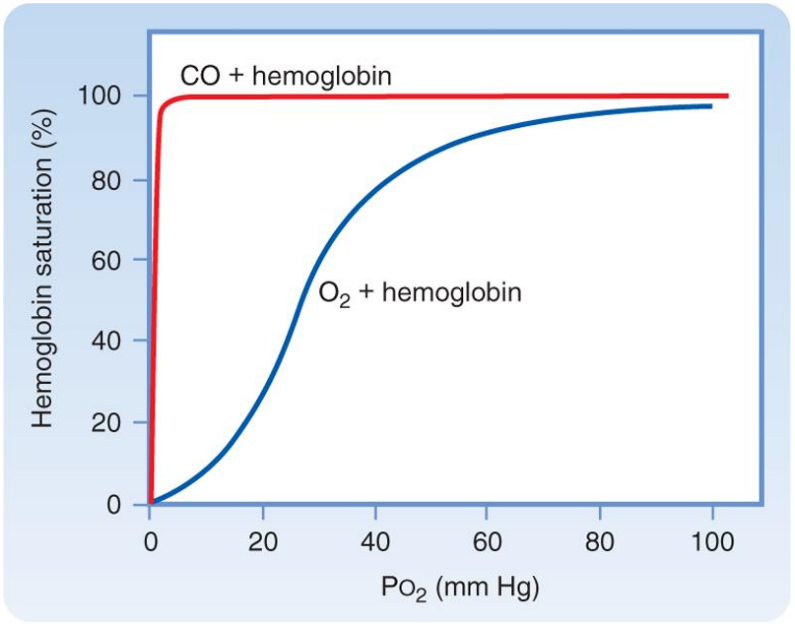
When Hb binds H^+ and CO_2, it changes the molecular structure of hemoglobin and makes it more likely to offload O_2




Percent Hb saturation
100
80
60
40
20
0
20
40
60
80
100
7.6
7.4
7.2
P_{O_2} (mm Hg)
Effect of pH

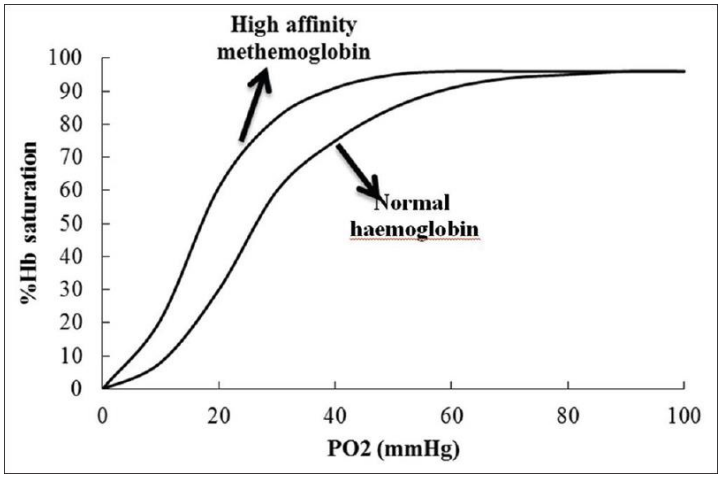

100
95.8
50
0
Percent saturation (sO_2, %)
Fetal hemoglobin
Adult hemoglobin
19 26.8
40
80
120
Oxygen partial pressure (pO_2, mmHg)


Percent Hb saturation
100
80
60
40
20
0
20
40
60
80
100
10°C
20°C
38°C
43°C
P_{O_2} (mm Hg)
Effect of temperature


CO + hemoglobin
O_2 + hemoglobin
Hemoglobin saturation (%)
100
80
60
40
20
0
0
20
40
60
80
100
P_{O_2} (mm Hg)

Methemoglobin

- When blood is exposed to various drugs and other oxidizing agents *in vitro* or *in vivo*, the ferrous iron (Fe^{2+}) that is normally present in hemoglobin is converted to ferric iron (Fe^{3+}) forming methemoglobin.
- Ferrous iron / reduced iron (Fe^{2+}) iron easily binds and unbounds oxygen and / or carbon dioxide
- **Ferric iron / oxidized iron** (Fe^{3+}) has high affinity for O_2 useless as an oxygen carrier

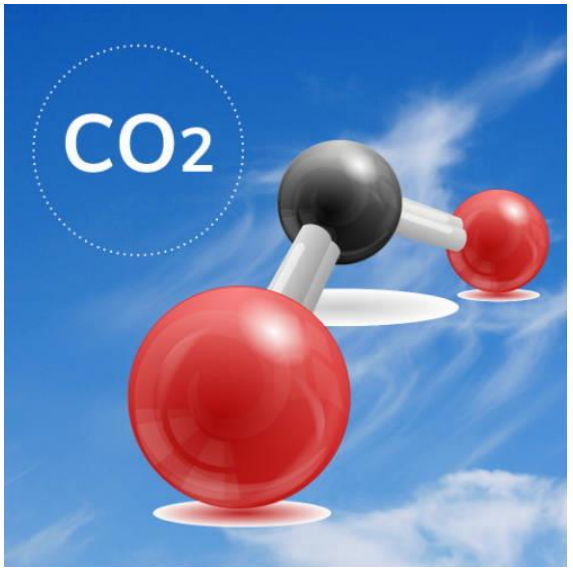


Methemoglobin

- ✓ Methemoglobin is dark-colored, and when it is present in large quantities in the circulation, it causes a dusky discoloration of the skin resembling cyanosis
- ✓ Normally, only **1% of hemoglobin** in human blood is in the structure of methemoglobin.
- ✓ There are 2 mechanisms that keep the iron of the Hb in reduced form:
  - Reduction of oxidized iron by **NADH** produced during **anaerobic glucose metabolism** (Embden-Meyerhof pathway) in RBCs: NADH-Methemoglobin reductase system
    - ✓ Congenital absence of methemoglobin reductase is one cause of hereditary methemoglobinemia.
  - Reduction of oxidized iron by **NADPH** produced during **Pentose Phospahte Pathway**

Carbon dioxide tranport in the blood

- Carbon dioxide is an important side product of both glycolysis and the citric acid cycle (Krebs cycle).
- This oxidized carbon represents an end product of metabolism that, ultimately, needs to be removed using transport to the lungs and subsequent expiration out into the surrounding environment.
- Together with renal regulation, this complex process of carbon dioxide production, transport, and elimination is the principal means by which the body regulates the blood's pH.



Carbondioxide tranport in the blood

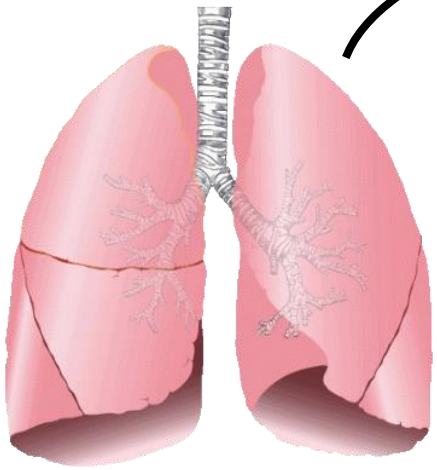
Method	**Percentage**
➢ Dissolved in the plasma	7 - 10%
➢ As $HCO3^-$ ion in the plasma	60 -70%
➢ Bound to hemoglobin	20 - 30%

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

Gas	Method of Transport in Blood	Percentage Carried in This Form
O_2	Physically dissolved	1.5
	Bound to hemoglobin	98.5
CO_2	Physically dissolved	10
	Bound to hemoglobin	30
	As bicarbonate (HCO_3^-)	60

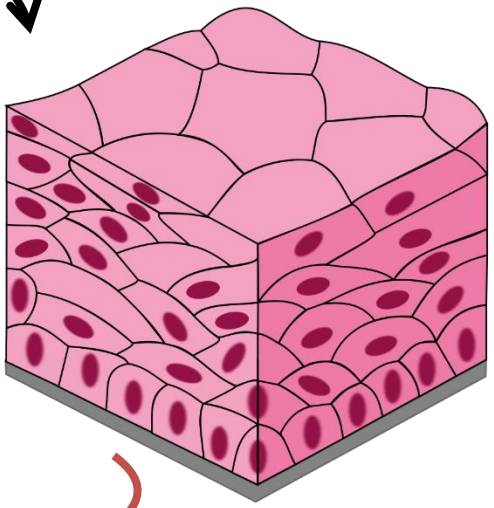
1. Dissolved O_2 in the plasma
2. HbO_2 (Oxyhemoglobin)

LUNGS



O_2

TISSUES



CO_2

BOHR EFFECT

CO_2 / H^+ are affecting the affinity of the hemoglobin for O_2

1. Dissolved CO_2 in the plasma
2. H^+Hb (HCO_3^-) → $CO_2 + H_2O$
3. $Hb\text{-}COO^- + H^+$

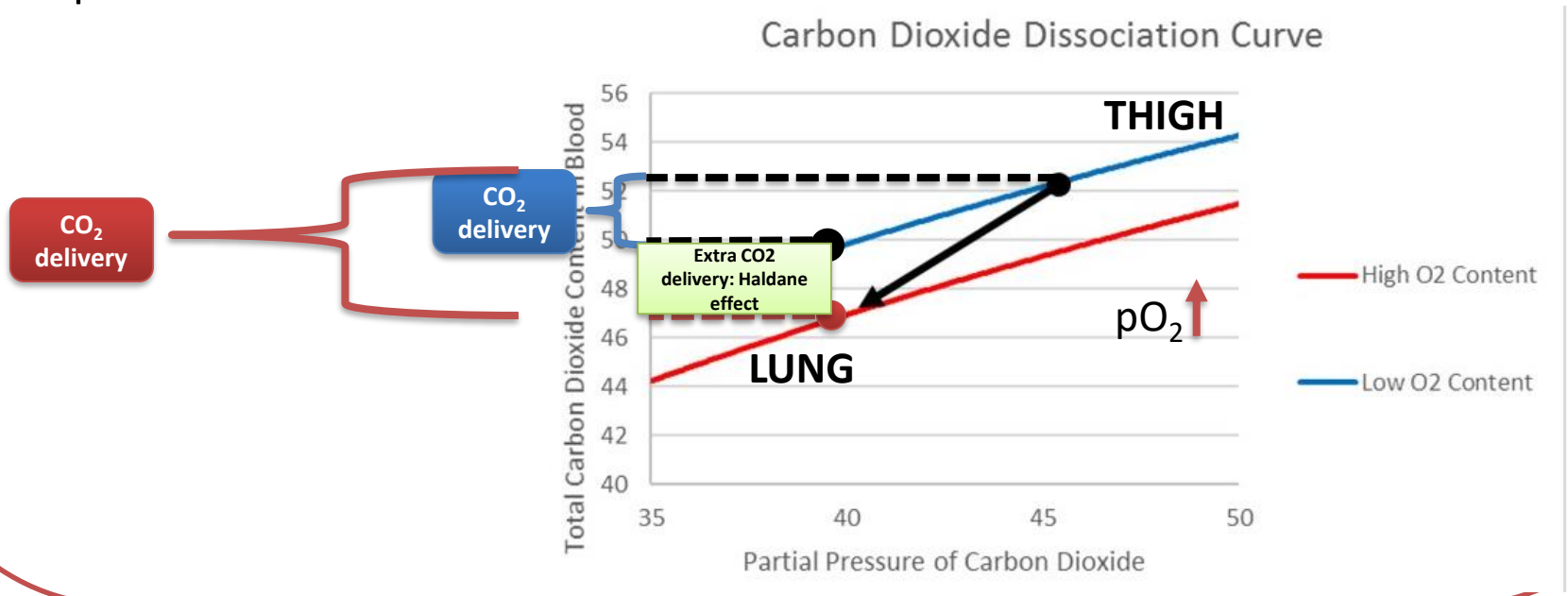
HALDANE EFFECT

O_2 is affecting the affinity of the hemoglobin for CO_2 / H^+

Haldane effect

O_2 is affecting the affinity of the hemoglobin for CO_2 / H^+

- ✓ Causes increased offloading of carbon dioxide upon oxygenation of the blood.
- ✓ As blood passes through the lungs, an influx of oxygen causes a right shift of the carbondioxide dissociation curve while the pCO_2 drops from 45-46 mmHg down to abot 40 mmHg.
- ✓ This serves to release a greater amount of carbon dioxide into the alveolar spaces.



Regulation of Oxygen and Carbon dioxide Concentrations in the Extracellular Fluid

- Because oxygen is one of the major substances required for chemical reactions in the cells, the body has a special control mechanism to maintain an almost exact and constant oxygen concentration in the extracellular fluid.
- This mechanism depends principally on the ***chemical characteristics of hemoglobin,*** which is present in all red blood cells.
- Hemoglobin combines with oxygen as the blood passes through the lungs.
- Then, as the blood passes through the tissue capillaries, hemoglobin, because of its own strong chemical affinity for oxygen, does not release oxygen into the tissue fluid if too much oxygen is already there.
- However, if the oxygen concentration in the tissue fluid is too low, sufficient oxygen is released to re-establish an adequate concentration.
- Thus regulation of oxygen concentration in the tissues is vested principally in the chemical characteristics of hemoglobin.
- This regulation is called **the *oxygen-buffering function of hemoglobin.***

Hemoglobin as a buffer

- Responsible for 50-60% of the total buffering capacity of the blood

- In the RBCs, hemoglobin is an important buffer, as follows:

$$H^+ + Hb \rightleftharpoons HHb$$

- Hemoglobin can accept H^+ as it has histidine, which is a basic amino acid. Moreover, deoxygenated haemoglobin has higher tendency to accept H^+

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