2.2 Ionization of Water, Weak Acids, and Weak Bases

Although many of the solvent properties of water can be explained in terms of the uncharged H_2O molecule, the small degree of ionization of water to hydrogen ions (H^+) and hydroxide ions (OH^-) must also be taken into account. Like all reversible reactions, the ionization of water can be described by an equilibrium constant. When weak acids are dissolved in water, they contribute H^+ by ionizing; weak bases consume H^+ by becoming protonated. These processes are also governed by equilibrium constants. The total hydrogen ion concentration from all sources is experimentally measurable and is expressed as the pH of the solution. To predict the state of ionization of solutes in water, we must take into account the relevant equilibrium constants for each ionization reaction. We therefore turn now to a brief discussion of the ionization of water and of weak acids and bases dissolved in water.

Pure Water Is Slightly Ionized

Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium

$$H_2O \rightleftharpoons H^+ + OH^- \qquad (2\text{--}1)$$

Although we commonly show the dissociation product of water as H^+, free protons do not exist in solution; hydrogen ions formed in water are immediately hydrated to **hydronium ions** (H_3O^+). Hydrogen bonding between water molecules makes the hydration of dissociating protons virtually instantaneous:

$$H{-}O{\cdots}H{-}O \rightleftharpoons H{-}O^+{-}H + OH^-$$
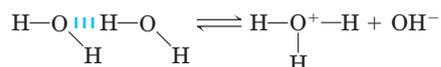
The ionization of water can be measured by its electrical conductivity; pure water carries electrical current as H^+ migrates toward the cathode and OH^- toward the anode. The movement of hydronium and hydroxide ions in the electric field is anomalously fast compared with that of other ions such as Na^+, K^+, and Cl^-. This high ionic mobility results from the kind of "proton hopping" shown in Figure 2–14. No individual proton moves very far through the bulk solution, but a series of proton hops between hydrogen-bonded water molecules causes the net movement of a proton over a long distance in a remarkably short time. As a result of the high ionic mobility of H^+ (and of OH^-, which also moves rapidly by proton hopping, but in the opposite direction), acid-base reactions in aqueous solutions are generally exceptionally fast. As noted above, proton hopping very likely also plays a role in biological proton-transfer reactions (Fig. 2–10; see also Fig. 19–XX).

Because reversible ionization is crucial to the role of water in cellular function, we must have a means of
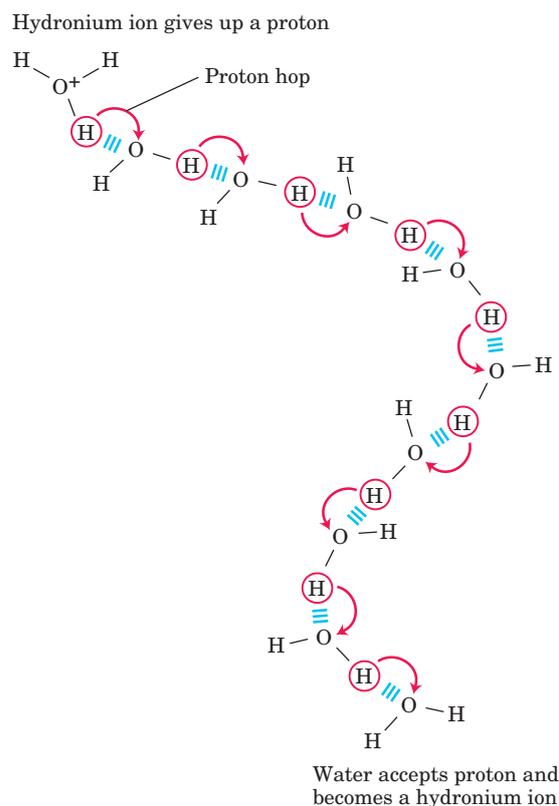

Hydronium ion gives up a proton

FIGURE 2–14 Proton hopping. Short "hops" of protons between a series of hydrogen-bonded water molecules effect an extremely rapid net movement of a proton over a long distance. As a hydronium ion (upper left) gives up a proton, a water molecule some distance away (lower right) acquires one, becoming a hydronium ion. Proton hopping is much faster than true diffusion and explains the remarkably high ionic mobility of H^+ ions compared with other monovalent cations such as Na^+ or K^+.

expressing the extent of ionization of water in quantitative terms. A brief review of some properties of reversible chemical reactions shows how this can be done.

The position of equilibrium of any chemical reaction is given by its **equilibrium constant, K_{eq}** (sometimes expressed simply as K). For the generalized reaction

$$A + B \rightleftharpoons C + D \qquad (2\text{--}2)$$

an equilibrium constant can be defined in terms of the concentrations of reactants (A and B) and products (C and D) at equilibrium:

$$K_{eq} = \frac{[C][D]}{[A][B]}$$

Strictly speaking, the concentration terms should be the *activities,* or effective concentrations in nonideal solutions, of each species. Except in very accurate work, however, the equilibrium constant may be approxi-

mated by measuring the *concentrations* at equilibrium. For reasons beyond the scope of this discussion, equilibrium constants are dimensionless. Nonetheless, we have generally retained the concentration units (M) in the equilibrium expressions used in this book to remind you that molarity is the unit of concentration used in calculating K_{eq}.

The equilibrium constant is fixed and characteristic for any given chemical reaction at a specified temperature. It defines the composition of the final equilibrium mixture, regardless of the starting amounts of reactants and products. Conversely, we can calculate the equilibrium constant for a given reaction at a given temperature if the equilibrium concentrations of all its reactants and products are known. As we will show in Chapter 13, the standard free-energy change ($\Delta G°$) is directly related to K_{eq}.

The Ionization of Water Is Expressed by an Equilibrium Constant

The degree of ionization of water at equilibrium (Eqn 2–1) is small; at 25 °C only about two of every 10^9 molecules in pure water are ionized at any instant. The equilibrium constant for the reversible ionization of water (Eqn 2–1) is

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} \qquad (2–3)$$

In pure water at 25 °C, the concentration of water is 55.5 M (grams of H_2O in 1 L divided by its gram molecular weight: $(1{,}000 \text{ g/L})/(18.015 \text{ g/mol})$) and is essentially constant in relation to the very low concentrations of H^+ and OH^-, namely, 1×10^{-7} M. Accordingly, we can substitute 55.5 M in the equilibrium constant expression (Eqn 2–3) to yield

$$K_{eq} = \frac{[H^+][OH^-]}{55.5 \text{ M}},$$

which, on rearranging, becomes

$$(55.5 \text{ M})(K_{eq}) = [H^+][OH^-] = K_w \qquad (2–4)$$

where K_w designates the product $(55.5 \text{ M})(K_{eq})$, the **ion product of water** at 25 °C.

The value for K_{eq}, determined by electrical-conductivity measurements of pure water, is 1.8×10^{-16} M at 25 °C. Substituting this value for K_{eq} in Equation 2–4 gives the value of the ion product of water:

$$K_w = [H^+][OH^-] = (55.5 \text{ M})(1.8 \times 10^{-16} \text{ M})$$
$$= 1.0 \times 10^{-14} \text{ M}^2$$

Thus the product $[H^+][OH^-]$ in aqueous solutions at 25 °C always equals 1×10^{-14} M^2. When there are exactly equal concentrations of H^+ and OH^-, as in pure water, the solution is said to be at **neutral pH.** At this pH, the concentration of H^+ and OH^- can be calculated from the ion product of water as follows:

$$K_w = [H^+][OH^-] = [H^+]^2$$

Solving for $[H^+]$ gives

$$[H^+] = \sqrt{K_w} = \sqrt{1 \times 10^{-14} \text{ M}^2}$$
$$[H^+] = [OH^-] = 10^{-7} \text{ M}$$

As the ion product of water is constant, whenever $[H^+]$ is greater than 1×10^{-7} M, $[OH^-]$ must become less than 1×10^{-7} M, and vice versa. When $[H^+]$ is very high, as in a solution of hydrochloric acid, $[OH^-]$ must be very low. From the ion product of water we can calculate $[H^+]$ if we know $[OH^-]$, and vice versa (Box 2–2).

The pH Scale Designates the H^+ and OH^- Concentrations

The ion product of water, K_w, is the basis for the **pH scale** (Table 2–6). It is a convenient means of designating the concentration of H^+ (and thus of OH^-) in any aqueous solution in the range between 1.0 M H^+ and 1.0 M OH^-. The term **pH** is defined by the expression

$$pH = \log \frac{1}{[H^+]} = -\log [H^+]$$

The symbol p denotes "negative logarithm of." For a precisely neutral solution at 25 °C, in which the concentration of hydrogen ions is 1.0×10^{-7} M, the pH can be calculated as follows:

$$pH = \log \frac{1}{1.0 \times 10^{-7}} = \log (1.0 \times 10^{7})$$
$$= \log 1.0 + \log 10^{7} = 0 + 7 = 7$$

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, $pH + pOH = 14$.

BOX 2–2   WORKING IN BIOCHEMISTRY

The Ion Product of Water: Two Illustrative Problems

The ion product of water makes it possible to calculate the concentration of H^+, given the concentration of OH^-, and vice versa; the following problems demonstrate this.

1. What is the concentration of H^+ in a solution of 0.1 M NaOH?

$$K_w = [H^+][OH^-]$$

Solving for $[H^+]$ gives

$$[H^+] = \frac{K_w}{[OH^-]} = \frac{1 \times 10^{-14} \text{ M}^2}{0.1 \text{ M}} = \frac{10^{-14} \text{ M}^2}{10^{-1} \text{ M}}$$

$$= 10^{-13} \text{ M} \quad (answer)$$

2. What is the concentration of OH^- in a solution with an H^+ concentration of 1.3×10^{-4} M?

$$K_w = [H^+][OH^-]$$

Solving for $[OH^-]$ gives

$$[OH^-] = \frac{K_w}{[H^+]} = \frac{1.0 \times 10^{-14} \text{ M}^2}{1.3 \times 10^{-4} \text{ M}}$$

$$= 7.7 \times 10^{-11} \text{ M} \quad (answer)$$

When doing these or any other calculations, be sure to round your answers to the correct number of significant figures.

The value of 7 for the pH of a precisely neutral solution is not an arbitrarily chosen figure; it is derived from the absolute value of the ion product of water at 25 °C, which by convenient coincidence is a round number. Solutions having a pH greater than 7 are alkaline or basic; the concentration of OH^- is greater than that of H^+. Conversely, solutions having a pH less than 7 are acidic.

Note that the pH scale is logarithmic, not arithmetic. To say that two solutions differ in pH by 1 pH unit means that one solution has ten times the H^+ concentration of the other, but it does not tell us the absolute magnitude of the difference. Figure 2–15 gives the pH of some common aqueous fluids. A cola drink (pH 3.0) or red wine (pH 3.7) has an H^+ concentration approximately 10,000 times that of blood (pH 7.4).

The pH of an aqueous solution can be approximately measured using various indicator dyes, including litmus, phenolphthalein, and phenol red, which undergo color changes as a proton dissociates from the dye molecule. Accurate determinations of pH in the chemical or clinical laboratory are made with a glass electrode that is selectively sensitive to H^+ concentration but insensitive to Na^+, K^+, and other cations. In a pH meter the signal from such an electrode is amplified and compared with the signal generated by a solution of accurately known pH.

Measurement of pH is one of the most important and frequently used procedures in biochemistry. The pH affects the structure and activity of biological macromolecules; for example, the catalytic activity of enzymes is strongly dependent on pH (see Fig. 2–21). Measurements of the pH of blood and urine are commonly used in medical diagnoses. The pH of the blood plasma of people
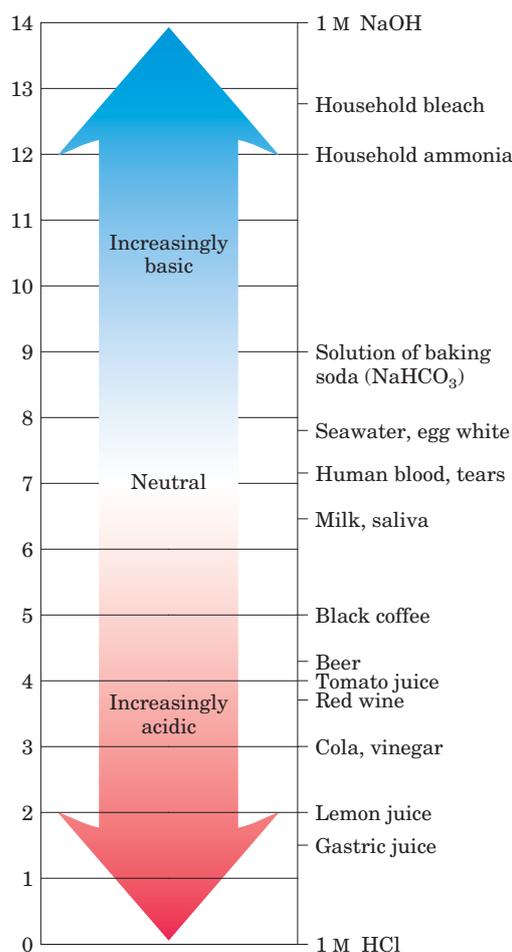

FIGURE 2–15 The pH of some aqueous fluids.

with severe, uncontrolled diabetes, for example, is often below the normal value of 7.4; this condition is called acidosis. In certain other disease states the pH of the blood is higher than normal, the condition of alkalosis.

Weak Acids and Bases Have Characteristic Dissociation Constants

Hydrochloric, sulfuric, and nitric acids, commonly called strong acids, are completely ionized in dilute aqueous solutions; the strong bases NaOH and KOH are also completely ionized. Of more interest to biochemists is the behavior of weak acids and bases—those not completely ionized when dissolved in water. These are common in biological systems and play important roles in metabolism and its regulation. The behavior of aqueous solutions of weak acids and bases is best understood if we first define some terms.

Acids may be defined as proton donors and bases as proton acceptors. A proton donor and its corresponding proton acceptor make up a **conjugate acid-base pair** (Fig. 2–16). Acetic acid (CH_3COOH), a proton donor, and the acetate anion (CH_3COO^-), the corre-

sponding proton acceptor, constitute a conjugate acid-base pair, related by the reversible reaction

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$
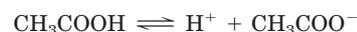
Each acid has a characteristic tendency to lose its proton in an aqueous solution. The stronger the acid, the greater its tendency to lose its proton. The tendency of any acid (HA) to lose a proton and form its conjugate base (A^-) is defined by the equilibrium constant (K_{eq}) for the reversible reaction

$$HA \rightleftharpoons H^+ + A^-,$$

which is

$$K_{eq} = \frac{[H^+][A^-]}{[HA]} = K_a$$

Equilibrium constants for ionization reactions are usually called ionization or **dissociation constants,** often designated K_a. The dissociation constants of some acids are given in Figure 2–16. Stronger acids, such as phosphoric and carbonic acids, have larger dissociation constants; weaker acids, such as monohydrogen phosphate (HPO_4^{2-}), have smaller dissociation constants.

Monoprotic acids
Acetic acid
($K_a = 1.74 \times 10^{-5}$ M)

Ammonium ion
($K_a = 5.62 \times 10^{-10}$ M)

Diprotic acids
Carbonic acid
($K_a = 1.70 \times 10^{-4}$ M);
Bicarbonate
($K_a = 6.31 \times 10^{-11}$ M)

Glycine, carboxyl
($K_a = 4.57 \times 10^{-3}$ M);
Glycine, amino
($K_a = 2.51 \times 10^{-10}$ M)

Triprotic acids
Phosphoric acid
($K_a = 7.25 \times 10^{-3}$ M);
Dihydrogen phosphate
($K_a = 1.38 \times 10^{-7}$ M);
Monohydrogen phosphate
($K_a = 3.98 \times 10^{-13}$ M)

$pK_a = 4.76$

$NH_4^+ \rightleftharpoons NH_3 + H^+$
$pK_a = 9.25$

$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$
$pK_a = 3.77$

$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$
$pK_a = 10.2$

$pK_a = 2.34$

$pK_a = 9.60$

$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+$
$pK_a = 2.14$

$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$
$pK_a = 6.86$

$HPO_4^{2-} \rightleftharpoons PO_4^{3-} + H^+$
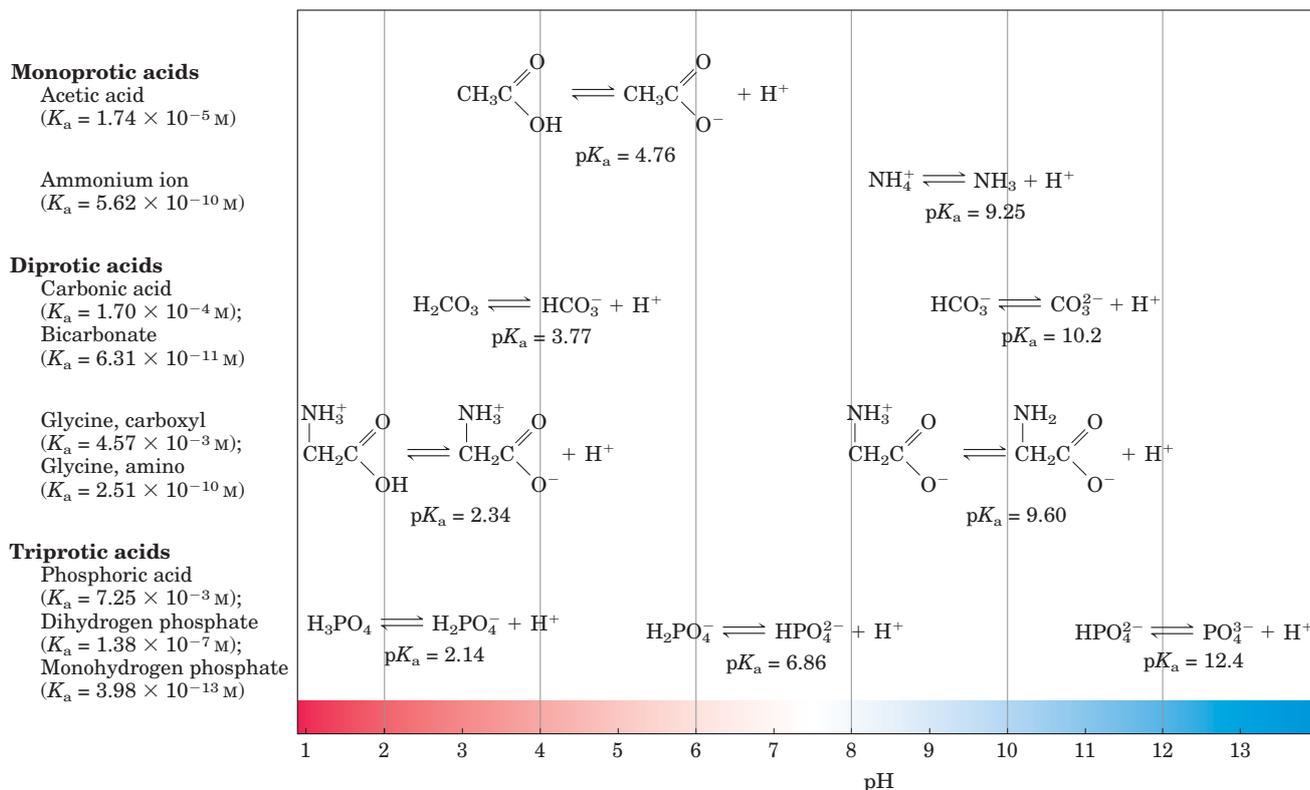$pK_a = 12.4$

pH

FIGURE 2–16 Conjugate acid-base pairs consist of a proton donor and a proton acceptor. Some compounds, such as acetic acid and ammonium ion, are monoprotic; they can give up only one proton. Others are diprotic (H_2CO_3 (carbonic acid) and glycine) or triprotic (H_3PO_4 (phosphoric acid)). The dissociation reactions for each pair are shown where they occur along a pH gradient. The equilibrium or dissociation constant (K_a) and its negative logarithm, the pK_a, are shown for each reaction.

Also included in Figure 2–16 are values of **pK_a,** which is analogous to pH and is defined by the equation

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pK_a. As we shall now see, the pK_a of any weak acid can be determined quite easily.

Titration Curves Reveal the pK_a of Weak Acids

Titration is used to determine the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration. The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added.

A plot of pH against the amount of NaOH added (a **titration curve**) reveals the pK_a of the weak acid. Consider the titration of a 0.1 M solution of acetic acid (for simplicity denoted as HAc) with 0.1 M NaOH at 25 °C (Fig. 2–17). Two reversible equilibria are involved in the process:

$$H_2O \rightleftharpoons H^+ + OH^- \tag{2–5}$$

$$HAc \rightleftharpoons H^+ + Ac^- \tag{2–6}$$

The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,

$$K_w = [H^+][OH^-] = 1 \times 10^{-14}\ M^2 \tag{2–7}$$

$$K_a = \frac{[H^+][Ac^-]}{[HAc]} = 1.74 \times 10^5\ M \tag{2–8}$$

At the beginning of the titration, before any NaOH is added, the acetic acid is already slightly ionized, to an extent that can be calculated from its dissociation constant (Eqn 2–8).

As NaOH is gradually introduced, the added OH$^-$ combines with the free H$^+$ in the solution to form H$_2$O, to an extent that satisfies the equilibrium relationship in Equation 2–7. As free H$^+$ is removed, HAc dissociates further to satisfy its own equilibrium constant (Eqn 2–8). The net result as the titration proceeds is that more and more HAc ionizes, forming Ac$^-$, as the NaOH is added. At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added, one-half of the original acetic acid has undergone dissociation, so that the concentration of the proton donor, [HAc], now equals that of the proton acceptor, [Ac$^-$]. At this midpoint a very important relationship holds: the pH of the equimolar solution of acetic acid and acetate is ex-
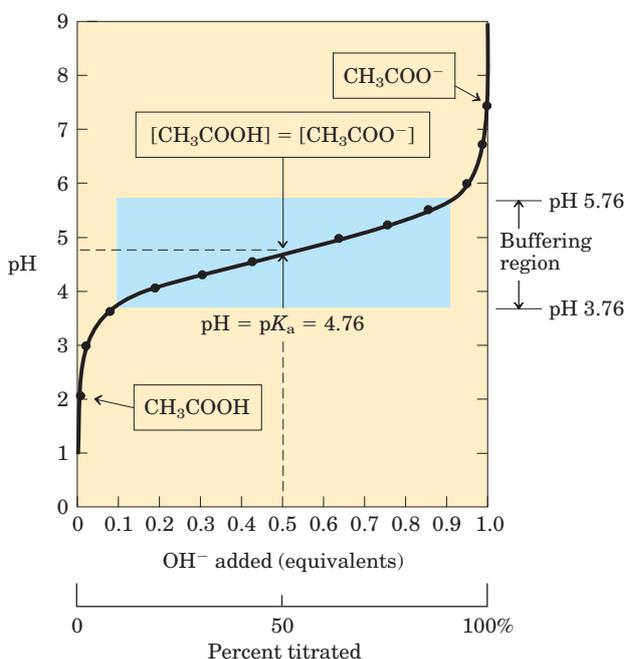

FIGURE 2–17 The titration curve of acetic acid. After addition of each increment of NaOH to the acetic acid solution, the pH of the mixture is measured. This value is plotted against the amount of NaOH expressed as a fraction of the total NaOH required to convert all the acetic acid to its deprotonated form, acetate. The points so obtained yield the titration curve. Shown in the boxes are the predominant ionic forms at the points designated. At the midpoint of the titration, the concentrations of the proton donor and proton acceptor are equal, and the pH is numerically equal to the pK_a. The shaded zone is the useful region of buffering power, generally between 10% and 90% titration of the weak acid.

actly equal to the pK_a of acetic acid (pK_a = 4.76; Figs 2–16, 2–17). The basis for this relationship, which holds for all weak acids, will soon become clear.

As the titration is continued by adding further increments of NaOH, the remaining nondissociated acetic acid is gradually converted into acetate. The end point of the titration occurs at about pH 7.0: all the acetic acid has lost its protons to OH$^-$, to form H$_2$O and acetate. Throughout the titration the two equilibria (Eqns 2–5, 2–6) coexist, each always conforming to its equilibrium constant.

Figure 2–18 compares the titration curves of three weak acids with very different dissociation constants: acetic acid (pK_a = 4.76); dihydrogen phosphate, H$_2$PO$_4^-$ (pK_a = 6.86); and ammonium ion, NH$_4^+$ (pK_a = 9.25). Although the titration curves of these acids have the same shape, they are displaced along the pH axis because the three acids have different strengths. Acetic acid, with the highest K_a (lowest pK_a) of the three, is the strongest (loses its proton most readily); it is al-
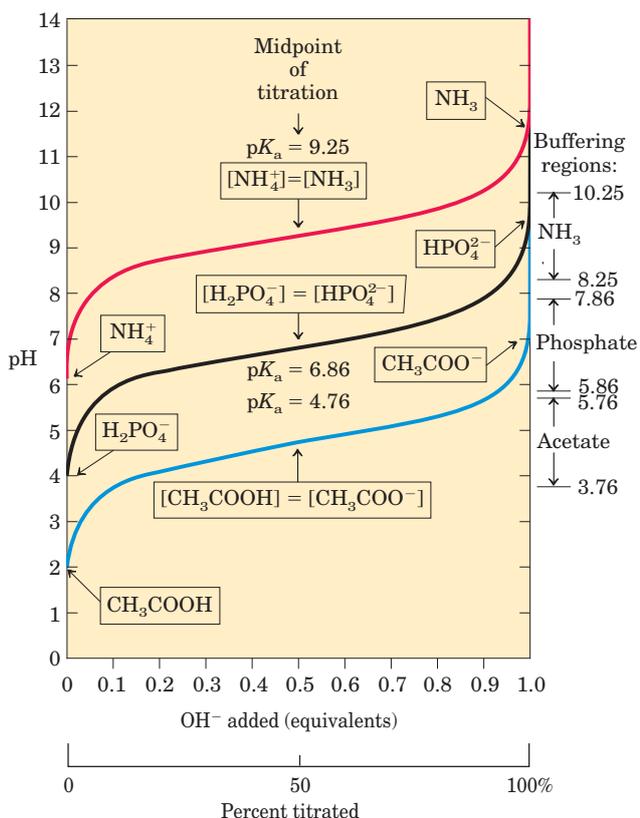
FIGURE 2–18 Comparison of the titration curves of three weak acids.
Shown here are the titration curves for CH_3COOH, $H_2PO_4^-$, and NH_4^+. The predominant ionic forms at designated points in the titration are given in boxes. The regions of buffering capacity are indicated at the right. Conjugate acid-base pairs are effective buffers between approximately 10% and 90% neutralization of the proton-donor species.

ready half dissociated at pH 4.76. Dihydrogen phosphate loses a proton less readily, being half dissociated at pH 6.86. Ammonium ion is the weakest acid of the three and does not become half dissociated until pH 9.25.

The most important point about the titration curve of a weak acid is that it shows graphically that a weak acid and its anion—a conjugate acid-base pair—can act as a buffer.

SUMMARY 2.2 Ionization of Water, Weak Acids, and Weak Bases

■ Pure water ionizes slightly, forming equal numbers of hydrogen ions (hydronium ions, H_3O^+) and hydroxide ions. The extent of ionization is described by an equilibrium constant, $K_{eq} = \dfrac{[H^+][OH^-]}{[H_2O]}$, from which the ion product of

water, K_w, is derived. At 25 °C, $K_w = [H^+][OH^-] = (55.5\ \text{M})(K_{eq}) = 10^{-14}\ \text{M}^2$.

■ The pH of an aqueous solution reflects, on a logarithmic scale, the concentration of hydrogen ions: $\text{pH} = \log \dfrac{1}{[H^+]} = -\log [H^+]$.

■ The greater the acidity of a solution, the lower its pH. Weak acids partially ionize to release a hydrogen ion, thus lowering the pH of the aqueous solution. Weak bases accept a hydrogen ion, increasing the pH. The extent of these processes is characteristic of each particular weak acid or base and is expressed as a dissociation constant, K_a: $K_{eq} = \dfrac{[H^+][A^-]}{[HA]} = K_a$.

■ The pK_a expresses, on a logarithmic scale, the relative strength of a weak acid or base: $pK_a = \log \dfrac{1}{K_a} = -\log K_a$.

■ The stronger the acid, the lower its pK_a; the stronger the base, the higher its pK_a. The pK_a can be determined experimentally; it is the pH at the midpoint of the titration curve for the acid or base.

2.3 Buffering against pH Changes in Biological Systems

Almost every biological process is pH dependent; a small change in pH produces a large change in the rate of the process. This is true not only for the many reactions in which the H^+ ion is a direct participant, but also for those in which there is no apparent role for H^+ ions. The enzymes that catalyze cellular reactions, and many of the molecules on which they act, contain ionizable groups with characteristic pK_a values. The protonated amino and carboxyl groups of amino acids and the phosphate groups of nucleotides, for example, function as weak acids; their ionic state depends on the pH of the surrounding medium. As we noted above, ionic interactions are among the forces that stabilize a protein molecule and allow an enzyme to recognize and bind its substrate.

Cells and organisms maintain a specific and constant cytosolic pH, keeping biomolecules in their optimal ionic state, usually near pH 7. In multicellular organisms, the pH of extracellular fluids is also tightly regulated. Constancy of pH is achieved primarily by biological buffers: mixtures of weak acids and their conjugate bases.

We describe here the ionization equilibria that account for buffering, and we show the quantitative relationship between the pH of a buffered solution and the pK_a of the buffer. Biological buffering is illustrated by the phosphate and carbonate buffering systems of humans.

Buffers Are Mixtures of Weak Acids and Their Conjugate Bases

Buffers are aqueous systems that tend to resist changes in pH when small amounts of acid (H^+) or base (OH^-) are added. A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor). As an example, a mixture of equal concentrations of acetic acid and acetate ion, found at the midpoint of the titration curve in Figure 2–17, is a buffer system. The titration curve of acetic acid has a relatively flat zone extending about 1 pH unit on either side of its midpoint pH of 4.76. In this zone, an amount of H^+ or OH^- added to the system has much less effect on pH than the same amount added outside the buffer range. This relatively flat zone is the buffering region of the acetic acid–acetate buffer pair. At the midpoint of the buffering region, where the concentration of the proton donor (acetic acid) exactly equals that of the proton acceptor (acetate), the buffering power of the system is maximal; that is, its pH changes least on addition of H^+ or OH^-. The pH at this point in the titration curve of acetic acid is equal to its pK_a. The pH of the acetate buffer system does change slightly when a small amount of H^+ or OH^- is added, but this change is very small compared with the pH change that would result if the same amount of H^+ or OH^- were added to pure water or to a solution of the salt of a strong acid and strong base, such as NaCl, which has no buffering power.

Buffering results from two reversible reaction equilibria occurring in a solution of nearly equal concentrations of a proton donor and its conjugate proton acceptor. Figure 2–19 explains how a buffer system works. Whenever H^+ or OH^- is added to a buffer, the result is a small change in the ratio of the relative concentrations of the weak acid and its anion and thus a small change in pH. The decrease in concentration of one component of the system is balanced exactly by an increase in the other. The sum of the buffer components does not change, only their ratio.

Each conjugate acid-base pair has a characteristic pH zone in which it is an effective buffer (Fig. 2–18). The $H_2PO_4^-/HPO_4^{2-}$ pair has a pK_a of 6.86 and thus can serve as an effective buffer system between approximately pH 5.9 and pH 7.9; the NH_4^+/NH_3 pair, with a pK_a of 9.25, can act as a buffer between approximately pH 8.3 and pH 10.3.

A Simple Expression Relates pH, pK_a, and Buffer Concentration

The titration curves of acetic acid, $H_2PO_4^-$, and NH_4^+ (Fig. 2–18) have nearly identical shapes, suggesting that these curves reflect a fundamental law or relationship. This is indeed the case. The shape of the titration curve of any weak acid is described by the **Henderson-**
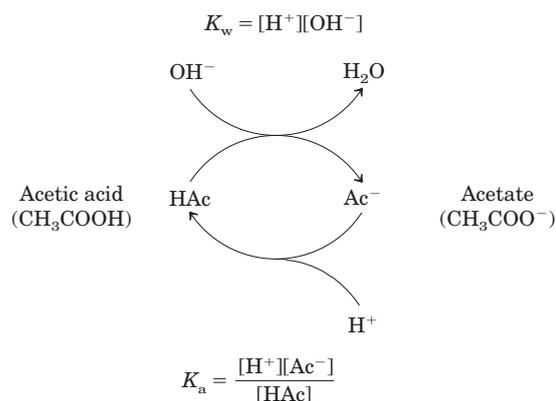
$$K_w = [H^+][OH^-]$$

$$K_a = \frac{[H^+][Ac^-]}{[HAc]}$$

FIGURE 2–19 The acetic acid–acetate pair as a buffer system. The system is capable of absorbing either H^+ or OH^- through the reversibility of the dissociation of acetic acid. The proton donor, acetic acid (HAc), contains a reserve of bound H^+, which can be released to neutralize an addition of OH^- to the system, forming H_2O. This happens because the product $[H^+][OH^-]$ transiently exceeds K_w (1×10^{-14} M^2). The equilibrium quickly adjusts so that this product equals 1×10^{-14} M^2 (at 25 °C), thus transiently reducing the concentration of H^+. But now the quotient $[H^+][Ac^-] / [HAc]$ is less than K_a, so HAc dissociates further to restore equilibrium. Similarly, the conjugate base, Ac^-, can react with H^+ ions added to the system; again, the two ionization reactions simultaneously come to equilibrium. Thus a conjugate acid-base pair, such as acetic acid and acetate ion, tends to resist a change in pH when small amounts of acid or base are added. Buffering action is simply the consequence of two reversible reactions taking place simultaneously and reaching their points of equilibrium as governed by their equilibrium constants, K_w and K_a.

Hasselbalch equation, which is important for understanding buffer action and acid-base balance in the blood and tissues of vertebrates. This equation is simply a useful way of restating the expression for the dissociation constant of an acid. For the dissociation of a weak acid HA into H^+ and A^-, the Henderson-Hasselbalch equation can be derived as follows:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

First solve for $[H^+]$:

$$[H^+] = K_a \frac{[HA]}{[A^-]}$$

Then take the negative logarithm of both sides:

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

Substitute pH for $-\log [H^+]$ and pK_a for $-\log K_a$:

$$pH = pK_a - \log \frac{[HA]}{[A^-]}$$

Now invert $-\log$ [HA]/[A$^-$], which involves changing its sign, to obtain the Henderson-Hasselbalch equation:

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \qquad (2\text{–}9)$$

Stated more generally,

$$\text{pH} = \text{p}K_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

This equation fits the titration curve of all weak acids and enables us to deduce a number of important quantitative relationships. For example, it shows why the pK_a of a weak acid is equal to the pH of the solution at the midpoint of its titration. At that point, [HA] equals [A$^-$], and

$$\text{pH} = \text{p}K_a + \log 1 = \text{p}K_a + 0 = \text{p}K_a$$

As shown in Box 2–3, the Henderson-Hasselbalch equation also allows us to (1) calculate pK_a, given pH and the molar ratio of proton donor and acceptor; (2) calculate pH, given pK_a and the molar ratio of proton donor and acceptor; and (3) calculate the molar ratio of proton donor and acceptor, given pH and pK_a.

Weak Acids or Bases Buffer Cells and Tissues against pH Changes

The intracellular and extracellular fluids of multicellular organisms have a characteristic and nearly constant
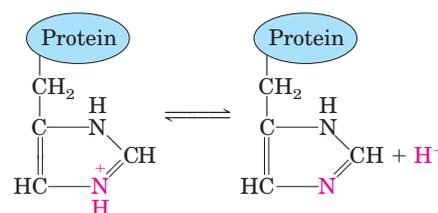

FIGURE 2–20 The amino acid histidine, a component of proteins, is a weak acid. The pK_a of the protonated nitrogen of the side chain is 6.0.

pH. The organism's first line of defense against changes in internal pH is provided by buffer systems. The cytoplasm of most cells contains high concentrations of proteins, which contain many amino acids with functional groups that are weak acids or weak bases. For example, the side chain of histidine (Fig. 2–20) has a pK_a of 6.0; proteins containing histidine residues therefore buffer effectively near neutral pH. Nucleotides such as ATP, as well as many low molecular weight metabolites, contain ionizable groups that can contribute buffering power to the cytoplasm. Some highly specialized organelles and extracellular compartments have high concentrations of compounds that contribute buffering capacity: organic acids buffer the vacuoles of plant cells; ammonia buffers urine.

BOX 2–3   WORKING IN BIOCHEMISTRY

Solving Problems Using the Henderson-Hasselbalch Equation

1. Calculate the pK_a of lactic acid, given that when the concentration of lactic acid is 0.010 M and the concentration of lactate is 0.087 M, the pH is 4.80.

$$\text{pH} = \text{p}K_a + \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$\text{p}K_a = \text{pH} - \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$= 4.80 - \log \frac{0.087}{0.010} = 4.80 - \log 8.7$$

$$= 4.80 - 0.94 = 3.9 \quad (answer)$$

2. Calculate the pH of a mixture of 0.10 M acetic acid and 0.20 M sodium acetate. The pK_a of acetic acid is 4.76.

$$\text{pH} = \text{p}K_a + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$= 4.76 + \log \frac{0.20}{0.10} = 4.76 + 0.30$$

$$= 5.1 \quad (answer)$$

3. Calculate the ratio of the concentrations of acetate and acetic acid required in a buffer system of pH 5.30.

$$\text{pH} = \text{p}K_a + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$\log \frac{[\text{acetate}]}{[\text{acetic acid}]} = \text{pH} - \text{p}K_a$$

$$= 5.30 - 4.76 = 0.54$$

$$\frac{[\text{acetate}]}{[\text{acetic acid}]} = \text{antilog } 0.54 = 3.5 \quad (answer)$$

To see the effect of pH on the degree of ionization of a weak acid, see the Living Graph for Equation 2–9.

Two especially important biological buffers are the phosphate and bicarbonate systems. The phosphate buffer system, which acts in the cytoplasm of all cells, consists of $H_2PO_4^-$ as proton donor and HPO_4^{2-} as proton acceptor:

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$
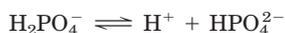
The phosphate buffer system is maximally effective at a pH close to its pK_a of 6.86 (Figs 2–16, 2–18) and thus tends to resist pH changes in the range between about 5.9 and 7.9. It is therefore an effective buffer in biological fluids; in mammals, for example, extracellular fluids and most cytoplasmic compartments have a pH in the range of 6.9 to 7.4.

Blood plasma is buffered in part by the bicarbonate system, consisting of carbonic acid (H_2CO_3) as proton donor and bicarbonate (HCO_3^-) as proton acceptor:
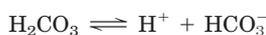
$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

This buffer system is more complex than other conjugate acid-base pairs because one of its components, carbonic acid (H_2CO_3), is formed from dissolved (d) carbon dioxide and water, in a reversible reaction:

$$CO_2(d) + H_2O \rightleftharpoons H_2CO_3$$

$$K_2 = \frac{[H_2CO_3]}{[CO_2(d)][H_2O]}$$

Carbon dioxide is a gas under normal conditions, and the concentration of dissolved CO_2 is the result of equilibration with CO_2 of the gas (g) phase:

$$CO_2(g) \rightleftharpoons CO_2(d)$$

$$K_3 = \frac{[CO_2(d)]}{[CO_2(g)]}$$

The pH of a bicarbonate buffer system depends on the concentration of H_2CO_3 and HCO_3^-, the proton donor and acceptor components. The concentration of H_2CO_3 in turn depends on the concentration of dissolved CO_2, which in turn depends on the concentration of CO_2 in the gas phase, called the **partial pressure** of CO_2. Thus the pH of a bicarbonate buffer exposed to a gas phase is ultimately determined by the concentration of HCO_3^- in the aqueous phase and the partial pressure of CO_2 in the gas phase (Box 2–4).

Human blood plasma normally has a pH close to 7.4. Should the pH-regulating mechanisms fail or be overwhelmed, as may happen in severe uncontrolled diabetes when an overproduction of metabolic acids causes acidosis, the pH of the blood can fall to 6.8 or below, leading to irreparable cell damage and death. In other diseases the pH may rise to lethal levels.
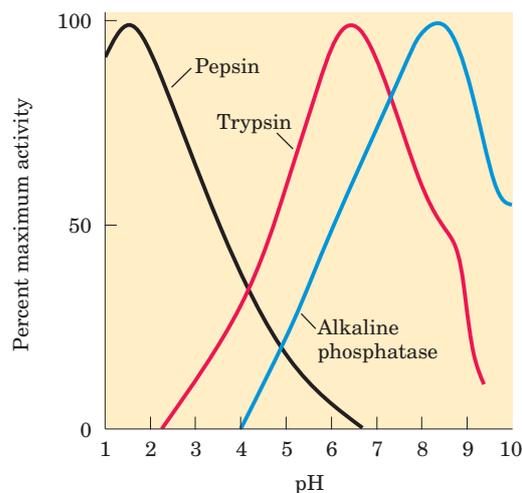

FIGURE 2–21 **The pH optima of some enzymes.** Pepsin is a digestive enzyme secreted into gastric juice; trypsin, a digestive enzyme that acts in the small intestine; alkaline phosphatase of bone tissue, a hydrolytic enzyme thought to aid in bone mineralization.

Although many aspects of cell structure and function are influenced by pH, it is the catalytic activity of enzymes that is especially sensitive. Enzymes typically show maximal catalytic activity at a characteristic pH, called the **pH optimum** (Fig. 2–21). On either side of the optimum pH their catalytic activity often declines sharply. Thus, a small change in pH can make a large difference in the rate of some crucial enzyme-catalyzed reactions. Biological control of the pH of cells and body fluids is therefore of central importance in all aspects of metabolism and cellular activities.

SUMMARY 2.3 Buffering against pH Changes in Biological Systems

- A mixture of a weak acid (or base) and its salt resists changes in pH caused by the addition of H^+ or OH^-. The mixture thus functions as a buffer.

- The pH of a solution of a weak acid (or base) and its salt is given by the Henderson-Hasselbalch equation: $pH = pK_a - \log \frac{[HA]}{[A^-]}$.

- In cells and tissues, phosphate and bicarbonate buffer systems maintain intracellular and extracellular fluids at their optimum (physiological) pH, which is usually close to pH 7. Enzymes generally work optimally at this pH.

BOX 2–4 BIOCHEMISTRY IN MEDICINE

Blood, Lungs, and Buffer: The Bicarbonate Buffer System

In animals with lungs, the bicarbonate buffer system is an effective physiological buffer near pH 7.4, because the H_2CO_3 of blood plasma is in equilibrium with a large reserve capacity of $CO_2(g)$ in the air space of the lungs. This buffer system involves three reversible equilibria between gaseous CO_2 in the lungs and bicarbonate (HCO_3^-) in the blood plasma (Fig. 1).

When H^+ (from lactic acid produced in muscle tissue during vigorous exercise, for example) is added to blood as it passes through the tissues, reaction 1 proceeds toward a new equilibrium, in which the concentration of H_2CO_3 is increased. This increases the concentration of $CO_2(d)$ in the blood plasma (reaction 2) and thus increases the pressure of $CO_2(g)$ in the air space of the lungs (reaction 3); the extra CO_2 is exhaled. Conversely, when the pH of blood plasma is raised (by NH_3 production during protein catabolism, for example), the opposite events occur: the H^+ concentration of blood plasma is lowered, causing more H_2CO_3 to dissociate into H^+ and HCO_3^-. This in turn causes more $CO_2(g)$ from the lungs to dissolve in the blood plasma. The rate of breathing—that is, the rate of inhaling and exhaling CO_2—can quickly adjust these equilibria to keep the blood pH nearly constant.
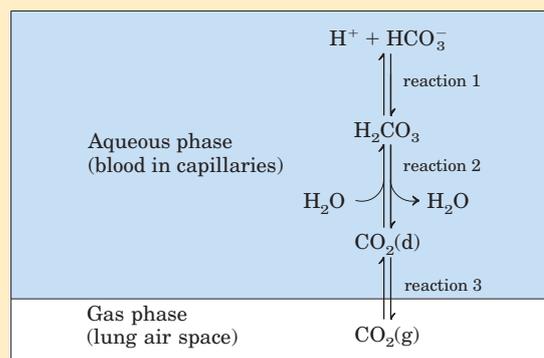
FIGURE 1 The CO_2 in the air space of the lungs is in equilibrium with the bicarbonate buffer in the blood plasma passing through the lung capillaries. Because the concentration of dissolved CO_2 can be adjusted rapidly through changes in the rate of breathing, the bicarbonate buffer system of the blood is in near-equilibrium with a large potential reservoir of CO_2.

2.4 Water as a Reactant

Water is not just the solvent in which the chemical reactions of living cells occur; it is very often a direct participant in those reactions. The formation of ATP from ADP and inorganic phosphate is an example of a **condensation reaction** in which the elements of water are eliminated (Fig. 2–22a). The reverse of this reaction—cleavage accompanied by the addition of the elements of water—is a **hydrolysis reaction.** Hydrolysis reactions are also responsible for the enzymatic depolymerization of proteins, carbohydrates, and nucleic acids. Hydrolysis reactions, catalyzed by enzymes called

FIGURE 2–22 Participation of water in biological reactions. (a) ATP is a phosphoanhydride formed by a condensation reaction (loss of the elements of water) between ADP and phosphate. R represents adenosine monophosphate (AMP). This condensation reaction requires energy. The hydrolysis of (addition of the elements of water to) ATP to form ADP and phosphate releases an equivalent amount of energy. Also shown are some other condensation and hydrolysis reactions common in biological systems **(b), (c), (d).**