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FOURTH EDITION

BASIC BIOCHEMISTRY

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Basic Biochemistry

Fourth Edition

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PREFACE TO THE FOURTH EDITION

BASIC BIOCHEMISTRY in its fourth edition retains as its purpose the introduction to the student in life sciences and allied disciplines the principles and viewpoints of biochemistry and a core of essential facts, without presenting an overwhelming amount of factual material. To accomplish this goal, the authors have assumed that the reader has an adequate background in biology and chemistry. As a consequence, the definitions of most basic biologic and chemical terms have not been emphasized. In addition, the authors have purposely excluded material that some instructors might have wished included. However, we believe that sufficient areas have been presented in depth to allow the student to obtain a basic understanding of the functions of biologic systems at the molecular and cellular levels and to progress subsequently to other topics in biochemistry. Students in diverse disciplines (medicine, osteopathy, dentistry, pharmacy, podiatry, veterinary science, nursing, and other health professions) as well as those preparing for medical board and other examinations should find this text helpful.

The book has been rewritten to include new material of importance and to delete sections of lesser significance. Some changes have been made in the general organization. These include new, separate chapters on bioenergetic principles (Chapter 2), oxidative phosphorylation (Chapter 7), chemistry and metabolism of nucleotides (Chapter 9), structure and functions of nucleic acids (Chapter 10), and integration of metabolism (Chapter 12). Chapters that have been retained from the third edition have been rewritten. Metabolic regulation has been expanded and updated and is, as in previous editions, integrated throughout the appropriate areas of metabolism, with an overview presented in Chapter 12.

As in previous editions the water-soluble vitamins are dealt with as cofactors in the development of metabolic pathways, rather than in a separate chapter. Hormones

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that have well-established metabolic roles are considered in the appropriate areas of metabolism where possible and discussed in terms of their overall integrative effects on metabolism in Chapter 12. Fat-soluble vitamins and hormones, for which clear-cut biochemical mechanisms may not as yet be known, are discussed in the sections on descriptive chemistry. Rather than starting with detailed mechanisms and combining them into a total picture, metabolism is usually presented first from the overall point of view, after which detailed mechanisms are developed to fit the larger picture. Because of the importance of control mechanisms and protein synthesis, the authors have included more material on these subjects. Considerable additional material of clinical interest has also been included.

Suggested readings are limited to a select few that will lead the reader to other helpful sources of information. The authors hope that students will continue to use, for reference purposes, one or more of the excellent comprehensive texts* that provide extensive bibliographies.

The two remaining authors from the third edition (M. E. R. and J. A. H.) regret the retirement of Stephen B. Binkley and will miss his broad knowledge and good judgment, his splendid cooperation, and his friendship. As a new coauthor we welcome Anatoly Bezkorovainy, who brings both extensive knowledge and teaching experience to the venture, and look forward to a productive and pleasant relationship.

Continued suggestions, criticisms, and corrections of the book are appreciated as guides and aids to future revisions.

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M. E. R.
J. A. H.
A. B.

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BASIC BIOCHEMISTRY

CONTENTS

ACIDS, BASES, AND BUFFERS

MOST biologic substances contain functional residues that can accept or donate protons, and the biologic activity of many such substances depends on this phenomenon. According to the **Bronstedt** definition, these substances are either acids or bases. An **acid** is a substance that donates protons to the medium, whereas a **base** is a substance that accepts protons. For example, in the equation HB $\rightleftharpoons$ H$^+$ + B$^-$, HB is an acid and B$^-$ is a base (also called conjugate base). Water reacts with protons, H$^+$ + H$_2$O $\rightleftharpoons$ H$_3$O$^+$, to form the **hydronium ion.** In this case, water is a base and hydronium ion is an acid. In future discussions, H$^+$ will be used, though it should be understood that the hydrogen ion actually exists in solution in the form of hydronium ions.

Biologic fluids such as blood and intracellular fluid contain numerous substances that can be classified as acids or bases, and H$^+$ constantly enters these fluids as a result of metabolic activity. In order to maintain a constant pH within the organism, the various biologic fluids contain buffering systems that serve to blunt the effects of H$^+$ and other substances. When such buffering systems are disturbed, homeostasis, with respect to pH maintenance, is disrupted and severe consequences may overtake the patient. A thorough understanding of the basic properties of acids, bases, and buffers is crucial to the understanding of homeostasis and structure-function relationships in biomolecules.

METHODS OF EXPRESSING CONCENTRATIONS OF ACIDS

Acids may differ in strength, and this strength may be expressed in terms of hydrogen ion concentration as grams (g) per liter (L).

$$0.1 \text{ M HCl has } 0.1 \text{ g H}^+/\text{L}$$
$$\text{H}_2\text{O has } 0.000,0001 \text{ g H}^+/\text{L } (10^{-7})$$

It would be time consuming and complicated to make calculations with such unwieldy numbers. Thus, it is common practice to use the convenient designation **pH,** the negative logarithm of the hydrogen ion concentration, which is expressed symbolically as $pH = -\log H^+$. For water, then

$$\log 10^{-7} = -7$$
$$-\log 10^{-7} = 7$$
$$pH = 7$$

Intracellular pH has been determined by various methods. Depending on the method used and the condition of the cell, researchers obtained values ranging from about 5.2 (slightly acid) to 8.5 (slightly alkaline). The pH of fluids from most cells appears to be close to neutral—that is, pH 7.0.

Problem 1–1

Blood has a $[H^+]^* = 5 \times 10^{-8}$. Calculate its pH.

$$\begin{aligned} pH &= -\log [H^+] \\ &= -\log (5 \times 10^{-8}) \\ &= -(\log 5 + \log 10^{-8}) \\ &= -(0.7 - 8) \\ &= 7.3 \end{aligned}$$

Problem 1–2

Calculate the pH of 0.15 M acetic acid.

$$K_a = 2 \times 10^{-5}$$
$$HAc \rightleftharpoons H^+ + Ac^-$$
$$(0.15 - x) \rightleftharpoons (x) + (x)$$

$$\frac{[x][x]}{[0.15 - x]} = 2 \times 10^{-5}$$

Neglect the concentration of x in the denominator since it is much smaller than 0.15. Then

$$[x]^2 = 0.3 \times 10^{-5} = 3 \times 10^{-6}$$
$$[x] = 1.73 \times 10^{-3} \text{ moles } H^+/L$$
$$pH = -\log [H^+] = -(\log 1.73 + \log 10^{-3}) = -(0.24 - 3.0) = 2.76$$

IONIZATION OF WATER

The pH of water, 7, may be derived from the following relationships.

$$H_2O \rightleftharpoons H^+ + OH^-$$
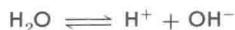
$$\frac{[H^+][OH^-]}{[H_2O]} = K \qquad [H_2O] \text{ is constant}$$

therefore $\qquad [H^+][OH^-] = K_w = 10^{-14}$

since $\qquad [H^+] = [OH^-]$

then $\qquad [H^+]^2 = 10^{-14}$

and $\qquad [H^+] = 10^{-7}$

therefore $\qquad pH = 7$

*Square brackets around an ion indicate molar concentration.

Since $[H^+] = [OH^-]$, we may also write pOH = 7 for water, and hence, pH + pOH = 14. It is thus possible to calculate the hydrogen ion concentration from a pOH, that is a knowledge of hydroxide ion concentration and vice versa. Thus, in a 0.1 M NaOH solution, pOH is 1. pH will then be $14 - 1 = 13$.

The pH values for some common fluids are

0.1 N NaOH	13.0	Urine (average)	4.8–7.5
0.1 N NH₄OH	11.1	0.1 N CH₃COOH	2.9
Pancreatic juice	7.5–8.0	Gastric juice	1.0
Bile	5.5–7.0	0.1 N HCl	1.0
Blood	7.35–7.45		

STRENGTH OF ACIDS

Strong acids are completely ionized into hydrogen ions and their conjugate bases. Thus for HCl, dissociation produces 1 mole of H^+ and 1 mole of Cl^- from 1 mole of HCl. Weak acids dissociate to a much smaller extent, and the amount of hydrogen ion produced is very low compared to the same quantity of a strong acid. Thus, a 1 M acetic acid solution will only produce approximately 0.0045 M H^+. The rest of the acetic acid remains undissociated. For a weak acid HA we can write a dissociation expression

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

and a **dissociation constant**, K_a,

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

The dissociation constant is characteristic for each weak acid or weak base. Thus, for acetic acid, $K_a = 2 \times 10^{-5}$, whereas for ammonium ion, $NH_4^+ \rightleftharpoons NH_3 + H^+$, it is 5×10^{-10}.

It is clear that there is a frequent need to determine or calculate the pH of certain weak acids and bases, and the use of the dissociation constant expression for this purpose is somewhat cumbersome. An equation has been derived to overcome some of these difficulties, which has been called the **Henderson-Hasselbalch** equation in honor of its developers. The basic expression $K_a = [H^+][A^-]/[HA]$ may be rewritten in terms of logarithms as follows:

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

and rearranged to yield

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

Since $-\log [H^+] = $ pH, and if we define $-\log K_a$ as pK, then the final form of the Henderson-Hasselbalch equation can be written as

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

This equation then indicates that the pH of a system will depend on its pK, which is constant, and the ratio of the conjugate base to acid. Another way to state the latter is to think of it as the ratio of the unprotonated form of the acid to the protonated form. If this ratio should be 1, regardless of the absolute concentrations of A^- and HA, then $\log [A^-]/[HA] = 0$, and $pH = pK$. This gives us another definition of pK as being that pH at which the concentrations of the undissociated (protonated) acid and its unprotonated forms are equal. The same situation exists in case of a weak base, where $pH = pK$ when, in case of ammonia, $[NH_3]/[NH_4^+] = 1$.

The situation where $pH = pK$ can never be achieved if one merely has a solution of a weak acid or a weak base. In the case of the weak acid, the concentration of HA will always exceed that of A^- (that is, $pH < pK$); and in the case of weak bases, the pH is always greater than the pK. A pH equal to or exceeding the pK can be achieved for a weak acid by the addition of exogenous A^- in the form of a salt such as NaA. The mixture of HA and A^- is then called a **buffer.**

BUFFERS

Buffers are defined as solutions that resist changes in pH upon the addition of acids or bases. They consist of mixtures of either a weak acid and its conjugate base, or a weak base and its conjugate acid. A buffer has the greatest capacity to resist such changes when its $pH = pK$. The buffering action of various substances is due to the **law of mass action.** If we consider the equilibrium $HA \rightleftharpoons H^+ + A^-$, then, if exogenous H^+ is added, the equilibrium must shift to the left to form HA from most of the H^+ added and any A^- that is present in order to maintain the equilibrium constant as it must be. On the other hand, if OH$^-$ ions are added, these will combine with the H^+ present to form poorly dissociated water. As H^+ is removed from the medium, the equilibrium shifts to the right, resulting in dissociation of additional HA to replenish the H^+ lost. The factor that limits the ability of a buffer to neutralize H^+ will depend on the availability of A^-, whereas the ability of a buffer to neutralize OH$^-$ will depend on the availability of HA.

The Henderson-Hasselbalch equation is extremely useful in defining buffer systems in terms of their pH values and concentrations of buffering molecular species present. (See Problem 1–4.)

Problem 1–3

How many moles of sodium acetate and how many moles of acetic acid are needed to prepare 1 L of a 0.1 M solution having a pH 5.0?

$$pH = 4.6 + \log \frac{[Ac^-]}{[HAc]}$$

$$5.0 = 4.6 + \log \frac{[Ac^-]}{[HAc]}$$

$$\log \frac{[Ac^-]}{[HAc]} = 0.4$$

$$\frac{[Ac^-]}{[HAc]} = 2.5$$

Therefore 2.5 moles of salt would be required for each mole of acid. Since a 0.1 M solution is desired

$$\frac{2.5}{3.5} \times 0.1 = 0.071 \text{ mole sodium acetate}$$

$$\frac{1}{3.5} \times 0.1 = 0.029 \text{ mole acetic acid}$$

Dissolve 0.071 mole sodium acetate and 0.029 mole acetic acid in water and dilute to 1 L.

Problem 1–4

What quantity of 1 M NaOH must be added to 100 ml of a solution of 0.1 M phosphoric acid (H_3PO_4) to give a buffer solution at pH 7.5?

Using the Henderson-Hasselbalch equation, we have
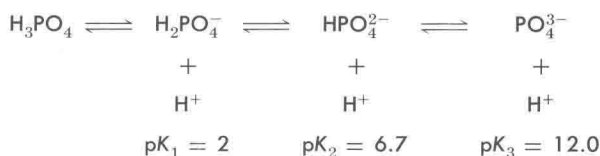
$$7.5 = 6.7 + \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

and $\qquad \dfrac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 6.3 \quad$ or $\quad [HPO_4^{2-}] = 6.3 \times [H_2PO_4^-]$

Since $[HPO_4^{2-}] + [H_2PO_4^-] = 10$ mmoles, that is, 100×0.1, and substituting $6.3 \times [H_2PO_4^-]$ for $[HPO_4^{2-}]$ in $[HPO_4^{2-}] + [H_2PO_4^-] = 10$, we have $7.3 \, [H_2PO_4^-] = 10$, and $[H_2PO_4^-] = 10/7.3 = 1.37$ mmoles, whereas $[HPO_4^{2-}] = 8.63$ mmoles. Now, to convert H_3PO_4 to $H_2PO_4^-$, one would require 10 mmoles of NaOH, and to convert 8.57 mmoles of $H_2PO_4^-$ to HPO_4^{2-}, an additional 8.63 mmoles of NaOH is required, for a total of 18.63 mmoles. This would represent 18.63 ml of a 1 M NaOH solution.

TITRATION CURVES

A titration curve is obtained when the pH of a weak acid or base is measured as a function of added increments of a strong base or acid, respectively. A series of titration curves is shown in Figure 1–1. If attention is focused on the titration curve of acetic acid (designated as HAc), it is seen that the pH is near 3 in the beginning. As NaOH is added, the pH rises rather rapidly until pH 4 is reached. The pH then rises very gradually until a pH of approximately 5.4 is attained. This gradual rise in pH is called a **plateau region** and corresponds to the pH where acetic acid is a good buffering agent. After pH 5.4, the pH resumes its rapid ascent. Notice that the $pK = pH = 4.7$, where 0.5 equivalent of NaOH had been added. It also corresponds to the mathematical inflection point of the titration curve.

A more complicated situation is observed with phosphoric acid. This acid has three hydrogen ions that are titrable per each phosphoric acid molecule, and therefore 3 moles of NaOH would be required to neutralize completely 1 mole of H_3PO_4. The stepwise dissociation of phosphoric acid with the corresponding pK values are

$$H_3PO_4 \;\rightleftharpoons\; H_2PO_4^- \;\rightleftharpoons\; HPO_4^{2-} \;\rightleftharpoons\; PO_4^{3-}$$
$$+ \qquad\qquad + \qquad\qquad +$$
$$H^+ \qquad\qquad H^+ \qquad\qquad H^+$$
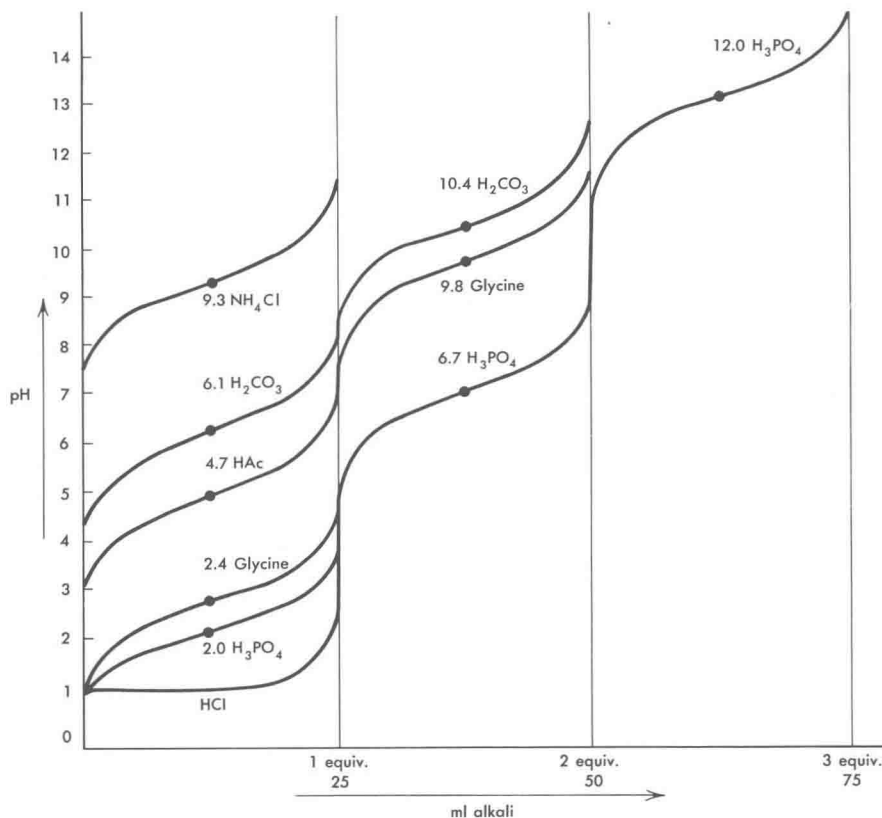$$pK_1 = 2 \qquad pK_2 = 6.7 \qquad pK_3 = 12.0$$

Figure 1-1. Titration curves of several acids. The titrations of 25 ml 0.1 M acid with 0.1 M NaOH.

Thus, where 0.5 equivalents of NaOH are added (Figure 1-1), the pH = pK_1 = 2.0, and there is an equimolar quantity of H_3PO_4 and $H_2PO_4^-$. After the addition of 1 equivalent of NaOH, only $H_2PO_4^-$ is present. At pK_2 = 6.7, there are equal amounts of $H_2PO_4^-$ and HPO_4^{2-}, and after the addition of 2 equivalents of NaOH, only HPO_4^{2-} is present. At pH 12 (2.5 equivalents of NaOH added), there are equal amounts of HPO_4^{2-} and PO_4^{3-}, whereas after the addition of 3 equivalents of NaOH, only PO_4^{3-} can be found.

SUMMARY

The understanding of acid, base, and buffer chemistry is crucial to the understanding of the biological activities of biomolecules and homeostatic mechanisms. Acids and bases are defined as those substances that donate and accept protons, respectively. For weakly dissociated acids, it is possible to write an expression showing its reversible dissociation into hydrogen ions and its conjugate base. The Henderson-Hasselbalch equation, pH = pK + log unprotonated form/protonated form, may be derived from such an expression. The Henderson-Hasselbalch equation is used to determine pH values and solute concentrations of buffer solutions. Buffers are solutions that resist changes in pH and consist of either a weak acid and its

conjugate base, or a weak base and its conjugate acid. The pH of a buffer solution depends on its pK value, defined as that pH at which the concentration of the protonated and the unprotonated forms of a weak acid or base are equal, and the ratio of the unprotonated to the protonated forms is equal to 1. Titration curves of buffers indicate their pK values, as well as their buffering capacities.

SELECTED READINGS

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Dawes, E. A.: *Quantitative Problems in Biochemistry,* 4th and 5th eds. Williams and Wilkins, Baltimore, 1969 and 1975.

Chapter 2

BIOENERGETICS

MANY of the chemical reactions taking place in the organism are concerned with the generation of energy in the form of heat and the so-called **"high energy"** compounds. The fuel for the generation of such energy is the food that the organism ingests, the most important examples being carbohydrates, fats, and proteins. The processes whereby food is converted into energy are collectively called **metabolism,** though the term is also applied to processes that are not concerned with the production of energy, such as biosynthetic processes. A more precise name for metabolic processes leading to energy production is **catabolism,** which indicates degradation of biomolecules as opposed to **anabolism,** which indicates biosynthesis. The topic of bioenergetics deals with the various forms of energy in the biologic systems and the quantitative relationships among them and other physical-chemical parameters.

The standard unit of energy is the **calorie** (cal), defined as that amount of energy necessary to raise the temperature of 1 g water from 14.5 to 15.5°C; 1000 cal equals 1 kilocalorie (kcal); 1 cal is also equal to 4.18 joules (J).

FIRST LAW OF THERMODYNAMICS

This law states that energy can be neither created nor destroyed. Mathematically it may be stated

$$q = \Delta E + w \quad \text{or} \quad \Delta E = q - w$$

which simply means that if a quantity of energy, q, is added to a system, the system will do work, w, and/or increase its internal energy by a quantity defined as ΔE. When the system is enclosed so that the volume cannot change, there will be no work

performed of the pressure-volume type; then $\Delta E = q_v$. On the other hand, where the system is maintained at a constant pressure, that is, volume changes are possible, it is possible to write $q_p = \Delta E + p\,\Delta V$, where p is pressure and ΔV is the volume change. The sum of $\Delta E + p\,\Delta V$ is defined as ΔH, also called **enthalpy** or **heat content,** so that $q_p = \Delta H$.

Both ΔE and ΔH depend only on the initial and final states of the system and not on the pathway taken to reach the final state. Because of this, they may be measured in **calorimeters,** that is, devices that record the absorption or release of heat. A constant volume calorimeter will give ΔE, whereas a constant pressure calorimeter gives ΔH. ΔE and ΔH may be positive or negative. If heat is absorbed by a chemical reaction, ΔE or ΔH are positive. If heat is given off, as in a combustion process, ΔE or ΔH is negative. Such reactions are called **endothermic** and **exothermic,** respectively. ΔH and ΔE are related to each other via $\Delta H = \Delta E + \Delta nRT$ where Δn is the change in the number of moles of gaseous reactants and products, R is the gas constant, and T is absolute temperature.

The first law of thermodynamics is the basis for thermochemical calculations that utilize heats of combustion and heats of formation of various organic and inorganic compounds. More important for biochemists, however, the first law allows the determination of energy equivalents for foods and various substances in the human organism, thus providing valuable nutritional information. Thus, for the oxidation of 1 mole of glucose, as determined in a constant-pressure calorimeter,

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,H_2O + 6\,CO_2 \tag{2-1}$$

there are 673 kcal released, or $\Delta H = -673$ kcal. It is assumed that this represents the calorie value of ingested glucose. Table 2–1 lists the caloric values available to man of the three major food types and alcohol.

The exact value of energy provided by a protein will depend on its amino acid content; for example, glycine gives 234 kcal/mole, whereas tyrosine gives 1070 kcal/mole upon combustion in a calorimeter. The same applies to fats, though to a much smaller extent. An adult male leading a sedentary lifestyle requires approximately 2000 kcal/day, whereas a female requires about 1800 kcal/day. The requirements are, of course, greater for persons performing strenuous work, for example, for lumberjacks the recommended daily intake is 5000 kcal/day.

Calorimeters to measure heat production have been devised for animals and even human beings. Clinically, this type of an apparatus has been used to determine a

Table 2–1
Energies Provided by
Major Types of Food Substances

Carbohydrate (starch)	4.1 kcal/g
Carbohydrate (glucose)	3.8 kcal/g
Fats	9.4 kcal/g
Proteins (average)	4.0 kcal/g
Ethanol	7.0 kcal/g