other physiological systems, however. The solubility of the buffer components may also limit the concentration that can be employed.

Table 1.3 lists the pK_a for some buffers commonly employed in biochemistry.

1.15 PHYSIOLOGICAL BUFFERS

The ability to ionize is an essential property of many biological compounds. Organic acids, amino acids, proteins, nucleotides, and other phosphate esters are examples of biochemicals that are ionized to varying degrees in biological systems. Since the pH of most biological fluids is near 7, the extent of dissociation of some of these compounds may be complete there. For example, the first ionization of H_3PO_4 will be complete; the second ionization ($pK_{a2} = 7.2$) will be approximately half-complete. Other examples will be encountered later in the book. One may ask which of these are physiologically significant as buffers in the intact organism. The answer is dependent on several factors including those listed in the preceding section; that is, the molar concentration of the buffer components and the ratio of the concentration of the conjugate base to that of the weak acid. The former of these factors would appear to rule out many of the compounds encountered in intermediary metabolism in which the concentrations of such metabolites are seldom large. This would include the phosphate esters of glycolysis, the organic acids of the Krebs cycle, and the free amino acids. In plants, however, certain of the organic acids—malic, citric, and isocitric—can accumulate in the vacuoles and, in that case, play a major role in determining the pH of that part of the cell. Yeasts can also accumulate relatively large concentrations of phosphate esters during glycolysis.

In animals, a complex system of pH control, with both buffering and active pH controlling elements, is found in the circulating blood. The components of this system include CO_2 and HCO_3, NaH_2PO_4 and Na_2HPO_4, the oxygenated and nonoxygenated forms of hemoglobin, and the plasma proteins. Two of these components deserve further comment. Since the pK_{a1} for H_2CO_3 is 6.1, the ratio of conjugate base to weak acid is approximately 20 : 1 in the normal pH range of 7.35 to 7.45 of blood. Consequently, one would expect that the H_2CO_3–HCO_3^- pair is not very effective as a buffer. Nevertheless, the carbonic acid–bicarbonate pair does have an important role in pH control, because blood is subject to CO_2 exchange with tissues and the atmosphere. The weak acid H_2CO_3 is in rapid equilibrium with dissolved CO_2 in the plasma (Equation 1.23). This equilibrium is catalyzed by the enzyme **carbonic anhydrase** that is found in red blood cells.

$$H_2CO_3 \rightleftharpoons CO_{2,\text{diss}} + H_2O \qquad (1.23)$$

The dissolved CO_2 is, in turn, in equilibrium with CO_2 in the atmosphere and, depending on the partial pressure of CO_2 in the gas phase, will either escape into the air phase (as in the lungs where CO_2 is expired) or will enter the blood (as in the peripheral tissues where CO_2 is produced by respiring cells). Thus, the H_2CO_3–HCO_3^- buffer system is less important than the rapid $CO_2 \rightleftharpoons H_2CO_3$ equilibrium that makes CO_2 into what is essentially a very volatile acid, the total amount of which is controlled in the blood to be within certain limits in order to maintain the ratio of 20 : 1 for conjugate base (HCO_3^-) to weak acid (H_2CO_3).

The two forms of hemoglobin found in the blood (oxygenated hemoglobin, $HHbO_2$, and unoxygenated hemoglobin, HHb) constitute the other major pH control system of blood. The buffering capacity of the hemoglobins, the most abundant protein in blood, is due in large measure to the imidazole group of histidine residues (see Section 3.14.3). The hemoglobin in 1 liter of blood can buffer 27.5 meq of H^+ (i.e., theoretically 27.5 meq of acid would be required to change the pH by one unit). In contrast, the plasma proteins can buffer only 4.24 meq of H^+. Active pH control by hemoglobins occurs because the two forms of hemoglobin differ in their pK_a's; $HHbO_2$ is the stronger acid and dissociates with a pK_{a1} of 6.2.

$$HHbO_2 \rightleftharpoons H^+ + HbO_2^-, \qquad pK_{a1} = 6.2$$

Therefore, in the lungs where the partial pressure of O_2 is high, $HHbO_2$ will predominate over the unoxygenated form and the blood tends to become more acidic. In the peripheral tissues where the partial pressure O_2 is relatively lower, HHb with the higher pK_{a1} of 7.7 will predominate, and the pH will tend to increase, since the equilibrium shown below shifts to the left.

$$HHb \rightleftharpoons H^+ + Hb^-, \qquad pK_{a1} = 7.7$$

The two effects compensate for the low concentration of CO_2 in the lungs relative to that in the peripheral tissues, and the two effects working together provide for a minimum change in pH. The pH control of the blood, then, is one example of the biochemical machinery (see Section 1.1) that is absolutely essential to the proper functioning of biological systems.

REFERENCES

1. *L. J. Henderson,* The Fitness of the Environment. *Boston: Beacon Press, 1958.*
2. *I. H. Segel,* Biochemical Calculations, *2nd ed. New York: Wiley, 1976.*

REVIEW PROBLEMS

1. What constitutes the earth's biosphere?
2. What are some of the potential advantages and disadvantages of studying a particular biochemical reaction in isolation, with purified cell components in a test tube, rather than with and in intact cells?
3. What properties of water make it particularly suited to the support of life on our planet?
4. Approximately what would be the pH of a solution that was prepared by dissolving 0.02 moles of formic acid and 0.012 moles of NaOH in water to give a final volume of 100 ml?
5. Calculate the compositions of two buffers. Each buffer is to be 0.10*M* in ethanolamine ($H_2N{-}CH_2{-}CH_2{-}OH$) and to contain acetic acid. One buffer is to be pH 5.0, the other 9.2. Which of these two buffers will have the greater buffering capacity?