

Chemical and Biological Foundations of Biochemistry

The discipline of biochemistry developed as chemists began to study the molecules of cells, tissues, and body fluids and physicians began to look for the molecular basis of various diseases. Today, the practice of medicine depends on understanding the roles and interactions of the enormous number of different chemicals enabling our bodies to function. The task is less overwhelming if one knows the properties, nomenclature and function of classes of compounds, such as carbohydrates and enzymes. The intent of this section is to review some of this information in a context relevant to medicine. Students enter medical school with different scientific backgrounds and some of the information in this section will, therefore, be familiar to many students.

We begin by discussing the relationship of metabolic acids and buffers to blood pH in Chapter 4. Chapter 5 focuses on the nomenclature, structure, and some of the properties of the major classes of compounds found in the human body. The structure of a molecule determines its function and its fate, and the common name of a compound can often tell you something about its structure.

Proteins are linear chains of amino acids that fold into complex 3-dimensional structures. They function in the maintenance of cellular and tissue structure and the transport and movement of molecules. Some proteins are enzymes, which are catalysts that enormously increase the rate of chemical reactions in the body. Chapters 6 and 7 describe the amino acids and their interactions within proteins that provide proteins with a flexible and functional 3-dimensional structure. Chapters 8 and 9 describe the properties, functions, and regulation of enzymes.

Our proteins and other compounds function within a specialized environment defined by their location in cells or body fluids. Their ability to function is partially dependent on membranes that restrict the free movement of molecules. Chapter 10 includes a brief review of the components of cells, their organization into subcellular organelles, and the manner in which various types of molecules move into cells and between compartments within a cell.

In a complex organism like the human, different cell types carry out different functions. This specialization of function requires cells to communicate with each other. One of the ways they communicate is through secretion of chemical messengers that carry a signal to another cell. In Chapter 11, we consider some of the principles of cell signaling and describe some of the chemical messenger systems.

Both in this book and in medical practice, you will need to interconvert different units used for the weight and size of compounds and for their concentration in blood and other fluids. Table 1 provides definitions of some of the units used for these interconversions.



The nomenclature used to describe patients may include the name of a class of compounds. For example a patient with diabetes mellitus who has hyperglycemia has hyper (high) concentrations of carbohydrates (glyc) in her blood (emia).



From a biochemist's point of view, most metabolic diseases are caused by enzymes and other proteins that malfunction and the pharmacological drugs used to treat these diseases correct that malfunction. For example, individuals with atherosclerosis, who have elevated blood cholesterol levels, are treated with a drug that inhibits an enzyme in the pathway for cholesterol synthesis. Even a bacterial infection can be considered a disease of protein function, if one considers the bacterial toxins that are proteins, the enzymes in our cells affected by these toxins, and the proteins involved in the immune response when we try to destroy these bacteria.



Di Abietes had an elevated blood glucose level of 684 mg/dL. What is the molar concentration of glucose in Di's blood? (Hint: the molecular weight of glucose ($C_6H_{12}O_6$) is 180 grams per mole.)



mg/dL is the common way clinicians in the United States express blood glucose concentration. A concentration of 684 mg/dL is 684 mg per 100 mL of blood, or 6,480 mg per one liter (L), or 6.48 g/L. If 6.84 g/L is divided by 180 grams per mole, one obtains a value of 0.038 mol/L, which is 0.038 M, or 38 mM.

Table 1 Common Units Expressed in Equivalent Ways

1 M	1 mol/L	molecular weight in g/L
1 mM	1 millimol/L	10^{-3} mol/L
1 mM	1 micromol/L	10^{-6} mol/L
1 μ M	1 nanomol/L	10^{-9} mol/L
1 mL	1 milliliter	10^{-3} L
1 mg%	1 mg/100 mL	10^{-3} g/100 mL
1 mg/dL	1 mg/100 mL	10^{-3} g/100 mL
1 mEq/L	1 milliequivalent/L	mM X valence of ion
1 kg	1000 g	2.2 lbs (pounds)
1 cm	10^{-2} m	0.394 inches

4 Water, Acids, Bases, and Buffers

Approximately 60% of our body is water. It acts as a solvent for the substances we need, such as K^+ , glucose, adenosine triphosphate (ATP), and proteins. It is important for the transport of molecules and heat. Many of the compounds produced in the body and dissolved in water contain chemical groups that act as acids or bases, releasing or accepting hydrogen ions. The hydrogen ion content and the amount of body water are controlled to maintain a constant environment for the cells called homeostasis (same state) (Fig. 4.1). Significant deviations from a constant environment, such as acidosis or dehydration, may be life-threatening. This chapter describes the role of water in the body and the buffer systems used by the body to protect itself from acids and bases produced from metabolism.

Water. Water is distributed between intracellular and extracellular compartments, the latter comprising interstitial fluids, blood, and lymph. Because water is a **dipolar molecule** with an uneven distribution of electrons between the hydrogen and oxygen atoms, it forms hydrogen bonds with other polar molecules and acts as a **solvent**.

The pH of water. Water dissociates to a slight extent to form hydrogen (H^+) and hydroxyl (OH^-) ions. The concentration of hydrogen ions determines the acidity of the solution, which is expressed in terms of **pH**. The pH of a solution is the negative log of its hydrogen ion concentration.

Acids and Bases. An **acid** is a substance that can **release hydrogen ions** (protons), and a **base** is a substance that can **accept hydrogen ions**. When dissolved in water, almost all the molecules of a **strong acid** dissociate and release their hydrogen ions, but only a small percentage of the total molecules of a weak acid dissociate. A **weak acid** has a characteristic **dissociation constant, K_a** . The relationship between the pH of a solution, the K_a of an acid, and the extent of its dissociation are given by the **Henderson-Hasselbalch equation**.

Buffers. A buffer is a mixture of an **undissociated acid** and its **conjugate base** (the form of the acid having lost its proton). It causes a solution to resist changes in pH when either H^+ or OH^- is added. A buffer has its greatest buffering capacity in the pH range near its **pK_a** (the negative log of its K_a). Two factors determine the effectiveness of a buffer, its pK_a relative to the pH of the solution and its concentration.

Metabolic Acids and Bases. Normal metabolism generates CO_2 , **metabolic acids** (e.g., **lactic acid** and **ketone bodies**) and **inorganic acids** (e.g., **sulfuric acid**). The major source of acid is CO_2 , which reacts with water to produce **carbonic acid**. To maintain the pH of body fluids in a range compatible with life, the body has **buffers** such as **bicarbonate, phosphate, and hemoglobin** (see Fig 4.1). Ultimately, **respiratory mechanisms** remove carbonic acid through the **expiration of CO_2** , and the **kidneys excrete acid** as **ammonium ion (NH_4^+)** and other ions.

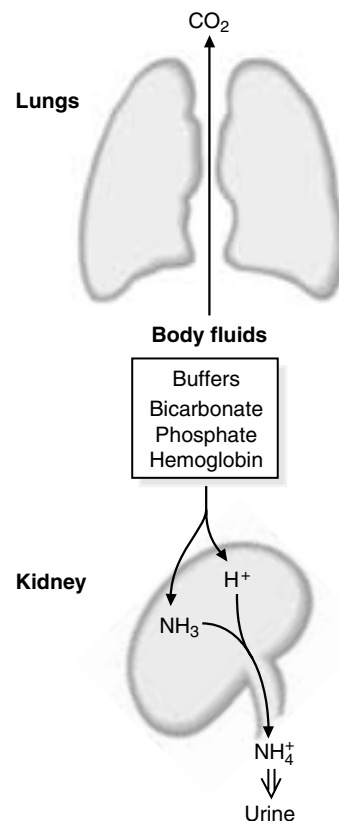


Fig. 4.1. Maintenance of body pH. The body produces approximately 13 to 22 moles of acid per day from normal metabolism. The body protects itself against this acidity by buffers that maintain a neutral pH and by the expiration of CO_2 through the lungs and the excretion of NH_4^+ and other ions through the kidney.



Di Abietes has a ketoacidosis. When the amount of insulin she injects is inadequate, she remains in a condition similar to a fasting state even though she ingests food (see Chapters 2 and 3). Her liver continues to metabolize fatty acids to the ketone bodies acetoacetic acid and β -hydroxybutyric acid. These compounds are weak acids that dissociate to produce anions (acetate and β -hydroxybutyrate, respectively) and hydrogen ions, thereby lowering her blood and cellular pH below the normal range.



Percy Veere is a 59-year-old school teacher who persevered through a period of malnutrition associated with mental depression precipitated by the death of his wife (see Chapters 1 and 3). Since his recovery, he has been looking forward to an extended visit from his grandson.



THE WAITING ROOM



Dianne (Di) Abietes is a 26-year-old woman who was diagnosed with type 1 diabetes mellitus at the age of 12 years. She has an absolute insulin deficiency resulting from autoimmune destruction of the β -cells of her pancreas. As a result, she depends on daily injections of insulin to prevent severe elevations of glucose and ketone bodies in her blood.

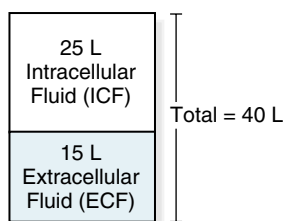
When **Di Abietes** could not be aroused from an afternoon nap, her roommate called an ambulance, and Di was brought to the emergency room of the hospital in a coma. Her roommate reported that Di had been feeling nauseated and drowsy and had been vomiting for 24 hours. Di is clinically dehydrated, and her blood pressure is low. Her respirations are deep and rapid, and her pulse rate is rapid. Her breath has the “fruity” odor of acetone.

Blood samples are drawn for measurement of her arterial blood pH, arterial partial pressure of carbon dioxide (P_{aCO_2}), serum glucose, and serum bicarbonate (HCO_3^-). In addition, serum and urine are tested for the presence of ketone bodies, and Di is treated with intravenous normal saline and insulin. The laboratory reports that her blood pH is 7.08 (reference range = 7.36–7.44) and that ketone bodies are present in both blood and urine. Her blood glucose level is 648 mg/dL (reference range = 80–110 after an overnight fast, and no higher than 200 in a casual glucose sample taken without regard to the time of a last meal).



Dennis “the Menace” Veere, age 3 years, was brought to the emergency department by his grandfather, **Percy Veere**. While Dennis was visiting his grandfather, he climbed up on a chair and took a half-full 500-tablet bottle of 325-mg aspirin (acetylsalicylic acid) tablets from the kitchen counter. Mr. Veere discovered Dennis with a mouthful of aspirin, which he removed, but he could not tell how many tablets Dennis had already swallowed. When they arrived at the emergency room, the child appeared bright and alert, but Mr. Veere was hyperventilating.

A. Total body water



B. Extracellular fluid



Fig. 4.2. Fluid compartments in the body based on an average 70-kg man.

I. WATER

Water is the solvent of life. It bathes our cells, dissolves and transports compounds in the blood, provides a medium for movement of molecules into and throughout cellular compartments, separates charged molecules, dissipates heat, and participates in chemical reactions. Most compounds in the body, including proteins, must interact with an aqueous medium function. In spite of the variation in the amount of water we ingest each day and produce from metabolism, our body maintains a nearly constant amount of water that is approximately 60% of our body weight (Fig. 4.2).

A. Fluid Compartments in the Body

Total body water is roughly 50 to 60% of body weight in adults and 75% of body weight in children. Because fat has relatively little water associated with it, obese people tend to have a lower percentage of body water than thin people, women tend to have a lower percentage than men, and older people have a lower percentage than younger people.

Approximately 40% of the total body water is intracellular and 60% extracellular. The extracellular water includes the fluid in plasma (blood after the cells have been removed) and interstitial water (the fluid in the tissue spaces, lying between

cells). Transcellular water is a small, specialized portion of extracellular water that includes gastrointestinal secretions, urine, sweat, and fluid that has leaked through capillary walls because of such processes as increased hydrostatic pressure or inflammation.

B. Hydrogen Bonds in Water

The dipolar nature of the water (H_2O) molecule allows it to form hydrogen bonds, a property that is responsible for the role of water as a solvent. In H_2O , the oxygen atom has two unshared electrons that form an electron dense cloud around it. This cloud lies above and below the plane formed by the water molecule (Fig. 4.3). In the covalent bond formed between the hydrogen and oxygen atoms, the shared electrons are attracted toward the oxygen atom, thus giving the oxygen atom a partial negative charge and the hydrogen atom a partial positive charge. As a result, the oxygen side of the molecule is much more electronegative than the hydrogen side, and the molecule is dipolar.

Both the hydrogen and oxygen atoms of the water molecule form hydrogen bonds and participate in hydration shells. A hydrogen bond is a weak noncovalent interaction between the hydrogen of one molecule and the more electronegative atom of an acceptor molecule. The oxygen of water can form hydrogen bonds with two other water molecules, so that each water molecule is hydrogen-bonded to approximately four close neighboring water molecules in a fluid three-dimensional lattice (see Fig. 4.3).

1. WATER AS A SOLVENT

Polar organic molecules and inorganic salts can readily dissolve in water because water also forms hydrogen bonds and electrostatic interactions with these molecules. Organic molecules containing a high proportion of electronegative atoms (generally oxygen or nitrogen) are soluble in water because these atoms participate in hydrogen bonding with water molecules (Fig. 4.4). Chloride (Cl^-), bicarbonate (HCO_3^-), and other anions are surrounded by a hydration shell of water molecules arranged with their hydrogen atoms closest to the anion. In a similar fashion, the oxygen atom of water molecules interacts with inorganic cations such as Na^+ and K^+ to surround them with a hydration shell.

Although hydrogen bonds are strong enough to dissolve polar molecules in water and to separate charges, they are weak enough to allow movement of water and solutes. The strength of the hydrogen bond between two water molecules is only approximately 4 kcal, roughly 1/20th of the strength of the covalent O–H bond in the water molecule. Thus, the extensive water lattice is dynamic and has many strained bonds that are continuously breaking and reforming. The average hydrogen bond between water molecules lasts only about 10 psec (1 picosecond is 10^{-12} sec), and each water molecule in the hydration shell of an ion stays only 2.4 nsec (1 nanosecond = 10^{-9} sec). As a result, hydrogen bonds between water molecules and polar solutes continuously dissociate and reform, thereby permitting solutes to move through water and water to pass through channels in cellular membranes.

2. WATER AND THERMAL REGULATION

The structure of water also allows it to resist temperature change. Its heat of fusion is high, so a large drop in temperature is needed to convert liquid water to the solid state of ice. The thermal conductivity of water is also high, thereby facilitating heat dissipation from high energy-using areas such as the brain into the blood and the total body water pool. Its heat capacity and heat of vaporization are remarkably high; as liquid water is converted to a gas and evaporates from the

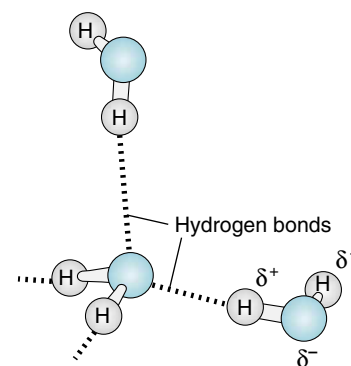


Fig. 4.3. Hydrogen bonds between water molecules. The oxygen atoms are shown in blue.

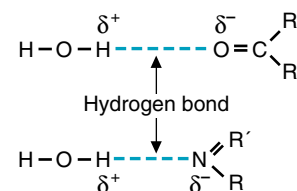


Fig. 4.4. Hydrogen bonds between water and polar molecules. R denotes additional atoms.

Table 4.1. Distribution of ions in body fluids.

	ECF* mmol/L	ICF
Cations		
Na ⁺	145	12
K ⁺	4	150
Anions		
Cl ⁻	105	5
HCO ₃ ⁻	25	12
Inorganic Phosphate	2	100

*The content of inorganic ions is very similar in plasma and interstitial fluid, the two components of the extracellular fluid. (ECF, extracellular fluid; ICF, intracellular fluid).



In the emergency room, **Di Abietes** was rehydrated with intravenous saline, which is a solution of 0.9% NaCl. Why was saline used instead of water?



Di Abietes has an osmotic diuresis. Because her blood levels of glucose and ketone bodies are so high, these compounds are passing from the blood into the glomerular filtrate in the kidneys and then into the urine. As a consequence of the high osmolality of the glomerular filtrate, much more water is being excreted in the urine than usual. Thus, Di has polyuria (increased urine volume). As a result of water lost from the blood into the urine, water passes from inside cells into the interstitial space and into the blood, resulting in an intracellular dehydration. The dehydrated cells in the brain are unable to carry out their normal functions. As a result, Di is in a coma.

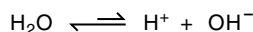


Fig. 4.5. The dissociation of water.



The laboratory reported that **Di Abietes'** blood pH was 7.08 (reference range = 7.37–7.43) What was the [H⁺] in her blood compared with the concentration at a normal pH of 7.4?

Equation 4.1. Definition of pH.

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

skin, we feel a cooling effect. Water responds to the input of heat by decreasing the extent of hydrogen bonding and to cooling by increasing the bonding between water molecules.

C. Electrolytes

Both extracellular fluid (ECF) and intracellular fluid (ICF) contain electrolytes, a general term applied to bicarbonate and inorganic anions and cations. The electrolytes are unevenly distributed between compartments; Na⁺ and Cl⁻ are the major electrolytes in the ECF (plasma and interstitial fluid), and K⁺ and phosphates such as HPO₄⁻² are the major electrolytes in cells (Table 4.1) This distribution is maintained principally by energy-requiring transporters that pump Na⁺ out of cells in exchange for K⁺ (see Chapter 10).

D. Osmolality and Water Movement

Water distributes between the different fluid compartments according to the concentration of solutes, or osmolality, of each compartment. The osmolality of a fluid is proportionate to the total concentration of all dissolved molecules, including ions, organic metabolites, and proteins (usually expressed as milliosmoles (mOsm)/kg water). The semipermeable cellular membrane that separates the extracellular and intracellular compartments contains a number of ion channels through which water can freely move, but other molecules cannot. Likewise, water can freely move through the capillaries separating the interstitial fluid and the plasma. As a result, water will move from a compartment with a low concentration of solutes (lower osmolality) to one with a higher concentration to achieve an equal osmolality on both sides of the membrane. The force it would take to keep the same amount of water on both sides of the membrane is called the osmotic pressure.

As water is lost from one fluid compartment, it is replaced with water from another to maintain a nearly constant osmolality. The blood contains a high content of dissolved negatively charged proteins and the electrolytes needed to balance these charges. As water is passed from the blood into the urine to balance the excretion of ions, the blood volume is repleted with water from interstitial fluid. When the osmolality of the blood and interstitial fluid is too high, water moves out of the cells. The loss of cellular water also can occur in hyperglycemia, because the high concentration of glucose increases the osmolality of the blood.

II. ACIDS AND BASES

Acids are compounds that donate a hydrogen ion (H⁺) to a solution, and bases are compounds (such as the OH⁻ ion) that accept hydrogen ions. Water itself dissociates to a slight extent, generating hydrogen ions (H⁺), which are also called protons, and hydroxide ions (OH⁻) (Fig. 4.5). The hydrogen ions are extensively hydrated in water to form species such as H₃O⁺, but nevertheless are usually represented as simply H⁺. Water itself is neutral, neither acidic nor basic.

A. The pH of Water

The extent of dissociation by water molecules into H⁺ and OH⁻ is very slight, and the hydrogen ion concentration of pure water is only 0.0000001 M, or 10⁻⁷ mol/L. The concentration of hydrogen ions in a solution is usually denoted by the term *pH*, which is the negative log₁₀ of the hydrogen ion concentration expressed in mol/L (Equation 4.1). Therefore, the pH of pure water is 7.

The dissociation constant for water, K_d, expresses the relationship between the hydrogen ion concentration [H⁺], the hydroxide ion concentration [OH⁻],

and the concentration of water $[H_2O]$ at equilibrium (Equation 4.2). Because water dissociates to such a small extent, $[H_2O]$ is essentially constant at 55.5 M. Multiplication of the K_d for water (approximately 1.8×10^{-16} M) by 55.5 M gives a value of approximately 10^{-14} (M)², which is called the ion product of water (K_w) (Equation 4.3). Because K_w , the product of $[H^+]$ and $[OH^-]$, is always constant, a decrease of $[H^+]$ must be accompanied by a proportionate increase of $[OH^-]$.

A pH of 7 is termed neutral because $[H^+]$ and $[OH^-]$ are equal. Acidic solutions have a greater hydrogen ion concentration and a lower hydroxide ion concentration than pure water (pH < 7.0), and basic solutions have a lower hydrogen ion concentration and a greater hydroxide ion concentration (pH > 7.0).

B. Strong and Weak Acids

During metabolism, the body produces a number of acids that increase the hydrogen ion concentration of the blood or other body fluids and tend to lower the pH (Table 4.2). These metabolically important acids can be classified as weak acids or strong acids by their degree of dissociation into a hydrogen ion and a base (the anion component). Inorganic acids such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) are strong acids that dissociate completely in solution (Fig. 4.6). Organic acids containing carboxylic acid groups (e.g., the ketone bodies acetoacetic acid and β -hydroxybutyric acid) are weak acids that dissociate only to a limited extent in water. In general, a weak acid (HA), called the conjugate acid, dissociates into a hydrogen ion and an anionic component (A^-), called the conjugate base. The name of an undissociated acid usually ends in “ic acid” (e.g., acetoacetic acid) and the name of the dissociated anionic component ends in “ate” (e.g., acetoacetate).

The tendency of the acid (HA) to dissociate and donate a hydrogen ion to solution is denoted by its K_a , the equilibrium constant for dissociation of a weak acid (Equation 4.4). The higher the K_a , the greater is the tendency to dissociate a proton.

In the Henderson-Hasselbalch equation, the formula for the dissociation constant of a weak acid is converted to a convenient logarithmic equation (Equation 4.5). The term pK_a represents the negative log of K_a . If the pK_a for a weak acid is known, this

Equation 4.2. Dissociation of water.

$$K_d = \frac{[H^+][OH^-]}{[H_2O]}$$

Equation 4.3. The ion product of water.

$$K_w = [H^+][OH^-] = 1 \times 10^{-14}$$

Equation 4.4. The K_a acid

For the reaction
 $HA \leftrightarrow A^- + H^+$

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



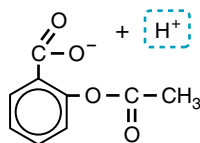
0.9% NaCl is 0.9 g NaCl/100 mL, equivalent to 9 g/L. NaCl has a molecular weight of 58 g/mole, so the concentration of NaCl in isotonic saline is 0.155 M, or 155 mM. If all of the NaCl were dissociated into Na^+ and Cl^- ions, the osmolality would be 310 mOsm/kg water. Because NaCl is not completely dissociated and some of the hydration shells surround undissociated NaCl molecules, the osmolality of isotonic saline is approximately 290 mOsm/kg H_2O . The osmolality of plasma, interstitial fluids, and ICF is also approximately 290 mOsm/kg water, so that no large shifts of water or swelling occur when isotonic saline is given intravenously.

Equation 4.5. The Henderson-Hasselbalch equation

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



Dennis Veere has ingested an unknown number of acetylsalicylic acid (aspirin) tablets. Acetylsalicylic acid is rapidly converted to salicylic acid in the body. The initial effect of aspirin is to produce a respiratory alkalosis caused by a stimulation of the “metabolic” central respiratory control center in the hypothalamus. This increases the rate of breathing and the expiration of CO_2 . This is followed by a complex metabolic acidosis caused partly by the dissociation of salicylic acid (salicylic acid \leftrightarrow salicylate⁻ + H^+ , $pK_a = \sim 3.5$).



Acetylsalicylate

Salicylate also interferes with mitochondrial ATP production, resulting in increased generation of CO_2 and accumulation of lactate and other organic acids in the blood. Subsequently, salicylate may impair renal function, resulting in the accumulation of strong acids of metabolic origin, such as sulfuric acid and phosphoric acid. Usually, children who ingest toxic amounts of aspirin are acidotic by the time they arrive in the emergency room.



From inspection, you can tell that her $[H^+]$ is greater than normal, but less than 10 times higher. A 10-fold change in $[H^+]$ changes the pH by 1 unit. For Di, the pH of 7.08 = $-\log [H^+]$, and therefore her $[H^+]$ is $1 \times 10^{-7.08}$. To calculate her $[H^+]$, express -7.08 as $-8 + 0.92$. The antilog to the base 10 of 0.92 is 8.3. Thus, her $[H^+]$ is 8.3×10^{-8} compared to 4.0×10^{-8} at pH 7.4, or slightly more than double the normal value.

Table 4.2. Acids in the Blood of a Healthy Individual.

Acid	Anion	pK _a	Major Sources
Strong acid Sulfuric acid (H ₂ SO ₄)	Sulfate SO ₄ ⁻²	Completely Dissociated	Dietary sulfate and S-containing amino acids
Weak acid Carbonic acid (R-COOH)	Bicarbonate (R-COO ⁻)	3.80	CO ₂ from TCA cycle
Lactic acid (R-COOH)	Lactate (R-COO ⁻)	3.73	Anaerobic glycolysis
Pyruvic acid (R-COOH)	Pyruvate (R-COO ⁻)	2.39	Glycolysis
Citric acid (R-3COOH)	Citrate (R-3COO ⁻)	3.13; 4.76; 6.40	TCA cycle and diet (e.g., citrus fruits)
Acetoacetic acid (R-COOH)	Acetoacetate (R-COO ⁻)	3.62	Fatty acid oxidation to ketone bodies
β-Hydroxybutyric acid (R-COOH)	β-Hydroxybutyrate (R-COO ⁻)	4.41	Fatty acid oxidation to ketone bodies
Acetic acid (R-COOH)	Acetate (R-COO ⁻)	4.76	Ethanol metabolism
Dihydrogen phosphate (H ₂ PO ₄ ⁻)	Monohydrogen phosphate (HPO ₄ ⁻²)	6.8	Dietary organic phosphates
Ammonium ion (NH ₄ ⁺)	Ammonia (NH ₃)	9.25	Dietary nitrogen-containing compounds

equation can be used to calculate the ratio of the unprotonated to the protonated form at any pH. From this equation, you can see that a weak acid is 50% dissociated at a pH equal to its pK_a.

Most of the metabolic carboxylic acids have pK_as between 2 and 5, depending on the other groups on the molecule (see Table 4.2). The pK_a reflects the strength of an acid. Acids with a pK_a of 2 are stronger acids than those with a pK_a of 5 because, at any pH, a greater proportion is dissociated.

III. BUFFERS

Buffers, which consist of a weak acid and its conjugate base, cause a solution to resist changes in pH when hydrogen ions or hydroxide ions are added. In Figure 4.7, the pH of a solution of the weak acid acetic acid is graphed as a function of the

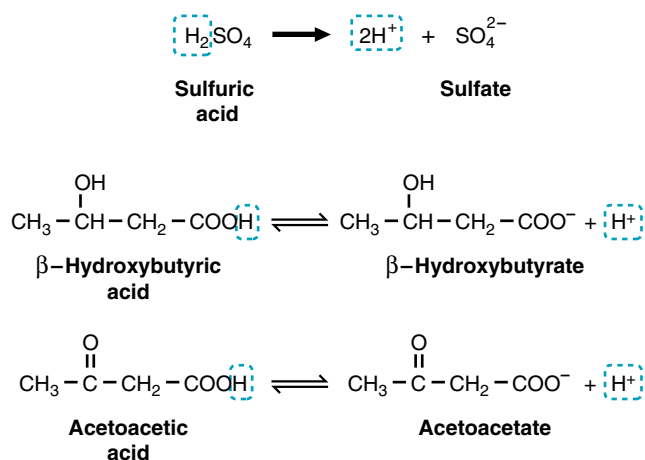


Fig. 4.6. Dissociation of acids. Sulfuric acid is a strong acid that dissociates into H⁺ ions and sulfate. The ketone bodies acetoacetic acid and β-hydroxybutyric acid are weak acids that partially dissociate into H⁺ and their conjugate bases.

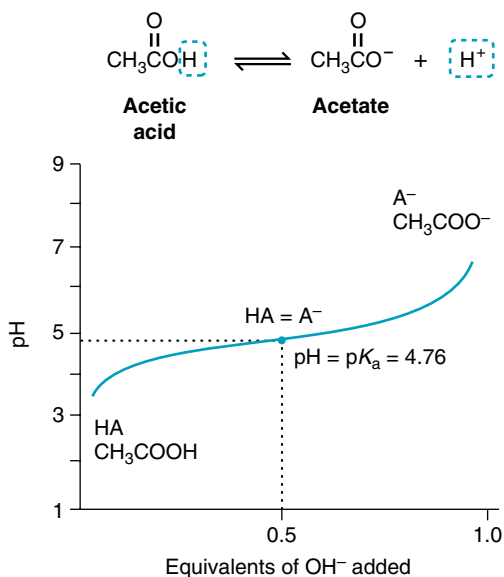


Fig. 4.7. The titration curve for acetic acid.

amount of OH^- that has been added. The OH^- is expressed as equivalents of total acetic acid present in the dissociated and undissociated forms. At the midpoint of this curve, 0.5 equivalents of OH^- have been added, and half of the conjugate acid has dissociated so that $[\text{A}^-]$ equals $[\text{HA}]$. This midpoint is expressed in the Henderson-Hasselbalch equation as the pK_a , defined as the pH at which 50% dissociation occurs. As you add more OH^- ions and move to the right on the curve, more of the conjugate acid molecules (HA) dissociate to generate H^+ ions, which combine with the added OH^- ions to form water. Consequently, only a small increase in pH results. If you add hydrogen ions to the buffer at its pK_a (moving to the left of the midpoint in Fig. 4.7), conjugate base molecules (A^-) combine with the added hydrogen ions to form HA, and almost no decrease in pH occurs.

As can be seen from Figure 4.7, a buffer can only compensate for an influx or removal of hydrogen ions within approximately 1 pH unit of its pK_a . As the pH of a buffered solution changes from the pK_a to one pH unit below the pK_a , the ratio of $[\text{A}^-]$ to HA changes from 1:1 to 1:10. If more hydrogen ions were added, the pH would fall rapidly because relatively little conjugate base remains. Likewise, at 1 pH unit above the pK_a of a buffer, relatively little undissociated acid remains. More concentrated buffers are more effective simply because they contain a greater total number of buffer molecules per unit volume that can dissociate or recombine with hydrogen ions.

IV. METABOLIC ACIDS AND BUFFERS

An average rate of metabolic activity produces roughly 22,000 mEq acid per day. If all of this acid were dissolved at one time in unbuffered body fluids, their pH would be less than 1. However, the pH of the blood is normally maintained between 7.36 and 7.44, and intracellular pH at approximately 7.1 (between 6.9 and 7.4). The widest range of extracellular pH over which the metabolic functions of the liver, the beating of the heart, and conduction of neural impulses can be maintained is 6.8 to 7.8. Thus, until the acid produced from metabolism can be excreted as CO_2 in expired air and as ions in the urine, it needs to be buffered in the body fluids. The major buffer systems in the body are: the bicarbonate–carbonic acid buffer system, which operates principally in extracellular fluid; the hemoglobin buffer system in red blood cells; the phosphate buffer system in all types of cells; and the protein buffer system of cells and plasma.

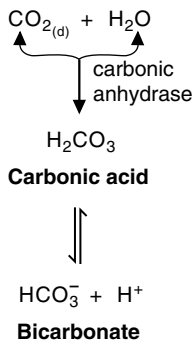


Fig. 4.8. The bicarbonate buffer system.



The pK_a for dissociation of bicarbonate anion (HCO_3^-) into H^+ and carbonate (CO_3^{2-}) is 9.8; therefore, only trace amounts of carbonate exist in body fluids.

Equation 4.6: The Henderson-Hasselbalch equation for the bicarbonate buffer system

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3] + [\text{CO}_2(\text{d})]}$$

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{0.03\text{Paco}_2}$$

where $[\text{CO}_2(\text{d})]$ is the concentration of dissolved CO_2 , the $[\text{HCO}_3^-]$ is expressed as mEq/mL, and Paco_2 as mm Hg. The constant of 0.03 is a solubility constant that also corrects for the use of different units.

A. The Bicarbonate Buffer System

The major source of metabolic acid in the body is the gas CO_2 , produced principally from fuel oxidation in the TCA cycle. Under normal metabolic conditions, the body generates more than 13 moles of CO_2 per day (approximately 0.5–1 kg). CO_2 dissolves in water and reacts with water to produce carbonic acid, H_2CO_3 , a reaction accelerated by the enzyme carbonic anhydrase (Fig. 4.8). Carbonic acid is a weak acid that partially dissociates into H^+ and bicarbonate anion, HCO_3^- .

Carbonic acid is both the major acid produced by the body, and its own buffer. The pK_a of carbonic acid itself is only 3.8, so at the blood pH of 7.4 it is almost completely dissociated and theoretically unable to buffer and generate bicarbonate. However, carbonic acid can be replenished from CO_2 in body fluids and air because the concentration of dissolved CO_2 in body fluids is approximately 500 times greater than that of carbonic acid. As base is added and H^+ is removed, H_2CO_3 dissociates into hydrogen and bicarbonate ions, and dissolved CO_2 reacts with H_2O to replenish the H_2CO_3 (see Fig. 4.8). Dissolved CO_2 is in equilibrium with the CO_2 in air in the alveoli of the lungs, and thus the availability of CO_2 can be increased or decreased by an adjustment in the rate of breathing and the amount of CO_2 expired. The pK_a for the bicarbonate buffer system in the body thus combines K_h (the hydration constant for the reaction of water and CO_2 to form H_2CO_3) with the chemical pK_a to obtain the value of 6.1 used in the Henderson-Hasselbalch equation (Equation 4.6). To use the terms for blood components measured in the emergency room, the dissolved CO_2 is expressed as a fraction of the partial pressure of CO_2 in arterial blood, Paco_2 .

B. Bicarbonate and Hemoglobin in the Red Blood Cell

The bicarbonate buffer system and hemoglobin in red blood cells cooperate in buffering the blood and transporting CO_2 to the lungs. Most of the CO_2 produced from tissue metabolism in the TCA cycle diffuses into the interstitial fluid and the blood plasma and then into red blood cells (Fig. 4.9, circle 1). Although no carbonic anhydrase can be found in blood plasma or interstitial fluid, the red blood cells contain high amounts of this enzyme, and CO_2 is rapidly converted to carbonic acid (H_2CO_3) within these cells (circle 2). As the carbonic acid dissociates (circle 3), the H^+ released is also buffered by combination with hemoglobin (Hb, circle 4). The side chain of the amino acid histidine in hemoglobin has a pK_a of 6.7 and is thus able to accept a proton. The bicarbonate anion is transported out of the red blood cell into the blood in exchange for chloride anion, and thus bicarbonate is relatively high in the plasma (circle 5) (see Table 4.1).

As the red blood cell approaches the lungs, the direction of the equilibrium reverses. CO_2 is released from the red blood cell, causing more carbonic acid to dissociate into



The partial pressure of CO_2 (Paco_2) in **Di Abietes'** arterial blood was 28 mm Hg (reference range = 37–43), and her serum bicarbonate level was 8 mEq/L (reference range = 24–28). Elevated levels of ketone bodies had produced a ketoacidosis, and **Di Abietes** was exhaling increased amounts of CO_2 by breathing deeply and frequently (Kussmaul's breathing) to compensate. Why does this occur? Ketone bodies are weak acids that partially dissociate, increasing H^+ levels in the blood and the interstitial fluid surrounding the "metabolic" respiratory center in the hypothalamus that controls the rate of breathing. As a result of the decrease in pH, her respiratory rate increased, causing a fall in the partial pressure of arterial CO_2 (Paco_2). As bicarbonate and increased protons combined to form carbonic acid and produce more CO_2 , her bicarbonate concentration decreased ($\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$). As shown by Di's low arterial blood pH of 7.08, the Kussmaul's breathing was unable to fully compensate for the high rate of acidic ketone body production.

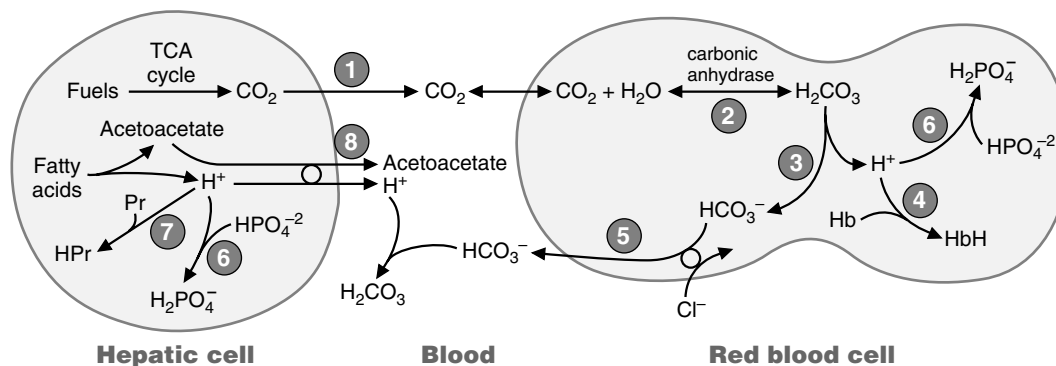


Fig. 4.9. Buffering systems of the body. CO₂ produced from cellular metabolism is converted to bicarbonate and H⁺ in the red blood cells. Within the red blood cell, the H⁺ is buffered by hemoglobin (Hb) and phosphate (HPO₄⁻²). The bicarbonate is transported into the blood to buffer H⁺ generated by the production of other metabolic acids, such as the ketone body acetoacetic acid. Other proteins (Pr) also serve as intracellular buffers.

CO₂ and water and more hydrogen ions to combine with bicarbonate. Hemoglobin loses some of its hydrogen ions, a feature that allows it to bind oxygen more readily (see Chapter 7). Thus, the bicarbonate buffer system is intimately linked to the delivery of oxygen to tissues.

The respiratory center within the hypothalamus, which controls the rate of breathing, is sensitive to changes in pH. As the pH falls, individuals breathe more rapidly and expire more CO₂. As the pH rises, they breathe more shallowly. Thus, the rate of breathing contributes to regulation of pH through its effects on the dissolved CO₂ content of the blood.

Bicarbonate and carbonic acid, which diffuse through the capillary wall from the blood into interstitial fluid, provide a major buffer for both plasma and interstitial fluid. However, blood differs from interstitial fluid in that the blood contains a high content of extracellular proteins, such as albumin, which contribute to its buffering capacity through amino acid side chains that are able to accept and release protons. The protein content of interstitial fluid is too low to serve as an effective buffer.

C. Intracellular pH

Phosphate anions and proteins are the major buffers involved in maintaining a constant pH of intracellular fluids. The inorganic phosphate anion H₂PO₄⁻ dissociates to generate H⁺ and the conjugate base, HPO₄⁻² with a pK_a of 7.2 (see Fig. 4.9, circle 6). Thus, phosphate anions play a major role as an intracellular buffer in the red blood cell and in other types of cells, where their concentration is much higher than in blood and interstitial fluid (See Table 4.1, extracellular fluid). Organic phosphate anions, such as glucose 6-phosphate and ATP, also act as buffers. Intracellular fluid contains a high content of proteins that contain histidine and other amino acids that can accept protons, in a fashion similar to hemoglobin (see Fig. 4.9, circle 7).

The transport of hydrogen ions out of the cell is also important for maintenance of a constant intracellular pH. Metabolism produces a number of other acids in addition to CO₂. For example, the metabolic acids acetoacetic acid and β-hydroxybutyric acid are produced from fatty acid oxidation to ketone bodies in the liver, and lactic acid is produced by glycolysis in muscle and other tissues. The pK_a for most metabolic carboxylic acids is below 5, so these acids are completely dissociated at the pH of blood and cellular fluid. Metabolic anions are transported out of the cell together with H⁺ (see Fig. 4.9, circle 8). If the cell becomes too acidic, more H⁺ is transported out in exchange for Na⁺ ions by a different transporter. If the cell becomes too alkaline, more bicarbonate is transported out in exchange for Cl⁻ ions.



Both **Di Abietes** and **Percy Veere** were hyperventilating when they arrived at the emergency room:

Ms. Abietes in response to her primary metabolic acidosis and Mr. Veere in response to anxiety. Hyperventilation raised blood pH in both cases: In Ms. Abietes case it partially countered the acidosis and in Mr. Veere's case it produced a respiratory alkalosis (abnormally high arterial blood pH).



Phosphoric acid (H₃PO₄) dissociates into H⁺ ions and dihydrogen phosphate (H₂PO₄⁻) with a pK_a of 2.15. The dissociation of H₂PO₄⁻ into H⁺ and monohydrogen phosphate (HPO₄⁻²) occurs with a pK_a of 7.20. Monohydrogen phosphate dissociates into H⁺ and phosphate anions (PO₄⁻³) with a pK_a of 12.4.

D. Urinary Hydrogen, Ammonium, and Phosphate Ions

The nonvolatile acid that is produced from body metabolism cannot be excreted as expired CO_2 and is excreted in the urine. Most of the nonvolatile acid hydrogen ion is excreted as undissociated acid that generally buffers the urinary pH between 5.5 and 7.0. A pH of 5.0 is the minimum urinary pH. The acid secretion includes inorganic acids such as phosphate and ammonium ions, as well as uric acid, dicarboxylic acids, and tricarboxylic acids such as citric acid (see Table 4.2). One of the major sources of nonvolatile acid in the body is sulfuric acid (H_2SO_4). Sulfuric acid is generated from the sulfate-containing compounds ingested in foods and from metabolism of the sulfur-containing amino acids, cysteine and methionine. It is a strong acid that is dissociated into H^+ and sulfate anion (SO_4^{-2}) in the blood and urine (see Fig. 4.6). Urinary excretion of H_2PO_4^- helps to remove acid. To maintain metabolic homeostasis, we must excrete the same amount of phosphate in the urine that we ingest with food as phosphate anions or organic phosphates such as phospholipids. Whether the phosphate is present in the urine as H_2PO_4^- or HPO_4^{-2} depends on the urinary pH and the pH of blood.

Ammonium ions are major contributors to buffering urinary pH, but not blood pH. Ammonia (NH_3) is a base that combines with protons to produce ammonium (NH_4^+) ions ($\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$), a reaction that occurs with a pK_a of 9.25. Ammonia is produced from amino acid catabolism or absorbed through the intestine, and kept at very low concentrations in the blood because it is toxic to neural tissues. Cells in the kidney generate NH_4^+ and excrete it into the urine in proportion to the acidity (proton concentration) of the blood. As the renal tubular cells transport H^+ into the urine, they return bicarbonate anions to the blood.



Ammonium ions are acids that dissociate to form the conjugate base, ammonia, and hydrogen ions.

What is the form present in blood? In urine?



Diabetes mellitus is diagnosed by the concentration of plasma glucose (plasma is blood from which the red blood cells have been removed by centrifugation). Because plasma glucose normally increases after a meal, the normal reference ranges and the diagnostic level are defined relative to the time of consumption of food, or to consumption of a specified amount of glucose during an oral glucose tolerance test. After an overnight fast, values for the fasting plasma glucose below 110 mg/dL are considered normal and above 126 mg/dL define diabetes mellitus. Fasting plasma glucose values greater or equal to 110 and less than 126 mg/dL define an intermediate condition termed impaired fasting glucose. Normal casual plasma glucose levels (casual is defined as any time of day without regard to the time since a last meal) should not be above 200 mg/dL. A 2-hour postprandial (after a meal or after an oral glucose load) plasma glucose level between 140 and 199 mg/dL defines a condition known as impaired glucose tolerance. A level above 200 mg/dL defines “overt” diabetes mellitus.

E. Hydrochloric Acid

Hydrochloric acid (HCl), also called gastric acid, is secreted by parietal cells of the stomach into the stomach lumen, where the strong acidity denatures ingested proteins so they can be degraded by digestive enzymes. When the stomach contents are released into the lumen of the small intestine, gastric acid is neutralized by bicarbonate secreted from pancreatic cells and by cells in the intestinal lining.

CLINICAL COMMENTS



Dianne Abietes. Di Abietes has type 1 diabetes mellitus (formerly called juvenile or insulin-dependent diabetes mellitus, IDDM). She maintains her insulin level through two daily subcutaneous (under the skin) injections of insulin. If her blood insulin levels fall too low, free fatty acids leave her adipocytes (fat cells) and are converted by the liver to the ketone bodies acetoacetic acid and β -hydroxybutyric acid. As these acids accumulate in the blood, a metabolic acidosis known as diabetic ketoacidosis (DKA) develops. Until insulin is administered to reverse this trend, several compensatory mechanisms operate to minimize the extent of the acidosis. One of these mechanisms is a stimulation of the respiratory center in the hypothalamus induced by the acidosis, which leads to deeper and more frequent respiration (Kussmaul’s respiration). CO_2 is expired more rapidly than normal, and the blood pH rises. The results of the laboratory studies performed on **Di Abietes** in the emergency room were consistent with a moderately severe DKA. Her arterial blood pH and serum bicarbonate were low, and ketone bodies were present in her blood and urine (normally, ketone bodies are not present in the urine). In addition, her serum glucose level was 648 mg/dL (reference range = 80 – 110 fasting and no higher than 200 in a random glucose sample). Her hyperglycemia, which induces an osmotic diuresis, contributed to her dehydration and the hyperosmolality of her body fluids.

Treatment was initiated with intravenous saline solutions to replace fluids lost with the osmotic diuresis and hyperventilation. The osmotic diuresis resulted from increased urinary water volume to dilute the large amounts of glucose and ketone bodies excreted in the urine. Hyperventilation increased the water of respiration lost with expired air. A loading dose of regular insulin was given as an intravenous bolus followed by additional insulin each hour as needed. The patient's metabolic response to the treatment was monitored closely.

Dennis Veere. Dennis remained alert in the emergency room. While awaiting the report of his initial serum salicylate level, his stomach was lavaged, and several white tablets were found in the stomach aspirate. He was examined repeatedly and showed none of the early symptoms of salicylate toxicity, such as respiratory stimulation, upper abdominal distress, nausea, or headache.

His serum salicylate level was reported as 92 $\mu\text{g}/\text{mL}$ (the usual level in an adult receiving a therapeutic dosage of 4–5 g/day is 120–350 $\mu\text{g}/\text{mL}$, and a level of 800 $\mu\text{g}/\text{mL}$ is considered potentially lethal). He was admitted for overnight observation and continued to do well. A serum salicylate level the following morning was 24 $\mu\text{g}/\text{mL}$. He was discharged later that day.

Percy Veere. At the emergency room, Mr. Veere complained of light-headedness and “pins and needles” (paresthesias) in his hands and around his lips. These symptoms resulted from an increase in respiratory drive mediated in this case through the “behavioral” rather than the “metabolic” central respiratory control system (as seen in **Di Abietes** when she was in diabetic ketoacidosis). The behavioral system was activated in Mr. Veere's case by his anxiety over his grandson's potential poisoning. His alveolar hyperventilation caused Mr. Veere's PaCO_2 to decrease below the normal range of 37 to 43 mm Hg. The alkalemia caused his neurologic symptoms. After being reassured that his grandson would be fine, Mr. Veere was asked to breathe slowly into a small paper bag placed around his nose and mouth, allowing him to reinhale the CO_2 being exhaled through hyperventilation. Within 20 minutes, his symptoms disappeared.

BIOCHEMICAL COMMENTS

Body water and dehydration. Dehydration, or loss of water, occurs when salt and water intake is less than the combined rates of renal plus extrarenal volume loss (Fig. 4.10). In a true hypovolemic state, total body water, functional ECF volume, and ICF volume are decreased. One of the causes of hypovolemia is an intake of water that is inadequate to resupply the daily excretion volume (maintenance of fluid homeostasis). The amount of water lost by the kidneys is determined by the amount of water necessary to dilute the ions, acids, and other solutes excreted. Both urinary solute excretion and water loss in expired air, which amount to almost 400 mL/day, occur during fasting as well as during periods of normal food intake. Thus, people who are lost in the desert or shipwrecked continue to lose water in air and urine, in addition to their water loss through the skin and sweat glands. Comatose patients and patients who are debilitated and unable to swallow also continue to lose water and become dehydrated. Dysphagia (loss of appetite), such as **Percy Veere** experienced during his depression (see Chapters 1 and 3), can result in dehydration because food intake is normally a source of fluid. Cerebral injuries that cause a loss of thirst also can lead to dehydration.

Dehydration also can occur secondary to excessive water loss through the kidneys, lungs, and skin. Excessive renal loss could be the result of impaired kidney function, or impaired response by the hormones that regulate water balance (e.g.,

A: The pK_a for dissociation of ammonium ions is 9.25. Thus, the undissociated conjugate acid form, NH_4^+ , predominates at the physiologic pH of 7.4 and urinary pH of 5.5 to 7.0. Ammonia is an unusual conjugate base because it is not an anion.

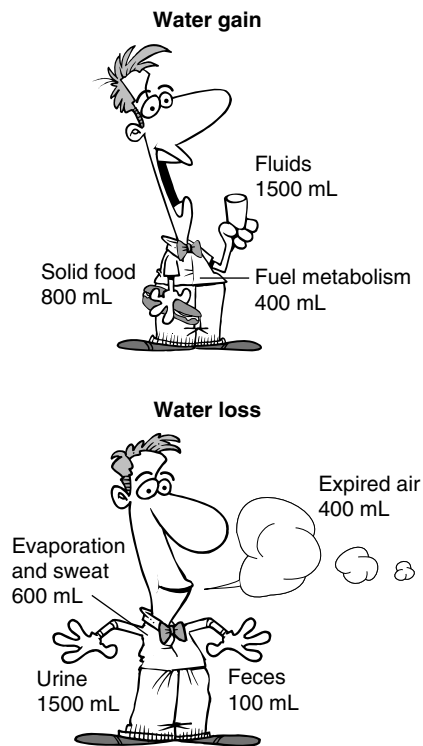


Fig. 4.10. Body fluid homeostasis (constant body water balance). Intake is influenced by availability of fluids and food, thirst, hunger, and the ability to swallow. The rates of breathing and evaporation and urinary volume influence water loss. The body adjusts the volume of urinary excretion to compensate for variations in other types of water loss and for variations in intake. The hormones aldosterone and antidiuretic hormone (ADH) help to monitor blood volume and osmolality through mechanisms regulating thirst and sodium and water balance.

antidiuretic hormone [ADH] and aldosterone). However, in individuals such as **Di Abietes** with normal kidney function, dehydration can occur as a response to water dilution of the high concentrations of ketone bodies, glucose, and other solutes excreted in the urine (osmotic diuresis). If the urinary water loss is associated with an acidosis, hyperventilation occurs, increasing the amount of water lost in expired air. Water loss in expired air (pulmonary water loss) could occur also as a result of a tracheotomy or cerebral injury. An excessive loss of water and electrolytes through the skin can result from extensive burns.

Gastrointestinal water losses also can result in dehydration. We secrete approximately 8 to 10 L fluid per day into our intestinal lumen. Normally, more than 90% of this fluid is reabsorbed in the intestines. The percentage reabsorbed can be decreased by vomiting, diarrhea, tube drainage of gastric contents, or loss of water into tissues around the gut via bowel fistulas.

Suggested References

Many physiology textbooks have good in-depth descriptions of acid-base balance. A very useful reference for medical students is: Brensilver J, Goldberger, E. *A Primer of Water, Electrolyte, and Acid-Base Syndromes*. 8th Ed. Philadelphia: F.A. Davis, 1996 .

For additional information on toxic effects of aspirin, see Roberts LJ II, Morrow JD. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman JG, Limbird LE, Goodman AG, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 2001:697–701.



REVIEW QUESTIONS—CHAPTER 4

1. A decrease of blood pH from 7.5 to 6.5 would be accompanied by which of the following changes in ion concentration?
 - (A) A 10-fold increase in hydrogen ion concentration.
 - (B) A 10-fold increase in hydroxyl ion concentration.
 - (C) An increase in hydrogen ion concentration by a factor of 7.5/6.5.
 - (D) A decrease in hydrogen ion concentration by a factor of 6.5/7.5.
 - (E) A shift in concentration of buffer anions, with no change in hydrogen ion concentration.
2. Which of the following describes a universal property of buffers?
 - (A) Buffers are usually composed of a mixture of strong acids and strong bases.
 - (B) Buffers work best at the pH at which they are completely dissociated.
 - (C) Buffers work best at the pH at which they are 50% dissociated.
 - (D) Buffers work best at one pH unit lower than the pK_a .
 - (E) Buffers work equally well at all concentrations.
3. A patient with an enteropathy (intestinal disease) produced large amounts of ammonia (NH_3) from bacterial overgrowth in the intestine. The ammonia was absorbed through the intestine into the portal vein and entered the circulation. Which of the following is a likely consequence of his ammonia absorption?
 - (A) A decrease of blood pH
 - (B) Conversion of ammonia to ammonium ion in the blood
 - (C) A decreased concentration of bicarbonate in the blood
 - (D) Kussmaul's respiration
 - (E) Increased expiration of CO_2

4. Which of the following physiologic/pathologic conditions is most likely to result in an alkalosis, provided that the body could not fully compensate?
- (A) Production of lactic acid by muscles during exercise
 - (B) Production of ketone bodies by a patient with diabetes mellitus
 - (C) Repeated vomiting of stomach contents, including HCl
 - (D) Diarrhea with loss of the bicarbonate anions secreted into the intestine
 - (E) An infection resulting in a fever and hypercatabolism
5. Laboratory tests on the urine of a patient identified the presence of methylmalonate (${}^{-}\text{OOC-CH}(\text{CH}_3)\text{-COO}^{-}$). Methylmalonate
- (A) is a strong acid.
 - (B) is the conjugate base of a weak acid.
 - (C) is 100% dissociated at its pK_a .
 - (D) is 50% dissociated at the pH of the blood.
 - (E) is a major intracellular buffer.