Respiratory Pigments

- **High molecular weights (10^5 to nearly 10^7 D)**
- ***Conjugated proteins* - proteins complexed w/ other organic molecules or metal ions (Fe^{2+} or Cu^{2+})**
  - **Color produced by these complexes**
  - **Pigment is not *oxidized* by O_2 , it is *oxygenated* (reversible reaction)**
- **Necessary because of low solubility of O_2 in aqueous solution (about 0.3 ml O_2/100 ml)**
  - **Allow transport of *molecular* oxygen, pickup of O_2 at sites of high O_2 tension and deposition at sites of low O_2 tension**
  - **Allow blood to carry a much greater quantity of O_2**
  - **Allow quick removal of O_2 from respiratory surfaces, thus maintaining a concentration gradient down which O_2 can diffuse**

Types of Respiratory Pigments

***Hemocyanin* - gastropods and cephalopods, crustaceans, arachnids, and horseshoe crabs**

- **Cu-containing protein, MW 1-7 million D**
- **One hemocyanin molecule contains two Cu atoms and can combine with 1 O_2 molecule**
- **Blue in deoxygenated state, colorless or white in oxygenated state**
- **Never present inside cells, but always in suspension in blood**

***Hemerythrin* - sipunculids (peanut worms), polychaetes, priapulids, and branchiopods**

- **Fe-containing protein, MW about 100,000 D**
- **One hemerythrin molecule contains several Fe atoms and each O_2 molecule can combine with 2 or 3 Fe atoms**
- **Brownish in deoxygenated state, purple in oxygenated state**
- **Contained within coelomocytes that circulate within the coelomic fluid**

Types of Respiratory Pigments

***Chlorocruotin* - two families of marine polychaetes (Serpulidae and Sabellidae)**

- **Fe-containing protein, MW about 3 million D**
- **Similar structure to hemoglobin, except that in the heme group one vinyl group is replaced by a formyl group**
- **As with hemoglobin, one O_2 molecule combines with each of the 4 heme groups**
- **Green in both deoxygenated and oxygenated states**
- **Occurs in suspension in blood**

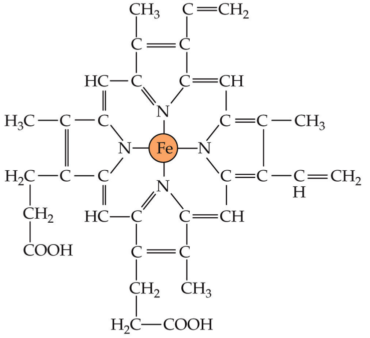
Types of Respiratory Pigments

***Hemoglobin* (Hb) - all vertebrates and many invertebrates (most annelids, nemerteans, phoronids, and echiurids)**

- **Fe-containing protein, MW 68,000 D in humans. Composed of two pairs of polypeptide chains (α and β-Hb). Size ranges from 17,000 D to 3,000,000 D (annelids).**
- **Similar in structure to cytochromes, and thus is also found in plants**
- **Contains four heme groups, each of which can combine with one O_2 molecule, when all four heme groups are complexed with O_2, the molecule is said to be *fully saturated***
- **Dark red in the deoxygenated state, bright red in the oxygenated state**
- **May occur within RBCs or in suspension in blood. Effectiveness greatly increased when packed into cells, as is true of most vertebrates**

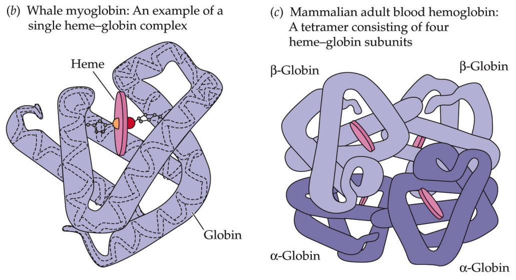
Figure 23.1 The chemical structure of hemoglobin (Part 1)

(a) Heme



Animal Physiology 2e, Figure 23.1 (Part 1)

Figure 23.1 The chemical structure of hemoglobin (Part 2)



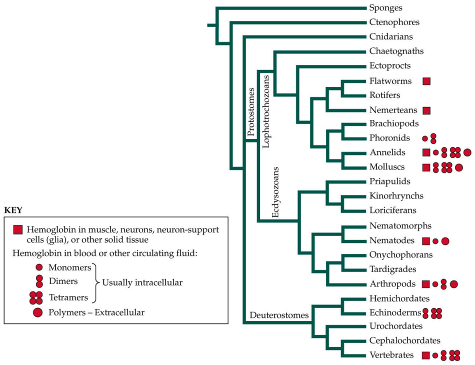
(b) Whale myoglobin: An example of a single heme–globin complex

(c) Mammalian adult blood hemoglobin: A tetramer consisting of four heme–globin subunits

Animal Physiology 2e, Figure 23.1 (Part 2)

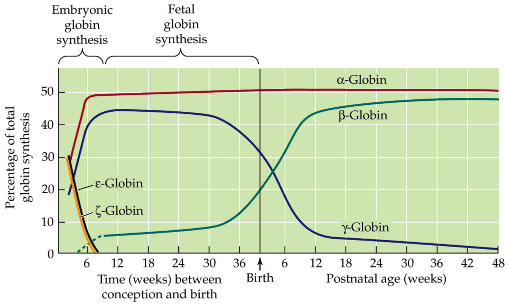
Respiratory Pigments

Figure 23.3 The distribution of hemoglobins in animals



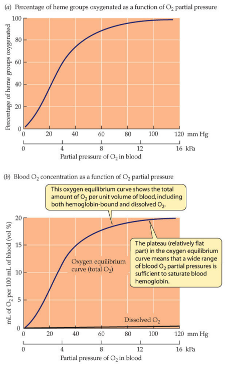
Animal Physiology 2e, Figure 23.3

Figure 23.2 Human developmental changes in the types of globins synthesized for incorporation into blood hemoglobins



Animal Physiology 2e, Figure 23.2

Figure 23.4 A typical oxygen equilibrium curve for human arterial blood presented in two different ways


(a) Percentage of heme groups oxygenated as a function of O_2 partial pressure

(b) Blood O_2 concentration as a function of O_2 partial pressure

This oxygen equilibrium curve shows the total amount of O_2 per unit volume of blood, including both hemoglobin-bound and dissolved O_2.

The plateau (relatively flat part) in the oxygen equilibrium curve means that a wide range of blood O_2 partial pressures is sufficient to saturate blood hemoglobin.

Animal Physiology 2e, Figure 23.4

Functions of Respiratory Pigments

- **At a given pH, the amount of O_2 that a given quantity of respiratory pigment can pick up depends on P_{O_2} (usually in mm Hg), and the loading of the pigment is given in percentage of saturation with O_2**
- **These relationships are called *oxygen dissociation curves*. The s-shape allows more O_2 to be delivered a a given P_{O_2}**
  - **Slight changes in P_{O_2} permit a relatively large amount of O_2 to be released (at P_{O_2}' s typical of tissue cells)**
  - **At higher P_{O_2}' s (typical of respiratory surfaces), pigment is completely saturated**

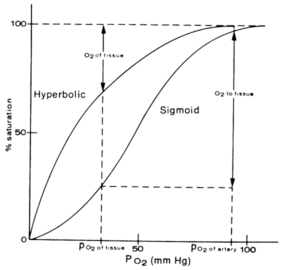
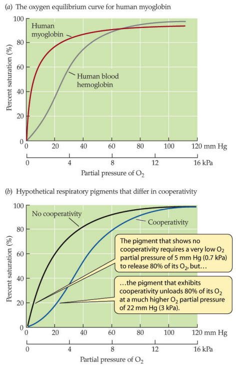


Figure 23.7 Respiratory pigments display hyperbolic or sigmoid oxygen equilibrium curves depending on whether they exhibit cooperativity in O_2 binding


(a) The oxygen equilibrium curve for human myoglobin

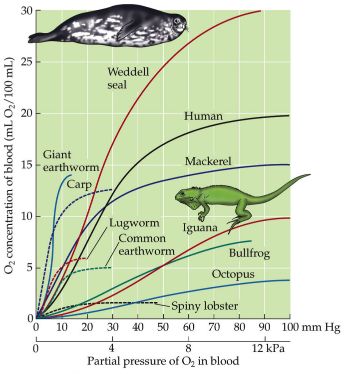
(b) Hypothetical respiratory pigments that differ in cooperativity

The pigment that shows no cooperativity requires a very low O_2 partial pressure of 5 mm Hg (0.7 kPa) to release 80% of its O_2, but…

…the pigment that exhibits cooperativity unloads 80% of its O_2 at a much higher O_2 partial pressure of 22 mm Hg (3 kPa).

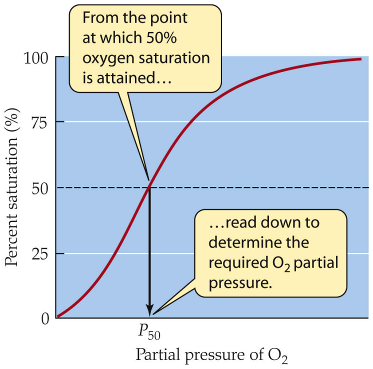
Animal Physiology 2e, Figure 23.7

Figure 23.8 A diversity of blood oxygen equilibrium curves



Animal Physiology 2e, Figure 23.8

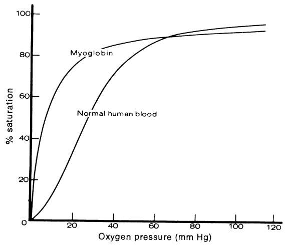
Figure 23.9 How to measure P_{50}



Animal Physiology 2e, **Figure 23.9**

Functions of Respiratory Pigments

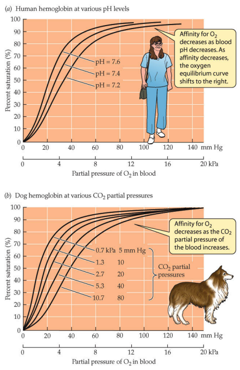
- *Half-saturation pressure* is the P_{O_2} at which a respiratory pigment is only half-saturated with O_2
  - Pigments with a lower half-saturation pressure have a higher *affinity* for O_2
  - For example, myoglobin has a half-saturation pressure of about 6 mm Hg, compared with human hemoglobin's 34 mm Hg. This allows myoglobin to remain complexed with O_2, while the O_2 of hemoglobin is 98% dissociated
  - Myoglobin's dissociation curve is thus not sigmoidal, and resembles the hyperbaric curve on the previous slide
  - This is because myoglobin has only one protein chain and one heme group, and can thus combine with only one O_2



Environmental Effects on Respiratory Pigment Function

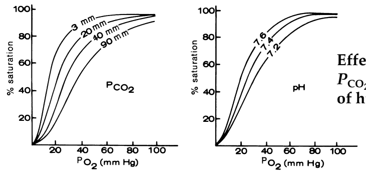
- In hemoglobin, the interaction of one heme group with an O_2 molecule increases the affinity of the other heme groups
  - Since the 3-D structure of hemoglobin suggests that the heme groups are widely separated, it is though that the combination of O_2 with one heme group affects the entire Hb molecule shape
- O_2 dissociation curves also affected by temperature, blood pH, and blood P_{CO_2}
  - Low pH or high P_{CO_2} generally shift the dissociation curve to the right (*Bohr effect*). These effects are slightly different, although a high P_{CO_2} does produce a lower blood pH
  - This important in O_2 transport, in that the P_{CO_2} in the capillaries around active tissues is elevated and the blood slightly acid. Under these conditions, a shift to the right facilitates O_2 unloading
  - Explains why sudden shifts in temperature, or decreased pH from acid deposition, can kill aquatic animals

Figure 23.10 The Bohr effect: Affinity for O_2 decreases as pH decreases or CO_2 partial pressure increases



Animal Physiology 2e, **Figure 23.10**

Environmental Effects on Respiratory Pigment Function



Effects of blood pH and blood P_{CO_2} on O_2 dissociation curves of human hemoglobin

Effects of pH and temperature on O_2 dissociation curves of horseshoe crabs. Note the _reverse_ *Bohr effect* for pH.

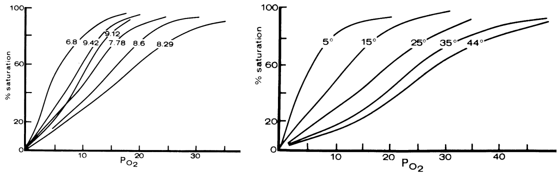
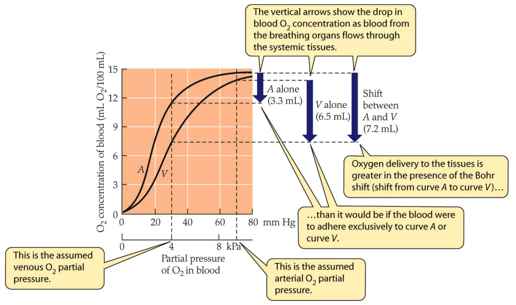


Figure 23.11 The Bohr effect typically enhances O_2 delivery in an animal



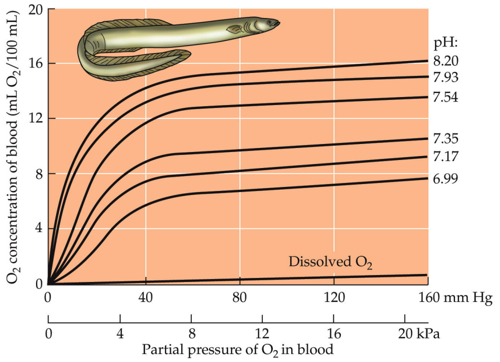
Animal Physiology 2e, **Figure 23.11**

Reverse Bohr Effect

Why would an organism have a reverse Bohr effect and high O_2 affinity?

- **Actually, a given pigment may not exhibit the Bohr effect in a given animal, but all of the respiratory pigments have been shown to exhibit this behavior, especially at high P_{CO_2}'s**
- **Typical of animals that live in stagnant, low O_2 environments**
- **Reverse Bohr shift increases the loading capacity of blood under these conditions**
- **In the case of horseshoe crabs, it is to provide enough O_2 for reproduction.**
  - **Long copulatory period in shallow bays**
  - **High temperatures low dissolved O_2, etc.**

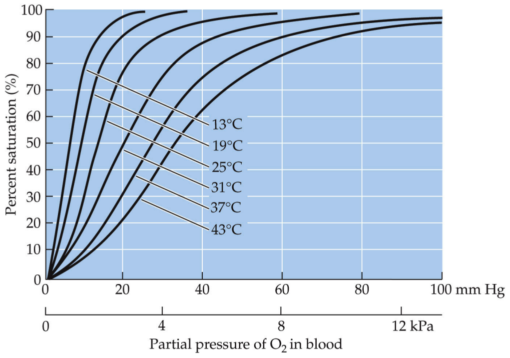
Figure 23.12 The Root effect in eels: Acidification lowers the oxygen-carrying capacity of hemoglobin



Animal Physiology 2e, Figure 23.12

© 2008 Sinauer Associates, Inc.

Figure 23.13 An increase in temperature typically causes a decrease in O_2 affinity



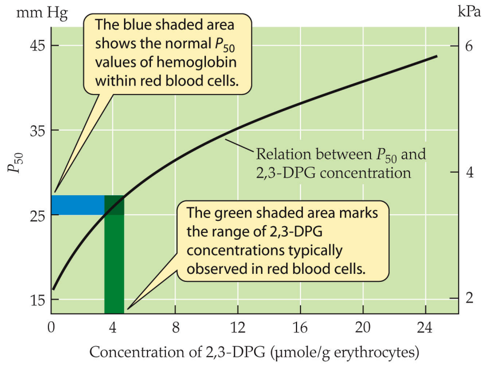
Animal Physiology 2e, Figure 23.13

© 2008 Sinauer Associates, Inc.

Other Effects on O_2 Affinities

- **O_2 affinity decreases at low salinity, probably due to conformational effects on pigment proteins**
- **O_2 affinity of hemoglobin is decreased by phosphate compounds in vertebrate blood cells: ATP, GTP, diphoshoglycerate (DPG), inocetyl pentaphosphate (IP5)**
  - **Fishes: high GTP**
  - **Reptiles: high ATP**
  - **Birds: high IP5**
  - **Mammals: high DPG**
- **These phosphate compounds increase O_2 loading, and can increase *or* decrease O_2 unloading**

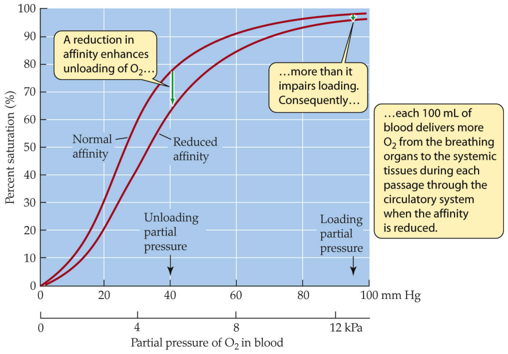
Figure 23.14 The normal P_{50} of human hemoglobin within red blood cells depends on a normal intracellular concentration of 2,3-DPG



Animal Physiology 2e, Figure 23.14

© 2008 Sinauer Associates, Inc.

Figure 23.15 A decrease in the O_2 affinity of hemoglobin can aid O_2 delivery to the systemic tissues when the O_2 partial pressure in the breathing organs remains high



Animal Physiology 2e, Figure 23.15

© 2008 Sinauer Associates, Inc.

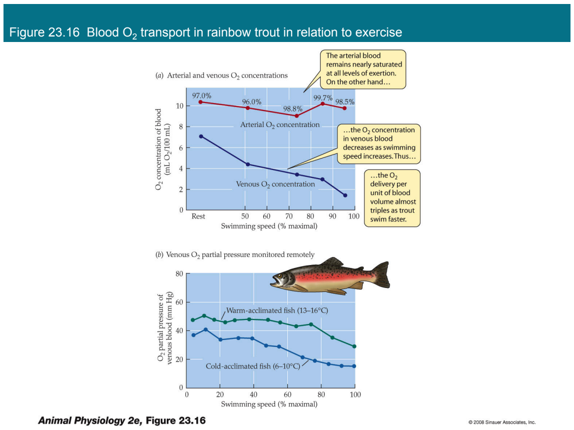


Figure 23.16 Blood O_2 transport in rainbow trout in relation to exercise

The arterial blood remains nearly saturated at all levels of exertion. On the other hand…

…the O_2 concentration in venous blood decreases as swimming speed increases. Thus…

…the O_2 delivery per unit of blood volume almost triples as trout swim faster.

Animal Physiology 2e, Figure 23.16

© 2008 Sinauer Associates, Inc.

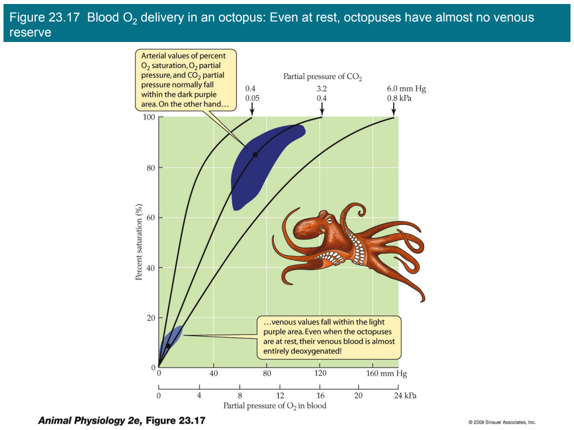


Figure 23.17 Blood O_2 delivery in an octopus: Even at rest, octopuses have almost no venous reserve

Arterial values of percent O_2 saturation, O_2 partial pressure, and CO_2 partial pressure normally fall within the dark purple area. On the other hand…

…venous values fall within the light purple area. Even when the octopuses are at rest, their venous blood is almost entirely deoxygenated!

Animal Physiology 2e, Figure 23.17

© 2008 Sinauer Associates, Inc.

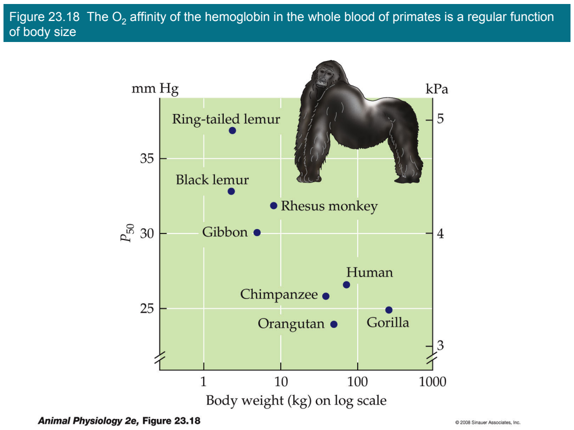


Figure 23.18 The O_2 affinity of the hemoglobin in the whole blood of primates is a regular function of body size

Animal Physiology 2e, Figure 23.18

© 2008 Sinauer Associates, Inc.

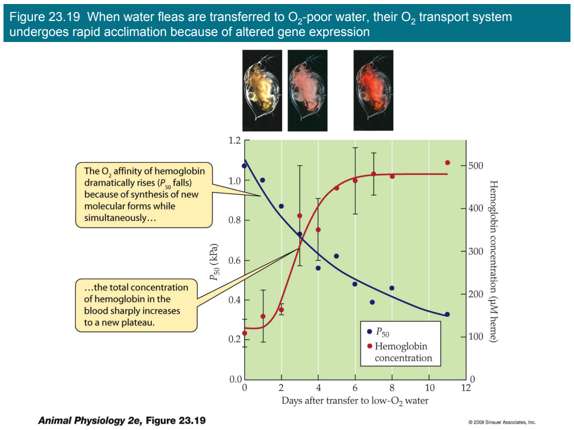


Figure 23.19 When water fleas are transferred to O_2-poor water, their O_2 transport system undergoes rapid acclimation because of altered gene expression

The O_2 affinity of hemoglobin dramatically rises (P_{50} falls) because of synthesis of new molecular forms while simultaneously…

…the total concentration of hemoglobin in the blood sharply increases to a new plateau.

Animal Physiology 2e, Figure 23.19

© 2008 Sinauer Associates, Inc.

CO_2 Transport

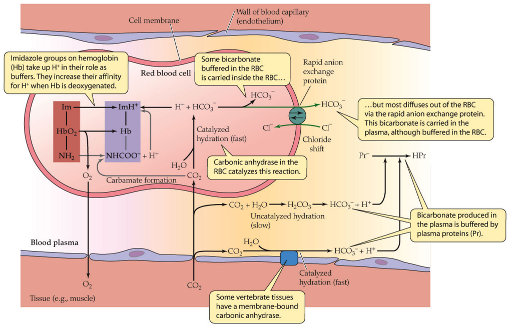
- CO_2 produced by tissue cells is transported to the lungs or other respiratory surfaces for excretion
- Only small amounts of CO_2 are in solution in the blood because of reactions that occur within the plasma, or within erythrocytes of vertebrates
- CO_2 in aqueous solution forms *carbonic acid* (H_2CO_3), a reaction speeded by the enzyme *carbonic anhydrase*, which is found in the blood of many animals (in vertebrates it is mostly within the erythrocytes)
- Carbonic acid can dissociate into H^+ and HCO_3^-, and these *bicarbonate* ions can then enter the acid-base regulating mechanisms
- However, about 15-20% of the CO_2 reacts with hemoglobin

Hemoglobin and CO_2 Transport

$$HbNH_2 + CO_2 \Leftrightarrow HbNHCOOH \Leftrightarrow HbNHCOO^-$$

- Where NH_2 represents the amino groups of the amino acids of the hemoglobin (the complex is known as *carbaminohemoglobin*)
- At the respiratory surface, CO_2 diffuses from the blood into the air or water. Carbonic anhydrase catalyzes the reverse reaction and aids in forming CO_2, which can be eliminated
- Direction of the reaction depends on P_{CO_2}: where CO_2 is low (as near the respiratory exchange surfaces) H_2CO_3 is split into H_2O and CO_2 , and the CO_2 move down its concentration gradient to the exterior

Figure 23.22 The processes of CO_2 uptake by the blood in a systemic blood capillary of a vertebrate



Imidazole groups on hemoglobin (Hb) take up H$^+$ in their role as buffers. They increase their affinity for H$^+$ when Hb is deoxygenated.

Some bicarbonate buffered in the RBC is carried inside the RBC…

…but most diffuses out of the RBC via the rapid anion exchange protein. This bicarbonate is carried in the plasma, although buffered in the RBC.

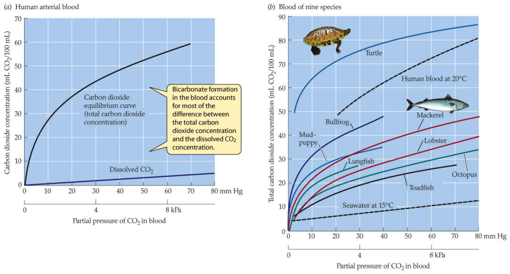
Carbonic anhydrase in the RBC catalyzes this reaction.

Bicarbonate produced in the plasma is buffered by plasma proteins (Pr).

Some vertebrate tissues have a membrane-bound carbonic anhydrase.

***Animal Physiology 2e,* Figure 23.22**

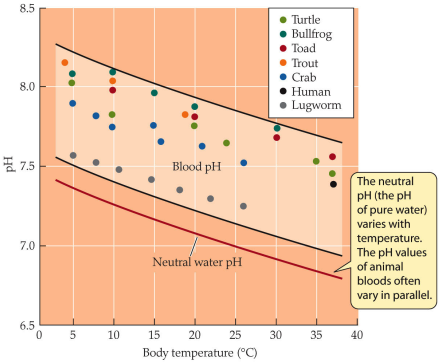
Figure 23.20 Carbon dioxide equilibrium curves



Bicarbonate formation in the blood accounts for most of the difference between the total carbon dioxide concentration and the dissolved CO_2 concentration.

***Animal Physiology 2e,* Figure 23.20**

Figure 23.23 Normal blood pH is a temperature-dependent variable



The neutral pH (the pH of pure water) varies with temperature. The pH values of animal bloods often vary in parallel.

***Animal Physiology 2e,* Figure 23.23**