Chap. 1 "Introduction to Biochemistry"

Reading Assignment: pp. 3-25.

Review: Read the Chap. 1 Appendix section & sections concerning cell biology in the textbook. Also review the supplemental chemistry information at the end of this part of the notes.

I. The science of biochemistry.

       The ultimate goal of biochemistry is to explain all life processes in molecular detail. Because life processes are performed by organic molecules the discipline of biochemistry relies heavily on fundamental principles of organic chemistry and other basic sciences. It is of no surprise that the first "biochemists" actually were organic chemists who specialized in the chemistry of compounds derived from living organisms. The text provides an historical overview of some of the key contributions of the early chemists, and of modern 20th century biochemists who have lead the discipline to where it is today. Research endeavors such as the human genome project ultimately owe their success to basic discoveries about the structure of the DNA "double helix" by Watson & Crick and the development of DNA sequencing methods by Fredrick Sanger.

II. The chemical basis of life.

       The biomolecules such as proteins that are present in living organisms are carbon-based compounds. Carbon is the third most abundant element in living organisms (relative abundance H > O > C > N > P > S). **Fig. 1.1.** shows the 29 elements found in living organisms. The most common ions are Ca^{+2}, K^{+}, Na^{+}, Mg^{+2}, and Cl^{-}. The properties of biomolecules, such as shape and chemical reactivity, are best described by the discipline of organic chemistry.

A. Representations of molecular structures.

       Your text will use skeletal, ball & stick, and space-filling models to show molecular structures. Therefore, you must be familiar with each of these types of representations. Skeletal and ball & stick models are good for showing the positions of nuclei in organic compounds. Space-filling models show van der Waals radii of the atoms in molecules, i.e., the surfaces of closest possible approach by neighboring molecules.

B. Chemical bonding.

       If necessary, please review the supplemental notes at the end of this section concerning the atomic and molecular (bonding) orbitals of carbon, nitrogen, and oxygen. sp^3 and sp^2 molecular orbitals are the most prevalent in biomolecules. The orientations of bonding orbitals in space ultimately determine the shapes of biomolecules.

C. Functional groups.

The chemical reactions of biomolecules are dictated by the functional groups they contain. **Fig. 1.2.** shows the general formulas of common organic compounds and functional groups that will be encountered constantly in the proteins, carbohydrates, nucleic acids and simple metabolites you will study. You should be familiar with the structure, charge properties, polarity, and basic chemical reactivity of all of these compounds and functional groups.

III. Many biomolecules are polymers.

The principle biomolecules in cells (proteins, polysaccharides, and nucleic acids) are polymer chains of amino acids, monosaccharides, and nucleotides, respectively. Biopolymers are formed by condensation reactions in which water is removed from the reacting monomer units. Each monomer unit of a biopolymer is referred to as a residue.

A. Proteins.

Most of the chemical reactions of the cell are carried out by proteins. Proteins also are the major structural components of most cells and tissues. Proteins are often called polypeptides in reference to the fact that they are composed of amino acids held together by peptide bonds (**Fig. 1.3**). Peptide bonds actually are amide bonds which are formed by the condensation of the carboxyl groups and amino groups of consecutive amino acids in the polymer chain. The so-called peptide backbone of a protein is a monotonous, regularly repeating structure. Projecting out from the backbone are the R-groups which are the side-chains of the amino acids. In a later chapter, we will discuss how the R-groups play a significant role in determining the 3D structure of a protein, i.e., its active conformation.

The enzymes comprise one subclass of proteins. These proteins carry out chemical reactions with extraordinary specificity and speed (up to 10^{17}-fold enhancement in reaction rate). Specificity is achieved because the binding site for reactants--the active site--is highly complementary in shape to the reactants and products. A stereoview of the active site of lysozyme is shown in **Fig. 1.4.** This enzyme binds to and cleaves the polysaccharide portion of the bacterial cell wall. Cleavage leads to osmotic lysis of the affected bacterium. Lysozyme is present in tears and egg whites where it helps protect against unwanted bacterial growth and infection. We will discuss the structure and function of many medically and otherwise relevant proteins and enzymes such as myoglobin, hemoglobin, collagen, trypsin, insulin receptor, glycogen phosphorylase, plasma lipoproteins, and DNA polymerase in this course. Many of these proteins and enzymes are the targets of poisons and drugs whose actions also will be discussed.

B. Polysaccharides.

Polysaccharides are polymers of simples sugars known as monosaccharides (e.g., glucose). Different polysaccharides perform either structural (cellulose) or energy storage (glycogen, starch) functions. Polysaccharide and monosaccharides were some of the first biomolecules that were studied by organic chemists. You should be familiar with the different types of representations used to describe the structures of monosaccharides (**Fig. 1.5**). A

comparison of the polysaccharides starch and cellulose provides an excellent example of how structure is crucial to biological function. Namely, the structure of the glycosidic bonds linking the glucose units in cellulose and starch are very similar, yet the subtle difference in bond configuration determines whether the polymer is digestible (starch) or not (cellulose). We will spend a number of lectures on polysaccharide and general carbohydrate metabolism. The medical relevance of these topics cannot be overemphasized. For example, more than 1 in 30 Americans will become diabetic during their lifetimes and suffer consequences attributable to energy imbalance and monosaccharide-based tissue damage.

C. Nucleic acids.

Nucleic acids are composed of nucleotide monomer units. Nucleotides themselves are composed of a monosaccharide, a nitrogenous base, and one or more phosphate groups (**Fig. 1.8**). The nucleotide ATP is the major energy currency of the cell which is used to power a huge variety of energy-requiring reactions. ATP and other ribonucleotides (containing ribose) also make up the biopolymer RNA. Deoxyribonucleotides (containing deoxyribose) make up DNA. All nucleotides are held together by phosphodiester linkages where one phosphate group is attached to 2 sugar units in the backbone of the polymer (**Fig. 1.9**). Nucleotides play key roles in information transfer in all organisms (DNA → RNA → protein). RNA also can carry out structural and enzymatic functions. For example, the formation of peptide bonds during protein synthesis actually is performed by one of the RNA constituents of the ribosome. In addition the main structural component of ribosomes is RNA. Lastly, a number of nucleic acid analogs are used to inhibit DNA synthesis and are extremely important in management of cancers and virally caused diseases such as AIDS.

D. Lipids and membranes.

Lipids are a diverse collection of biomolecules that are composed mostly of carbon and hydrogen, i.e., hydrocarbons. Lipids contain relatively few polar functional groups. They typically are more soluble in organic solvents than in water. The primary building block of many lipids is a fatty acid. The most common structural lipid in cell membranes--glycerophospholipid--contains 2 fatty acids, glycerol and a polar head group (**Fig. 1.11 & 1.12**). When collected as assemblies of millions of molecules, the classical biological structure known as a membrane is formed (**Fig. 1.13**). Biological membranes usually contain proteins, and protein content and composition is highly variable and determined by membrane function. Although discussed here along with true biopolymers, membranes are actually molecular aggregates. In later chapters, we will cover the functions of membrane-bound proteins, enzymes and receptors. We'll discuss how membranes serve as the primary sites of energy production in aerobic tissues such as the brain and liver, how membrane-bound hormone receptors signal metabolic changes in cells, and how many toxins act to impair membrane protein function.

IV. The energetics of life.

Living organisms are highly complicated at the molecular level. A large amount of energy is invested in maintaining the ordered and complicated state of cells and tissues. In humans and animals, energy needed for work and biosynthesis of cellular structures is derived

from organic molecules in the diet. Often these come from plant sources, who derived their energy for synthesis of biomolecules from sunlight. In animals, energy is derived from the breakdown of fuel molecules by processes referred to as catabolism. In turn, the energy released from catabolism is used to drive biosynthetic processes collectively referred to as anabolism.

The flow of energy in biological systems is covered in the discipline known as bioenergetics. Bioenergetics is a sub-discipline of classical thermodynamics, which has been covered in your physics courses. Most of our use of thermodynamics will be concerned with the calculation of free energy changes (ΔG) which can be used to determine the direction of metabolic reactions and their equilibrium constants. ΔG values are determined by the enthalpy (ΔH, heat transfer) and entropy (ΔS, change in randomness) changes associated with a reaction through the equation $\Delta G = \Delta H - T\Delta S$. Negative values of ΔG signify favorable reactions, whereas positive values of ΔG are associated with unfavorable reactions. K_{eq} is based on the DG value and gives the ratio of products to reacts once equilibrium is reached. This is important because it will show how a cell can ratio reactions in different directions. Kinetics measures the rate at which a reaction takes place and how the reaction gets from start to finish. So reactions can be thermodynamically favorable but kinetically unfavorable. So cells use enzymes that can only affect the kinetics not the thermodynamics. Bioenergetics is one of the tools used in animal and human nutrition. Weight gain or loss ultimately depend on the difference between caloric intake and expenditure. In this course, we will discuss energy metabolism in different physiological states such as exercise and fasting, and in diseases such as diabetes.

V. Biochemistry and evolution.

Biochemistry has greatly extended our knowledge of phylogeny and evolution that was acquired originally through the disciplines of comparative anatomy, population genetics and paleontology. In fact, only through biochemistry have we come to appreciate that all living organisms are similar at the molecular level. Namely, they share similar means of replication, cellular structure, and often energy utilization & production. For this reason, much of what we can learn about simple organisms such as *Escherichia coli* can be applied to the study of higher organisms such as us.

The similarity of organisms at a molecular level indicates that all are derived from a common ancestor. Carl Woese determined by comparisons of ribosomal RNA (rRNA) sequences that it is possible to construct a highly accurate tree of life showing the evolutionary relationship between all life forms. rRNA analysis has proven that living organisms are best divided into 3 domains of life--the Archaea, the Bacteria, and the Eukarya. At the end of the course we will discuss the properties of one of the most important enzymes used in modern biochemistry laboratories, Taq polymerase. This enzyme is derived from the Archaean species *Thermus aquaticus*, which was isolated from a hotspring located in Yellowstone Natl. Pk. One common use of this enzyme is in sequencing rRNA genes from newly isolated microorganisms.

Chap. 2. "Water"

Reading Assignment: pp. 26-49.

Problem Assignment: 1-5, 7, 8, 11, 13, 15 and 16

I. Introduction.

While modern biochemistry tends to focus on the structure and function of molecules such as proteins and DNA, it is important to keep in mind that biomolecular structure and function are dictated by the properties of the medium in which they are dissolved. Therefore, this chapter presents an overview of the properties of water that are germane to the structure and function of biomolecules. As an illustration of the importance of water in biological systems, consider the formation of biological membranes. Cell membranes ultimately form due to the fact that the acyl chains of glycerophospholipids are not soluble in water. As a consequence, glycerophospholipids and other membrane lipids cluster together leading to structures such as the cytoplasmic membrane and membranes of organelles. In this chapter, we will review fundamental properties of water such as solvation of polar and nonpolar molecules, water ionization and pH, and acid-base chemistry and buffering systems. These topics are essential for understanding everything that will be discussed in later chapters of the text.

II. General properties of water molecules.

The oxygen atom in a water molecule has an sp^3 arrangement of bonding orbitals in which the 2 H atoms and 2 unshared pairs of electrons are located in a tetrahedral arrangement around the oxygen. This arrangement results in a net dipole in which the end of the molecule containing the unshared electrons has partial negative character and the end containing the 2 hydrogens has partial positive character (**Fig. 2.1**). In addition, each H-O- bond also has dipolar character due to unequal sharing of electrons between hydrogen and oxygen. Due to the fact that a net dipole exists in individual water molecules, water is regarded as a polar solvent. The polarity of other small molecules is considered in **Fig. 2.2**.

III. H-bonding in water.

Neighboring molecules in bulk water are held together by non-covalent bonds known as H-bonds. The configuration of atoms in an H-bond is illustrated in **Fig. 2.3**. In the figure, the H-bond is the non-covalent attraction (dashed line) between the partially positively charged H atom attached to the left oxygen atom and one of the unshared electron pairs (not shown) of the oxygen atom on the right. Each water molecule has 2 unshared electron pairs and 2 hydrogens that can participate in H-bonding. Thus, each water molecule can H-bond to 4 neighbors. Since sp^3 molecular orbitals are tetrahedrally oriented, neighboring water molecules surrounding a given water molecule are located in a tetrahedral arrangement (**Fig. 2.4**). In ice, water molecules are organized in a rigid, precisely tetrahedral crystalline lattice (**Fig. 2.5**) where each molecule is H-bonded to 4 others. In liquid water, each water molecule is H-bonded to ~3.4 others on average. Local groups, i.e., "flickering clusters," of molecules only exist for nanoseconds. While a roughly tetrahedral arrangement of molecules is present, liquid water is more dense than ice

because the somewhat irregular packing of molecules allows them to fit together a bit closer. Due to extensive H-bonding, water is highly cohesive. The cohesiveness of molecules confers a high melting point and boiling point in spite of the low molecular weight of water (18 g/mol). The high specific heat and heat of evaporation make water an excellent thermal buffer for actively metabolizing cells and tissues. It also explains why cold water can quickly conduct heat away from a swimmer leading to hypothermia and possibly death.

IV. Behavior of ionic and polar substances in water.

Because water molecules are polar, ionic compounds (electrolytes) and polar molecules are relatively soluble in water. Substances that can dissolve readily in water are referred to as hydrophilic. For salts (e.g., NaCl), both the cationic (Na^+) and anionic (Cl^-) components of the salt can be solvated via interactions with the negative and positive, respectively, ends of dipolar water molecules (**Fig. 2.6**). Because the interactions are energetically favorable, the salt dissolves. Dissolved ions are considered to be "solvated" or "hydrated." The shells of surrounding water molecules shield the ions preventing them from strongly interacting and reforming the crystal. Water molecules also form H-bonds to polar functional groups in polar biomolecules such as sugars and amino acids. The different types of H-bonds that can form are discussed below. It should be noted that many biomolecules contain a combination of polar and nonpolar groups. Thus, the actual solubility of biomolecules is quite variable and depends on the relative proportions of polar and nonpolar regions (**Table 2.1**).

V. Behavior of nonpolar substances.

Nonpolar substances are relatively insoluble in water and therefore are referred to as hydrophobic. Such molecules typically are hydrocarbons containing methylene, methyl, and aromatic ring functional groups. They generally lack polar groups that can interact with water molecules. Because water molecules cannot form H-bonds to a nonpolar substance, water molecules become highly ordered in the immediate vicinity of the compound forming ice-like bonds to one another. Indeed cage-like structures known as clathrates are formed which can be viewed as rigid geodesic domes surrounding the nonpolar molecule. All this structuring decreases the disorder or entropy of the water, which is an energetically unfavorable process. To avoid this situation as much as possible, the suspended hydrophobic substances coalesce which reduces the surface area of the nonpolar molecules in contact with water. The term hydrophobic interactions refers to the clustering together of nonpolar molecules such as membrane lipids to avoid the entropically unfavorable process of ordering neighboring water molecules. It is important to note that hydrophobic interactions are not a type of chemical bond per se.

One other important class of molecules--the amphiphiles--deserves mention. These molecules have significant proportions of both hydrophilic and hydrophobic functional groups. Typical examples are detergents such as sodium dodecyl sulfate (SDS) (**Fig. 2.8**) which contains a highly water soluble sulfate group and a very insoluble 12-carbon alkyl group. This schizophrenic combination results in the hydrocarbon chains clustering together away from water contact when SDS is added to water. In this case, the clusters formed are spherical structures known as micelles (**Fig. 2.9**). At an air-water surface SDS molecules actually line up with their hydrocarbon tails pointing up into the air and the sulfate groups in contact with water. SDS is a useful detergent. Its hydrocarbon tail will bind to nonpolar surfaces, such as greasy dirt,

and dissolve it within the interior of the micelle. After the dirt-filled micelles are suspended in water by agitation, the dirt and detergent can be rinsed away.

VI. Noncovalent interactions in biomolecules.

Weak, reversible bonds (noncovalent bonds or noncovalent interactions) mediate interactions between biomolecules. Noncovalent bonds are "individually weak, but collectively strong" and together stabilize the complex structures of biomolecules such as proteins. However, because they are individually weak, biomolecules exhibit flexibility which is important in processes such as enzyme catalysis. Furthermore, non-covalent interactions allow reversible binding of small biomolecules to enzymes and nucleic acids. Generally, noncovalent interactions are less than 1/10 th as strong as covalent bonds such as the -C-H bond. The general properties of each of type of noncovalent interaction and the energy required to break them (i.e., the strength) are summarized in **Fig. 2.13**.

A. Charge-charge interactions.

Charge-charge interactions occur between oppositely charged functional groups or ions. These bonds are also known as ion pairing interactions and salt-bridges. The strength of these bonds is inversely dependent on the square of the distance separating the charges. Strength also depends on the medium in which they occur, with polar media such as water weakening interactions through solvation of interacting ions. Repulsive forces between like charges also can play an important role in biological processes.

B. H-bonds.

The H-bonds that occur between water molecules are just one example of the many types found in biomolecules. In general, an H-bond is defined as a dipolar attraction between the hydrogen atom attached to one electronegative atom, and a second electronegative atom. The H atom must be covalently bonded to an electronegative atom such as O or N to generate a molecular dipole. Common types of H-bonds are shown in **Fig. 2.10**. The atom with the covalently bound hydrogen atom is called the hydrogen donor, and the other atom is the hydrogen acceptor. The distance between the two electronegative atoms in an H-bond is ~0.3 nm (3 Å). H-bond strength is highly dependent on the alignment of molecular orbitals in the interacting molecules and is strongest when they are lined up properly. As a result, H-bonds are very important in establishing specificity in molecular interactions, e.g., A-T and G-C base pairing in DNA (**Fig. 2.11**).

C. van der Waals forces.

These forces are attractions between oppositely oriented dipoles that are transiently induced in the electron clouds of closely interacting molecules. The strength of these forces is maximal when the interacting molecules are just touching. In fact, these forces become destabilizing and push molecules apart if molecules are compressed more tightly together (**Fig. 2.12**). Note that the van der Waals contact radius is defined as the distance at which the attraction of molecules is maximal. van der Waals forces typically are the weakest of the noncovalent

interactions (**Fig. 2.13**). However, van der Waals bonds often are important in the packing of amino acids inside a folded protein and in the interactions between adjacent bases stacked within the DNA double helix. They also can mediate specific interactions because they become collectively strong if the interacting molecules have precisely complementary shapes and can approach one another closely.

VII. Water is a nucleophile.

Water often is a reactant in biochemical reactions. The unshared pairs of electrons in water molecules can behave as nucleophiles which can attack an electrophilic center in another molecule. A good example where water serves as a nucleophile is in the hydrolysis of peptide bonds (**Fig. 2.14**). Although this is a favorable reaction, peptide bonds are actually quite stable due to the fact that the activation energy for this reaction is quite high. Thus the reaction is very slow at physiological temperatures and pH unless catalyzed by an enzyme.

VIII. Ionization of water.

As a prelude to our discussion of pH, we need to discuss the ionization of water, as it is through this reaction that solution pH ultimately is established. A water molecule has a slight tendency to undergo a dissociation reaction whereby a proton is lost to another water molecule. The products of this reaction are a hydronium ion (H_3O^+) and a hydroxyl ion (OH^-).

$$H_2O + H_2O \quad H_3O^+ + OH^-$$

A hydronium ion can donate its proton to another molecule and hence is considered to be an acid (proton donor). A hydroxyl ion can accept a proton from an acid and thus is called a base (proton acceptor). The ionization reaction commonly is written as

$$H_2O \quad H^+ + OH^-$$

Water has a finite and defined capacity to ionize, and the ionization process has a characteristic equilibrium constant at a given temperature.

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

K_{eq} for water has been experimentally determined by measuring the electrical conductivity of pure water. (Note, electrical conductivity is proportional to the levels of ions in the water). The value for $K_{eq} = 1.8 \times 10^{-16}$ M. This K_{eq} value and the value of the concentration of water ($[H_2O] = 55.5$ M) can be substituted into the equilibrium equation to derive another equation which specifies the amounts of $[H^+]$ and $[OH^-]$ in any water sample or biochemical buffer:

$$1.8 \times 10^{-16} \text{ M} = \frac{[H^+][OH^-]}{(55 \text{ M})}$$

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

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

The constant, K_W, is called the ion product of water. The derivation indicates that the product of the $[H^+]$ and $[OH^-]$ concentrations in any water sample or buffer always will equal $1 \times 10^{-14} \text{ M}^2$.

This result leads directly to a definition of a "neutral solution" and to a definition of the pH scale. A neutral solution is defined as one in which $[H^+] = [OH^-]$. When these concentrations are equivalent, $[H^+] = [OH^-] = 1 \times 10^{-7}$ M. Due to the fact that this equilibrium reaction always is obeyed, the addition of a base which consumes protons leads to an excess of hydroxyl ions and a basic solution. Likewise the addition of an acid which consumes hydroxyl ions leads to an excess of hydronium ions and an acidic solution.

IX. The pH scale.

pH is simply a more convenient (if logarithms are convenient!) way of specifying the concentration of H^+ ions in solution. pH is defined as

$$pH = -\log [H^+] = \log (1/[H^+])$$

By using logarithms, concentrations in the range of 1 M to 10^{-14} M H^+ are converted to numbers between 0 and 14. For example, the pH of a neutral solution is

$$pH = -\log (1 \times 10^{-7}) = 7.0$$

A table relating the pH scale to the concentrations of H^+ and OH^- in solution is presented in **Table 2.3**. Solutions in which pH = 7.0 are defined as neutral. Solutions with pH < 7.0 are called "acidic," and solutions in which pH > 7.0 are called "basic." In **Fig. 2.16** are shown the pH values of some common fluids.

X. Acid dissociation constants of weak acids.

A. Strong and weak acids and bases.

Most reactions are reversible, and equilibrium is achieved when the rate of the forward reaction becomes equal to the rate of the reverse. As in the water ionization example shown above, the equilibrium constant (K_{eq}) for a general reaction is defined as the ratio of products to reactants at equilibrium.

$$A + B \rightleftharpoons C + D$$

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

Remember, all reactions have a characteristic K_{eq} at a defined temperature.

Many biomolecules (such as amino acids) are weak acids. Unlike strong acids (HCl, H_2SO_4, etc.) which completely dissociate when dissolved in water, weak acids only partially dissociate. Equilibrium reactions for the dissociation of strong acids, strong bases, and the weak acid, acetic acid (CH_3COOH) are shown below.

$HCl \rightarrow H^+ + Cl^-$ (100% dissociated)

$NaOH \rightarrow Na^+ + OH^-$ (100% dissociated)

$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$ (<<1% dissociated)
(conj. acid)   (conj. base)
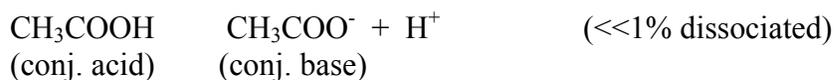
In the case of a weak acid, the two species in solution at equilibrium are called the conjugate acid-conjugate base pair.

B. Equilibrium constant (K_{eq}) and the pK_a.

The equilibrium constant for dissociation of a weak acid (HA) is

$$HA \rightleftharpoons H^+ + A^-$$
(conj. acid)   (conj. base)
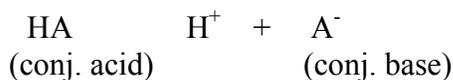
$$K_{eq} = \frac{[H^+][A^-]}{[HA]}$$

The equilibrium constant for acid dissociation is more commonly called the acid dissociation constant, K_a, and $K_a = K_{eq}$. Note, "the higher the K_a, the stronger the acid."

As in the case of pH, biochemists typically use "pK_a" values instead of K_a values for weak acids. pK_a is defined in the same manner as pH,

$$pK_a = -\log K_a = \log (1/K_a)$$

When comparing pK_as, "the lower the pK_a, the stronger the acid." pK_a values for a number of weak acids are listed in **Table 2.4**.

C. Henderson-Hasselbalch equation.

The Henderson-Hasselbalch equation describes the quantitative relationship between pH and pK_a in buffer solutions. In fact, a titration curve can be plotted using it. The HH equation will be derived in class starting from the equation specifying the equilibrium constant for ionization of a weak acid, $K_a = [H^+][A^-]/[HA]$. The final form of the HH eq is

$$pH = pK_a + log\ ([A^-]/[HA])$$

or

$$pH = pK_a + log\ \frac{[\text{conjugate base}]}{[\text{conjugate acid}]}$$

The equation indicates that the pH of a solution depends on the pK_a and the ratio of conjugate base to conjugate acid components present. The equation can be used to calculate the pH of a solution of a weak acid when the ratio of $[A^-]/[HA]$ is known, or alternatively, to calculate the ratio of $[A^-]/[HA]$ when the pH is known.

D. Measurement of pK_a by titration.

pK_a values are measured experimentally by titration. In a titration experiment, a weak acid in solution is converted to its conjugate base by the addition of a strong base. Remember that a strong base (e.g., NaOH) will stoichiometrically (1 part-to-1 part) convert a weak acid to its conjugate base form. Thus, the numbers of moles of weak acid in solution is the same as the number of moles of strong base needed to convert all conjugate acid molecules to their conjugate base form. A sample equation for titration of acetic acid is shown below.

$$CH_3COOH\ +\ Na^+\ +\ OH^-\ \rightarrow\ CH_3COO^-\ +\ Na^+\ +\ H_2O$$
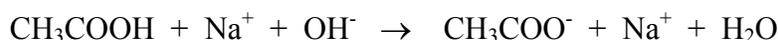
During the titration, two equilibrium reactions are occurring simultaneously.

$$1)\quad H^+\ +\ OH^-\quad H_2O$$

and

$$2)\quad CH_3COOH\quad H^+\ +\ CH_3COO^-$$
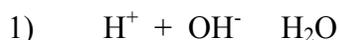
That is, as an OH^- ion is added to the solution, it combines with an H^+ ion present in solution. When the H^+ ion is removed, a molecule of the conjugate acid form of acetic acid dissociates a proton to restore equilibrium. The end result of the titration is that the base converts all of the CH_3COOH present to CH_3COO^-.

Likewise, a strong acid (e.g., HCl) will stoichiometrically convert a weak base to its conjugate acid.

$$CH_3COO^-\ +\ H^+\ +\ Cl^-\ \rightarrow\ CH_3COOH\ +\ Cl^-$$
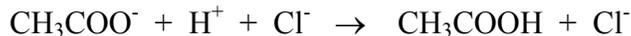
The pK_a turns out (as will be proven mathematically using the Henderson-Hasselbalch equation, see below) to be the midpoint of the plot of a titration curve.

An example of a titration curve is shown using acetic acid in **Fig. 2.17.** Note, that the pH changes rapidly at the ends of the titration curve, and modestly in the middle for a given amount of base added. The change in pH is least at the midpoint of the curve, i.e., when 0.5 equivalents of strong base have been added. This middle region of the curve is the optimum buffering region for the weak acid, i.e., the pH changes least on addition of a strong base or strong acid. At the midpoint $[CH_3COOH] = [CH_3COO^-]$.

Problem 1. Why does $pH = pK_a$ at the midpoint of the titration curve?
At the midpoint, $[A^-] = [HA]$. Thus,

$$pH = pK_a + \log (1/1)$$
$$pH = pK_a + 0$$
$$pH = pK_a$$

Problem 2. What is the ratio of $[A^-]/[HA]$ for a weak acid ($pK_a = 7.2$) at $pH = 8.4$?

$$8.4 = 7.2 + \log ([A^-]/[HA])$$
$$1.2 = \log ([A^-]/[HA])$$
$$10^{1.2} = [A^-]/[HA]$$
$$15.8/1 = [A^-]/[HA]$$

Problem 3. How does pH vary as a function of the ratio of $[A^-]/[HA]$?

$[A^-][HA]$	pH
100/1	$pH = pK_a + 2$
10/1	$pH = pK_a + 1$
1/1	$pH = pK_a$
1/10	$pH = pK_a - 1$
1/100	$pH = pK_a - 2$

The titration behavior and its quantitative treatment using the HH equation are similar when dealing with weak acids that have more than one dissociable proton, e.g., phosphoric acid (**Fig. 2.19**). The only difference is that instead of having one plateau, the titration curve of a "polyprotic" compound shows a plateau for each of its dissociable protons. The midpoint of each plateau is the pK_a for the acid group that is giving up the proton, and one conjugate acid/base pair predominates in solution across each plateau.

XI. Buffers.

A buffer is "a solution that tends to resist a change in pH on addition of a small amount of strong acid or base." As shown above for the acetic acid titration curve, the pH of a solution undergoing titration changes minimally near the midpoint of the curve. The optimum buffering power of the solution occurs at the midpoint (where pH = pK_a, and $[CH_3COOH] = [CH_3COO^-]$). In practice, the optimum buffering region extends about 1 pH unit on either side of the pK_a. In this pH range, buffering power is best because the concentrations of <u>both</u> buffering species, HA and A^-, are the highest. Buffers are selected based on their pK_a values and the range of pHs to be buffered. For example, acetic acid (pK_a = 4.76) is a good buffer for the range 3.76 < pH < 5.76, whereas the compound "Tris" (pK_a ~ 8.0) is a good buffer for the range 7.0 < pH < 9.0.

The main buffering agent inside cells is phosphate. In this case it is the second dissociation reaction with pK_2 = 7.2 (**Fig. 2.19**) that provides buffering.

$$H_2PO_4^- \quad H^+ + HPO_4^{2-}$$

In the blood, the CO_2-carbonic acid-bicarbonate system (**Figs. 2.20 & 2.21**) is used for buffering. Here the major forms responsible for buffering are carbonic acid and bicarbonate. While the pK_a for the reaction

$$H_2CO_3 \quad H^+ + HCO_3^-$$
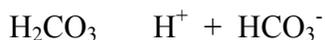
is only 6.4, H_2CO_3 readily converts to CO_2 and H_2O which tends to shift the carbonic acid-bicarbonate equilibrium to the left (to a higher pK_a) which is closer to the pH of blood. Interestingly, hemoglobin also is a buffering agent in the blood, and the mechanism by which it acts as a buffer will be described in Chap. 4.

Logarithms and Antilogarithms.

Definition of a logarithm:

The logarithm of number is the value of the exponent that is needed to express the number as a power of 10.

Relationship between logarithmic and exponential equations:

A logarithmic equation is just another form of the more familiar exponential equation. That is

$$\log_{10} y = x$$

expresses the same relationship as

$$y = 10^x$$

When you take the logarithm of a number, you are calculating the exponent x. In taking the antilogarithm of a number, you are converting the number back to its exponential form, i.e., the number written in scientific notation.

Relationships between scientific notation and logarithms.

y	y written in scientific notation	$x = \log_{10} y$
0.0000001	1×10^{-7}	-7
0.000001	1×10^{-6}	-6
0.00001	1×10^{-5}	-5
0.0001	1×10^{-4}	-4
0.001	1×10^{-3}	-3
0.01	1×10^{-2}	-2
0.1	1×10^{-1}	-1
1	1×10^{0}	0
10	1×10^{1}	1
100	1×10^{2}	2
1,000	1×10^{3}	3
10,000	1×10^{4}	4
100,000	1×10^{5}	5
1,000,000	1×10^{6}	6
10,000,000	1×10^{7}	7

Application to pH.

By analogy to the above, $pH = - \log_{10} [H^+]$ expresses the same relationship as $10^{-pH} = [H^+]$.