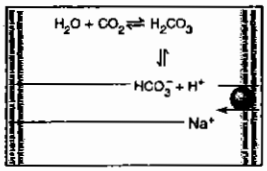
CHAPTER

25

GAS TRANSPORT AND pH REGULATION

James Baggott



25.1 INTRODUCTION TO GAS TRANSPORT

Large organisms, especially terrestrial ones, require a relatively tough, impermeable outer covering to help shield them from dust, twigs, nonisotonic fluids like rain and seawater, and other elements in the environment that might be harmful to living cells. One of the consequences of being large and having an impermeable covering is that individual cells of the organism cannot exchange gases directly with the atmosphere. Instead there must exist a specialized exchange surface, such as a lung or a gill, and a system to circulate the gases (and other materials, such as nutrients and waste products) in a manner that will meet the needs of every living cell in the body.

The existence of a system for the transport of gases from the atmosphere to cells deep within the body is not merely necessary, it has definite advantages. Oxygen is a good oxidizing agent, and at its partial pressure in the atmosphere, about 160 mmHg or 21.3 kPa, it would oxidize and inactivate many components of the cells, such as essential sulfhydryl groups of enzymes. By the time O_2 gets through the transport system of the body its partial pressure is reduced to a much less damaging 20 mmHg (2.67 kPa) or less. In contrast, CO_2 is relatively concentrated in the body and becomes diluted in transit to the atmosphere. In the tissues, where it is produced, its partial pressure is 46 mmHg (6.13 kPa) or more. In the lungs it is 40 mmHg (5.33 kPa), and in the atmosphere only 0.2 mmHg (0.03 kPa), less abundant than the rare gas, argon. Its relatively high concentration in the body permits it to be used as one component of a physiologically important buffering system, a system that is particularly useful because, upon demand, the concentration of CO_2 in the extracellular fluid can be varied over a rather wide range. This is discussed in more detail later in the chapter.

Oxygen and CO_2 are carried between the lungs and the other tissues by the blood. In the blood some of each gas is present in simple physical solution, but mostly each is involved in some sort of interaction with hemoglobin, the major protein of the red blood cell. There is a reciprocal relation between hemoglobin's affinity for O_2 and CO_2, so that the relatively high level of O_2 in the lungs aids the release of CO_2, which is to be expired, and the high CO_2 level in other tissues aids the release of O_2 for their use. Thus a description of the physiological transport of O_2 and CO_2 is the story of the interaction of these two compounds with hemoglobin.

25.2 NEED FOR A CARRIER OF OXYGEN IN BLOOD

An O_2 carrier is needed in blood because O_2 is not soluble enough in blood plasma to meet the body's needs. At 38°C, 1 L of plasma dissolves only 2.3 mL of O_2. Whole blood, because of its **hemoglobin,** has a much greater oxygen capacity (see Clin. Corr. 25.1). One liter of blood normally contains about 150 g of hemoglobin (contained within the erythrocytes), and each gram of hemoglobin can combine with 1.34 mL of O_2. Thus the hemoglobin in 1 L of blood can carry 200 mL of O_2, 87 times as much as plasma alone would carry. Without an O_2 carrier, the blood would have to circulate 87 times as fast to provide the same amount of O_2. As it is, the blood makes a complete circuit of the body in 60 s under resting conditions, and in the aorta it flows at the rate of about 18.6 m s^{-1}. An 87-fold faster flow would require a fabulous high-pressure pump, would produce tremendously turbulent flow and high shear forces in the plasma, would result in uncontrollable bleeding from wounds, and would not even allow the blood enough time in the lungs to take up O_2. The availability of a carrier not only permits us to avoid these impracticalities, but also gives us a way of controlling oxygen delivery, since the O_2 affinity of the carrier is responsive to changing physiological conditions.

CLINICAL CORRELATION 25.1

Diaspirin Hemoglobin

Shock is a condition of inadequate tissue perfusion due, for example, to loss of blood. Hemorrhagic shock is a major cause of death following trauma. Rapid blood transfusion can be life-saving, but cross-matching must be done before transfusing blood, and transfusion is associated with a significant risk of disease. In addition, blood (or blood of the correct type) may be in short supply under certain circumstances. Hence there is considerable interest in developing a safe, effective blood substitute.

Hemoglobin in plasma has a very short lifetime. It rapidly dissociates into $\alpha\beta$ dimers, which bind to the plasma protein, haptoglobin, and are removed from circulation. Hemoglobin can be specifically cross-linked with bis(3,5-dibromosalicyl) fumarate at the Lys 99 of the α chains; the product is called diaspirin cross-linked hemoglobin (DCLHb). DCLHb has a longer lifetime in plasma than hemoglobin, and its lifetime can be extended still further by polymerizing the DCLHb. DCLHb has performed well as a blood replacement in experimental animals, and the possibility of using it in humans is being pursued.

:spiratory System Anatomy Affects Blood Gas Concentration

ıe respiratory system includes the trachea, in the neck, which bifurcates in
e thorax into right and left bronchi, as shown schematically in Figure 25.1.
ıe bronchi continue to bifurcate into smaller and smaller passages, ending
.th tiny bronchioles, which open into microscopic gas-filled sacs called alveoli.
is in the alveoli that gas exchange takes place with the alveolar capillary blood.

As we inhale and exhale, the alveoli do not appreciably change in size.
.ther, it is the airways that change in length and diameter as the air is pumped
to and out of the lungs. Gas exchange between the airways and the alveoli
en proceeds simply by diffusion. These anatomical and physiological facts
.ve two important consequences. In the first place, since the alveoli are at
e ends of long tubes that constitute a large dead space, and the gases in the
veoli are not completely replaced by fresh air with each breath, the gas
mposition of the alveolar air differs from that of the atmosphere, as shown
Table 25.1. Oxygen concentration is lower in the alveoli because it is removed
the blood. Carbon dioxide concentration is higher because it is added. Since
e do not usually breathe air that is saturated with water vapor at 38°C, water
.por is generally added in the airways. The concentration of nitrogen is lower
the alveoli, not because it is taken up by the body, but simply because it is
luted by the CO_2 and water vapor.

A second consequence of the existence of alveoli of essentially constant
ze is that the blood that flows through the pulmonary capillaries during expira-
on, as well as the blood that flows through during inspiration, can exchange
.ses. This would not be possible if the alveoli collapsed during expiration and
ontained no gases, in which case the composition of the blood gases would
ıctuate widely, depending on whether the blood passed through the lungs
ıring an inspiratory or expiratory phase of the breathing cycle.

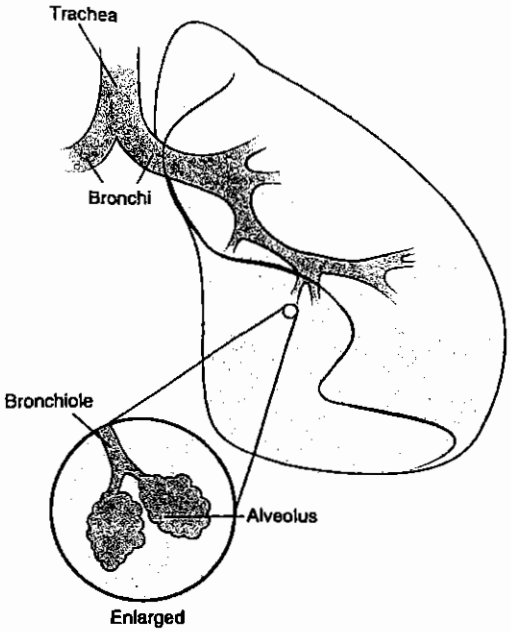


FIGURE 25.1
Diagram showing the respiratory tract.

Physiological Oxygen Carrier Must Have Unusual Properties

'e have seen that an O_2 carrier is necessary. Clearly this carrier would have
be able to bind oxygen at an O_2 tension of about 100 mmHg (13.3 kPa), the
ırtial pressure of oxygen in the alveoli. The carrier must also be able to release
$_2$ to the extrapulmonary tissues. The O_2 tension in the capillary bed of an
:tive muscle is about 20 mmHg (2.67 kPa). In resting muscle it is higher, but
ıring extreme activity it is lower. These O_2 tensions represent the usual limits
ithin which an oxygen carrier must work. An efficient carrier would be nearly
lly saturated in the lungs but should be able to give up most of this to a
orking muscle.

Let us first see whether a carrier that binds O_2 in a simple equilibrium
presented by

$$\text{Oxygen} + \text{carrier} \leftrightharpoons \text{oxygen} \cdot \text{carrier}$$

ABLE 25.1 Partial Pressures of Important Gases Given in Millimeters of Hg (kPa)

	In the Atmosphere		*In the Alveoli of the Lungs*	
Gas	*mmHg*	*kPa*	*mmHg*	*kPa*
O_2	159	21.2	100	13.3
N_2	601	80.1	573	76.4
CO_2	0.2	0.027	40	5.33
H_2O	0	0	47	6.27
Total	760	101	760	101

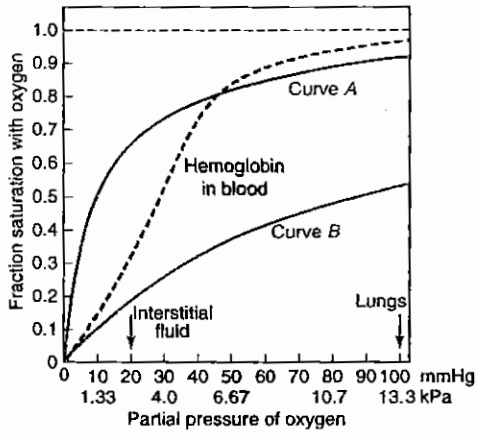


FIGURE 25.2
Oxygen saturation curves for two hypothetical oxygen carriers and for hemoglobin.
Curve *A*: Hypothetical carrier with hyperbolic saturation curve (a simple carrier), 90% saturated in the lungs and 66% saturated at the partial pressure found in interstitial fluid. Curve *B*: Hypothetical carrier with hyperbolic saturation curve (another simple carrier), 56% saturated in the lungs and 20% saturated at the partial pressure found in interstitial fluid. Dashed curve: Hemoglobin in whole blood.

would be satisfactory. For this type of carrier the dissociation constant woul be given by the simple expression

$$K_d = \frac{[\text{oxygen}][\text{carrier}]}{[\text{oxygen} \cdot \text{carrier}]}$$

and the saturation curve would be a **rectangular hyperbola.** This mode would be valid even for a carrier with several oxygen-binding sites per molecule which we know is the case for hemoglobin, as long as each site were indepen dent and not influenced by the presence or absence of O_2 at adjacent sites.

If such a carrier had a dissociation constant that permitted 90% saturatio in the lungs, then, as shown in Figure 25.2, curve *A*, at a partial pressure of 2 mmHg (2.67 kPa) it would still be 66% saturated and would have delivere only 24% of its O_2 load. This would not be very efficient.

What about some other simple carrier, one that bound O_2 less tightly an therefore released most of it at low partial pressure, so that the carrier was, say only 20% saturated at 20 mmHg (2.67 kPa)? Again, as shown in Figure 25.2 curve *B*, it would be relatively inefficient; in the lungs this carrier could fill onl 56% of its maximum O_2 capacity and would deliver only 36% of what it coul carry. It appears then that the mere fivefold change in O_2 tension between th lungs and the unloading site is not compatible with efficient operation of a simple carrier. Simple carriers are not sensitive enough to respond massively to a signal as small as a fivefold change.

Figure 25.2 also shows the oxygen-binding curve of hemoglobin in norma blood. The curve is **sigmoid,** not hyperbolic, and it cannot be described by a simple equilibrium expression. Hemoglobin, however, is a very good physiolog ical O_2 carrier. It is 98% saturated in the lungs and only about 33% saturatec in the working muscle. Under these conditions it delivers about 65% of the O it can carry.

It can be seen in Figure 25.2 that hemoglobin is 50% saturated with O_2 a a partial pressure of 27 mmHg (3.60 kPa). The partial pressure corresponding to 50% saturation is called the P_{50}. The term P_{50} is the most common way o expressing hemoglobin's O_2 affinity. By analogy with K_m for enzymes, a relatively high P_{50} corresponds to a relatively low O_2 affinity.

The Steep Part of the Curve Lies in the Physiological Range

Note that the steep part of hemoglobin's saturation curve lies in the range of O_2 tensions that prevail in the extrapulmonary tissues. This means that relatively small decreases in oxygen tension in these tissues will result in large increases in O_2 delivery, this effect becoming more pronounced as the partial pressure of O_2 diminishes within the physiological range. Furthermore, small shifts of the curve to the left or right will also strongly influence O_2 delivery. In Sections 25.3, 25.5, and 25.6 we see how physiological signals effect such shifts and result in enhanced delivery under conditions of increased O_2 demand. Small decreases of O_2 tension in the lungs, however, such as occur at moderately high altitudes, do not seriously compromise hemoglobin's ability to bind oxygen. This will be true as long as the alveolar partial pressure of O_2 remains in a range that corresponds to the relatively flat region of hemoglobin's O_2 dissociation curve (see Clin. Corr. 25.2).

Finally, we can see from Figure 25.2 that the binding of oxygen by hemoglobin is cooperative. At very low O_2 tension the hemoglobin curve tends to follow the hyperbolic curve, which represents relatively weak O_2 binding, but at higher tensions it actually rises above the hyperbolic curve that represents tight binding. Thus hemoglobin binds O_2 weakly at low oxygen tension and tightly at high tension. The binding of the first O_2 to each hemoglobin molecule enhances the binding of subsequent O_2 molecules.

CLINICAL CORRELATION 25.2

Cyanosis

Cyanosis is a condition in which a patient's skin or mucous membrane appears gray or (in severe cases) purple-magenta. It is due to an abnormally high concentration of deoxyhemoglobin below the surface, which is responsible for the observed color. The familiar blue of superficial veins is due to their deoxyhemoglobin content and is a normal manifestation of this color effect.

Cyanosis is most commonly caused by diseases of the cardiac or pulmonary systems, resulting in inadequate oxygenation of the blood. It can also be caused by certain hemoglobin abnormalities. Severely anemic individuals cannot become cyanotic; they do not have enough hemoglobin in their blood for the characteristic color of its deoxy form to be apparent.

Albert, R. K. Approach to the patient with cyanosis and/or hypoxemia. In: W. N. Kelley (Ed.), *Textbook of Internal Medicine.* Philadelphia: Lippincott, 1989, pp. 2041–2044.

Hemoglobin's ability to bind O_2 cooperatively is reflected in its **Hill coeffi-**
ıt, which has a value of about 2.7. (The Hill equation is derived and inter-
ed on p. 119.) Since the maximum value of the Hill coefficient for a system
quilibrium is equal to the number of cooperating binding sites, a value of
neans that hemoglobin, with its four oxygen-binding sites, is more coopera-
than would be possible for a system with only two cooperating binding
but it is not as cooperative as it could be.

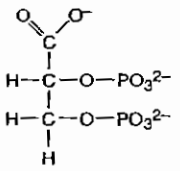


FIGURE 25.3
2,3-Bisphosphoglycerate (BPG).

HEMOGLOBIN AND ALLOSTERISM: EFFECT OF 2,3-BISPHOSPHOGLYCERATE

›globin's binding of O_2 was the original example of a **homotropic effect**
ıerativity and allosterism are discussed in Chapter 4), but hemoglobin also
its a **heterotropic effect** of great physiological significance. This involves
eraction with **2,3-bisphosphoglycerate** (BPG) (Figure 25.3), which is
y related to the glycolytic intermediate, 1,3-bisphosphoglycerate, from
it is biosynthesized.

had been known for many years that hemoglobin in the red cell bound
ı less tightly than purified hemoglobin could (Figure 25.4). It had also
:nown that the red cell contained high levels of BPG, nearly equimolar
:moglobin. Finally, the appropriate experiment was done to demonstrate
ationships between these two facts. It was shown that the addition of
· purified hemoglobin produced a shift to the right of its oxygen-binding
bringing it into congruence with the curve observed for whole blood.
›rganic polyphosphates, such as ATP and inositol pentaphosphate, also
is effect. Inositol pentaphosphate is the physiological effector in birds,
t replaces BPG, and ATP plays a similar role in some fish.

nod's model of allosterism explains heterotropic interaction. Applying
del to hemoglobin, in the deoxy conformation (the **T state)** a cavity
ıough to admit BPG exists between the β chains of hemoglobin. This
lined with positively charged groups and firmly binds one molecule
:gatively charged BPG. In the oxy conformation (the **R state)** this cavity
:r, and it no longer accommodates BPG as easily. The result is that the
of BPG to oxyhemoglobin is much weaker. Since BPG binds preferen-
he T state, the presence of BPG shifts the R–T equilibrium in favor of
ıte; the deoxyhemoglobin conformation is thus stabilized over the
›globin conformation (Figure 25.5). For oxygen to overcome this and
ıemoglobin, a higher concentration of oxygen is required. Oxygen
ı the lungs is sufficiently high under most conditions to saturate hemo-
nost completely, even when BPG levels are high. The physiological
3PG can, therefore, be expected to be upon release of oxygen to the
ıonary tissues, where O_2 tensions are low.

ignificance of a high BPG concentration is that the efficiency of O_2
ı increased. Concentrations of BPG in the red cell rise in conditions
l with **tissue hypoxia,** such as various anemias, cardiopulmonary
ıcy, and high altitude. These high levels of BPG enhance the formation
emoglobin at low partial pressures of oxygen; hemoglobin then deliv-
›f its O_2 to the tissues. This effect can result in a substantial increase
›unt of O_2 delivered because the venous blood returning to the heart
ıl individual is (at rest) at least 60% saturated with O_2. Much of this
ıociate in the peripheral tissues if the BPG concentration rises.

²G mechanism works very well as a compensation for tissue hypoxia
he partial pressure of oxygen in the lungs remains high enough that
ıding in the lungs is not compromised. Since, however, BPG shifts
-binding curve to the right, the mechanism will not compensate for
xia when the partial pressure of O_2 in the lungs falls too low. Then

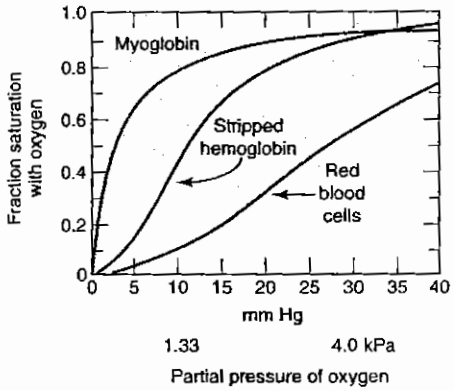


FIGURE 25.4
Oxygen dissociation curves for myoglobin, for hemoglobin that has been stripped of CO_2 and organic phosphates, and for whole red blood cells.
Data from Brenna, O., Luzzana, M., Pace, M., et al. Adv. Exp. Biol. Med. *28:19, 1972. Adapted from McGilvery, R. W.* Biochemistry: A Functional Approach, *2nd ed. Philadelphia: Saunders, 1979, p. 236.*

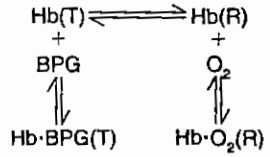
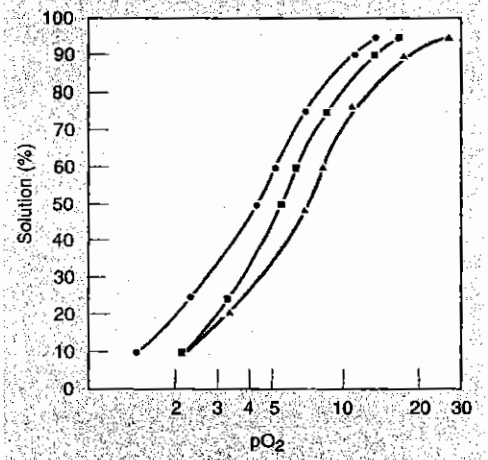


FIGURE 25.5
Schematic representation of equilibria among BPG, O_2, and the T and R states of hemoglobin.

CLINICAL CORRELATION 25.3

Chemically Modified Hemoglobins: Methemoglobin and Sulfhemoglobin

Methemoglobin is a form of hemoglobin in which the iron is oxidized from the iron (II) state to the iron (III) state. A tendency for methemoglobin to be present in excess of its normal level of about 1% may be due to a hereditary defect of the globin chain or to exposure to oxidizing drugs or chemicals. Sulfhemoglobin is a species that forms when a sulfur atom is incorporated into the porphyrin ring of hemoglobin. Exposure to certain drugs or to soluble sulfides produces it. Sulfhemoglobin is green. Hemoglobin subunits containing these modified hemes do not bind oxygen, but they change the oxygen-binding characteristics of the normal subunits in hybrid hemoglobin molecules containing some normal subunits and one or more modified subunits. The accompanying figure shows the oxygen-binding curve of normal HbA, 15% methemoglobin and 12% sulfhemoglobin. The presence of methemoglobin shifts the curve to the left, impairing the delivery of the decreased amount of bound oxygen. In contrast, the sulfhemoglobin curve is shifted to the right, a BPG-like effect. As a result, oxygen delivery is enhanced, partially compensating for the inability of the sulfur-modified hemes to bind oxygen.



Oxygenation curves of unmodified hemoglobin A (squares) of a 15% oxidized hemolysate (circles) and of a hemolysate containing 12% sulfhemoglobin (triangles) in 0.1 M phosphate, pH 7.35, at 20°C.
Data from Park, C. M., and Nagel, R. L., N. Engl. J. Med. *310:1579, 1984.*

the increased efficiency of O_2 unloading to the tissues is counterbalanced by a decrease in the efficiency of loading in the lungs. This may be a factor in determining the maximum altitude at which people choose to establish permanent dwellings, which is about 18,000 ft (~5500 m). There is evidence that a better adaptation to extremely low ambient partial pressures of O_2 would be a shift of the curve to the left.

25.4 OTHER HEMOGLOBINS

Although hemoglobin A is the major form of hemoglobin in adults and in children over seven months of age, accounting for about 90% of their total hemoglobin, it is not the only normal hemoglobin species. Normal adults also have 2–3% of **hemoglobin A_2,** which is composed of two α chains like those in hemoglobin A and two δ chains. It is represented as $\alpha_2\delta_2$. The δ chains differ in amino acid sequence from the β chains and are under independent genetic control. Hemoglobin A_2 does not appear to be important in normal individuals.

Several species of modified hemoglobin A also occur normally. These are designated A_{1a1}, A_{1a2}, A_{1b}, and A_{1c}. They are adducts of hemoglobin with various sugars, such as glucose, glucose 6-phosphate, and fructose 1,6-bisphosphate. The quantitatively most significant is **hemoglobin A_{1c},** formed by covalent binding of a glucose residue to the N terminal of the β chain at a rate that depends on the concentration of glucose. As a result, hemoglobin A_{1c} forms more rapidly in uncontrolled diabetics and can comprise up to 12% of their total hemoglobin. Hemoglobin A_{1c} or total glycosylated hemoglobin levels are a useful measure of how well diabetes has been controlled during the days and weeks before the measurement is taken; measurement of blood glucose only indicates how well diabetes is under control when the blood sample is taken. Chemical modification of hemoglobin A can also occur from interaction with drugs or environmental pollutants (see Clin. Corr. 25.3).

Fetal hemoglobin, **hemoglobin F,** is the major hemoglobin in newborn infants. It contains two γ chains in place of the β chains and is represented as $\alpha_2\gamma_2$. Shortly before birth γ-chain synthesis diminishes and β-chain synthesis is initiated, and by the age of seven months well over 90% of the infant's hemoglobin is hemoglobin A.

Hemoglobin F is adapted to the environment of the fetus, who gets oxygen from maternal blood, a source that is far poorer than the atmosphere. To compete with the maternal hemoglobin for O_2, fetal hemoglobin must bind O_2 more tightly; its oxygen-binding curve is thus shifted to the left relative to hemoglobin A. This is accomplished through a difference in the influence of BPG upon the maternal and fetal hemoglobins. In hemoglobin F two of the groups that line the BPG-binding cavity have neutral side chains instead of the positively charged ones that occur in hemoglobin A. Consequently, hemoglobin F binds BPG less tightly and thus binds oxygen more tightly than hemoglobin A does. Also, about 15–20% of the hemoglobin F is acetylated at the N terminals; this is referred to as hemoglobin F_1. Hemoglobin F_1 does not bind BPG, and its affinity for oxygen is not affected at all by BPG. The postnatal change from hemoglobin F to hemoglobin A, combined with a rise in red cell BPG that peaks three months after birth, results in a gradual shift to the right of the infant's oxygen-binding curve (Figure 25.6). The result is greater delivery of oxygen to the tissues at this age than at birth, in spite of a 30% decrease in the infant's total hemoglobin concentration.

In many inherited anomalies of hemoglobin synthesis there is formation of a structurally abnormal hemoglobin; these are called **hemoglobinopathies.** They may involve the substitution of one amino acid in one type of polypeptide chain for some other amino acid or they may involve absence of one or more amino acid residues of a polypeptide chain. In some cases the change is clinically insignificant, but in others it causes serious disease (see Clin. Corr. 25.4).

.5 PHYSICAL FACTORS THAT AFFECT OXYGEN BINDING

gh Temperature Weakens Hemoglobin's Oxygen Affinity

mperature has a significant effect on O_2 binding by hemoglobin (Figure 25.7). below-normal temperatures the binding is tighter, resulting in a leftward shift the curve; at higher temperatures the binding becomes weaker, and the rve is shifted to the right. The effect of elevated temperature is like that of ;h levels of BPG, in that both enhance unloading of oxygen. The temperature ect is physiologically useful, as it makes additional O_2 available to support : high metabolic rate found in fever or in exercising muscle with its elevated nperature. The relative insensitivity to temperature of O_2 binding at high rtial pressure of oxygen minimizes compromise of O_2 uptake in the lungs der these conditions.

The tighter binding of O_2 that occurs in hypothermic conditions is not portant in hypothermia induced for surgical purposes. Decreased O_2 utiliza- n by the body and increased solubility of O_2 in plasma at lower temperatures, well as the increased solubility of CO_2, which acidifies the blood, compensate hemoglobin's diminished ability to release O_2.

w pH Weakens Hemoglobin's Oxygen Affinity

drogen ion concentration influences hemoglobin's O_2 binding. As shown in ure 25.8, low pH shifts the curve to the right, enhancing O_2 delivery, whereas h pH shifts the curve to the left. It is customary to express O_2 binding by noglobin as a function of plasma pH because it is this value, not the pH hin the erythrocyte, that is usually measured. Erythrocyte cell sap pH is ver than the plasma pH, but these two fluids are in equilibrium, and changes one reflect changes in the other.

The influence of pH upon O_2 binding is physiologically significant, since ecrease in pH is often associated with increased oxygen demand. Increased tabolic rate increases production of carbon dioxide and, as in muscular rcise and hypoxic tissue, lactic acid. These acids produced by metabolism p release oxygen to support that metabolism.

The increase in acidity of hemoglobin as it binds O_2 is known as the **Bohr ect;** an equivalent statement is that the Bohr effect is the increase in basicity of noglobin as it releases oxygen. The effect may be expressed by the equation

$$HHb + O_2 \leftrightharpoons HbO_2 + H^+$$

s equation gives the same information as Figure 25.8—that increases in lrogen ion concentration favor formation of free oxygen from oxyhemoglo- , and conversely, that oxygenation of hemoglobin lowers the pH of the so- on.

6 CARBON DIOXIDE TRANSPORT

: carbon dioxide we produce is excreted by the lungs, to which it is trans- ted by the blood. Carbon dioxide transport is closely tied to hemoglobin l to the problem of maintaining a constant pH in the blood, a problem that be discussed subsequently.

od CO_2 Is Present in Three Major Forms

bon dioxide is present in the blood in three major forms, as dissolved CO_2, HCO_3^- (formed by ionization of H_2CO_3 produced when CO_2 reacts with H_2O), as carbaminohemoglobin (formed when CO_2 reacts with amino groups of tein). Each of these is present both in arterial blood and in venous blood

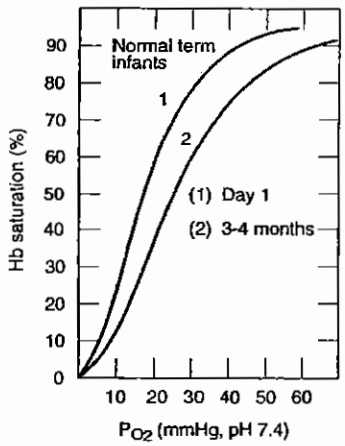


FIGURE 25.6
Oxygen dissociation curves after birth.
Adapted from Oski, F. A., and Delivoria-Papadopoulos, M. J. Pediatr. *77:941, 1970.*

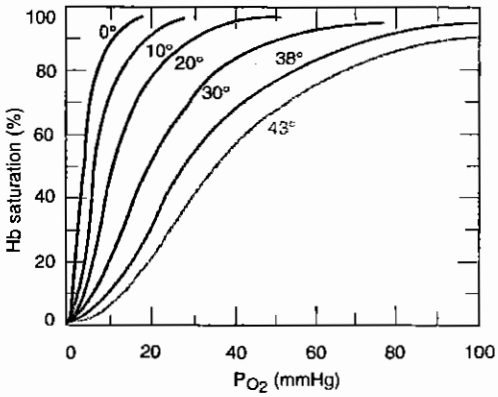


FIGURE 25.7
Oxygen dissociation curve for whole blood at various temperatures.
From Lambertson, C. J. In: P. Bard (Ed.), Medical Physiology, *11th ed. St. Louis, MO: Mosby, 1961, p. 596.*

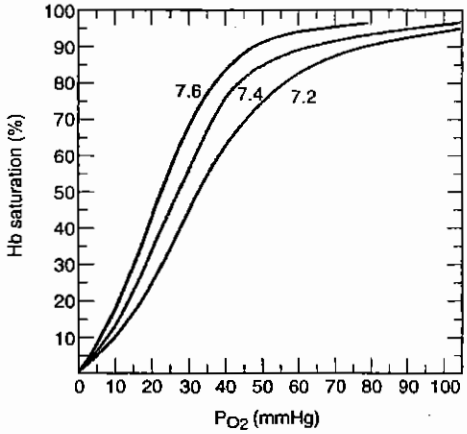


FIGURE 25.8
Oxygen dissociation curve for whole blood at various values of plasma pH.
Adapted from Lambertson, C. J. In: P. Bard (Ed.), Medical Physiology, *11th ed. St. Louis, MO: Mosby, 1961, p. 596.*

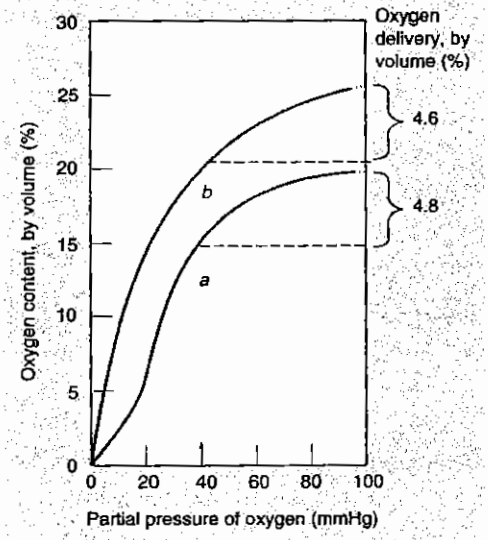
CLINICAL CORRELATION 25.4

Hemoglobins with Abnormal Oxygen Affinity

Some abnormal hemoglobins have an altered affinity for oxygen. If oxygen affinity is increased (P_{50} decreased), oxygen delivery to the tissues will be diminished unless some sort of compensation occurs. Typically, the body responds by producing more erythrocytes (polycythemia) and more hemoglobin. Hb Rainier is an abnormal hemoglobin in which the P_{50} is 12.9 mmHg, far below the normal value of 27 mmHg.

In the accompanying figure the oxygen content in volume percent (mL of O_2 per 100 mL of blood) is plotted versus partial pressure of oxygen, both for normal blood (curve *a*) and for the blood of a patient with Hb Rainier (curve *b*). Obviously, the patient's blood carries more oxygen; this is because it contains 19.5 g of Hb per 100 mL instead of the usual 15 g per 100 mL.

Since the partial pressure of oxygen in mixed venous blood is about 40 mmHg, the volume of oxygen the blood of each individual can deliver may be obtained from the graph by subtracting the oxygen content of the blood at 40 mmHg from its oxygen content at 100 mmHg. As shown in the figure, the blood of the patient with Hb Rainier delivers nearly as much oxygen as normal blood does, although Hb Rainier delivers a significantly smaller fraction of the total amount it carries. Evidently, polycythemia is an effective compensation for this condition, at least in the resting state.



Oxygen content plotted against partial pressure of oxygen. (continued)

(see the top three lines of Table 25.2). Net transport to the lungs for excretion is represented by the concentration difference between arterial and venous blood, shown in the last column. Note that for each form of carbon dioxide the arterial–venous difference is only a small fraction of the total amount present; venous blood contains only about 10% more **total carbon dioxide** (total CO_2 is the sum of HCO_3^-, dissolved CO_2, and carbaminohemoglobin) than arterial blood.

After carbon dioxide enters the bloodstream for transport, it generates hydrogen ions. Most come from formation of bicarbonate ion, which occurs in the following manner.

Bicarbonate Formation

Carbon dioxide enters the blood and diffuses into erythrocytes, whose membranes, like most biological membranes, are freely permeable to dissolved CO_2. Within the erythrocytes most of the carbon dioxide is acted on by the intracellular enzyme, **carbonic anhydrase,** which catalyzes the reaction

$$CO_2 + H_2O \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} H_2CO_3$$

This reaction proceeds in the absence of a catalyst, as is well known to all who drink carbonated beverages. Without the catalyst, however, it is too slow to meet the body's needs, taking over 100 s to reach equilibrium. Recall that at rest the blood makes a complete circuit of the body in 60 s. Carbonic anhydrase is a very active enzyme, having a turnover number of the order of 10^6, and inside the erythrocytes the reaction reaches equilibrium within 1 s, less than the time spent by the blood in the capillary bed. The enzyme contains zinc and accounts in part for our dietary requirement for this metal.

The ionization of carbonic acid, $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$, is a rapid, spontaneous reaction. It produces equivalent amounts of H^+ and HCO_3^-. Since, as shown in the last column of line 2 in Table 25.2, 1.69 meq of bicarbonate was added to each liter of blood by this process, 1.69 meq of H^+ must also have been generated per liter of blood. Addition of this much acid, over 10^{-3} equiv of H^+, to 1 L of water would give a final pH below 3. Since the pH of venous plasma averages 7.37, most of the H^+ generated during HCO_3^- production must be consumed by buffer action and/or other processes. This is discussed below.

Because of the compartmentalization of carbonic anhydrase, essentially all conversion of CO_2 to H_2CO_3, and ultimately to HCO_3^-, occurs inside the erythrocyte. Negligible amounts of CO_2 react nonenzymatically in the plasma. Thus virtually all of the increase in HCO_3^- in venous as compared to arterial blood is generated in erythrocytes. Most of this diffuses into the plasma, so that venous plasma HCO_3^- is higher than the arterial, but the erythrocyte was the site of its formation.

Carbaminohemoglobin Formation

It has been observed that in the presence of carbonic anhydrase inhibitors, such as acetazolamide or cyanide, blood will still take up a certain amount of carbon dioxide rapidly. This is due to the reaction of carbon dioxide with amino groups of proteins within erythrocytes to form **carbamino groups** (Figure 25.9). Hemoglobin is quantitatively the most important protein involved in this reaction. Deoxyhemoglobin forms **carbamino hemoglobin** more readily than oxyhemoglobin. Oxygenation causes release of CO_2 in carbaminohemoglobin.

Carbaminohemoglobin formation occurs only with uncharged aliphatic amino groups, not with the charged form, $R{-}NH_3^+$. The pH within erythrocytes is normally about 7.2, somewhat more acidic than the plasma. Since protein amino groups have pK values well to the alkaline side of 7.2, they will be mostly in the charged (undissociated acid) form. Removal of some of the un-

charged form via carbamino group formation shifts the equilibrium, generating more uncharged amino groups and an equivalent amount of H^+, as shown in Figure 25.10. Carbamination, like HCO_3^- formation, generates H^+.

The N-terminal α-amino groups of proteins have pK values in the range of 7.6–8.4. The N terminals of hemoglobin's polypeptide chains are the principal sites of carbamination. If they are blocked chemically by reaction with cyanate, carbamino formation does not occur.

The N-terminal amino groups of the β-globin chains are part of the binding site for BPG. Since they cannot bind BPG and also form carbamino groups, a competition arises. Carbon dioxide diminishes the effect for BPG and, conversely, BPG diminishes the ability of hemoglobin to form carbaminohemoglobin. Ignorance of the latter interaction led to a major overestimation of the role of carbaminohemoglobin in carbon dioxide transport. Prior to the discovery of the BPG effect, careful measurements were made of the capacity of purified hemoglobin (no BPG present) to form carbaminohemoglobin. The results were assumed to be applicable to hemoglobin in the erythrocyte, leading to the erroneous conclusion that carbaminohemoglobin accounted for 25–30% or more of CO_2 transport. It now appears that 13–15% of CO_2 transport is via carbaminohemoglobin. Table 25.3 summarizes the contribution of each major form of blood carbon dioxide to overall CO_2 transport.

Curve *a* shows the oxygen dissociation curve of normal blood with a hemoglobin of 15 g dL^{-1}, P_{50} 27 mmHg, n 2.8, at pH 7.4, 37°C. Curve *b* shows that of blood from a patient with Hb Rainier, having a hemoglobin of 19.5 g dL^{-1}, P_{50} 12.9 mmHg, n 1.2, at the same pH and temperature. (1 mmHg ≈ 133.3 Pa.) On the right is shown the oxygen delivery. The compensatory polycythemia and hyperbolic curve of Hb Rainier result in practically normal arterial and venous oxygen tensions. Arrow indicates normal mixed venous oxygen tension.
From Bellingham, A. J. Br. Med. Bull. *32:234, 1976.*

Two Processes Regulate [H^+] Derived from CO_2 Transport

Buffering

Hemoglobin, besides carrying O_2 and CO_2 in the covalently bound form of a carbamino group, also plays the major role in handling the H^+ produced in CO_2 transport. It does this by buffering and by the isohydric mechanism (discussed below). **Hemoglobin's buffering** power resides in its ionizable groups with pK values close to the intraerythrocyte pH. These include the four N-terminal amino groups and the imidazole side chains of the histidine residues. There are 38 histidines per hemoglobin tetramer; these provide most of hemoglobin's buffering ability.

In whole blood, buffering takes up about 60% of the acid generated in normal carbon dioxide transport. Although hemoglobin is by far the most important nonbicarbonate buffer in blood, the organic phosphates in the eryth-

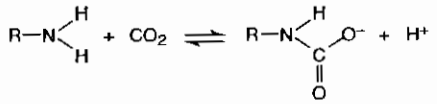


FIGURE 25.9
Carbamino formation from a free amino group and carbon dioxide.

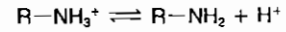


FIGURE 25.10
Dissociation of an ammonium ion to yield a free amino group and H^+.

TABLE 25.2 Properties of Blood of Humans at Rest[a]

	Arterial			*Venous*			*A–V Difference*		
	Serum	***Cells***	***Blood***	***Serum***	***Cells***	***Blood***	***Serum***	***Cells***	***Blood***
Hb carbamino groups (meq L^{-1} of blood)		1.13	1.13		1.42	1.42		+0.29	+0.29
HCO_3^- (meq L^{-1} of blood)	13.83	5.73	19.56	14.84	6.41	21.25	+1.01	+0.68	+1.69
Dissolved CO_2 (meq L^{-1} of blood)	0.71	0.48	1.19	0.82	0.56	1.38	+0.11	+0.08	+0.19
Total CO_2 (meq L^{-1} of blood)	14.54	7.34	21.88	15.66	8.39	24.05	+1.12	+1.05	+2.17
Free O_2 (mmol L^{-1} of blood)			0.10			0.04			−0.06
Bound O_2 (mmol L^{-1} of blood)			8.60			6.01			−2.59
Total O_2 (mmol L^{-1} of blood)			8.70			6.05			−2.65
P_{O_2} (mmHg)			88.0			37.2			−50.8
P_{CO_2} (mmHg)			41.0			47.5			+6.5
pH	7.40	7.19		7.37	7.17		−0.03	−0.02	
Volume (cc L^{-1} of blood)	551.7	448.3	1000	548.9	451.1	1000	−2.8	+2.8	0.0
H_2O (cc L^{-1} of blood)	517.5	322.8	840.0	514.7	325.6	840.0	−2.8	+2.8	0.0
Cl^- (meq L^{-1} of blood)	57.71	24.30	82.01	56.84	25.17	82.01	−0.88	+0.88	0.0

Source: From Baggott, J. *Trends Biochem. Sci.* 3:N207, 1978, with permission of the publisher.

[a] Hemoglobin, 9 mM; serum protein, 39.8 g L^{-1} of blood; respiratory quotient, 0.82.

TABLE 25.3 Major Forms of Carbon Dioxide Transport

Species	*Transport (%)*
HCO_3^-	78
CO_2 (dissolved)	9
Carbaminohemoglobin	13

TABLE 25.4 Processes Occurring at the N Terminals of the α Chains and β Chains of Hemoglobin

	N Terminals	
Process	*α Chains*	*β Chains*
Carbamino formation	Yes	Yes
BPG binding	No	Yes
H^+ binding in the Bohr effect	Yes	No

TABLE 25.5 Control of the Excess H^+ Generated During Normal Carbon Dioxide Transport

Buffering	
By hemoglobin	50%
By other buffers	10%
Isohydric mechanism (hemoglobin)	40%

rocytes, the plasma proteins, and so on also make a significant contribution. Buffering by these compounds accounts for about 10% of the H^+, leaving about 50% of acid control specifically attributable to buffering by hemoglobin. These buffer systems minimize the change in pH that occurs when acid or base is added but do not altogether prevent that change. A small difference in pH between arterial and venous blood is therefore observed.

Isohydric Mechanism

The remainder of the H^+ arising from carbon dioxide is taken up by hemoglobin, but not by buffering. Recall that when hemoglobin becomes oxygenated it becomes a stronger acid and releases H^+ (the Bohr effect). In the capillaries, where O_2 is released, the opposite occurs:

$$HbO_2 + H^+ \leftrightharpoons HHb + O_2$$

Simultaneously, CO_2 enters the capillaries and is hydrated:

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

Addition of these two equations gives

$$HbO_2 + CO_2 + H_2O \leftrightharpoons HHb + HCO_3^- + O_2$$

revealing that to some extent this system can take up H^+ arising from CO_2, and can do so without a change in H^+ concentration (i.e., with no change in pH). Hemoglobin's ability to do this, through the operation of the Bohr effect, is referred to as the **isohydric carriage of CO_2**. As already pointed out, there is a small A–V difference in plasma pH. This is because the isohydric mechanism cannot handle all the acid generated during normal CO_2 transport; if it could, no such difference would occur. Figure 25.11 is a schematic representation of

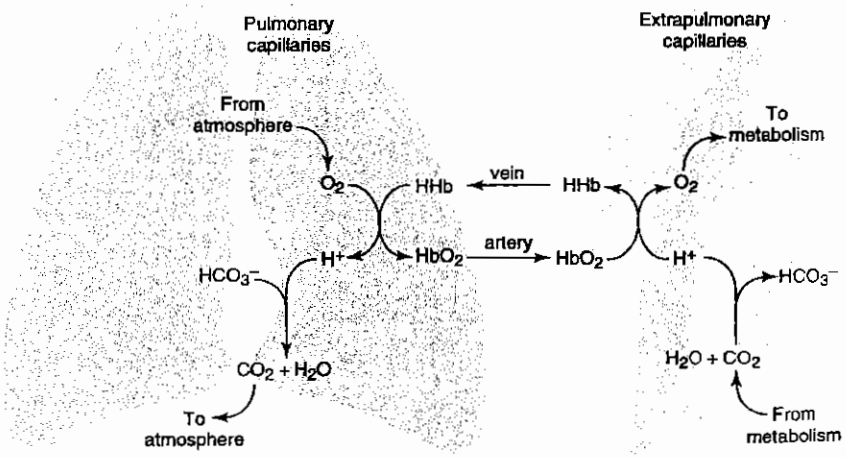


FIGURE 25.11
Schematic representation of oxygen transport and the isohydric carriage of CO_2 by hemoglobin.
In the lungs (left) O_2 from the atmosphere reacts with deoxyhemoglobin, forming oxyhemoglobin and H^+. The H^+ combines with the HCO_3^- to form H_2O and CO_2. The CO_2 is exhaled. Oxyhemoglobin is carried to extrapulmonary tissues (right), where it dissociates in response to low P_{O_2}. The O_2 is used by metabolic processes, and CO_2 is produced. CO_2 combines with H_2O to give HCO_3^- and H^+. H^+ can then react with deoxyhemoglobin to give HHb, which returns to the lungs, and the cycle repeats.

O_2 transport and the isohydric mechanism, showing what happens in the lungs and in the other tissues.

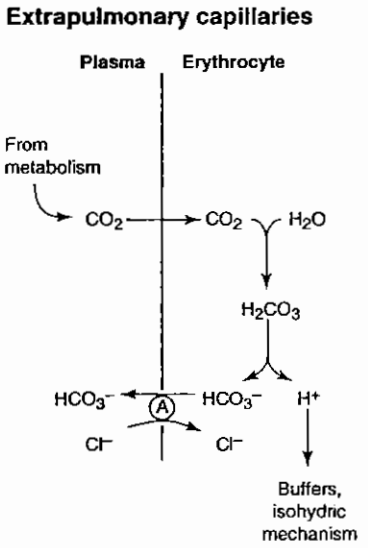
Estimates of the importance of the isohydric mechanism in handling normal respiratory acid production have changed upward and downward over the years. The older, erroneous estimates arose out of a lack of knowledge of the multiple interactions in which hemoglobin participates. The earliest experiments, titrations of purified oxyhemoglobin and purified deoxyhemoglobin, revealed that oxygenation of hemoglobin resulted in release of an average of 0.7 H^+ for every O_2 bound. This figure still appears in textbooks, and much is made of it. Authors point out that with a Bohr effect of this magnitude the isohydric mechanism alone could handle all of the acid produced by the metabolic oxidation of fat (RQ of fat is 0.7), and buffering would be unnecessary. Unfortunately, the experimental basis for this interpretation is physiologically unrealistic; the titrations were done in the total absence of carbon dioxide, which we now know binds to some of the Bohr groups, forming carbamino groups and diminishing the effect. When later experiments were carried out in the presence of physiological amounts of carbon dioxide, there was a drastic diminution of the Bohr effect, so much so that at pH 7.45 the isohydric mechanism was able to handle only the amount of acid arising from carbamino group formation. This work, however, was done prior to our appreciation of the competition between BPG and CO_2 for the same region of the hemoglobin molecule (see Table 25.4). Finally, in 1971, careful titrations of whole blood under presumably physiological conditions were carried out, yielding a value of 0.31 H^+ released per O_2 bound. This value is the basis of the present assertion that the isohydric mechanism accounts for about 40% of the H^+ generated during normal carbon dioxide transport. The quantitative contributions of various mechanisms to the handling of H^+ arising during carbon dioxide transport are summarized in Table 25.5. The major role of hemoglobin in handling this acid is obvious.

HCO_3^- Distribution Between Plasma and Erythrocytes

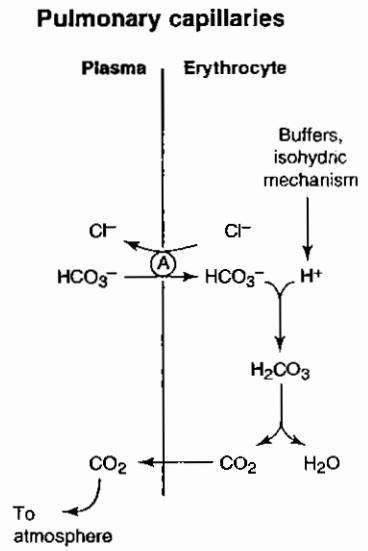
We have seen that essentially all of HCO_3^- formation is intracellular, catalyzed by carbonic anhydrase, and that the vast bulk of the H^+ generated by CO_2 is handled within the erythrocyte. These two observations bear upon the final distribution of HCO_3^- between plasma and the erythrocyte.

Intracellular formation of HCO_3^- increases its intracellular concentration. Since HCO_3^- and Cl^- exchange freely across the erythrocyte membrane, HCO_3^- will diffuse out of the erythrocyte, increasing the plasma HCO_3^- concentration. Electrical neutrality must be maintained across the membrane as this happens. Maintenance of neutrality can be accomplished in principle either by having a positively charged ion accompany HCO_3^- out of the cell or by having some other negatively charged ion enter the cell in exchange for the HCO_3^-. Since the distribution of the major cations, Na^+ and K^+, is under strict control, it is the latter mechanism that is seen, and the ion that is exchanged for HCO_3^- is Cl^-. Thus as HCO_3^- is formed in red cells during their passage through the capillary bed, it moves out into the plasma and Cl^- comes in to replace it. The increase in intracellular Cl^- is shown in the last line of Table 25.2. In the lungs, all events that occur in the peripheral capillary beds are reversed; HCO_3^- enters the erythrocytes to be converted to CO_2 for exhalation, and Cl^- returns to the plasma. The exchange of Cl^- and HCO_3^- between the plasma and the erythrocyte is called the **chloride shift** (Figure 25.12).

The intraerythrocytic buffering of H^+ from carbon dioxide causes these cells to swell, giving venous blood a slightly (0.6%) higher hematocrit than arterial blood. (Hematocrit is the volume percent of red cells in the blood.) This occurs because the charge on the hemoglobin molecule becomes more positive with every H^+ that binds to it. Each bound positive charge requires an accompanying negative charge to maintain neutrality. Thus as a result of buffering there is a net accumulation of HCO_3^- or Cl^- inside the erythrocyte.



(a) Bicarbonate efflux from and chloride influx into erythrocytes in extrapulmonary tissues.



(b) Chloride efflux from and bicarbonate influx into erythrocytes in pulmonary tissues.

FIGURE 25.12
Schematic representation of the chloride shift.
(a) In the capillaries of the extrapulmonary tissues, CO_2 produced by tissue metabolism is converted to HCO_3^- in the erythrocytes. This HCO_3^- exits the erythrocytes in exchange for Cl^-. *(b)* In the capillaries of the lungs, HCO_3^- enters the erythrocytes in exchange for Cl^-. Within the erythrocytes HCO_3^- is converted to CO_2. CO_2 subsequently diffuses out of the erythrocytes and is exhaled.

$$HHb\begin{matrix}BPG\\CO_2\end{matrix} + O_2 \rightleftharpoons HbO_2 + CO_2 + BPG + H^+$$

FIGURE 25.13
Interaction of H^+, BPG, CO_2, and O_2 with hemoglobin.
This is a schematic, intended to denote the direction of the equilibrium, not the stoichiometry of the reaction.

An increase in the osmotic pressure of the intracellular fluid results from this increase in concentration of particles. As a consequence, water enters the cells, causing them to swell slightly. Typically, an arterial hematocrit might be 44.8 and a venous hematocrit 45.1, as shown in Table 25.2 by the line labeled "volume (cc L^{-1} of blood)."

25.7 INTERRELATIONSHIPS AMONG HEMOGLOBIN, OXYGEN, CARBON DIOXIDE, HYDROGEN ION, AND 2,3-BISPHOSPHOGLYCERATE

By now it should be clear that multiple interrelationships of physiological significance exist among the ligands of hemoglobin. These interrelationships are summarized schematically in Figure 25.13. This equation shows that changes in the concentration of H^+, BPG, or CO_2 have similar effects on O_2 binding. The equation will help you remember the effect of changes in any one of these variables upon hemoglobin's O_2 affinity.

BPG levels in the erythrocytes are controlled by product inhibition of its synthesis and by pH. Hypoxia results in increased levels of deoxyhemoglobin on a time-averaged basis. Since deoxyhemoglobin binds BPG more tightly, in hypoxia there is less free BPG to inhibit its own synthesis, and so BPG levels will rise due to increased synthesis. The effect of pH is that high pH increases BPG synthesis and low pH decreases BPG synthesis; this reflects the influence of pH on the activity of **BPG mutase,** the enzyme that catalyzes BPG formation. Since changes in BPG levels take many hours to become complete, this means that the immediate effect of a decrease in blood pH is to enhance oxygen delivery by the Bohr effect. If the acidosis is sustained (most causes of chronic metabolic acidosis are not associated with a need for enhanced oxygen delivery), diminished BPG synthesis leads to a decrease in intracellular BPG concentration, and hemoglobin's oxygen affinity returns toward normal (Figure 25.14). This system can respond appropriately to acute conditions, such as vigorous exercise, but when faced with a prolonged abnormality of pH, it readjusts to restore normal (and presumably optimal) oxygen delivery.

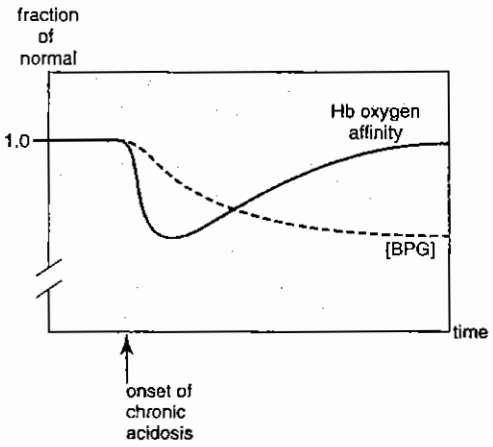


FIGURE 25.14
In chronic acidosis, BPG concentration decreases, returning hemoglobin's oxygen affinity toward normal.
This schematic diagram illustrates the rapid decrease in hemoglobin's oxygen affinity due to decreased pH. Lowering pH immediately lowers the activity of BPG mutase. In consequence, the concentration of BPG gradually diminishes as normal degradation proceeds. As BPG concentration diminishes, hemoglobin's oxygen affinity rises.

25.8 INTRODUCTION TO pH REGULATION

We have noted the large amount of H^+ generated by carbon dioxide transport, and we considered the ways in which the blood pH is controlled. This is important because changes in blood pH will affect intracellular pH, which in turn may profoundly alter metabolism. Protein conformation is affected by pH, as is enzyme activity. In addition, the equilibria of important reactions that consume or generate hydrogen ions, such as any of the oxidation–reduction reactions involving pyridine nucleotides, are shifted by changes in pH.

Normal arterial plasma pH is 7.40 ± 0.05; the pH range compatible with life is about 6.8–7.8. Intracellular pH varies with cell type; that of the erythrocyte is nearly 7.2, but that of most other cells is lower, about 7.0. Values as low as 6.0 have been reported for skeletal muscle.

It is fortunate for both diagnosis and treatment of diseases that the acid–base status of intracellular fluid influences and is influenced by the acid–base status of the blood. Blood is readily available for analysis, and when alteration of body pH becomes necessary, intravenous administration of acidifying or alkalinizing agents is efficacious.

25.9 BUFFER SYSTEMS OF PLASMA, INTERSTITIAL FLUID, AND CELLS

Each body water compartment is defined spatially by one or more differentially permeable membranes. Each contains characteristic kinds and concentrations

on the DNA target. Rather, the contacts from one monomer combine with those of the second monomer to form a continuous interaction through the single binding site in the DNA.

3.5 HEMOGLOBIN AND MYOGLOBIN

Hemoglobins are globular proteins, present in high concentrations in red blood cells, that bind oxygen in the lungs and transport the oxygen in blood to tissues and cells around the capillary beds of the vascular system. Hemoglobins also transport carbon dioxide and protons from the tissues to the lungs. Hemoglobins carry and release nitric oxide (NO), a potent vasodilator and inhibitor of platelet aggregation (see p. 995). In this section the structural and molecular aspects of hemoglobin and myoglobin are described. The physiological roles of these proteins are discussed in Chapter 25.

Human Hemoglobin Occurs in Several Forms

A hemoglobin molecule consists of four polypeptide chains, two each of two different amino acid sequences. The major form of human adult hemoglobin, **HbA_1**, consists of two α chains and two β chains ($\alpha_2\beta_2$). The α polypeptide has 141 and the β polypeptide has 146 amino acids. Other forms of hemoglobin predominate in the blood of the human fetus and early embryo (Figure 3.30). The fetal form **(HbF)** contains the same α chains found in HbA_1, but a second type of chain (γ chain) occurs in the tetramer molecule and differs in amino acid sequence from that of the β chain of adult HbA_1 (Table 3.8). Additional forms appear in the first months after conception (embryonic) in which the α chains are substituted by *zeta* (ζ) chains of different amino acid sequence and the ε chains serve as the β chains. A minor form of adult hemoglobin, HbA_2, comprises about 2% of normal adult hemoglobin and contains two α chains and two chains designated delta (δ) (Table 3.8).

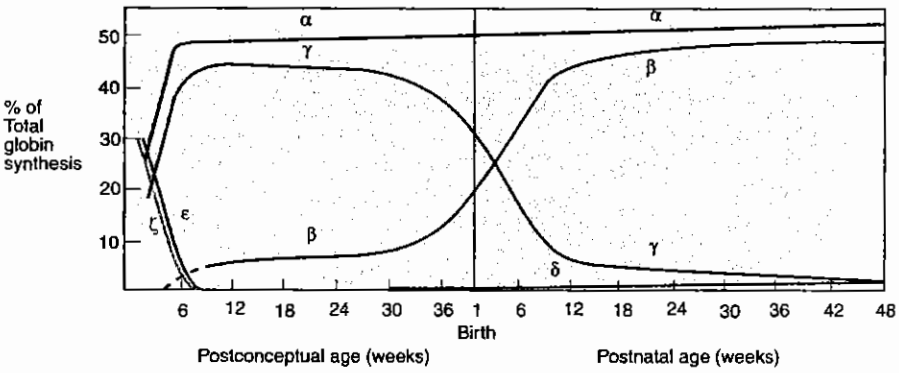


FIGURE 3.30
Changes in globin chain production during development.
Based on a figure in Nienhuis, A. W. and Maniatis, T. In: G. Stamatoyannopoulos, A. W. Nienhuis, P. Leder, and P. W. Majerus (Eds.), The Molecular Basis of Blood Diseases. *Philadelphia: Saunders, 1987, p. 68, in which reference of Weatherall, D. J., and Clegg, J. B.,* The Thalassemia Syndromes, *3rd ed. Oxford: Blackwell Scientific Publications, 1981, is acknowledged.*

TABLE 3.8 Chains of Human Hemoglobin

Developmental Stage	Symbol	Chain Designations
Adult	HbA_1	$\alpha_2\beta_2$
Adult	HbA_2	$\alpha_2\delta_2$
Fetus	HbF	$\alpha_2\gamma_2$
Embryo	Hb Gower-1	$\zeta_2\varepsilon_2$
Embryo	Hb Portland	$\zeta_2\gamma_2$

Myoglobin: A Single Polypeptide Chain with One O_2-Binding Site

Myoglobin (Mb) is an O_2-carrying protein that binds and releases O_2 with changes in the oxygen concentration in the sarcoplasm of skeletal muscle cells. In contrast to hemoglobin, which has four polypeptide chains and four O_2-binding sites, myoglobin contains only a single polypeptide chain and one O_2-binding site. Myoglobin is a model for what occurs when a single protomer molecule acts alone without the interactions exhibited among the four O_2-binding sites in the more complex tetramer molecule of hemoglobin.

A Heme Prosthetic Group Is at the Site of O_2 Binding

The four polypeptides of globin subunits in hemoglobin and the one of myoglobin each contain a heme prosthetic group. A **prosthetic group** is a nonpolypeptide moiety that forms a functional part of a protein. Without its prosthetic group, a protein is designated an **apoprotein.** With its prosthetic group it is a **holoprotein.**

Heme contains protoporphyrin IX (see Chapter 24) with an iron atom in its center (Figure 3.31). The iron atom is in the ferrous (2+ charge) oxidation state in functional hemoglobin and myoglobin. The ferrous atom in the heme can form five or six ligand bonds, depending on whether or not O_2 is bound to the molecule. Four bonds are to the pyrrole nitrogen atoms of the porphyrin. Since all pyrrole rings of porphyrin lie in a common plane, the four ligand bonds from the porphyrin to the iron atom will have a tendency to lie in the plane of the porphyrin ring. The fifth and the potentially sixth ligand bonds to the ferrous atom are directed along an axis perpendicular to the plane of the porphyrin ring (Figure 3.32). The fifth coordinate bond of the ferrous atom is to a nitrogen of a histidine imidazole. This is designated the **proximal histidine** in hemoglobin and myoglobin structures (Figures 3.32 and 3.33). O_2 forms a sixth coordinate bond to the ferrous atom when bound to hemoglobin. In the bonded position the O_2 is placed between the ferrous atom to which it is liganded and a second histidine imidazole, designated the **distal histidine.** In deoxyhemoglobin, the sixth coordination position of the ferrous atom is unoccupied.

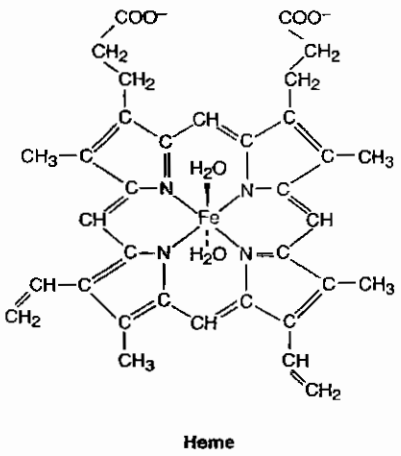


FIGURE 3.31
Structure of heme.

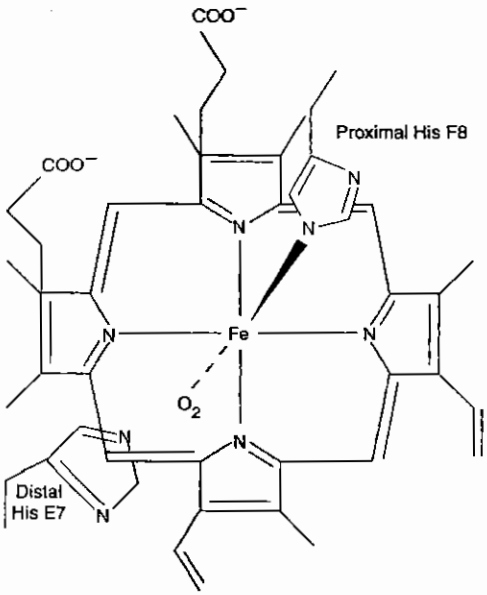


FIGURE 3.32
Ligand bonds to ferrous atom in oxyhemoglobin.

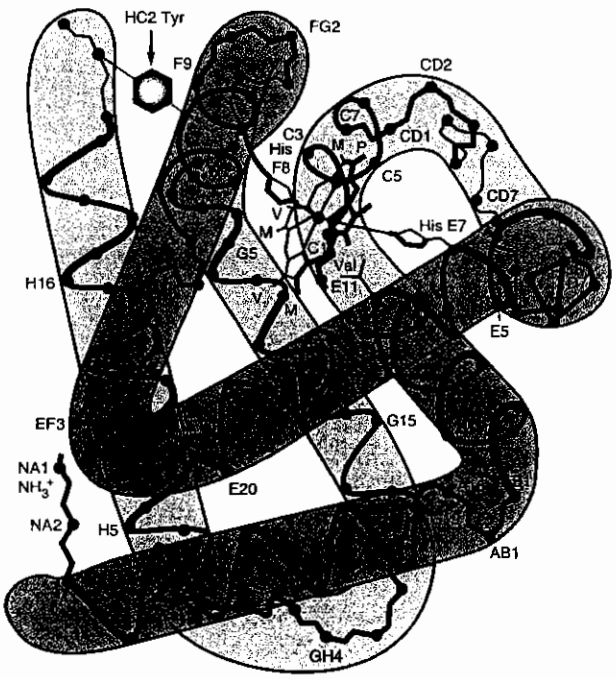


FIGURE 3.33
Secondary and tertiary structure characteristics of chains of hemoglobin.
Proximal His F8, distal His E7, and Val E11 side chains are shown. Other amino acids of polypeptide chain are represented by α-carbon positions only; the letters M, V, and P refer to the methyl, vinyl, and propionate side chains of the heme.
Reprinted with permission from Perutz, M. Br. Med. Bull. *32:195, 1976.*

The porphyrin part of the heme is positioned within a hydrophobic pocket of each globin subunit. In the heme pocket X-ray diffraction studies show that approximately 80 interactions are provided by approximately 18 residues to the heme. Most of these noncovalent interactions are between apolar side chains of amino acids and the apolar regions of the porphyrin. As discussed in Chapter 2, the driving force for these interactions is the expulsion of water of solvation on association of the hydrophobic heme with the apolar amino acid side chains in the heme pocket. In myoglobin additional noncovalent interactions are made between the negatively charged propionate groups of the heme and positively charged arginine and histidine side chains of the protein. However, in hemoglobin chains a difference in the amino acid sequence in this region of the heme-binding site leads to stabilization of the porphyrin propionates by interaction with an uncharged histidine imidazole and with water molecules of solvent toward the outer surface of the molecule.

X-Ray Crystallography Has Assisted in Defining the Structure of Hemoglobin and Myoglobin

The structure of deoxy and oxy forms of hemoglobin and myoglobin have been resolved by X-ray crystallography. In fact, sperm whale myoglobin was the first globular protein whose full three-dimensional structure was determined by this technique. This was followed by the X-ray structure of the more complex horse hemoglobin molecule. These structures show that each globin polypeptide in the hemoglobins and the single subunit of myoglobin are composed of multiple α-helical regions connected by turns of the polypeptide chain that allow the protein to fold into a spheroidal shape (Figure 3.33). The mechanism of cooperative associations of O_2, discussed below, is based on the X-ray structures of oxyhemoglobin, deoxyhemoglobin, and a variety of hemoglobin derivatives.

Primary, Secondary, and Tertiary Structures of Myoglobin and the Individual Hemoglobin Chains

The amino acid sequences of the polypeptide chain of myoglobin of 23 different animal species have been determined. All myoglobins contain 153 amino acids in their polypeptide chains, of which 83 are invariant. Only 15 of these invariant residues in the myoglobin sequence are identical to the invariant residues of the sequenced mammalian globins of hemoglobin. However, the changes are, in the great majority of cases, conservative and preserve the general physical properties of the residues (Table 3.9). Since myoglobin is active as a monomer, many of its surface positions interact with water and prevent another molecule of myoglobin from associating. In contrast, surface residues of the individual subunits in hemoglobin are designed to provide hydrogen bonds and nonpolar contacts with other subunits in the hemoglobin quaternary structure. Proximal and distal histidines are preserved in the sequences of all the polypeptide chains. Other invariant residues are in the hydrophobic heme pocket and form essential nonpolar contacts with the heme that stabilize the heme–protein complex.

While there is surprising variability in amino acid sequences among the different polypeptide chains, to a first approximation the secondary and tertiary structures of each of the subunits of hemoglobin and myoglobin are almost identical (Figure 3.34). Significant differences in physiological properties between α, β, γ, and δ chains of hemoglobins and the polypeptide chain of myoglobin are due to rather small specific changes in their structures. The similarity in tertiary structure, resulting from widely varied amino acid sequences, shows that the same tertiary structure for a protein can be arrived at by many different sequences.

TABLE 3.9 Amino Acid Sequences of Human Hemoglobin Chains and of Sperm Whale Myoglobin[a]

		NA 1	2	3	A 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	A 16	AB 1	B 1	2	3	4	5	6
MYOGLOBIN		Val	....	Leu	Ser	Glu	Gly	Glu	Trp	Gln	Leu	Val	Leu	His	Val	Trp	Ala	Lys	Val	Glu	Ala	Asp	Val	Ala	Gly	His	Gly
HEMOGLOBIN	Horse α	Val	....	Leu	Ser	Ala	Ala	Asp	Lys	Thr	Asn	Val	Lys	Ala	Ala	Trp	Ser	Lys	Val	Gly	Gly	His	Ala	Gly	Glu	Tyr	Gly
	β	Val	Gln	Leu	Ser	Gly	Glu	Glu	Lys	Ala	Ala	Val	Leu	Ala	Leu	Trp	Asp	Lys	Val	Asn	....	....	Glu	Glu	Glu	Val	Gly
	Human α	Val	....	Leu	Ser	Pro	Ala	Asp	Lys	Thr	Asn	Val	Lys	Ala	Ala	Trp	Gly	Lys	Val	Gly	Ala	His	Ala	Gly	Glu	Tyr	Gly
	β	Val	His	Leu	Thr	Pro	Glu	Glu	Lys	Ser	Ala	Val	Thr	Ala	Leu	Trp	Gly	Lys	Val	Asn	....	....	Val	Asp	Glu	Val	Gly
	γ	Gly	His	Phe	Thr	Glu	Glu	Asp	Lys	Ala	Thr	Ilu	Thr	Ser	Leu	Trp	Gly	Lys	Val	Asn	....	....	Val	Glu	Asp	Ala	Gly
	δ	Val	His	Leu	Thr	Pro	Glu	Glu	Lys	Thr	Ala	Val	Asn	Ala	Leu	Trp	Gly	Lys	Val	Asn	....	....	Val	Asp	Ala	Val	Gly

		7	8	9	10	11	12	13	14	15	16	C 1	2	3	4	5	6	7	CD 1	2	3	4	5	6	7	8	D 1
MYOGLOBIN		Gln	Asp	Ilu	Leu	Ilu	Arg	Leu	Phe	Lys	Ser	His	Pro	Glu	Thr	Leu	Glu	Lys	Phe	Asp	Arg	Phe	Lys	His	Leu	Lys	Thr
HEMOGLOBIN	Horse α	Ala	Glu	Ala	Leu	Glu	Arg	Met	Phe	Leu	Gly	Phe	Pro	Thr	Thr	Lys	Thr	Tyr	Phe	Pro	His	Phe	....	Asp	Leu	Ser	His
	β	Gly	Glu	Ala	Leu	Gly	Arg	Leu	Leu	Val	Val	Tyr	Pro	Trp	Thr	Gln	Arg	Phe	Phe	Asp	Ser	Phe	Gly	Asp	Leu	Ser	Gly
	Human α	Ala	Glu	Ala	Leu	Glu	Arg	Met	Phe	Leu	Ser	Phe	Pro	Thr	Thr	Lys	Thr	Tyr	Phe	Pro	His	Phe	....	Asp	Leu	Ser	His
	β	Gly	Glu	Ala	Leu	Gly	Arg	Leu	Leu	Val	Val	Tyr	Pro	Trp	Thr	Gln	Arg	Phe	Phe	Glu	Ser	Phe	Gly	Asp	Leu	Ser	Thr
	γ	Gly	Glu	Thr	Leu	Gly	Arg	Leu	Leu	Val	Val	Tyr	Pro	Trp	Thr	Gln	Arg	Phe	Phe	Asp	Ser	Phe	Gly	Asn	Leu	Ser	Ser
	δ	Gly	Glu	Ala	Leu	Gly	Arg	Leu	Leu	Val	Val	Tyr	Pro	Trp	Thr	Gln	Arg	Phe	Phe	Glu	Ser	Phe	Gly	Asp	Leu	Ser	Ser

		2	3	4	5	6	7	E 1	2	3	4	5	6	7	8	9	10	11	12	13	14	E 15	16	17	18	19	20
MYOGLOBIN		Glu	Ala	Glu	Met	Lys	Ala	Ser	Glu	Asp	Leu	Lys	Lys	His	Gly	Val	Thr	Val	Leu	Thr	Ala	Leu	Gly	Ala	Ilu	Leu	Lys
HEMOGLOBIN	Horse α	....	....	....	....	....	Gly	Ser	Ala	Gln	Val	Lys	Ala	His	Gly	Lys	Lys	Val	Ala	Asp	Gly	Leu	Thr	Leu	Ala	Val	Gly
	β	Pro	Asp	Ala	Val	Met	Gly	Asn	Pro	Lys	Val	Lys	Ala	His	Gly	Lys	Lys	Val	Leu	His	Ser	Phe	Gly	Glu	Gly	Val	His
	Human α	....	....	....	....	....	Gly	Ser	Ala	Gln	Val	Lys	Gly	His	Gly	Lys	Lys	Val	Ala	Asp	Ala	Leu	Thr	Asn	Ala	Val	Ala
	β	Pro	Asp	Ala	Val	Met	Gly	Asn	Pro	Lys	Val	Lys	Ala	His	Gly	Lys	Lys	Val	Leu	Gly	Ala	Phe	Ser	Asp	Gly	Leu	Ala
	γ	Ala	Der	Ala	Ilu	Met	Gly	Asn	Pro	Lys	Val	Lys	Ala	His	Gly	Lys	Lys	Val	Leu	Thr	Ser	Leu	Gly	Asp	Ala	Ilu	Lys
	δ	Pro	Asp	Ala	Val	Met	Gly	Asn	Pro	Lys	Val	Lys	Ala	His	Gly	Lys	Lys	Val	Leu	Gly	Ala	Phe	Ser	Asp	Gly	Leu	Ala

		EF 1	2	3	4	5	6	7	8	F 1	2	3	4	F 5	6	7	8	9	FG 1	2	3	4	5	G 1	2	3	4
MYOGLOBIN		Lys	Lys	Gly	His	His	Glu	Ala	Glu	Leu	Lys	Pro	Leu	Ala	Gln	Ser	His	Ala	Thr	Lys	His	Lys	Ilu	Pro	Ilu	Lys	Tyr
HEMOGLOBIN	Horse α	His	Leu	Asp	Asp	Leu	Pro	Gly	Ala	Leu	Ser	Asp	Leu	Ser	Asn	Leu	His	Ala	His	Lys	Leu	Arg	Val	Asp	Pro	Val	Asn
	β	His	Leu	Asp	Asn	Leu	Lys	Gly	Thr	Phe	Ala	Ala	Leu	Ser	Glu	Leu	His	Cys	Asp	Lys	Leu	His	Val	Asp	Pro	Glu	Asn
	Human α	His	Val	Asp	Asp	Met	Pro	Asn	Ala	Leu	Ser	Ala	Leu	Ser	Asp	Leu	His	Ala	His	Lys	Leu	Arg	Val	Asp	Pro	Val	Asn
	β	His	Leu	Asp	Asn	Leu	Lys	Gly	Thr	Phe	Ala	Thr	Leu	Ser	Glu	Leu	His	Cys	Asp	Lys	Leu	His	Val	Asp	Pro	Glu	Asn
	γ	His	Leu	Asp	Asp	Leu	Lys	Gly	Thr	Phe	Ala	Gln	Leu	Ser	Glu	Leu	His	Cys	Asp	Lys	Leu	His	Val	Asp	Pro	Glu	Asn
	δ	His	Leu	Asp	Asn	Leu	Lys	Gly	Thr	Phe	Ser	Gln	Leu	Ser	Glu	Leu	His	Cys	Asp	Lys	Leu	His	Val	Asp	pro	Glu	Asn

		5	6	7	8	G 9	10	11	12	13	14	15	16	17	18	19	GH 1	2	3	4	5	6	H 1	2	H 3	4	5
MYOGLOBIN		Leu	Glu	Phe	Ilu	Ser	Glu	Ala	Ilu	Ilu	His	Val	Leu	His	Ser	Arg	His	Pro	Gly	Asn	Phe	Gly	Ala	Asp	Ala	Gln	Gly
HEMOGLOBIN	Horse α	Phe	Lys	Leu	Leu	Ser	His	Cys	Leu	Leu	Ser	Thr	Leu	Ala	Val	His	Leu	Pro	Asn	Asp	Phe	Thr	Pro	Ala	Val	His	Ala
	β	Phe	Arg	Leu	Leu	Gly	Asn	Val	Leu	Ala	Leu	Val	Val	Ala	Arg	His	Phe	Gly	Lys	Asp	Phe	Thr	Pro	Glu	Leu	Gln	Ala
	Human α	Phe	Lys	Leu	Leu	Ser	His	Cys	Leu	Leu	Val	Thr	Leu	Ala	Ala	His	Leu	Pro	Ala	Glu	phe	Thr	Pro	Ala	Val	His	Ala
	β	Phe	Srg	Leu	Leu	Gly	Asn	Val	Leu	Val	Cys	Val	Leu	Ala	His	His	Phe	Gly	Lys	Glu	Phe	Thr	Pro	Pro	Val	Gln	Ala
	γ	Phe	Lys	Leu	Leu	Gly	Asn	Val	Leu	Val	Thr	Val	Leu	Ala	Ilu	His	Phe	Gly	Lys	Glu	Phe	Thr	Pro	Glu	Val	Gln	Ala
	δ	Phe	Arg	Leu	Leu	Gly	Asn	Val	Leu	Val	Cys	Val	Leu	Ala	Arg	Asn	Phe	Gly	Lys	Glu	Phe	Thr	Pro	Gln	Met	Gln	Ala

		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	H 21	22	23	2	HC 1	2	3	4	5
MYOGLOBIN		Ala	Met	Asn	Lys	Ala	Leu	Glu	Leu	Phe	Arg	Lys	Asp	Ilu	Ala	Ala	Lys	Tyr	Lys	Glu	Leu	Gly	Tyr	Gln	Gly
HEMOGLOBIN	Horse α	Ser	Leu	Asp	Lys	Phe	Leu	Ser	Ser	Val	Ser	Thr	Val	Leu	Thr	Ser	Lys	Tyr	Arg						
	β	Ser	Tyr	Gln	Lys	Val	Val	Ala	Gly	Val	Ala	Asn	Ala	Leu	Ala	His	Lys	Tyr	His						
	Human α	Ser	Leu	Asp	Lys	Phe	Leu	Ala	Ser	Val	Ser	Thr	Val	Leu	Thr	Ser	Lys	Tyr	Arg						
	β	Ala	Tyr	Gln	Lys	Val	Val	Ala	Gly	Val	Ala	Asn	Ala	Leu	Ala	His	Lys	Tyr	His						
	γ	Ser	Trp	Gln	Lys	Met	Val	Thr	Gly	Val	Ala	Ser	Ala	Leu	Ser	Ser	Arg	Tyr	His						
	δ	Ala	Tyr	Gln	Lys	Val	Val	Ala	Gly	Val	Ala	Asn	Ala	Leu	Ala	His	Lys	Tyr	His						

Source: Based on diagram in Dickerson, R. E., and Geis, I. *The Structure and Function of Proteins.* New York: Harper & Row, 1969, p. 52.

[a] Residues that are identical are enclosed in box. A, B, C, . . . designate different helices of tertiary structure (see text).

Approximately 70% of the residues participate in the α-helical secondary structures generating seven helical segments in the α chain and eight in the β chain. These latter eight helical regions are commonly lettered A–H, starting from the first (A) helix at the NH_2-terminal end. The interhelical regions are designated as AB, BC, CD, . . . , GH, respectively. The nonhelical region between the NH_2-terminal end and the A helix is designated the NA region; and the region between the COOH-terminal end and the H helix is designated the HC region (Figure 3.33). This naming system allows discussion of particular residues that have similar functional and structural roles in hemoglobin and myoglobin.

A Simple Equilibrium Defines O_2 Binding to Myoglobin

The association of oxygen to myoglobin is characterized by a simple equilibrium constant (Eqs. 3.1 and 3.2). In Eq. 3.2 $[MbO_2]$ is the solution concentration of oxymyoglobin, [Mb] is that of deoxymyoglobin, and $[O_2]$ is the concentration

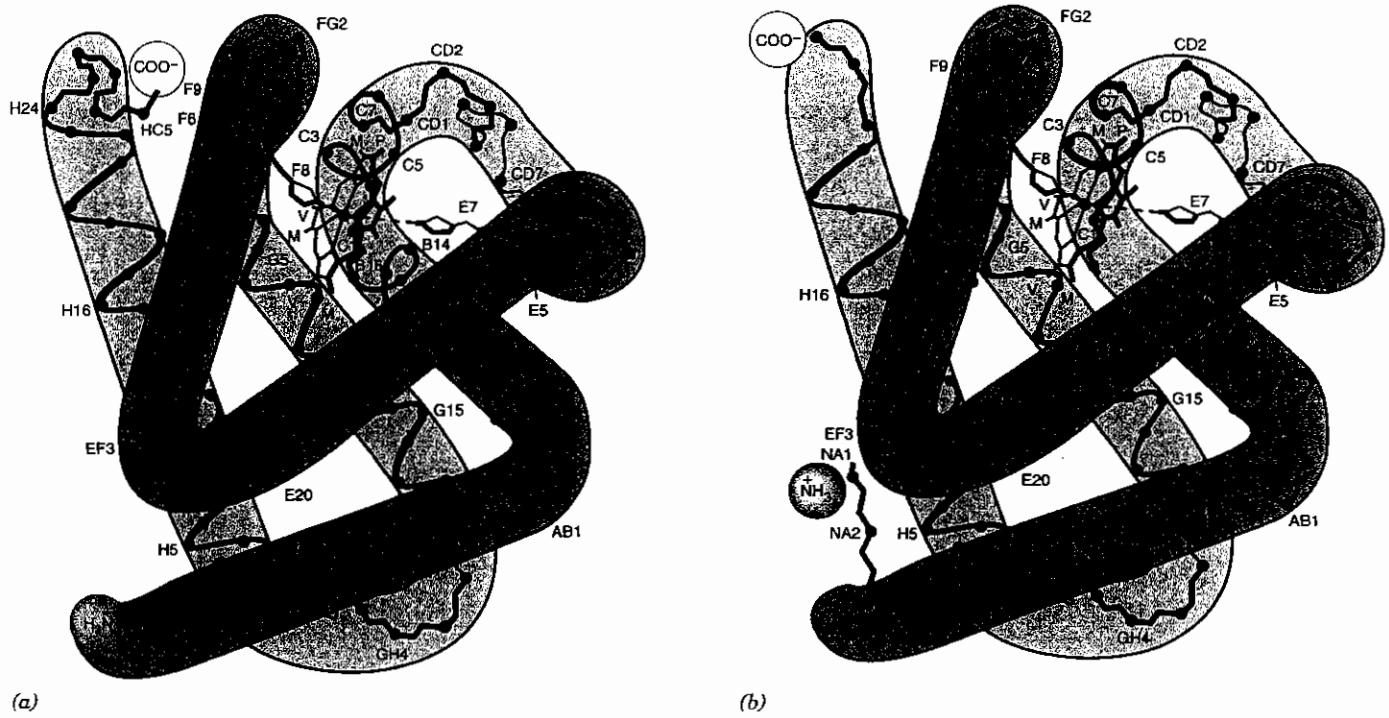


FIGURE 3.34
Comparison of conformation of (*a*) myoglobin and (*b*) β chain of HbA_1.
Overall structures are very similar, except at NH_2-terminal and COOH-terminal ends. *Reprinted with permission from Fersht, A.* Enzyme Structure and Mechanism. *San Francisco: Freeman, 1977, pp. 12, 13.*

of oxygen, in moles per liter. The equilibrium constant, K_{eq}, will also have the units of moles per liter. As for any true equilibrium constant, the value of K_{eq} is dependent on pH, ionic strength, and temperature.

$$Mb + O_2 \overset{K_{eq}}{\rightleftharpoons} MbO_2 \tag{3.1}$$

$$K_{eq} = \frac{[Mb][O_2]}{[MbO_2]} \tag{3.2}$$

Since oxygen is a gas, it is more convenient to express its concentration as the pressure of oxygen in torr (1 torr is equal to the pressure of 1 mmHg at 0°C and standard gravity). In Eq. 3.3 this conversion of units has been made: P_{50}, the equilibrium constant, and pO_2, the concentration of oxygen, being expressed in torr.

$$P_{50} = \frac{[Mb] \cdot pO_2}{[MbO_2]} \tag{3.3}$$

An oxygen-saturation curve characterizes the properties of an oxygen-binding protein. In this plot the fraction of oxygen-binding sites in solution that contain oxygen (Y, Eq. 3.4) is plotted on the ordinate *versus* pO_2 (oxygen concentration) on the abscissa. The Y value is simply defined for myoglobin by Eq. 3.5. Substitution into Eq. 3.5 of the value of $[MbO_2]$ obtained from Eq. 3.3, and then dividing through by [Mb], results in Eq. 3.6, which shows the dependence of Y on the value of the equilibrium constant P_{50} and the oxygen concentration. It is seen from Eqs. 3.3 and 3.6 that the value of P_{50} is equal to the oxygen concentration, pO_2, when $Y = 0.5$ (50% of the available sites occupied)—hence the designation of the equilibrium constant by the subscript 50.

$$Y = \frac{\text{number of binding sites occupied}}{\text{total number of binding sites in solution}} \quad (3.4)$$

$$Y = \frac{[MbO_2]}{[Mb] + [MbO_2]} \quad (3.5)$$

$$Y = \frac{pO_2}{P_{50} + pO_2} \quad (3.6)$$

A plot of Eq. 3.6 of Y versus pO_2 generates an oxygen-saturation curve for myoglobin in the form of a rectangular hyperbola (Figure 3.35).

A simple algebraic manipulation of Eq. 3.6 leads to Eq. 3.7. Taking the logarithm of both sides of Eq. 3.7 results in Eq. 3.8, the **Hill equation.** A plot of log $([Y/(1 - Y)]$ versus log pO_2, according to Eq. 3.8, yields a straight line with a slope equal to 1 for myoglobin (Figure 3.36). This is the Hill plot, and the slope (n_H) is the **Hill coefficient** (see Eq. 3.9).

$$\frac{Y}{1 - Y} = \frac{pO_2}{P_{50}} \quad (3.7)$$

$$\log \frac{Y}{1 - Y} = \log pO_2 - \log P_{50} \quad (3.8)$$

Binding of O_2 to Hemoglobin Involves Cooperativity Between the Hemoglobin Subunits

Whereas myoglobin has a single O_2-binding site per molecule, hemoglobins, with four monomeric subunits, have four heme-binding sites for O_2. Binding of the four O_2 molecules in hemoglobin is found to be **positively cooperative,** so that the binding of the first O_2 to deoxyhemoglobin facilitates the binding of O_2 to the other subunits in the molecule. Conversely, dissociation of the first O_2 from fully oxygenated hemoglobin, $Hb(O_2)_4$, will make the dissociation of O_2 from the other subunits of the tetramer easier.

Because of cooperativity in oxygen association and dissociation, the oxygen saturation curve for hemoglobin differs from that for myoglobin. A plot of Y versus pO_2 for hemoglobin is a sigmoidal line, indicating cooperativity in oxygen association (Figure 3.35). A plot of the Hill equation (Eq. 3.9) gives a value of the slope (n_H) equal to 2.8 (Figure 3.36).

$$\log \frac{Y}{1 - Y} = n_H \log pO_2 - \text{constant} \quad (3.9)$$

The meaning of the Hill coefficient to cooperative O_2 association can be evaluated quantitatively as presented in Table 3.10. A parameter known as the **cooperativity index,** R_x, is calculated, which shows the ratio of pO_2 required to change Y from a value of $Y = 0.1$ (10% of sites filled) to a value of $Y = 0.9$ (90% of sites filled) for designated Hill coefficient values found experimentally. For myoglobin, $n_H = 1$, and an 81-fold change in oxygen concentration is required to change from $Y = 0.1$ to $Y = 0.9$. For hemoglobin, where positive cooperativity is observed, $n_H = 2.8$ and only a 4.8-fold change in oxygen concentration is required to change the fractional saturation from 0.1 to 0.9.

The Molecular Mechanism of Cooperativity in O_2 Binding

X-ray diffraction data on deoxyhemoglobin show that the ferrous atoms actually sit out of the plane of their porphyrins by about 0.4–0.6 Å. This is thought to occur because of two factors. The electronic configuration of the five-coordinated ferrous atom in deoxyhemoglobin has a slightly larger radius than the distance from the center of the porphyrin to each of the pyrrole nitrogen atoms.

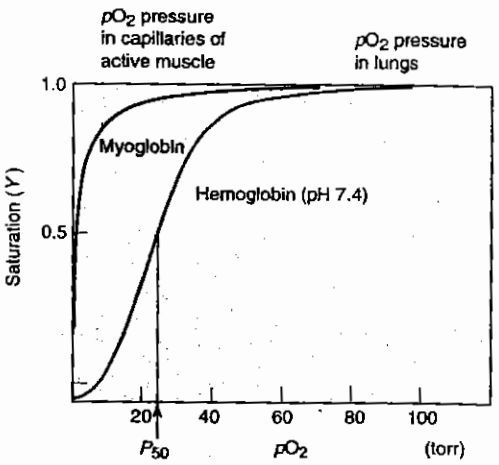


FIGURE 3.35
Oxygen-binding curves for myoglobin and hemoglobin.

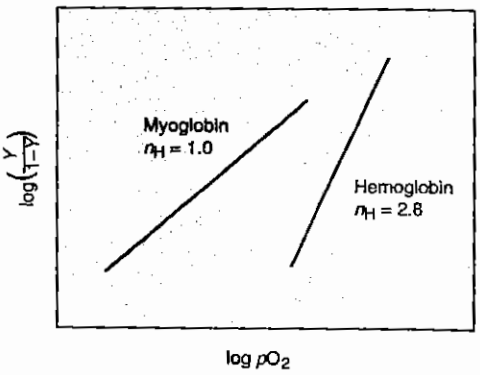


FIGURE 3.36
Hill plots for myoglobin and hemoglobin HbA_1.

TABLE 3.10 Relationship Between Hill Coefficient (n_H) and Cooperativity Index (R_x)

n_H	R_x	Observation
0.5	6560	Negative substrate cooperativity
0.6	1520	
0.7	533	
0.8	243	
0.9	132	
1.0	81.0	Noncooperativity
1.5	18.7	
2.0	9.0	
2.8	4.8	Positive substrate cooperativity
3.5	3.5	
6.0	2.1	
10.0	1.6	
20.0	1.3	

Source: Based on Table 7.1 in Cornish-Bowden, A. *Principles of Enzyme Kinetics.* London: Butterworths Scientific Publishers, 1976.

Accordingly, the iron can be placed in the center of the porphyrin only with some distortion of the porphyrin conformation. Probably a more important consideration is that if the iron atom sits in the plane of the porphyrin, the proximal His F8 imidazole will interact unfavorably with atoms of the porphyrin. The strength of this unfavorable steric interaction is due, in part, to conformational constraints on the His F8 and the porphyrin in the deoxyhemoglobin conformation that forces the approach of the His F8 toward the porphyrin to a particular path (Figure 3.37). These constraints become less significant in the oxy conformation of hemoglobin.

The conformation with the iron atom out of the plane of the porphyrin is unstrained and energetically favored for the five-coordinate ferrous atom. When O_2 binds the sixth coordinate position of the iron, however, this conformation becomes strained. A more energetically favorable conformation for the O_2 liganded iron is one in which the iron atom is within the plane of the porphyrin structure.

On binding of O_2 to a ferrous atom the favorable free energy of bond formation overcomes the repulsive interaction between His F8 and porphyrin, and the ferrous atom moves into the plane of the porphyrin ring. This is the most thermodynamically stable position for the now six-bonded iron atom; one axial ligand is on either side of the plane of the porphyrin ring, and the steric repulsion of one of the axial ligands with the porphyrin is balanced by the repulsion of the second axial ligand on the opposite side when the ferrous atom is in the center. If the iron atom is displaced from the center, the steric interactions of the two axial ligands with the porphyrin in the deoxy conformation are unbalanced, and the stability of the unbalanced structure will be lower than that of the equidistant conformation. Also, the radius of the iron atom with six ligands is reduced so that it can just fit into the center of the porphyrin without distortion of the porphyrin conformation.

Since steric repulsion between porphyrin and His F8 in the deoxy conformation must be overcome on O_2 association, binding of the first O_2 is characterized by a relatively low affinity constant. However, when O_2 association occurs to the first heme in deoxyhemoglobin, the change in position of the iron atom from above the plane of the porphyrin into the center of the porphyrin triggers

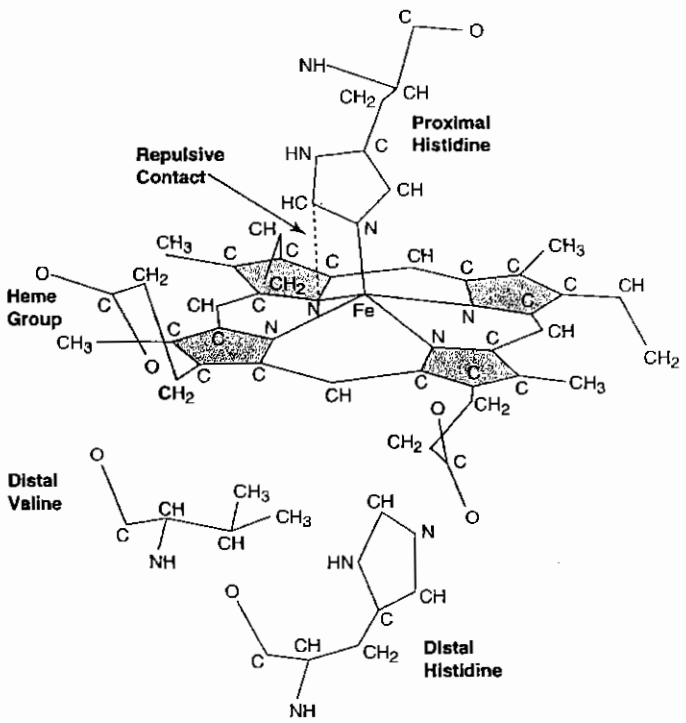


FIGURE 3.37
Steric hindrance between proximal histidine and porphyrin in deoxyhemoglobin.
From Perutz, M. Sci. Am., 239:92, 1978 Copyright © 1978 by Scientific American, Inc. All rights reserved.

a conformational change in the whole molecule. The change in conformation results in a greater affinity of O_2 to the other heme sites after the first O_2 has bound.

The conformation of deoxyhemoglobin is stabilized by noncovalent interactions of the quaternary structure at the interface between α and β subunits in which the FG corner of one subunit noncovalently binds to the C helix of the adjacent subunit (Figure 3.38). In addition, ionic interactions stabilize the deoxy

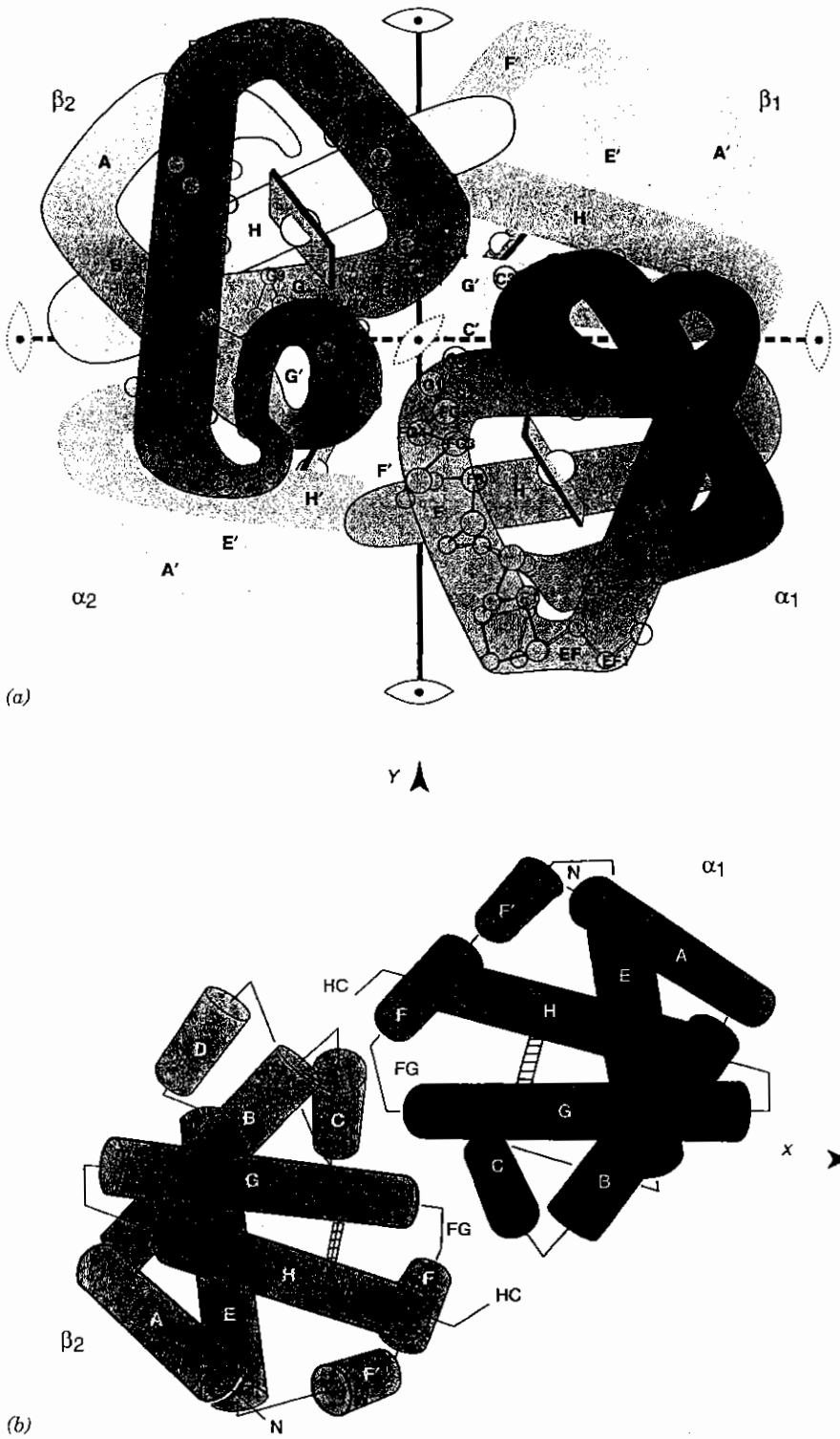


FIGURE 3.38
Quaternary structure of hemoglobin.
(*a*) $\alpha_1\beta_2$ interface contacts between FG corners and C helix are shown. (*b*) Cylinder representation of α_1 and β_2 subunits in hemoglobin molecule showing α_1 and β_2 interface contacts between FG corner and C helix, viewed from opposite side of x–y plane from (*a*).
(a) Reprinted with permission from Dickerson, R. E., and Geis, I. The Structure and Action of Proteins. *Menlo Park, CA: Benjamin, Inc., 1969, p. 56.*
(b) Reprinted with permission from Baldwin, J., and Chothia, C. J. Mol. Biol. *129:175, 1979. Copyright © 1979 by Academic Press, Inc. (London) Ltd.*

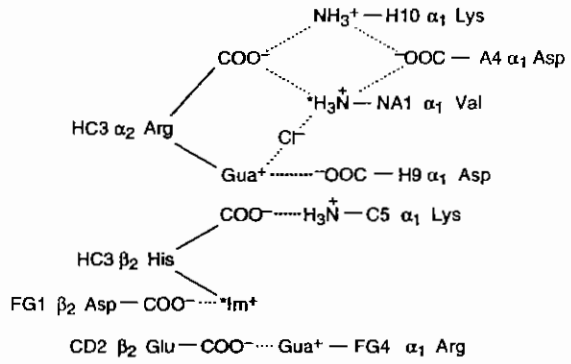


FIGURE 3.39
Salt bridges between subunits in deoxyhemoglobin.
Im^+ is imidazolium; Gua^+ is guanidinium; starred residues account for approximately 60% of alkaline Bohr effect.
Redrawn from Perutz, M. Br. Med. Bull. *32:195, 1976.*

conformation of the protein (Figure 3.39). These interactions of the deoxy conformation are now destabilized on the binding of O_2 to one of the heme subunits of a deoxyhemoglobin molecule. The binding of O_2 pulls the Fe^{2+} atom into the porphyrin plane and moves the His F8 toward the porphyrin and with it the F helix of which the His F8 is a part. Movement of the F helix, in turn, moves the FG corner of its subunit, destabilizing the FG noncovalent interaction with the C helix of the adjacent subunit at an $\alpha_1\beta_2$ or $\alpha_2\beta_1$ subunit interface (Figures 3.38 and 3.40).

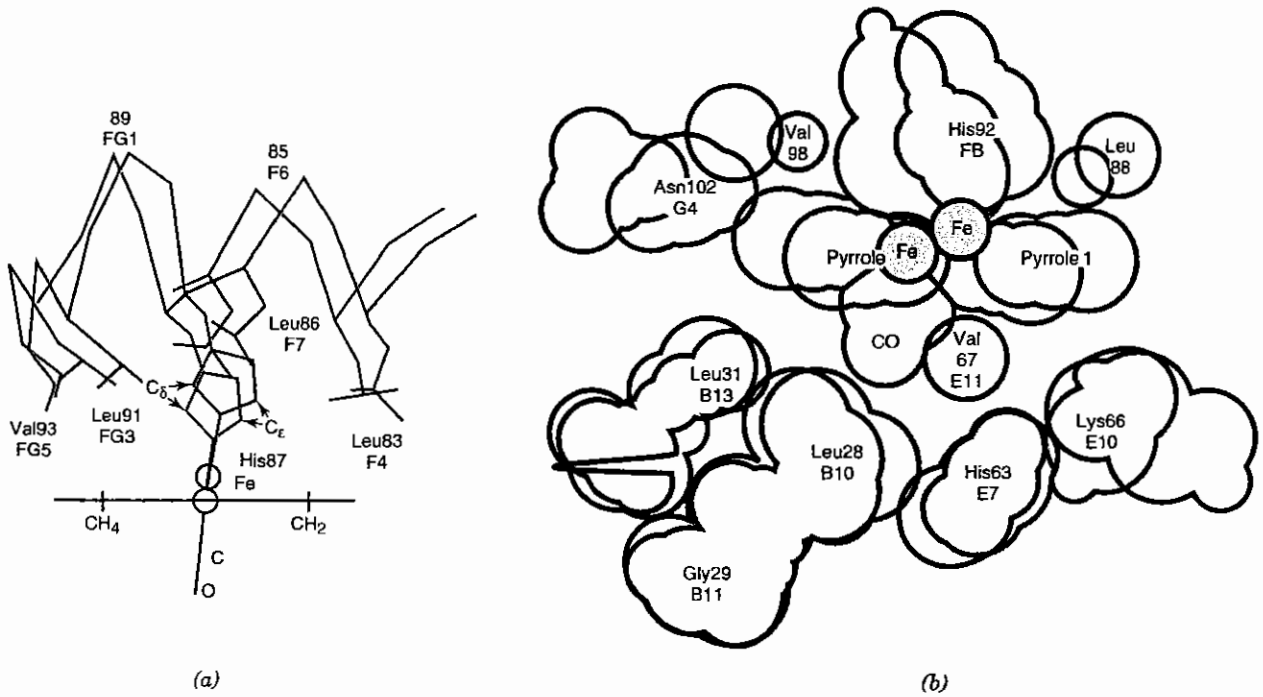


FIGURE 3.40
Stick and space-filling diagrams drawn by computer graphics showing movements of residues in heme environment on transition from deoxyhemoglobin to oxyhemoglobin.
(*a*) Black line outlines position of polypeptide chain and His F8 in carbon monoxide hemoglobin, a model for oxyhemoglobin. Red line outlines the same for deoxyhemoglobin. Position of iron atom shown by circle. Movements are for an α subunit. (*b*) Similar movements in a β subunit using space-filling diagram shown. Residue labels centered in density for the deoxyconformation.
Redrawn with permission from Baldwin, J., and Chothia, C. J. Mol. Biol. *129:175, 1979. Copyright © 1979 by Academic Press, Inc. (London) Ltd.*

The FG to C intersubunit contacts act as a "switch," because they exist in two different arrangements with different modes of contact between the FG corner of one subunit and the C helix of the adjacent subunit. The switch in noncovalent interactions between the two positions involves a relative movement of FG and C in adjacent subunits of about 6 Å. In the second position of the "switch," the tertiary conformation of the subunits participating in the FG to C intersubunit contact is less constrained and the adjacent subunit changes to a new tertiary conformation (oxy conformation) even without O_2 bound. This oxy conformation allows the His F8 residues to approach their porphyrins on O_2 association with a less significant steric repulsion than in the deoxy conformation (Figure 3.40). Thus an O_2 molecule binds to the empty hemes in the less constrained oxy conformation more easily than to a subunit conformation held by the quaternary interactions in the deoxy conformation.

In addition, Val E11 in the deoxy conformation of β subunits is at the entrance to the O_2-binding site, where it sterically impedes O_2 association to heme (see Figure 3.33). In the oxy conformation the heme in β subunits moves approximately 1.5 Å further into the heme-binding site, changing the geometric relationship of the O_2-binding site to the Val El1 side chain, so that the Val El1 no longer sterically interferes with O_2 binding. This is an important additional factor that increases affinity of O_2 for the oxy conformation of the β chain over that for the deoxy conformation.

The deoxy conformation of hemoglobin is referred to as the "tense" or **T conformational state.** The oxyhemoglobin conformational form is referred to as the "relaxed" or **R conformational state.** The allosteric mechanism shows how initial binding of the oxygen to one of the heme subunits of the tetrameric molecule pushes the molecular conformation from the T to R conformational state. The affinity constant of O_2 is greater for the R state hemes than the T state by a factor of 150–300, depending on the solution conditions.

The Bohr Effect Involves Dissociation of a Proton on Binding of Oxygen

The equilibrium expression for oxygen association to hemoglobin includes a term that indicates participation of H^+ in the equilibrium.

$$\underset{T}{Hb} + 4O_2 \rightleftharpoons \underset{R}{Hb(O_2)_4} + xH^+ \qquad \textbf{(3.10)}$$

Equation 3.10 shows that the R form is more acidic, and the H^+ dissociate when hemoglobin is changed to the R form. The equivalents of H^+ that dissociate per mole of hemoglobin depends on the pH of the solution and the concentration of other factors that can bind to hemoglobin, such as Cl^- and bisphosphoglycerate (see Chapter 25). At pH 7.4, the value of x may vary from 1.8 to 2.8, depending on the solution conditions. This production of H^+ at an alkaline pH (pH > 6), when deoxyhemoglobin is transformed to oxyhemoglobin, is known as the alkaline **Bohr effect.**

The H^+ are derived from the partial dissociation of acid residues with pK'_a values within 1.5 pH units of the solution pH, which change from a higher to lower pK'_a, on the change of the T to R conformation. For example, the HC3 His 146(β) in the deoxy (T) conformation is predominantly in its imidazolium form (positively charged acid form), which is stabilized by a favorable interaction with the negatively charged side chain of the FG1 Asp 94(β) (Figure 3.39). This ion pair makes it more difficult to remove the imidazolium proton and thus raises the pK'_a of the imidazolium to a higher value than normally found for a free imidazolium ion in solution, where a stabilization by a proximal negatively charged group does not normally occur. However, on conversion of the protein to the R conformation, the strength of this ionic interaction is broken and the imidazolium assumes a lower pK'_a. The decrease in pK'_a of histidine at blood pH results in conversion of some of its acid form to its conjugate base (imidazole) form, with dissociation of H^+ that forms a part of the Bohr effect. Breakage of

this ion pair with release of protons accounts for 50% of the H^+ released on conversion to the R conformation. Other acid groups in the protein contribute the additional H^+ due to analogous decreases in their pK'_a values on changing from the T to R conformation.

The equilibrium involving hydrogen ions produced by the Bohr effect has important physiological consequences. Cells metabolizing at high rates, with high requirements for molecular oxygen, produce carbonic acid and lactic acid, which act to increase the hydrogen ion concentration in the cell's environment. As the increase in hydrogen ion concentration forces the equilibrium of Eq. 3.10 to the left, from the higher O_2 affinity conformation (R) to the lower affinity conformation (T), an increased amount of oxygen is dissociated from the hemoglobin molecule.

BIBLIOGRAPHY

Immunoglobulins

Alzari, P. M., Lascombe, M.-B., and Poljak, R. J. Structure of antibodies. *Annu. Rev. Immunol.* 6:555, 1988.

Chothia, C., Lesk, A. M., Tramontano, A., Levitt, M., Smith-Gill, S. J., Air, G., Sheriff, S., Padlan, E. A., Davies, D., Tulip, W. R., Colman, P. M., Spinelli, S., Alzari, P. M., and Poljak, R. J. Conformations of immunoglobulin hypervariable regions. *Nature* 342:877, 1989.

Davies, D. R., Padlan, E. A., and Sheriff, S. Antibody–antigen complexes. *Acc. Chem. Res.* 26:421, 1993.

Guddat, L. W., Shan, L., Fan, Z.-C., Andersen, K. N., Rosauer, R., Linthicum, D. S., and Edmundson, A. B. Intramolecular signaling upon complexation. *FASEB J.* 9:101, 1995.

Hunkapiller, T., and Hood, L. Diversity of the immunoglobulin gene superfamily. *Adv. Immunol.* 44:1, 1989.

Padlan, E. A. Anatomy of the antibody molecule. *Mol. Immunol.* 31:169, 1994.

Rini, J. M., Schultze-Gahmen, U., Wilson, I. A. Structural evidence for induced fit as a mechanism for antibody–antigen recognition. *Science* 255:959, 1992.

Stanfield, R. L., Takimoto-Kaminmura, M., Rini, J. M., Profy, A. T., and Wilson, I. A. Major antigen-induced domain rearrangements in an antibody. *Structure* 1:83, 1993.

Serine Proteases

Birk, Y. Proteinase inhibitors. In: A. Neuberger and K. Brocklehurst (Eds.), *Hydrolytic Enzymes*. Amsterdam: Elsevier, 1987, p. 257.

Dufton, M. J. Could domain movements be involved in the mechanism of trypsin-like serine proteases? *FEBS Lett.* 271:9, 1990.

Greer, J. Comparative modeling methods: application to the family of the mammalian serine proteases. *Proteins* 7:317, 1990.

Liebman, M. N. Structural organization in the serine proteases. *Enzyme* 36:115, 1986.

Neurath, H. Proteolytic processing and physiological regulation. *Trends Biochem. Sci.* 14:268, 1989.

Perona, J. J., and Craik, C. S. Structural basis of substrate specificity in the serine proteases. *Protein Sci.* 4:337, 1995.

Polgar, L. Structure and function of serine proteases. In: A. Neuberger and K. Brocklehurst (Eds.), *Hydrolytic Enzymes*, series in *New Comprehensive Biochemistry*, Vol. 16. Amsterdam: Elsevier, 1987, p. 159.

Zwaal, R. F. A., and Hemker, H. C. (Eds.). *Blood Coagulation*, series in *New Comprehensive Biochemistry*, Vol. 13. Amsterdam: Elsevier, 1986.

DNA-Binding Proteins

Berger, J. M., Gamblin, S. J., Harrison, S. C., and Wang, J. C. Structure and mechanism of DNA topoisomerase II. *Nature* 379:225, 1996.

Cho, Y., Gorina, S., Jeffrey, P. D., Pavletich, N. P. Crystal structure of a p53 tumor suppressor–DNA complex: understanding tumorigenic mutations. *Science* 265:346, 1994.

Ellenberger, T. E., Brandl, C. J., Struhl, K., and Harrison, S. C. The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted α helices: crystal structure of the protein–DNA complex. *Cell* 71:1223, 1992.

Ghosh, G., Van Duyne, G., Ghosh, S., and Sigler, P. B. Structure of NF-κB p50 homodimer bound to a κB site. *Nature* 373:303, 1995.

Landschulz, W. H., Johnson, P. F., and McKnight, S. L. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* 240:1759, 1988.

Lee, M. S., Gippert, G. P., Soman, K. V., Case, D. A., and Wright, P. E. Three-dimensional solution structure of a single zinc finger DNA-binding domain. *Science* 245:635, 1989.

Lewis, M., Chang, G., Horton, N. C., Kercher, M. A., Pace, H. C., Schumacher, M. A., Brennan, R. G., and Lu, P. Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. *Science* 271:1247, 1996.

Müller, C. W., Rey, F. A., Sodeoka, M., Verdine, G. L., and Harrison, S. C. Structure of the NF-κB p50 homodimer bound to DNA. *Nature* 373:311, 1995.

Nikolov, D. B., Chen, H., Halay, E. D., Usheva, A. A., Hisatake, K., Lee, D. K., Roeder R. G., and Burley, S. K. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. *Nature* 377:119, 1995.

Pavletich, N. P., and Pabo, C. O. Zinc finger—DNA recognition: crystal structure of a Zif-268-DNA complex at 2.1 Å. *Science* 252:809, 1991.

Pellegrini, L., Tan, S., and Richmond, T. J. Structure of serum response factor core bound to DNA. *Nature* 376:490, 1995.

Hemoglobin

Baldwin, J., and Chothia, C. Haemoglobin: the structural changes related to ligand binding and its allosteric mechanism. *J. Mol. Biol.* 129:175, 1979.

Busch, M. R., Mace, J. E., Ho, N. T., and Ho, C. Roles of the *beta*-146 histidyl residue in the molecular basis of the Bohr effect of hemoglobin: a protein nuclear magnetic resonance study. *Biochemistry* 30:1865, 1991.

Dickerson, R. E., and Geis, I. *Hemoglobin: Structure, Function, Evolution and Pathology*. Menlo Park, CA: Benjamin-Cummings, 1983

Ho, C. Proton nuclear magnetic resonance studies on hemoglobin: cooperative interactions and partially ligated intermediates. *Adv. Protein Chem.* 43:154, 1992.

Jayaraman, V., Rodgers, K. R., Mukerji, I., and Spiro, T. G. Hemoglobin allostery: resonance Raman spectroscopy of kinetic intermediates. *Science* 269:1843, 1995.

Jia, L., Bonaventura, C., Bonaventura, J., and Stamler, J. S. *S*-Nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature* 380:221, 1996.

Mathews, A. J., Rohlfs, R. J., Olson, J. S., Tame, J., Renaud, J. P., and Nagai, K. The effects of E7 and E11 mutations on the kinetics of ligand binding to R state human hemoglobin, *J. Biol. Chem.* 264:16573, 1989.