Hemoglobin

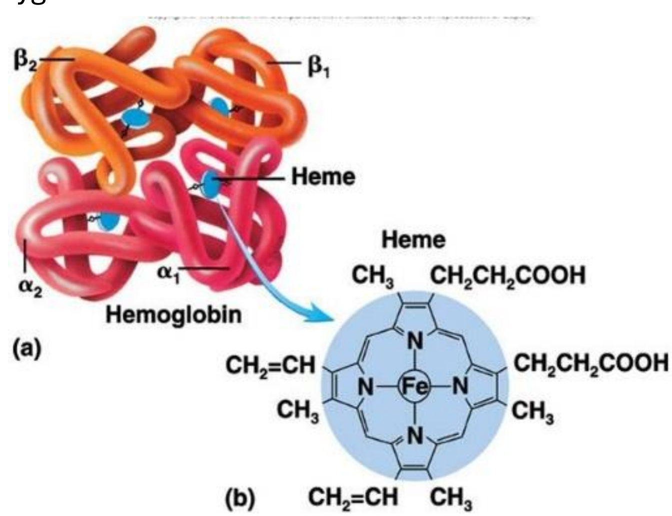
The big unique protein compound (come from words in Greek haima mean blood + globus mean sphere) have 64,000 daltons molecular weight, and formed 95% from dried RBC, but formed 34% from RBC during movement in blood vessels. It found inside erythrocytes and responsible for carries oxygen from lung to body tissues, also transport CO2 from tissues to the lungs. A healthy individual human has 12 to 20 grams of hemoglobin in every 100 mL of blood.

Hemoglobin (Hb) Composition

Each human red blood cell contains approximately 270 million hemoglobin molecules, it is a large tetrameric molecule, composed of four globular protein subunits. Each of four subunits contains; heme group and a globin chain (4 heme and 4 globin subunits).

Heme:

Is the prosthetic group of hemoglobin, is a complex of protoporphyrin IX ring and iron. Iron has to be in the ferrous state (Fe2+), otherwise it will not bind to Hb, ferrous iron located in the center of the protoporphyrin ring, each heme subunit can carry one molecule of oxygen bound to the central ferrous iron; thus, each hemoglobin molecule can carry four molecules of oxygen.



Globin:

The composition of the globin polypeptide chain is responsible for the different functional and physical properties of hemoglobin. Important amino acids that formed circular polypeptide chain of globin include Lysine, Leucine, Aspartate, Glutamate acid and Arginine.

Four types of globin chains, alpha α, beta β, gama γ and delta δ.

Adult hemoglobin HbA composed from 2 α chains and 2 β chains.

Alpha globin chain composed from 141 amino acids but other chains of globin contains 146 amino acids.

Hemoglobin types:

Hemoglobin is a heterotetramer composed of 2 α and 2 β polypeptide globin subunits, hemoglobin was one of the first proteins to be sequenced and the globin genes were among the earliest to be cloned, more than 1000 naturally occurring human hemoglobin variants with single amino acid substitutions throughout the molecule have been discovered, mainly through their clinical and/or laboratory manifestations.

Types of hemoglobin during different periods of life

Type of hemoglobin	*Globin chains*	*Period of life when predominant*
Hemoglobin Gower I	$\zeta\ 2\varepsilon 2$	Embryonic
Hemoglobin Gower II	$\alpha\ 2\varepsilon 2$	Embryonic
Hemoglobin Portland	$\zeta\ 2\gamma 2$	Embryonic
Hemoglobin F (Fetal hemoglobin)	$\alpha\ 2\gamma 2$	Fetal
Hemoglobin A (Adult hemoglobin)	$\alpha\ 2\beta 2$	Adult
Hemoglobin A_2	$\alpha\ 2\delta 2$	Adult

A-normal types

In adults, these are normal percentages of different hemoglobin molecules:

HbA: 95% to 98% /// **HbA2**: 2% to 3%

HbF: 0.8% to 2%

In infants and children, these are normal percentage of HbF molecules:

HbF (newborn): 50% to 80% (0.5 to 0.8), decrease after 6 months: 8%, and more than 6 months become 1 – 2%.

Normal value ranges may vary slightly among different laboratories. Some labs use different measurements or may test different samples.

B-abnormal types:

After electrophoresis of hemoglobin, inherited blood disorder in which the body makes an abnormal form of hemoglobin, such as HbH (thalassemia), HbSS (Sickle cell anemia), methemoglobin is hemoglobin with iron oxidized to the ferric state, which cannot carry oxygen.

Role in transport of oxygen and carbon dioxide

Haemoglobin (Hb) primarily carries oxygen (O_2) by binding it in the lungs to form **oxyhaemoglobin**, enabling massive O_2 transport (over 98%), and releases it in tissues where O_2 levels are low, facilitated by factors like pH, CO_2, and temperature (Bohr effect). It also transports carbon dioxide (CO_2) by binding it to its protein part (not iron site) as **carbaminohemoglobin**, and helps buffer blood pH, though most CO_2 travels as bicarbonate in plasma.

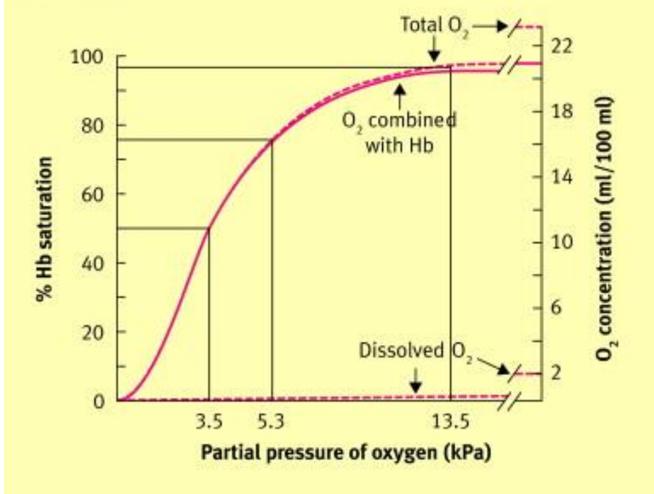
Role in Oxygen Transport

- **Binding:** In the lungs (high PO_2), O_2 binds to the iron in each of Hb's four subunits, forming oxyhaemoglobin (bright red).
- **Cooperative Binding:** Binding of one O_2 molecule increases Hb's affinity for more O_2, maximizing saturation in lungs.
- **Release:** In tissues (low PO_2), this binding is reversed, releasing O_2 for cellular respiration.
- **Oxygen-Hemoglobin Dissociation Curve:** Shows how Hb saturation changes with PO_2 and shifts due to factors like CO_2 and pH (Bohr Effect).

Role in Carbon Dioxide Transport

- **Carbaminohemoglobin:** CO_2 binds to the globin protein part (not the heme iron) of Hb to form carbaminohemoglobin in tissues.
- **Bicarbonate Formation:** Most CO_2 (around 70-80%) is converted to bicarbonate (HCO_3^-) in red blood cells (RBCs) and plasma, transported in plasma.
- **Buffering:** Hb binds to hydrogen ions (H^+) from carbonic acid, acting as a crucial blood pH buffer, preventing excessive acidity.

Hemoglobin oxygen dissociation curve



The normal haemoglobin-oxygen dissociation curve. Partial pressures of oxygen corresponding to saturations of 50%, 75% (mixed venous blood) and 97% (arterial blood) are shown

(Normal P50, measured at 37°C and an arterial pH of 7.40, is 27 mm/Hg).

When 50% of Hb saturated with O2 (SO2) = PO2 is 27 mm/hg

Venous blood: SO2=75 and PO2=40

Arterial blood: SO2 =100 and PO2 =100

By increasing the hydrogen ion concentration (and therefore the pH), the temperature, the carbon dioxide concentration or the amount of 2,3-BPG present in the red blood cell, we ultimately decrease the affinity of hemoglobin to oxygen, therefore these factors have negative (inversely) effect on affinity between O2 and Hb.

Bohr Effect

The Bohr Effect refers to the observation that increases in the carbon dioxide partial pressure of blood or decreases in blood pH result in a lower affinity of hemoglobin for oxygen. This manifests as a right-ward shift in the Oxygen-Hemoglobin Dissociation Curve described in Oxygen Transport and yields enhanced unloading of oxygen by hemoglobin.

Mechanism

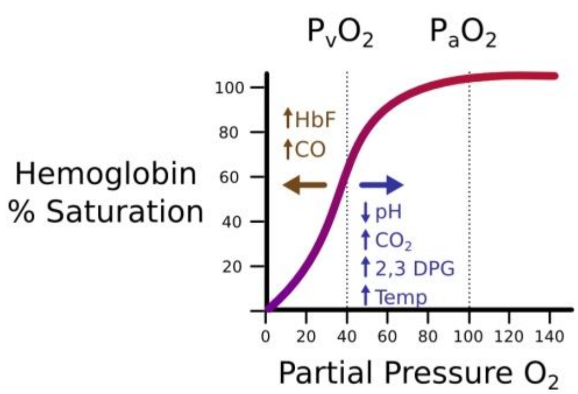
Decreases in blood pH, meaning increased H+ concentration, are likely the direct cause of lower hemoglobin affinity for oxygen. Specifically, the association of H+ ions with the amino acids of hemoglobin appear to reduce hemoglobin's affinity for oxygen. Because changes in the carbon dioxide partial pressure can modify blood pH, increased partial pressures of carbon dioxide can also result in right-ward shifts of the oxygen-hemoglobin dissociation curve. The relationship between carbon dioxide partial pressure and blood pH is mediated by carbonic anhydrase which converts gaseous carbon dioxide to carbonic acid that in turn releases a free hydrogen ion, thus reducing the local pH of blood.

Significance

The Bohr Effect allows for enhanced unloading of oxygen in metabolically active peripheral tissues such as exercising skeletal muscle. Increased skeletal muscle activity results in localized increases in the partial pressure of carbon dioxide which in turn reduces the local blood pH. Because of the Bohr Effect, this results in enhanced unloading of bound oxygen by hemoglobin passing through the metabolically active tissue and thus improves oxygen delivery. Importantly, the Bohr Effect enhances oxygen delivery proportionally to the metabolic activity of the tissue. As more metabolism takes place, the carbon dioxide partial pressure increases thus causing larger reductions in local pH and in turn allowing for greater oxygen unloading. This is especially true in exercising skeletal muscles which may also release lactic acid that further reduces local blood pH and thus enhances the Bohr Effect.

Modulation of the Oxygen-Hemoglobin Dissociation Curve

A variety of environmental factors can shift the Oxygen-Hemoglobin Dissociation Curve. Effects which are associated with increased peripheral tissue metabolism, such as reduced pH, increased CO2, increased temperature, shift the curve to the right, reducing hemoglobin’s affinity for oxygen and thus improving oxygen unloading. Chronic hypoxia increases the blood’s concentration of 2,3-DPG which also shifts the curve to the right. The presence of HbF and carbon monoxide (CO) shift the curve to the left, increasing the oxygen affinity of hemoglobin.



Transport of Carbon dioxide

When oxygen is used by the cells, virtually all of it becomes carbon dioxide (CO2), and this increases the intracellular PCO2; because of this high tissue cell PCO2, CO2 diffuses from the cells into the tissue capillaries and is then carried by the blood to the lungs. In the lungs, it diffuses from the pulmonary capillaries into the alveoli and is expired. Thus, at each point in the gas transport chain, CO2 diffuses in the direction exactly opposite to the diffusion of oxygen. Yet there is one major difference between diffusion of CO2 and of oxygen: carbon dioxide can diffuse about 20 times as rapidly as oxygen. Therefore, the pressure differences required to cause carbon dioxide diffusion are, in each instance, far less than the pressure differences required to cause oxygen diffusion.

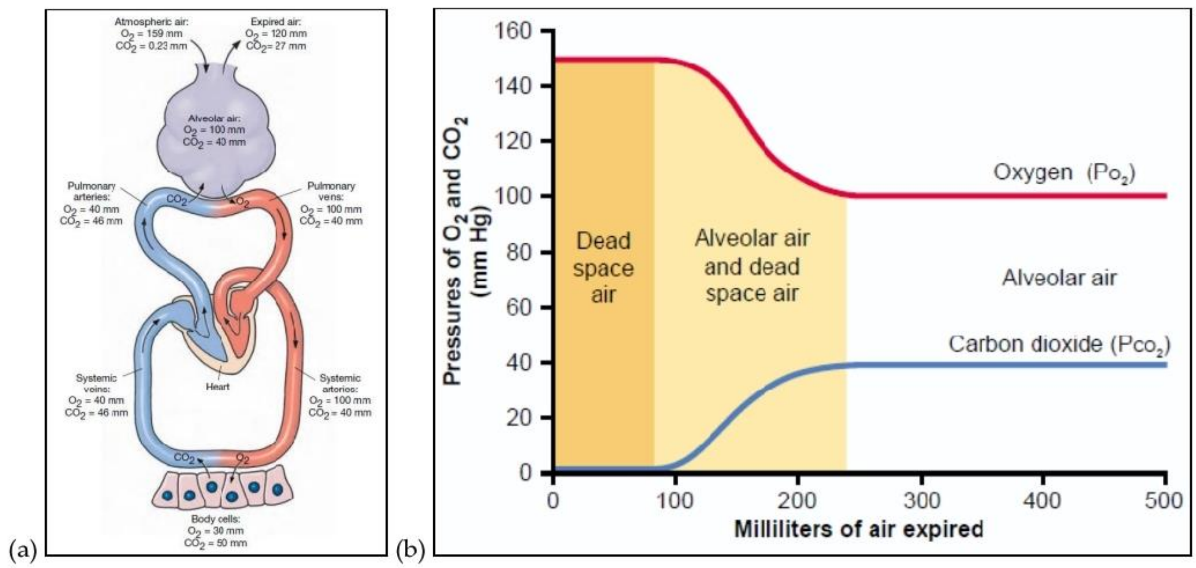


Figure: (a) Exchange of respiratory gases in lungs and tissue cells. Numbers present partial pressures in millimeters of mercury (mm Hg).(b)Oxygen and carbon dioxide partial pressures in the various portions of normal expired air.

TRANSPORT OF CO2

The phenomena of transport of CO2 takes place in the in following sequence of events:

A. <u>Diffusion of CO2 from cell to interstitial fluid:</u>
   CO2 diffuses about 20 times as rapidly as oxygen. Therefore, the pressure difference that causes the CO2 diffusion is far less than that is required for oxygen diffusion. Intracellular PCO2 is about 46 mm of Hg whereas the interstitial PCO2 is about 45 mm of Hg. This pressure difference of 1 mm of Hg is sufficient for the diffusion of CO2 from cell to the interstitial fluid.

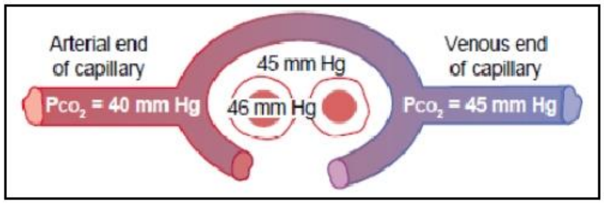


Figure: Uptake of carbon dioxide by the blood in the tissue capillaries. (P_{CO_2} in tissue cells = 46 mm Hg, and in interstitial fluid = 45 mm Hg.)

B. Diffusion of CO2 from interstitial fluid to blood:

PCO2 of arterial blood is 40 mm of Hg. The pressure difference of 5 mm of Hg causes CO2 to diffuse from interstitial fluid to blood.

C. Carriage of CO2 in blood:

Under normal resting condition, on an average about 4 ml of CO2 is transported from tissue to the lungs in each 100 ml of blood. The CO2 is carried in the blood in the following ways:

a. As physical solution:

PCO2 of venous blood is 45 mm of Hg and that of arterial blood is 40 mm of Hg. The amount of CO2 dissolved in the fluid of the blood at 95 mm of Hg is about 2.7 ml. The amount of CO2 dissolved at 40 mm of Hg is about 2.4 ml. So, 0.3 ml of CO2 is transported in the form of dissolved CO2 by each 100 ml of blood and that amounts to 5% of total CO2 transport.

Note: Here, = are the signs for reversible reaction.

b. As chemical compound:

- As bicarbonates:
    - In the corpuscles:

When carbon dioxide diffuses into the red blood cells (erythrocytes), in the presence of the catalyst carbonic anhydrase, most CO2 reacts with water (500 times faster) in the erythrocytes to form carbonic acid (H2CO3) and the following dynamic equilibrium is established.

$H_2O + CO_2 = H_2CO_3$

Carbonic acid, H2CO3, dissociates to form hydrogen ions and bicarbonate (HCO3-) ions. This is also a reversible reaction and undissociated carbonic acid, hydrogen ions and hydrogencarbonate ions exist in dynamic equilibrium with one another.

$H_2CO_3 = H^+ + HCO_3^-$

In the RBC, haemoglobin (Hb) remain in combination with K^+ ion in the form of KHb. KHb reacts with H_2CO_3 in the following way: $KHb + H^+ + HCO_3^- = HHb + KHCO_3$

- In the plasma:

Most of the CO_2 in the plasma combines with water to form carbonic acid. Carbonic acid dissociates in water into $H^+ + HCO_3$. The proteins present in the plasma combines with the H^+ and act as a buffer to maintain the acidity of the medium.

HCO_3^- becomes associated with plasma Na^+ to yield a reversible complex of $NaHCO_3$.

$H_2O + CO_2 = H_2CO_3 = H^+ + HCO_3^-$

H^+ + Protein = H-Protein

$Na^+ + HCO_3^- = HHb + NaHCO_3$

CO2 Dissociation curve:

The dependence of total blood CO_2 in free form or in combination with haemoglobin/ plasma protein on PCO_2 is depicted in the form of a curve called CO_2 dissociation curve.

Haldane effect:

The Haldane effect results from the simple fact that the combination of oxygen with hemoglobin in the lungs causes the hemoglobin to become a stronger acid. This displaces CO_2 from the blood and into the alveoli in two ways:

(1) The more highly acidic hemoglobin has less tendency to combine with carbon dioxide to form carbaminohemoglobin, thus displacing much of the carbon dioxide that is present in the carbamino form from the blood.

(2) The increased acidity of the hemoglobin also causes it to release an excess of hydrogen ions, and these bind with bicarbonate ions to form carbonic acid; this then dissociates into water and CO_2, and the CO_2 is released from the blood into the alveoli and, finally, into the air.

The Haldane effect on the transport of CO_2 from the tissues to the lungs is depicted in the figure below. This figure shows small portions of two CO_2 dissociation curves:

(1) when the PCO2 is 40 mm Hg, which is the case in the blood capillaries of the lungs, and

(2) when the PCO2is 45 mm Hg, which is the case in the tissue capillaries.

- Point A shows that the normal PCO2 of 45 mm Hg in the tissues causes 52 volumes per cent of CO2 to combine with the blood.
- On entering the lungs, the PCO2 falls to 40 mm Hg and the PCO2 rises to 100 mm Hg.
- If the CO2 dissociation curve did not shift because of the Haldane effect, the CO2 content of the blood would fall only to 50 volumes per cent, which would be a loss of only 2 volumes per cent of CO2.
- However, the increase in PCO2 in the lungs lowers the CO2 dissociation curve from the top curve to the lower curve of the figure, so that the CO2 content falls to 48 volumes per cent (point B).
- This represents an additional 2 volumes per cent loss of CO2. Thus, the Haldane effect approximately doubles the amount of CO2 released from the blood in the lungs and approximately doubles the pickup of CO2 in the tissues.

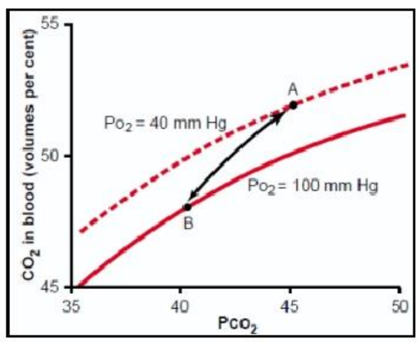


Figure: Portions of the CO_2 dissociation curve when the P_{O_2} is 100 mm Hg or 40 mm Hg. The arrow represents the Haldane effect on the transport of CO_2.