Chemical Foundations of Physiology II: Concentration and Kinetics 1.5

Learning Objectives

- Be able to calculate the molar concentration given the mass of solute, formula weight, and volume of solution
- Be able to calculate the number of moles of solute in an aliquot of solution given the volume and concentration
- Be able to determine the gram molecular weight of simple compounds
- Know the prefix notations for scientific notation
- Be able to calculate dilutions of stock solutions to form working solutions
- Be able to calculate the volume of distribution using Fick's dilution principle
- Define rate constant
- Be able to derive a first-order rate equation and calculate its half-life
- Define equilibrium constant
- Describe how an enzyme can change the rate of a reaction without changing its equilibrium
- Write the Michaelis–Menten equation and draw a saturation plot. Identify K_m and V_{max}
- Draw a double-reciprocal plot and identify K_m and V_{max} on this plot

AVOGADRO'S NUMBER COUNTS THE PARTICLES IN A MOLE

As described in Chapter 1.4, each chemical species is composed of a definite number of atoms of each element. This is Dalton's law of fixed proportions, which states that a molecule contains integral numbers of each kind of atom. Under ordinary chemical reactions, these atoms cannot be converted into each other. Each of these atoms contributes a tiny but definite mass to the molecule. The **atomic mass unit is defined as 1/12 of the mass of a carbon-12 atom**, the one containing 6 protons and 6 neutrons in its nucleus. This unit of mass is also called the **dalton**, **Da**. The atomic weight of a carbon-12 atom is *defined* as being 12 daltons. The atomic weight of most elements, including carbon, is not exactly integral because most elements consist of mixtures of **isotopes** that differ slightly in their atomic mass. These are atoms that possess the characteristic number of protons (the Z number) but differ in their numbers of neutrons.

Each molecule has a definite mass that depends on the atoms that make it up. If we add up the atomic masses of the constituent atoms, we get the **molecular weight** in daltons. If we express this molecular weight in grams, we will have the **gram molecular weight** (just scratch out "daltons" and replace it with "grams"). A pile of molecules whose mass in grams is the gram molecular weight is called a **mole**. Such a pile will consist of not one molecule but very many of them, and the number will be the number of daltons in a gram.

To see this, suppose we take a substance with a molecular weight of Z daltons. How many of these molecules will we have to pile up in order to make up a pile with a mass of Z grams? Let N be the number of molecules in the pile of Z grams. The mass of one molecule is Z daltons. The mass of N of them is just $N \times Z$ daltons. This mass will be the gram molecular weight. Thus, we have

$$Z \text{ daltons} \times N = Z \text{ grams}$$

$$N = \frac{Z \text{ grams}}{Z \text{ daltons}} = \frac{1 \text{ g}}{1 \text{ Da}}$$

The actual mass of 1 Da is 1.66×10^{-24} g. Thus, the number of molecules in a gram molecular weight is

$$N = \frac{1 \text{ g}}{1.66 \times 10^{-24} \text{ g}} = 6.02 \times 10^{23}$$

This is, of course, Avogadro's number. It is the number of molecules per mole. A mole is a pile of molecules of a single substance whose mass in grams is equal to its gram molecular weight and which contains Avogadro's number of molecules. We can turn this definition around and *define* the mole as Avogadro's number of particles. This definition is completely general. We did not specify how big the molecule is in the above calculation. No matter the size of the compound, one mole contains Avogadro's number of molecules.

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CONCENTRATION IS THE AMOUNT PER UNIT VOLUME

In Chapter 1.2, we defined extensive and intensive variables as those that depend on the extent of the system and those whose value is independent of the extent of the system, respectively. The amount of a solute, and the volume in which it is distributed, are both extensive variables. If you have twice the volume of solution, you have twice the amount of solute dissolved in it. The concentration, on the other hand, is an intensive variable which is given as the ratio of two extensive variables:

$$\text{Concentration} = \frac{\text{amount}}{\text{volume}} \qquad [1.5.1]$$

$$C = \frac{m}{V}$$

where C is the concentration, m is the amount of solute, and V is the volume in which that amount of solute is dissolved.

SCIENTIFIC PREFIXES INDICATE ORDER OF MAGNITUDE

Often concentrations of physiologically relevant materials in physiological fluids are quite small and must be expressed in scientific notation, as in the example of hemoglobin in Example 1.5.3. It is useful to know the established prefixes for units of volume, mass, or length. The standard units are the liter, L, the gram, g, and the meter, m, respectively. The standard prefixes are shown in Table 1.5.1.

EXAMPLE 1.5.1 Calculating Molar Concentration

Suppose we dissolve 18 g of NaCl in water in a 2-L volumetric flask and then add water to the mark so that the solution volume is 2 L. What is its concentration?

We can report this concentration in mass per liter as 9 g L^{-1}. For many solutes, this is an acceptable way of expressing the concentration. For example, the concentration of hemoglobin in whole blood is 15 g dL^{-1}. Here dL means "deciliter" and is one-tenth of a liter or 0.1 L. Since 1 L = 1000 mL, 1 dL = 1000 mL/10 = 100 mL. Thus, a hemoglobin concentration of 15 g dL^{-1} means that there is 15 g of hemoglobin in every 100 mL of whole blood.

The concentration of many solutes is not reported in these units, however, but in units of moles per liter, or **molar**. We can convert from mass to moles by dividing by the gram molecular weight. In the case of NaCl given above, the atomic weight of Na is 22.99 daltons and the atomic weight of chlorine is 34.45 daltons, giving a molecular weight for NaCl of 22.99 + 35.45 = 58.44 daltons; its gram molecular weight is 58.44 g. Thus 58.44 g of NaCl constitutes 1 mole of NaCl. The number of moles of NaCl in 18 g is calculated as

$$X \text{ moles} = \frac{18 \text{ g NaCl}}{58.44 \text{ g NaCl mol}^{-1}} = \mathbf{0.308\ mol}$$

Since this amount was dissolved in 2 L of solution, its concentration = amount/volume.

$$\text{Concentration} = \frac{0.308 \text{ mol}}{2 \text{ L}} = \mathbf{0.154\ M}$$

where M designates **molarity** or moles per liter.

EXAMPLE 1.5.2 How to Make Up a Solution

You need to make up 500 mL of a solution containing 0.3 M urea. How much urea do you need?

Here we rearrange the equation $C = m/V$ to read $m = C \times V$.

In this case, $C = 0.3$ M and $V = 0.5$ L. The number of moles of urea is thus

$$m = 0.3 \text{ M} \times 0.5 \text{ L} = \mathbf{0.15\ mol}$$

We need to convert this into grams so that we can accurately weigh out the required amount of urea on a good balance. To do this, we need the gram molecular weight of urea. We can find this out several ways. We can look it up in a CRC handbook or similar source. Another way is to write out the formula and add up all of the atomic weights of all the atoms in the molecule times their compositional stoichiometry. This could be tedious for a large molecule. We could look on the bottle, because most chemical companies publish the **formula weight** on the bottle. This formula weight may be different from the gram molecular weight because the chemical might have waters of hydration with it or ions to counterbalance charges on the chemical. ATP, for example, is usually sold as $Na_2ATP \cdot 3H_2O$ and its formula weight is not the gram molecular weight of ATP alone. Looking on the bottle seems like a winner, as we must go find the bottle to weigh out the urea. We find that the formula weight for urea is 60.08 g mol^{-1}. The mass of urea is found by multiplying the moles by the gram molecular weight:

$$m = 0.15 \text{ mol} \times 60.08 \text{ g mol}^{-1} = \mathbf{9.012\ g}$$

This is the amount you need to weigh on an accurate balance.

EXAMPLE 1.5.3 Blood Concentration of Hemoglobin

The blood content of hemoglobin is 15 g dL^{-1}. The molecular weight of hemoglobin is 66,500 daltons. What is its average molar concentration in the blood?

If the molecular weight is 66,500 daltons, then 1 mole has a mass of 66,500 g. The question asks for the average concentration because hemoglobin is not uniformly distributed within the blood, but is contained within red blood cells only. Therefore, its concentration in the red blood cells exceeds its average concentration in the blood. The concentration in blood is given by

$$[\text{Hb}] = \frac{15\ \text{g dL}^{-1} \times 1\ \text{dL}/0.1\ \text{L}}{66,500\ \text{g mol}^{-1}} = 0.00266\ \text{M} = \mathbf{2.66 \times 10^{-3}\ M}$$

These examples should enable you to calculate:

- the number of moles in a given volume of solution of given concentration;
- the mass of material in a given volume of solution of given concentration;
- the concentration of solution containing a known mass of material in a given volume.

EXAMPLE 1.5.4 Make a Dilution of a Stock Solution

Suppose we have a stock solution of 0.1 M $MgCl_2$ and we want to make up 50 mL of solution with a final concentration of 5 mM $MgCl_2$. How much of the stock solution should we add to a 50-mL volumetric flask?

We use Eqn [1.5.3] directly here:

$$0.1\ \text{M} \times X\ \text{mL} = 0.005\ \text{M} \times 50\ \text{mL}$$

Solving for X, we find $X = \mathbf{2.5\ mL}$

TABLE 1.5.1 Prefixes Used with Powers of Ten, in 10^3 Ratios

Atto	10^{-18}	Femto	10^{-15}	Pico	10^{-12}	Nano	10^{-9}
Micro	10^{-6}	Milli	10^{-3}	–	10^{0}	Kilo	10^{3}
Mega	10^{6}	Giga	10^{9}	Tera	10^{12}		

Thus we can write:

1 picoliter (1 pL) = 10^{-12} L	1 picogram (1 pg) = 10^{-12} g	1 picometer (1 pm) = 10^{-12} m
1 nanoliter (1 nL) = 10^{-9} L	1 nanogram (1 ng) = 10^{-9} g	1 nanometer (1 nm) = 10^{-9} m
1 microliter (1 μL) = 10^{-6} L	1 microgram (1 μg) = 10^{-6} g	1 micrometer (1 μm) = 10^{-6} m
1 milliliter (1 mL) = 10^{-3} L	1 milligram (1 mg) = 10^{-3} g	1 millimeter (1 mm) = 10^{-3} m
1 liter (1 L) = 10^{0} L	1 gram (1 g) = 10^{0} g 1 meter (1 m) = 10^{0} m	1 kilogram (1 kg) = 10^{3} g 1 kilometer (1 km) = 10^{3} m

The prefixes mega-, giga-, and tera- are not typically used for units of volume, mass, or distance, but often find use with other units such as hertz or watts. There are additional prefixes in the SI. These include:

centi	10^{-2}	deci	10^{-1}	deca	10^{1}	hecto	10^{2}

DILUTION OF SOLUTIONS IS CALCULATED USING CONSERVATION OF SOLUTE

Often it is necessary or easier to prepare solutions from more concentrated stock solutions by removing an **aliquot** (a fraction of the solution) of the more concentrated solution and placing it in a volumetric flask. We then add solvent to bring the volume up to the final solution volume, as shown in Figure 1.5.1.

Let V_1 be the volume of the aliquot of the more highly concentrated solution, with concentration C_1. The amount of solute in this aliquot is given by Eqn [1.5.2]:

$$[1.5.2] \qquad m = C_1 \times V_1$$

This amount is also the amount in the final solution with volume V_2 and final concentration C_2. Since the amount in the aliquot is still in the final solution, we can write

$$[1.5.3] \qquad C_1 V_1 = C_2 V_2$$

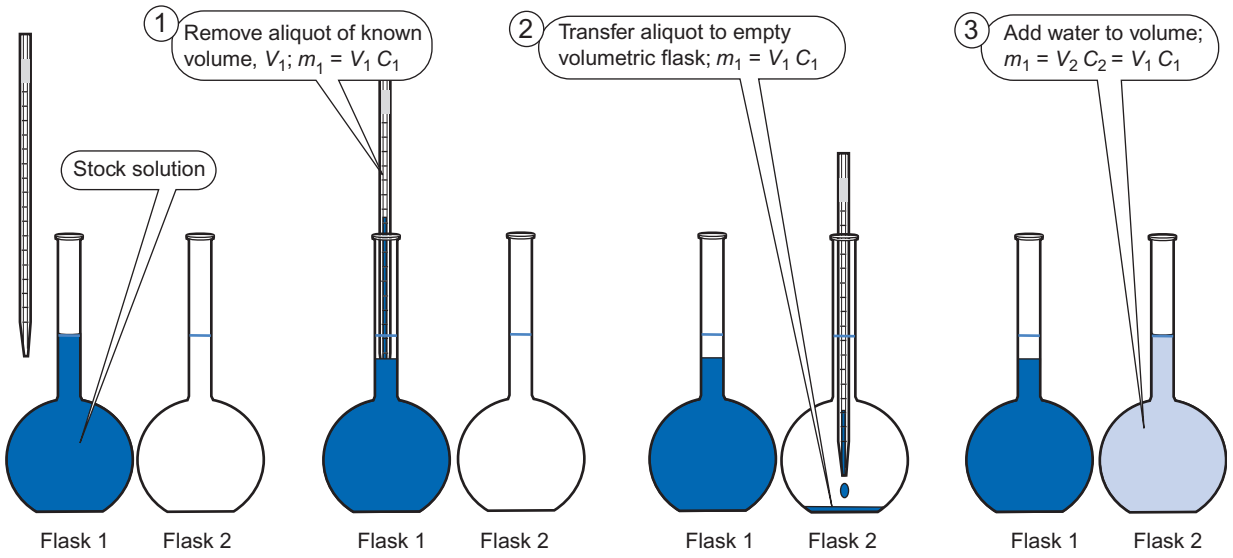

FIGURE 1.5.1 Dilution of a stock solution. Flask 1 contains a stock solution of concentration C_1. We remove a known volume, V_1, with a calibrated pipette and place it in an empty volumetric flask. We then add water, which adds solvent but no solute, to bring the volume up to the final volume. The amount of solute in the second flask is the same amount that we added in the aliquot, V_1, but now it is distributed in the volume V_2.

CALCULATION OF FLUID VOLUMES BY THE FICK DILUTION PRINCIPLE

The equation describing dilution of stock solutions can be used to determine the volume of distribution of materials in the body. Suppose we inject a known amount of a substance, Y, into a person and this substance Y remains in the plasma because it cannot get out of the vascular system and it cannot get into the cells suspended in the blood. We wait a few minutes for Y to become evenly distributed, and then take a sample of plasma and measure Y's concentration. Then we can calculate the volume of the plasma as

$$\text{Volume} = \frac{\text{amount}}{\text{concentration}} \qquad [1.5.4]$$

$$V = \frac{m}{C}$$

This is just a variant of Eqn [1.5.2] in which we solve for V instead of C or m. In our particular case in this example, we write

$$V = m_Y/[Y]$$

where m_Y is the amount of substance Y and $[Y]$ is its concentration.

CHEMICAL REACTIONS HAVE FORWARD AND REVERSE *RATE CONSTANTS*

Suppose that we observe a simple chemical reaction that can be described as

$$A + B \rightleftharpoons C \qquad [1.5.5]$$

where A, B, and C denote different chemicals and the arrows indicate that the reaction proceeds in the direction of the arrow. The reaction actually consists of two separate reactions:

$$A + B \longrightarrow C \qquad [1.5.6]$$
$$A + B \longleftarrow C$$

The top reaction is the **forward** reaction and the bottom reaction is the **reverse** reaction. Both are characterized by a **rate constant**. The rate constant gives the rate of the reaction when the rate constant is multiplied by the concentration of reactants:

$$J_f = k_f[A][B] \qquad [1.5.7]$$
$$J_r = k_r[C]$$

EXAMPLE 1.5.5 Estimation of Plasma Volume

Suppose that we inject a 50-kg person with 2 mL of a solution of Evans' Blue Dye, at 5 mg mL^{-1}. Evans' Blue Dye is restricted to the plasma because it tightly binds to a plasma protein, albumin, that ordinarily does not escape from the circulation and which only slowly is removed or added to the plasma. We wait 10 min and then obtain a sample of blood and measure the concentration of Evans' Blue Dye in the plasma and find that it is 0.4 mg dL^{-1}. What is the person's plasma volume?

The amount of Evans' Blue Dye that was injected is calculated as

$$m_{inj} = C_{inj} V_{inj} = 5 \text{ mg mL}^{-1} \times 2 \text{ mL} = \mathbf{10 \text{ mg}}$$

The concentration of Evans' Blue Dye after mixing was 0.4 mg dL^{-1}. We calculate its volume of distribution as

$$V = \frac{10 \text{ mg}}{0.4 \text{ mg dL}^{-1}} = 25 \text{ dL} = \mathbf{2.5 \text{ L}}$$

where J_f is the forward reaction rate, J_r is the reverse reaction rate, k_f is the forward reaction rate constant, and k_r is the reverse rate constant, and $[A]$, $[B]$, and $[C]$ are the concentrations of the indicated reactants.

Although this form of the reaction rate is true for elementary reactions, more complicated reactions could have a form that appears to have little to do with the overall reaction as written—its stoichiometric relation or accounting of the number of each kind of molecule that participates in the reaction. This is because complicated reactions occur with intermediary steps that may involve chemicals that do not appear in the overall balanced reaction. Nearly all enzymatic reactions, for example, do not obey Eqn [1.5.7] because the reaction rate is determined largely by the concentration of enzyme. For the moment, we will consider only elementary reactions that obey Eqn [1.5.7].

The rate of a reaction is the number of completed reactions that take place per unit time. You should recognize this as an extensive property. If we had twice the volume of a solution, with the same concentrations of reactants and products, we would have twice the number of reactions taking place per unit time. So we often convert reaction rates into intensive variables by dividing by the volume. The forward rate is thus the number of completed reactions per unit time per unit volume. We express these numbers of reactions in terms of moles, which are related by Avogadro's number to a real number of completed reactions. Thus the forward rate constant has units of $M^{-1}\,s^{-1}$. By similar reasoning, the reverse rate constant in Eqn [1.5.7] has the units of s^{-1}. The rate of change of reactants A and C can be given as

$$[1.5.8]\qquad \begin{aligned}\frac{d[A]}{dt} &= -k_f[A][B] + k_r[C]\\ \frac{d[C]}{dt} &= -k_f[A][B] - k_r[C]\end{aligned}$$

The negative sign before $J_f(=k_f[A][B])$ in the above equation indicates that this reaction reduces the concentration of reactant A; the positive sign of J_r indicates that this reaction flux adds to the concentration of reactant A. Similar reasoning gives us the rate of change of $[C]$. At equilibrium, the concentrations of A, B, and C are no longer changing. The values of $[A]$, $[B]$, and $[C]$ are altered from their original concentrations so that the forward and reverse rates are equal:

$$[1.5.9]\qquad \begin{aligned}\frac{d[A]}{dt} = 0 &= -k_f[A][B] + k_r[C]\\ k_f[A][B] &= k_r[C]\\ J_f &= J_r\end{aligned}$$

The rate constants, k_f and k_r, are characteristic of the reaction path involved and the experimental conditions such as ionic strength and temperature of the reactants. They typically do not vary with the concentrations of A, B, and C. Thus, at equilibrium, the middle expression in Eqn [1.5.9] is true for any set of $[A]$, $[B]$, and $[C]$. We rearrange this to get:

$$[1.5.10]\qquad \frac{k_f}{k_r} = \frac{[C]}{[A][B]} = K_{eq}$$

where K_{eq} is the **equilibrium constant**. From the units of k_f as $M^{-1}\,s^{-1}$ and k_r as s^{-1}, we see that K_{eq} has the units of M^{-1}.

FIRST-ORDER RATE EQUATIONS SHOW EXPONENTIAL DECAY

Some kinds of chemical reactions, such as decomposition reactions, obey the equation

$$[1.5.11]\qquad A \rightarrow B$$

Often this reaction is strongly directed to the right, meaning that $k_f >> k_r$. The reaction rate is approximated as

$$[1.5.12]\qquad J = -k_f[A]$$

Here we imagine that initially $[B] = 0$ and there is no reverse reaction. Some reactions occur with such completeness that the reverse reaction is negligible. We can rewrite Eqn [1.5.12] as

$$[1.5.13]\qquad \frac{d[A]}{dt} = -k_f[A]$$

This is called a first-order rate equation because the rate of reaction is proportional to the first order or the concentration of reactant—it is proportional to the first power of its concentration. We can separate variables and integrate this equation, as follows:

$$[1.5.14]\qquad \begin{aligned}\int_0^t \frac{d[A]}{[A]} &= \int_0^t -k\,dt\\ \ln[A] - \ln[A]_0 &= -kt\\ [A] &= [A]_0 e^{-kt}\end{aligned}$$

The last equation describes the concentration of A with time—it decays exponentially. This equation is described as a first-order exponential decay. Many reactions and processes are described by this type of analysis, such as radioactive decay and the disappearance of many different hormones from the circulation. This type of reaction is characterized by its half-life, the time required for $[A]$ to fall from its initial value, $[A]_0$, to one-half of its initial value, $[A]_0/2$. In this case, Eqn [1.5.13] gives

$$[1.5.15]\qquad \begin{aligned}\frac{[A]_0}{2} &= [A]_0 e^{-kt_{1/2}}\\ \ln\frac{1}{2} &= \ln e^{-kt_{1/2}}\\ -\ln 2 &= -kt_{1/2}\\ t_{1/2} &= \frac{\ln 2}{k}\end{aligned}$$

Thus the half-life of a first-order reaction is inversely proportional to the first-order rate constant.

RATES OF CHEMICAL REACTIONS DEPEND ON THE *ACTIVATION ENERGY*

The values of k_f and k_r depend on the reaction path taken for the reaction, but K_{eq} is not affected by the reaction path. We shall not prove this result here but will give some rationale for it. A more complete description is given in Appendix 1.5.A1.

The reactants (A and B) and the product, C, can be viewed as possessing some degree of energy. This energy is a potential energy that consists of the potential energy of all of the interactions of their orbital electrons with the positive nuclei. The set of nuclei has some spatial arrangement which changes during the course of the reaction. This is the essence of a chemical reaction, in which the relative positions of the nuclei are altered. The orbital electrons, of course, follow the nuclei so that the energy of the ensemble changes during the course of the reaction. We call this energy the potential energy, and it includes the potential energy of the electrons and the nuclei and the kinetic energy of the electrons, but does not include the kinetic energy of the nuclei. We can plot this potential energy against the "reaction coordinate," which is the actual distance along the minimum energy path from reactants to products. An example of such a graph is shown in Figure 1.5.2.

The rate constants that govern the rates of reaction depend on the energy required to reach the activated complex intermediate between reactants and products. Large activation constants are associated with small rate constants. The relationship is expressed by the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} \quad [1.5.16]$$

where k is the rate constant for the reaction, A is a *preexponential factor* that has to do with the orientation of the reaction and not its temperature dependence, E_a is the activation energy in $J\,mol^{-1}$, R is the gas constant ($=8.314\,J\,mol^{-1}\,K^{-1}$), and T is the absolute temperature (K). The equilibrium constant, however, depends only on the initial and final energy levels. Note that the Arrhenius equation means that a larger E_a would cause a lower rate constant, and a smaller E_a would increase the rate constant.

ENZYMES SPEED UP REACTIONS BY LOWERING E_A

It is possible for the reaction to take a different path. For example, A and B could be absorbed onto the surface of an enzyme. The forces that aid in this binding have been discussed in Chapter 1.4: electrostatic interaction between ions in the substrate and enzyme; hydrogen bonding; dipole–dipole interactions; and London dispersion forces. This binding to the enzyme changes the configuration of nuclei and alters the energy of the activated complex. In this way, the enzyme offers an alternative pathway for the reaction that involves far less activation energy. This increases the reaction rates without altering the final energetics of reactants and products. Thus the rates increase without changing the equilibrium constant. Figure 1.5.3 illustrates this idea.

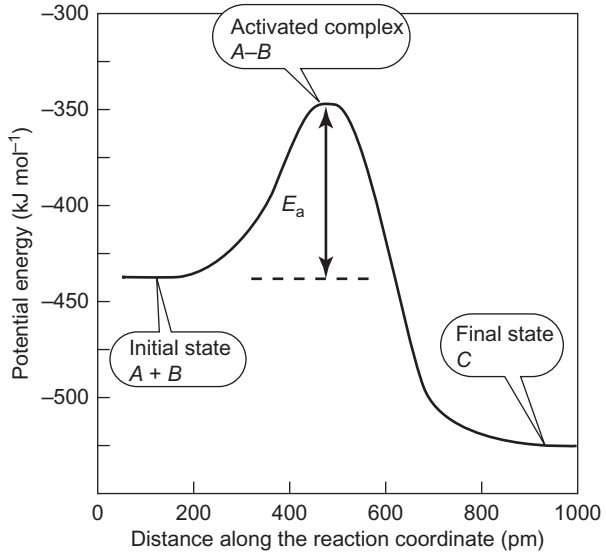

FIGURE 1.5.2 Potential energy along the reaction coordinate for the reaction $A + B \rightarrow C$. Reactants A and B are at low potential energy. The activated complex is a form intermediate between reactants and products, and can be attained by converting kinetic energy into potential energy by a collision between A and B. The difference between the energy of the activated complex and the reactants is the activation energy that must be supplied for the reaction to proceed. The "reaction coordinate" is the distance along the minimum free-energy path from reactants to products.

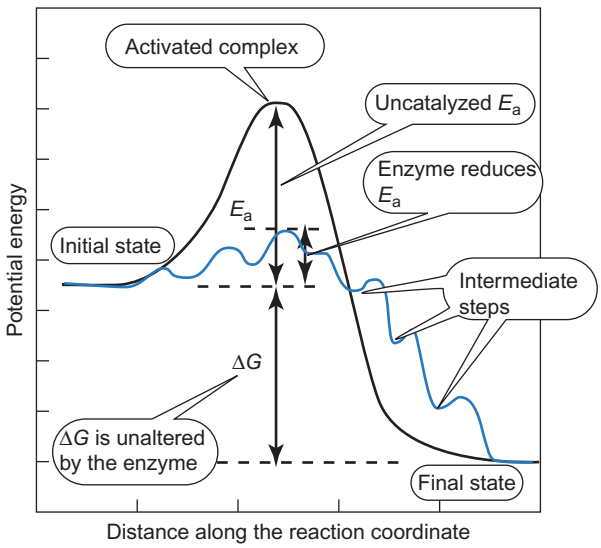

FIGURE 1.5.3 The effect of an enzyme on the overall activation energy for a reaction. The uncatalyzed reaction requires a large activation energy, E_a, and so the reaction occurs slowly. The enzyme offers an alternative path, often by breaking the reaction into a number of small steps, so that the reaction occurs more quickly. Enzymes do not change the overall energetics of the products with respect to the reactants (ΔG in the figure is the change in free energy in the transition between reactants and products) and so the equilibrium constant is unaffected.

THE MICHAELIS–MENTEN FORMULATION OF ENZYME KINETICS

Catalysis is the speeding up of a chemical reaction by a chemical species that does not enter into the stoichiometry of the reaction. Enzymes are catalysts because they speed up reactions without themselves being altered. Enzymes typically bind the reactants and alter their shape (the three-dimensional arrangement of all of the atomic nuclei in the reactants) by virtue of their being attracted to or bound to the surface of the enzyme. The enzyme changes the reaction from a homogeneous reaction in the solution to a heterogeneous reaction occurring on the surface of the enzyme. In this process, the enzyme itself is unchanged. It participates in the reaction but does not show up in an accounting of the reactants and products because it is unchanged.

Figure 1.5.3 shows that enzymes speed up reactions by providing an alternate path for the reaction to take, and that this path requires less activation energy. Michaelis and Menten provided a simple analysis of this reaction path by imagining it to take place in two steps:

$$[1.5.17] \qquad E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E - S \xrightarrow{k_3} E + P$$

where E is the enzyme, S is the substrate, $E{-}S$ is the substrate–enzyme complex, and P is the product. This is a very simple reaction mechanism. The rate of the enzyme-catalyzed reaction is defined by the rate of product release:

$$[1.5.18] \qquad J = k_3[E - S]$$

If we know $[E{-}S]$ and k_3, we can calculate the reaction rate. Here k_3 is in units of s^{-1}. If we keep $[S]$ and $[P]$ constant, we can define a steady-state rate in which S is converted to P at a constant rate. Under these conditions, we can solve for $[E{-}S]$. The rate of change of $[E{-}S]$ is given by

$$[1.5.19] \qquad \frac{d[E - S]}{dt} = k_1[E][S] - k_2[E - S] - k_3[E - S]$$

At steady state, $d[E - S]/dt = 0$, so that

$$[1.5.20] \qquad k_1[E][S] = (k_2 + k_3)[E - S]$$

Since the enzyme can exist in only two states, E and $E{-}S$, we have a conservation relation

$$[1.5.21] \qquad [E]_{total} = [E] + [E - S]$$

where $[E]_{total}$ is the total concentration of enzyme. Inserting this relation for $[E]$ in Eqn [1.5.20], we get

$$[1.5.22] \qquad k_1([E]_{total} - [E - S][S]) = (k_2 + k_3)[E - S]$$

Solving for $[E{-}S]$, we find

$$[1.5.23] \qquad [E - S] = \frac{k_1[E]_{total}[S]}{k_2 + k_3 + k_1[S]}$$

From Eqn [1.5.18], the rate of the reaction is just $k_3[E{-}S]$. Multiplying both sides of Eqn [1.5.23] by k_3, and dividing numerator and denominator by k_1, we obtain

$$[1.5.24] \qquad J = k_3[E - S] = \frac{k_3[E]_{total}[S]}{(k_2 + k_3)/k_1 + [S]}$$

The maximum velocity occurs when all of the enzyme is present as $[E{-}S]$. The maximum velocity or rate of the reaction is thus given as

$$[1.5.25] \qquad J_{max} = k_3[E]_{total}$$

Inserting this definition into Eqn [1.5.24], we finally obtain

$$[1.5.26] \qquad J = \frac{J_{max}[S]}{K_m + [S]}$$

where K_m is a newly defined constant called the Michaelis–Menten constant. It is given as

$$[1.5.27] \qquad K_m = \frac{k_2 + k_3}{k_1}$$

The units of k_2 and k_3 are both in s^{-1}, whereas the unit of k_1 is $M^{-1}\ s^{-1}$; thus, the unit of K_m is M. If $k_3 \ll k_2$, K_m approximates the value of k_2/k_1, which is the dissociation constant for binding of S to the enzyme. It can be obtained experimentally as the value of the substrate concentration at which the enzyme exhibits one-half maximal velocity. This can be seen from Eqn [1.5.26] by inserting $J = \frac{1}{2}J_{max}$ and finding that $[S] = K_m$ when the rate of the reaction is one-half maximal.

The saturation curve for a Michaelis–Menten type reaction is shown in Figure 1.5.4. In this case, J_{max} was 8 μmol min^{-1} mL^{-1}. At one-half of this maximal velocity, the substrate concentration was 1.5 mM.

The curve shown in Figure 1.5.4 often lacks sufficient points to accurately extrapolate the observed velocity

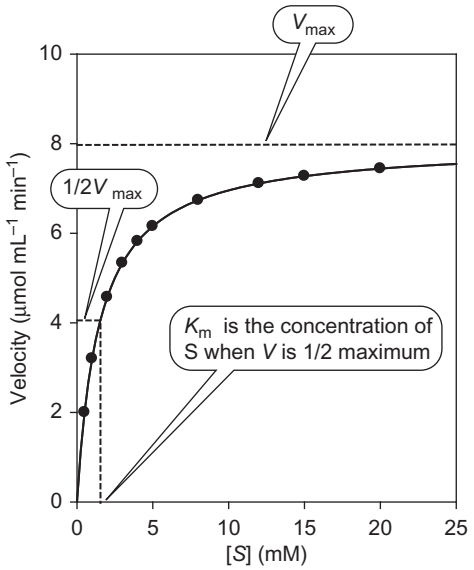

FIGURE 1.5.4 Saturation curve for an enzyme that obeys Michaelis–Menten kinetics. The velocity at low [S] is nearly linear with [S] but quickly levels off. This behavior is called saturation kinetics. The concentration of substrate at one-half maximal velocity (=one-half saturation) is equal to the K_m, the Michaelis–Menten constant.

to the maximal velocity. To aid in this graphical determination of enzyme kinetics, the Michaelis–Menten equation is transformed. If we take the inverse of Eqn [1.5.26], we obtain

$$J = \frac{J_{max}[S]}{K_m + [S]}$$

$$\frac{1}{J} = \frac{K_m + [S]}{J_{max}[S]} \quad [1.5.28]$$

$$\frac{1}{J} = \frac{1}{J_{max}} + \frac{K_m}{J_{max}}\frac{1}{[S]}$$

This equation suggests that a plot of $1/J$ versus $1/[S]$ should be linear with an intercept on the $1/J$ axis of $1/J_{max}$ and a slope of K_m/J_{max}. The intercept on the $1/[S]$-axis is $-1/K_m$. Such a plot is called a **double-reciprocal plot**, also known as a Lineweaver–Burk plot. An example for the results shown in Figure 1.5.4 is shown in Figure 1.5.5.

Throughout this discussion of enzyme rates, we have used J as the symbol of the rate of product release, or the rate of the reaction. J has the units of moles per unit volume, per unit time. Often this is taken to be the same as the reaction velocity, and the usual symbol in the Michaelis–Menten equation is V, and not J. Sometimes, the reaction velocity in these units is normalized by dividing by the enzyme concentration, so that the reaction velocity is given as a specific activity of the enzyme, in units of moles of reaction per unit enzyme per unit time. These various representations of the velocity all obey the same general form of equations and can be subjected to the same analysis.

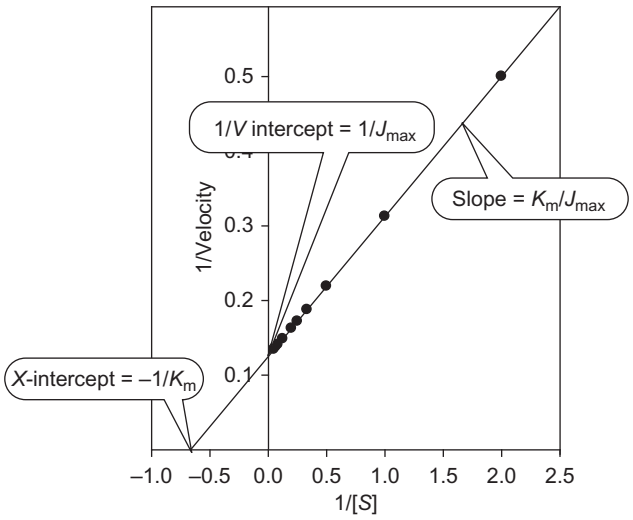

FIGURE 1.5.5 Lineweaver–Burk plot. Values of $1/[S]$ are plotted along the abscissa while values of $1/J$ are plotted on the ordinate. Here J is the velocity, or rate, of the enzyme reaction and $[S]$ is the substrate concentration. The slope of the line is K_m/J_{max}, where J is the enzyme flux ≈ enzyme velocity and J_{max} is the theoretical maximal rate. K_m is the Michaelis–Menten constant. It is also determined as the X-intercept $= -1/K_m$. The Y-intercept is $1/J_{max}$. Deviations from linearity on the Lineweaver–Burk plot suggest that the enzyme does not obey Michaelis–Menten kinetics.

PHYSIOLOGY IS ALL ABOUT SURFACES

As noted above, enzyme catalysis derives from the reaction occurring on the surface of the enzyme rather than in homogeneous solution. Physiology is all about surfaces and their interactions. Proteins have complicated three-dimensional shapes that closely appose the surfaces of some materials and not others—they are specific. Sometimes the fit is very close and therefore the binding strength is very high—the two surfaces form a tight association. Which proteins stick to which other proteins, or which other substrates, or ligands, determines the activity of the proteins, which determines the activities of the cells, and then the organs, and, finally, the organism. Ultimately, physiology is all about what happens on the surfaces of molecules.

SUMMARY

The concentration of a solute in solution is the amount of that solute per unit volume of solution. It can be expressed as the mass of the solute per unit volume or the number of moles of solute per unit volume. A mole of any substance is Avogadro's number of particles. Avogadro's number originates in the ratio of the mass of a carbon-12 atom to 12 g. The dalton is defined as 1/12 of the mass of a carbon-12 atom. The number of daltons in 1 g is Avogadro's number, 6.02×10^{23}.

The concentration in molar is the number of moles per liter of solution. Small concentrations use established prefixes to describe them, in increments of 1000. A millimolar solution is 10^{-3} M, micromolar is 10^{-6} M, nanomolar is 10^{-9} M, and picomolar is 10^{-12} M. These same prefixes are used for units of g, L, and m.

The relationship among concentration, amount of solute, and volume of solution can be used to determine the volume of physiological fluids. Evans' Blue Dye is an example of a solute that can be used to estimate plasma volume, because the dye enters the plasma but cannot leave it easily.

Elementary chemical reactions have forward and reverse rate constants that govern the rate of conversion in either the forward or reverse reaction. The rates of reaction have the units of moles per unit time per unit volume of solution. The ratio of the forward and reverse rate constants is the equilibrium constant. Conversion of reactants to products requires an activation energy, and because kinetic energy increases with temperature, the rate of the reaction also varies with the temperature. The relationship between temperature and rate is described by the Arrhenius equation, which incorporates the activation energy, E_a.

Enzymes speed chemical reactions by altering the path of the reaction by allowing it to proceed on the surface of the enzyme. Thus enzymes convert homogeneous reactions in the fluid phase to heterogeneous reactions on the surface of the enzyme. The alternate path reduces the activation energy for the reaction, thereby allowing it to proceed quicker.

The Michaelis–Menten formulation of enzyme kinetics describes the steady-state rate of enzyme reactions and is derived for a particular kind of enzyme mechanism. Nevertheless, the resulting equation often fits many enzyme-catalyzed reactions. It is given as

$$J = \frac{J_{max}[S]}{K_m + [S]}$$

where J is the reaction flux or velocity, in units of moles of completed reaction per unit time per unit volume, J_{max} is the asymptotic maximal rate achievable extrapolated at infinite concentration, K_m is the Michaelis–Menten constant, equal to the concentration of substrate at half maximal velocity, and $[S]$ is the substrate concentration. This equation describes **saturation kinetics**. It can be analyzed more easily using the inverse of the equation, rearranged, to give

$$\frac{1}{J} = \frac{1}{J_{max}} + \frac{K_m}{J_{max}}\frac{1}{[S]}$$

Plots of $1/J$ against $1/[S]$ yield $1/J_{max}$ as the intercept on the abscissa and $-1/K_m$ as the extrapolated intercept on the ordinate.

REVIEW QUESTIONS

1. What is a dalton? What is meant by molecular weight?
2. What is a mole? What is meant by the gram molecular weight? How would you determine the gram molecular weight of small compounds?
3. Write the relationship among concentration, volume, and amount.
4. What is meant by "first-order reaction"?
5. How do you calculate the half-life of a reaction?
6. In the plot of potential energy against reaction coordinate, what is meant by "reaction coordinate"? What units does it have?
7. What is the activation energy?
8. Why do reaction rates generally depend on the temperature?
9. How could you determine the activation energy for a reaction?
10. In general, how do enzymes speed up biochemical reactions?
11. How would you determine K_m and V_{max} for an enzyme?

APPENDIX 1.5.A1 TRANSITION STATE THEORY EXPLAINS REACTION RATES IN TERMS OF AN ACTIVATION ENERGY

TRANSITION STATE THEORY CALCULATES THE POTENTIAL ENERGY OF REACTANTS AS A FUNCTION OF SEPARATION

Transition state theory gives an expression for the rate constant of a chemical reaction by applying statistical mechanics to quantum mechanical calculations of various configurations of reactant and product. In this procedure, a collection of nuclei with their attendant electrons is treated as a "supermolecule." Quantum mechanical calculations are performed in which the potential energy of the supermolecule is calculated as a function of the relative positions of the nuclei. An exact solution requires solving for the total energy including the kinetic energy terms of all of the nuclei and electrons and the potential energy terms for all electron–electron, electron–nucleus, and nucleus–nucleus pairs. Since this solution is extremely difficult, some simplifying assumptions are made. One is that the electronic motion is extremely rapid compared to translation of the nuclei and that the electrons adjust instantly to any change in the positions of the nuclei. Thus the energy of the electrons and potential energy of the nuclei can be calculated as if the nuclei were at rest. The energy calculated in this way includes everything but the kinetic energy of the nuclei. It is called the potential energy even though it includes the kinetic energy of the electrons.

POTENTIAL ENERGY CAN BE GRAPHED AGAINST SEPARATION IN A SINGLE MOLECULE

The potential energy of a configuration of nuclei with their electrons may be represented as a point on an f-dimensional surface in an $f+1$-dimensional space, where f is the number of independent variables required to specify the relative positions of all nuclei. For a diatomic molecule (like H_2 or HF), $f=6$ because we need six variables to specify the Cartesian coordinates of the two nuclei. However, three of these can be considered to locate the center of mass of the molecule and another two specify the orientation of the molecular axis. As these do not really concern us, we have only one remaining variable, the internuclear distance, to specify the relative positions of the two nuclei. Since $f=1$, we have a potential surface which is the one-dimensional potential energy curve in the two-dimensional graph for a diatomic molecule (H_2) as shown in Figure 1.5.A1.1.

POTENTIAL ENERGY CAN BE GRAPHED AGAINST NUCLEI SEPARATION IN A CHEMICAL REACTION

In a configuration of three nuclei, the potential energy surface can be shown as a fixed-angle surface, where the angle is defined as the angle between one bond and the approaching reactant, as shown in Figure 1.5.A1.2. This allows us to plot the potential surface on paper; otherwise we need another physical dimension. The entire potential surface consists of an infinite number of these fixed-angle surfaces, one for each angle of approach.

Such a fixed-angle surface is shown in Figure 1.5.A1.3 for the reaction

$$F + H - H' \rightarrow F - H + H'$$

The surface itself is three-dimensional. What Figure 1.5.A1.3 shows is the two-dimensional projection of the surface onto the plane of the paper. Here every point on a given line has the same potential energy. Thus these lines represent potential energy contours in much

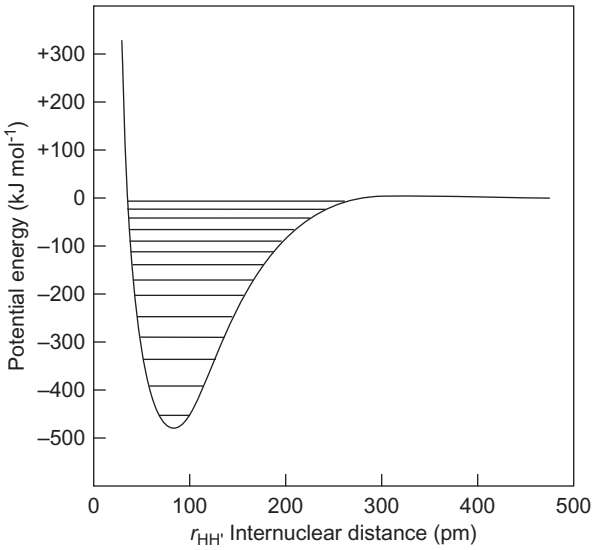

FIGURE 1.5.A1.1 Potential energy diagram for a diatomic molecule, H_2. Potential energy is plotted as a function of the separation of the two *H* nuclei. The average bond length corresponds to the minimum energy level, which in this case occurs at about 80 pm. The horizontal lines indicate quantum vibrational states. Rotation of the molecule alters the potential energy profile due to a centrifugal effect (rotation stretches the bond length). *Adapted from J.W. Moore and R.G. Pearson,* Kinetics and Mechanism, *John Wiley & Sons, New York, NY, 1981.*

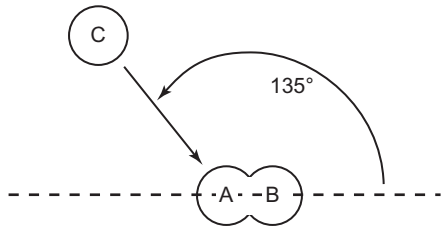

FIGURE 1.5.A1.2 Fixed angle of approach of reactant C to the diatomic molecule AB. The potential energy surface varies with the angle of approach.

the same way as contour lines on a topographic map represent lines of equal altitude.

In the contour map for the reaction $F + H - H' \rightarrow F - H + H'$, several regions are labeled. Region "W" corresponds to separated *F*, *H*, and *H′* nuclei and this is a plateau region where potential energy varies little with the distance between the nuclei. At region "X," *F* is far removed from *H*–*H′* and the potential energy, *U*, is affected little by changes in r_{FH}, the distance between the *F* nucleus and the *H* nucleus. The effect of $r_{HH'}$ on *U* is that shown earlier for the diatomic molecule, so that a vertical cross-section at "X" would show a deep valley with steep sides. At region "Y," all three nuclei are close together and the potential energy has increased due to van der Waals repulsion. A collision of *F* with *H*–*H′* thus corresponds to the movement of the configuration of nuclei from region "X" to region "Y" where potential energy is increased. If the reaction is completed, then the configuration moves on to region "Z." In this region, r_{FH} is short and $r_{HH'}$ is long, indicating that a bond has formed between *F*–*H* and the *H*–*H* is broken. The horizontal cross-section at "Z" gives the potential energy dependence of *F*–*H* on its bond distance.

THE ACTIVATED COMPLEX IS A METASTABLE COMPLEX OF REACTANTS

The movement of the configuration of nuclei from *X* to *Y* to *Z* in Figure 1.5.A1.3 traces a completed reaction from the initial reactants. At the point *Y*, there is a saddle point represented by higher potential energy, in this case, than for either reactants or products. The configuration of nuclei at this point is called the **activated complex** which is said to be in the **transition state**. Here all of the nuclei are close together, forming a complex. The path of steepest ascent from reactants to activated complex and on to products is called the **reaction coordinate**. This path is perpendicular to each contour it crosses. It is the minimum energy path from reactants to products.

Recall that the potential energy surface is just that—a potential energy. Collisions between reactants can overcome the potential energy barrier between the reactant and the activated complex if there is sufficient kinetic energy of the molecules that collide. The potential energy can be plotted against the distance along the reaction coordinate. This is the familiar diagram encountered in general chemistry, without any kind of explanation of what the reaction coordinate actually is. For our example reaction, $F + H{-}H' \rightarrow F{-}H + H'$, the potential energy versus reaction coordinate plot is shown in Figure 1.5.A1.4. This plot is the conceptual origin of Figure 1.5.2 in the text.

REACTIONS GENERALLY DO NOT FOLLOW A MINIMUM ENERGY PATH

Reactions in general do not follow the reaction coordinate. Two alternative trajectories are shown in Figure 1.5.A1.5. Because the H–H′ bond vibrates, the configuration of all three nuclei while *F* approaches *H*–*H′* oscillates. In Figure 1.5.A1.5A, the reaction ascends near to the activated complex but does not go to completion. This trajectory represents a nonreactive, inelastic collision between *F* and *H*–*H′*. In this case, there is a transfer of energy from translational kinetic energy to vibrational energy. In Figure 1.5.A1.5B, a completed reaction is shown. Here the oscillations correspond to vibrations of reactant *H*–*H′* and product *FH*.

THE TRANSITION STATE THEORY SAYS THAT THE RATE CONSTANT VARIES WITH THE EXPONENT OF THE ACTIVATION ENERGY

The derivation of a rate equation from the transition state theory is a bit complicated and we will not attempt it in any detail. However, an elementary understanding of it can be provided by thinking of the activated complex as a separate, transient species. Then the rate of completed reaction will be proportional to the amount of activated complex, and the rate per unit volume will be proportional to its concentration. If we imagine that the reactants and

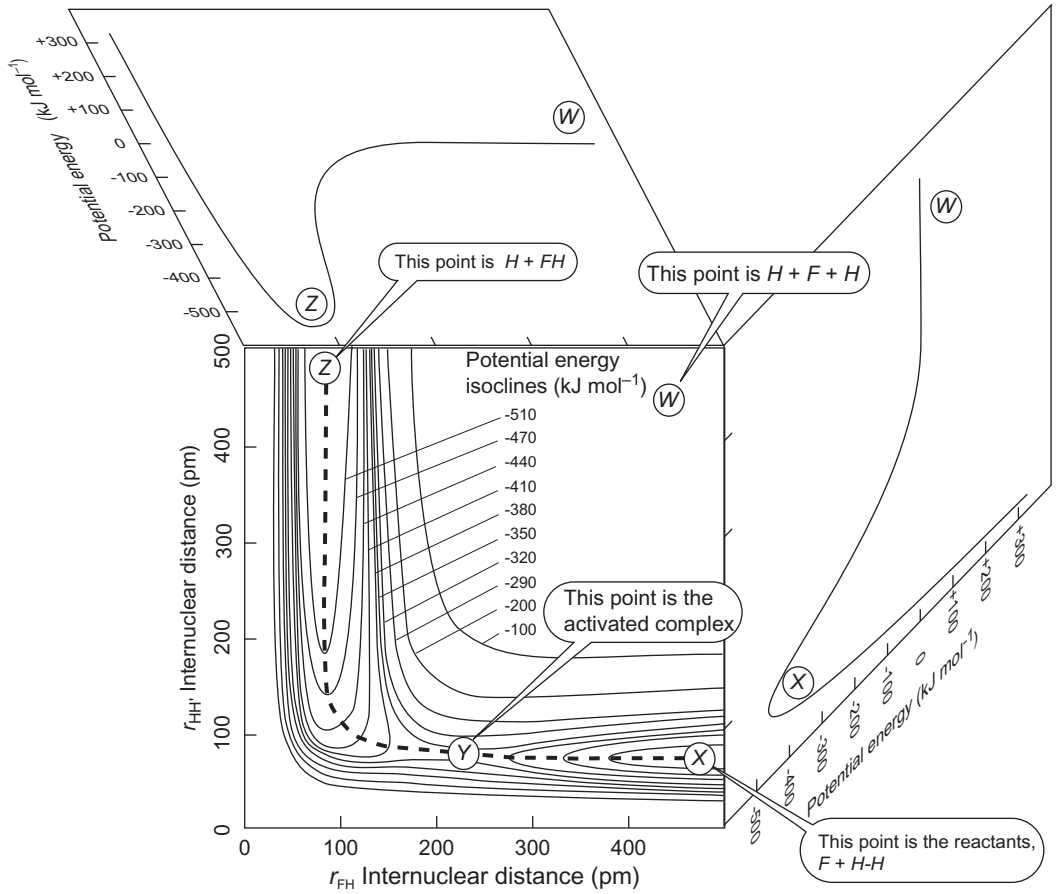

FIGURE 1.5.A1.3 Potential energy contour diagram for a fixed angle (180°) for the reaction $F + H{-}H' \rightarrow F{-}H + H'$. The lines represent a set of points of equal potential much like contour lines on a topographic map represent a set of points of equal altitude. The value of the potential energy (in kJ mol^{-1}) for each line is indicated. Point W lies on a plateau of high potential energy corresponding to dissociated *F*, *H*, and *H′* nuclei. *X* corresponds to the configuration in which *F* is far from *H*–*H′*; it is at a potential energy minimum. *Y* is the saddle point at which all three nuclei are close; it corresponds to the activated complex. *Z* corresponds to the configuration in which *H′* is far from *FH*. It represents the products of the completed reaction. The graph at the right is a vertical cross-section through the three-dimensional surface at point *X*. The graph at the top is a vertical cross-section (orthogonal to the one at the right) through the three-dimensional surface at point *Z*. *Adapted from J.W. Moore and R.G. Pearson,* Kinetics and Mechanism, *John Wiley & Sons, New York, NY, 1981.*

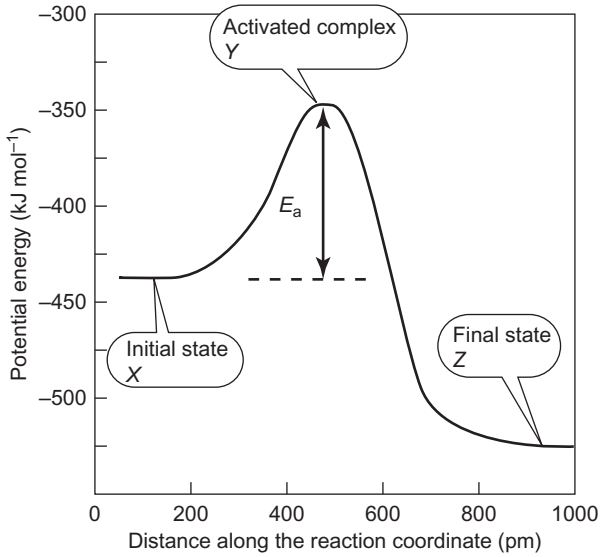

FIGURE 1.5.A1.4 Potential energy along the reaction coordinate for the reaction $F + H{-}H' \rightarrow F{-}H + H'$. Reactants $F + H{-}H'$ are at a low potential energy. The activated complex is at higher potential energy, which can be attained by converting kinetic energy into potential energy through a collision of *F* and *H*–*H′*. The reactants are at lower potential energy. The difference between the energy of the activated complex and the reactants is the activation energy that must be supplied for the reaction to proceed.

the activated complex are in equilibrium, then the rate constant for the overall reaction will be proportional to the equilibrium constant.

$$K_r = \kappa \frac{kT}{h} K^* \qquad [1.5.A1.1]$$

There are a lot of *k*'s in this equation. K_r is the rate constant for the reaction; κ is a **transmission coefficient** that tells us what fraction of activated complexes goes on to complete the reaction; *k* in *kT* is Boltzmann's constant, which is equal to the gas constant per molecule, or R/N_o, the gas constant divided by Avogadro's number; *h* is Planck's constant; and K^* is a constant that has the form of an equilibrium constant for the formation of the activated complex, but strictly speaking it is not an equilibrium constant. The equilibrium constant is related to the change in free energy for the reaction (see Chapter 1.7) according to

$$K^* = e^{\frac{-\Delta G^*}{RT}} \qquad [1.5.A1.2]$$

where ΔG^* is the free-energy change per mole for the formation of the activated complex from the reactants. This energy change is identified with the **activation energy**, E_a, as described in Figure 1.5.A1.4.

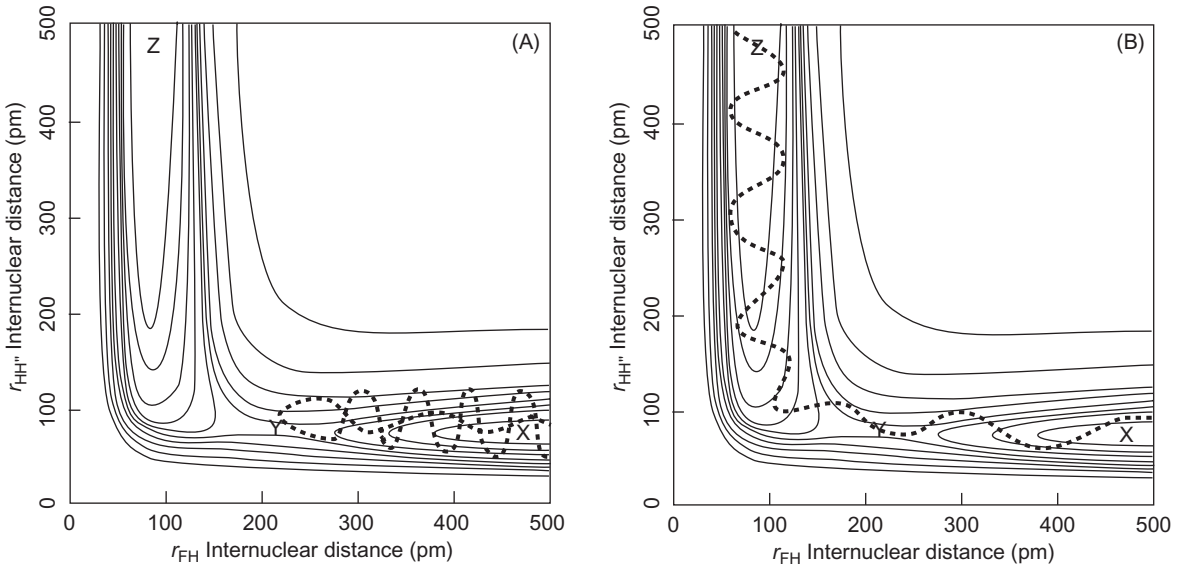

FIGURE 1.5.A1.5 Hypothetical trajectories of reactions. (A) An inelastic collision that does not result in a completed reaction. (B) A completed reaction. The oscillations are due to vibration of the molecule in which bond length oscillates around an average value.

In 1884, van't Hoff proposed that the temperature dependence of equilibrium constants could be described by

$$\frac{\mathrm{d}\ln K_{\mathrm{eq}}}{\mathrm{d}T} = \frac{\Delta G^0}{RT^2} \qquad [1.5.A1.3]$$

It was discovered shortly thereafter that the rates of chemical reactions approximately double for every 10°C increase in temperature. By analogy to the van't Hoff law, Arrhenius proposed in 1889 that the temperature dependence of the rate constant could be given as

$$\frac{\mathrm{d}\ln K_{\mathrm{r}}}{\mathrm{d}T} = \frac{\Delta E^0}{RT^2} \qquad [1.5.A1.4]$$

The integrated form of this equation is similar to what we see from transition state theory:

$$K_{\mathrm{r}} = A\mathrm{e}^{-E_{\mathrm{a}}/RT} \qquad [1.5.A1.5]$$

where A is the preexponential factor that incorporates the transmission coefficient. This is close to what one would expect by inserting the definition of K^* into Eqn [1.5.A1.1]. The natural log of both sides gives

$$\ln K_{\mathrm{r}} = \ln A - \frac{E_{\mathrm{a}}}{RT} \qquad [1.5.A1.6]$$

Thus the plots of the logarithm of the rate constant against $1/T$ should be linear with a slope $= -E_{\mathrm{a}}/R$. Such a plot is an **Arrhenius plot**. Although transition state theory does not predict a perfectly linear relation between $\ln K_{\mathrm{r}}$ and $1/T$, most reactions obey Eqn [1.5.A1.6] well.

THE ACTIVATION ENERGY DEPENDS ON THE PATH

The potential energy surfaces described in Figure 1.5.A1.3 pertain only to the collisions between the molecules $H{-}H'$ and F, which is a simple system. Similar potential energy surfaces could be described for more complicated reactions, but the surface becomes much more complicated because of the large number of atomic nuclei involved in typical biochemical reactions. However, the idea of the reaction coordinate and activation energy remains. By absorbing reactants onto their surfaces, enzymes completely alter the potential energy surfaces of a reaction. The net effect is to lower E_{a}. According to Eqn [1.5.A1.6], lower activation energies mean larger rate constants. Thus enzymes and other catalysts increase the speed of reactions by providing an alternate mechanism such that E_{a} is reduced. This idea is depicted graphically in Figure 1.5.3 in the text.

APPENDIX 1.5.A2 UNIDIRECTIONAL FLUXES OVER A SERIES OF INTERMEDIATES DEPEND ON ALL OF THE INDIVIDUAL UNIDIRECTIONAL FLUXES

UNIDIRECTIONAL FLUXES DIFFER FROM NET FLUXES

Consider the diagram shown in Figure 1.5.A2.1. Each node, indicated by a dot, represents a state, compartment, or individual chemical species in a series. Movement between nodes indicates either a flux of material between compartments or the transition of one chemical species into another, or the transition between intermediate states of an enzyme, for example.

We will treat all of the transitions between states as being an elementary reaction so that the flux obeys the relation

$$J_{ij} = \alpha_{ij} N_i \qquad [1.5.A2.1]$$

where J_{ij} is the flux from node i to node j, N_i is the population of node i, and α_{ij} is a pseudo-first order rate constant. It is called a pseudo-first order rate constant because it may incorporate the concentration of a ligand if the transition between node i and node j requires it.

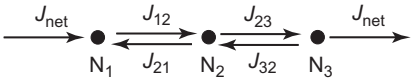

FIGURE 1.5.A2.1 Chemical species can be transformed into various intermediates here represented as three nodes, N1, N2, and N3. The fluxes between the nodes are indexed with the source node first and sink node second. Alternatively, the nodes can represent compartments into which the same chemical is transported, or different states of the same object, such as an enzyme.

At steady state, there could be a throughput of material such that sum flux, J_{net}, of material enters node 1 and the same flux exits the end, at node 3 in this case. At steady state, this requires that the net flux between any two nodes is the same, equal to J_{net}. However, there can be a higher unidirectional flux of material from node 1 to node 2 than this net flux. All that steady state requires is that

$$J_{ij} - J_{ji} = J_{net} \qquad [1.5.A2.2]$$

for each ij pair. The unidirectional flux, J_{ij}, can be determined by somehow labeling the population of state i and seeing how fast they are converted to state j. One way this can be done is by adding a pulse of radioactive material of state i, and watching its conversion to state j. We will note the population of radioactive tracer in each state or compartment with an asterisk, which is added into state i alone. We can write the following sets of equations that govern the time change in the population of radioactivity in each state:

$$\begin{aligned}\frac{dN_1^*}{dt} &= -J_{12}\frac{N_1^*}{N_1} + J_{21}\frac{N_2^*}{N_2}\\ \frac{dN_2^*}{dt} &= J_{12}\frac{N_1^*}{N_1} - (J_{21}+J_{23})\frac{N_2^*}{N_2} + J_{32}\frac{N_3^*}{N_3}\\ \frac{dN_3^*}{dt} &= J_{23}\frac{N_2^*}{N_2} - J_{32}\frac{N_3^*}{N_3}\end{aligned} \qquad [1.5.A2.3]$$

The idea about unidirectional flux is that there is no back flux. Thus, for the transition between state 1 and state 2, we can measure unidirectional flux J_{12} only when N_2^* is negligible. For the top equation in Eqn [1.5.A2.3], we have

$$\begin{aligned}\frac{dN_1^*}{dt} &= -J_{12}\frac{N_1^*}{N_1} + J_{21}\frac{N_2^*}{N_2}\\ \frac{N_2^*}{N_2} &\approx 0\\ \frac{dN_1^*}{dt} &= -J_{12}\frac{N_1^*}{N_1}\\ J_{12} &= -\frac{dN_1^*}{dt}\Big/\frac{N_1^*}{N_1}\end{aligned} \qquad [1.5.A2.4]$$

The last equation in Eqn [1.5.A2.4] defines what is meant by a unidirectional flux obtained by tracer means. In this case, dN_1^*/dt is negative—the amount of tracer in state 1 decreases with time—and thus the flux J_{12} is positive. The factor N_1^*/N_1 in the denominator is called the ***specific activity*** of the added radioactivity. The units of J_{12} are in units of N per unit time.

Measuring the unidirectional flux from state 1 to state 3 requires a similar condition as the unidirectional flux from state 1 to 2; that is, there must be negligible back flux during the measurement. In this case, this means that N_3^*/N_3 is negligible. We also require a steady state. In this case, steady state means $dN_2^*/dt = 0$. What we are searching for is an overall unidirectional flux that obeys the relation:

$$\frac{dN_3^*}{dt} = J_{13}\frac{N_1^*}{N_1} \qquad [1.5.A2.5]$$

Because $N_3^*/N_3 \approx 0$ as a criterion for measuring unidirectional flux, the bottom part of Eqn [1.5.A2.3] becomes

$$\frac{dN_3^*}{dt} = J_{23}\frac{N_2^*}{N_2} \qquad [1.5.A2.6]$$

If $dN_2^*/dt = 0$ as a condition of steady state, then by the middle part of Eqn [1.5.A2.3] we can write

$$\frac{N_1^*}{N_1} = \frac{(J_{21}+J_{23})}{J_{12}}\frac{N_2^*}{N_2} \qquad [1.5.A2.7]$$

Substituting in for N_2^*/N_2 from Eqn [1.5.A2.3] into Eqn [1.5.A2.7], we obtain

$$\frac{dN_3^*}{dt} = \frac{J_{12}J_{23}}{(J_{21}+J_{23})}\frac{N_1^*}{N_1} \qquad [1.5.A2.8]$$

Comparison of Eqn [1.5.A2.5] with Eqn [1.5.A2.8] allows us to identify J_{13} as

$$J_{13} = \frac{J_{12}J_{23}}{(J_{21}+J_{23})} = J_{12}\frac{J_{23}}{(J_{21}+J_{23})} \qquad [1.5.A2.9]$$

This result has an intuitive interpretation. It says that the unidirectional flux from node 1 to node 3 is the flux from node 1 to node 2 times the proportion of the flux that goes on to node 3. This proportion is the flux from node 2 to node 3 divided by the total flux away from node 2: the sum of J_{21} and J_{23}.

It can be seen readily that we could just as easily added radioactivity at node 3 and watch its appearance in node 1. From the symmetry, we can write J_{31} as

$$J_{31} = \frac{J_{32}J_{21}}{(J_{21}+J_{23})} = J_{32}\frac{J_{21}}{(J_{21}+J_{23})} \qquad [1.5.A2.10]$$

These results are completely general. Suppose that the sequence of nodes, states, or compartments was longer than just three, as shown in Figure 1.5.A2.2. We could use the unidirectional flux to reduce the diagram to one fewer states, and then do so again using the same principle that we discovered here. The results for J_{14} and J_{41} are given as

$$J_{14} = \frac{J_{13}J_{34}}{(J_{31}+J_{34})} = \frac{J_{12}\dfrac{J_{23}}{(J_{21}+J_{23})}J_{34}}{J_{32}\dfrac{J_{21}}{J_{21}+J_{23}}+J_{34}} = \frac{J_{12}J_{23}J_{34}}{J_{32}J_{21}+J_{21}J_{34}+J_{23}J_{34}} \qquad [1.5.A2.11]$$

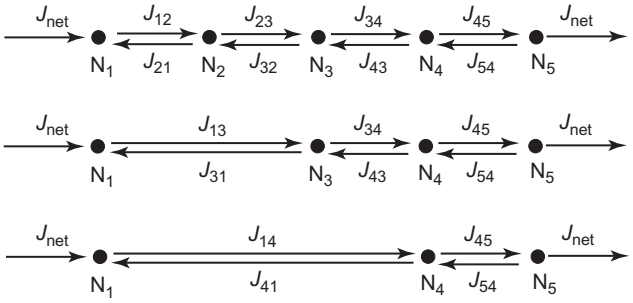

FIGURE 1.5.A2.2 Unidirectional fluxes over a series of intermediate states. The flux between states 1 and 3 can be replaced by two unidirectional fluxes. The process can be repeated over any number of intermediates.

$$J_{41} = \frac{J_{43}J_{31}}{(J_{31}+J_{34})} = \frac{J_{43}\dfrac{J_{21}}{(J_{21}+J_{23})}J_{32}}{J_{32}\dfrac{J_{21}}{J_{21}+J_{23}}+J_{34}} = \frac{J_{43}J_{32}J_{21}}{J_{32}J_{21}+J_{21}J_{34}+J_{23}J_{34}}$$

[1.5.A2.12]

In this way, the unidirectional flux over any number of intermediate states can be calculated from the set of individual unidirectional fluxes.

APPENDIX 1.5.A3 SIMPLE COMPARTMENTAL ANALYSIS

THE AGGREGATE OF A CHEMICAL SPECIES CONSTITUTES ITS BODILY "POOL"

Compounds in the body are normally in a dynamic state in which materials are converted from precursors to products. For example, there is a steady state between the phospholipids in the cytoplasm and in the mitochondria, with continuing interconversion between both. The aggregate of the cytoplasmic phospholipids in the its pool, as is the aggregate of the mitochondrial phospholipids. A "pool" or "compartment" is defined as the set of molecules of a specific compound or group of compounds found in a specific part of the organism. For example, we may speak of "liver glycogen" or "muscle glycogen" or "liver phospholipid" or "plasma phospholipid." In investigating these pools, we are most interested in the identity of the pools, their size, and the rate at which they exchange material with other pools. Radioactive labeling is an effective way of determining these parameters. Generally these pools change only slowly with time. Their maintenance at a fairly steady level is a consequence of homeostasis, the maintenance of a constant internal environment, which is the hallmark of physiological systems. Though blood glucose or blood $[Ca^{2+}]$ is maintained fairly constant, there is always material entering and leaving the plasma pool.

THE TURNOVER DESCRIBES THE RATE OF EXCHANGE OF A POOL

The rate of exchange of material is the rate at which material enters or leaves the pool, and can be given in units of amount per unit time. This is also called the turnover or the rate of renewal of the pool. The fractional turnover rate is the fraction of the total pool which is replaced per unit time. If A is the size of pool (in g or moles) and P g or moles of A are renewed each minute, then the fractional turnover rate is given as

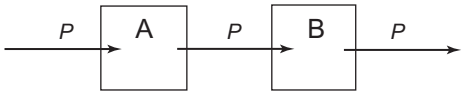

FIGURE 1.5.A3.1 Two-pool sequential model. Pool A of size A is converted to pool B of size B with a steady-state rate of P.

$$k_1 = \frac{P}{A} \qquad [1.5.A3.1]$$

where the units of k are reciprocal time. Here we consider pool A of size A which is a precursor of pool B of size B, as shown in Figure 1.5.A3.1. We will assume that the pools are in a steady state (their sizes do not change with time); there is rapid and uniform mixing within the pools and that the rate of transfer does not change with time. Suppose we inject an amount of radioactive compound A, which we denote as A^*, into pool A at time $t = 0$. If the radioactivity mixes well and uniformly, we may describe the change in radioactivity in pool A as

$$\frac{dA^*}{dt} = -P\frac{A^*}{A} \qquad [1.5.A3.2]$$

This equation derives from the idea that the radioactive portion of pool A will be turned over in proportion to its concentration in pool A. Thus when P unit of pool A turns over per unit time, PA^*/A unit is radioactive and $P(A - A^*)/A$ unit is not radioactive. The quantity A^*/A is called the specific activity of pool A, denoted as S_A. Equation [1.5.A3.2] can be rewritten as

$$\frac{1}{A}\frac{dA^*}{dt} = -\frac{P}{A}S_A \qquad [1.5.A3.3]$$

Since A is constant under steady-state conditions, we may rewrite Eqn [1.5.A3.3] as

$$\frac{d(A^*/A)}{dt} = -\frac{P}{A}S_A$$

$$\frac{dS_A}{dt} = -k_1 S_A \qquad [1.5.A3.4]$$

Here we have used the definition of the fractional turnover rate given in Eqn [1.5.A3.1]. The last equation is of the form of first-order decay and is easily integrated to give

$$S_A = S_{A_0}e^{-k_1 t} \qquad [1.5.A3.5]$$

where S_{A0} is the specific activity of pool A at time $t = 0$. Plots of $\ln S_A$ against time will allow the calculation of the fractional turnover rate as the negative of the slope. Extrapolation back to time zero gives the pool size: if A^*/A is known, and the amount of radioactivity injected, A^*, is known, then A can be calculated. It is important to note that the determination of the specific

activity, $S_A = A^*/A$, does not require knowing A; it does require knowing A^* and A in an aliquot of the pool. Thus the specific activity can be determined even if the pool size A cannot be directly measured. The validity of estimating pool sizes in this way depends on the assumptions of uniform and rapid mixing of the radioactive label with the endogenous material.

The specific activity of pool B is affected by influx from pool A and efflux from pool B. Here we write

[1.5.A3.6] $$\frac{dB^*}{dt} = P\,S_A - P\,S_B$$

We can divide both sides of the equation by B to obtain

$$\frac{1}{B}\frac{dB^*}{dt} = \frac{P}{B}(S_A - S_B)$$

[1.5.A3.7] $$\frac{dB^*/B}{dt} = k_2(S_A - S_B)$$

$$\frac{dS_B}{dt} = k_2(S_A - S_B)$$

We can rewrite the last part of this equation as

[1.5.A3.8] $$\frac{dS_B}{dt} + k_2\,S_B = k_2\,S_A$$

This equation cannot be solved by integration directly, because S_A is a function of time and we don't know S_B as a function of time. We can multiply both sides by an integrating factor, ρ, such that

[1.5.A3.9] $$d(\rho S_B) = \rho\,dS_B + \rho\,k_2\,S_B\,dt$$

We choose ρ such that ρS_B is an exact differential, so that

[1.5.A3.10] $$d(\rho S_B) = \rho\,dS_B + S_B\,d\rho$$

Comparison of Eqns [1.5.A3.9] and [1.5.A3.10] indicates that

[1.5.A3.11] $$d\rho = \rho k_2\,dt$$

Solution of this equation gives

[1.5.A3.12] $$\rho = e^{k_2 t}$$

Multiplying both sides of Eqn [1.5.A3.8] by this multiplication factor gives

[1.5.A3.13] $$\rho\,dS_B + \rho\,k_2\,S_B\,dt = \rho\,k_2\,S_A\,dt$$

We have chosen ρ so that Eqn [1.5.A3.9] is valid, so we can substitute $d(\rho S_B)$ for the left-hand side of Eqn [1.5.A3.13]:

[1.5.A3.14] $$d(\rho\,S_B) = \rho\,k_2 S_A\,dt$$

Integration of this equation is now possible because the left-hand side is a function of ρS_B alone and the right-hand side is a function of t alone. Substitution of ρ from Eqn [1.5.A3.12] and for S_A from Eqn [1.5.A3.5] gives

[1.5.A3.15] $$d(\rho\,S_B) = k_2\,e^{k_2 t} S_{A_0}\,e^{-k_1 t}\,dt$$

Integration from $t = t$ to $t = t$ gives

[1.5.A3.16]
$$\int_0^t d(\rho\,S_B) = \int_0^t k_2\,e^{k_2\,t}\,S_{A_0}\,e^{-k_1 t}\,dt$$
$$\rho\,S_B\Big|_0^t = \frac{k_2\,S_{A_0}}{k_2 - k_1} e^{(k_2 - k_1)t}\Big|_0^t$$
$$S_B e^{k_2 t} = \frac{k_2\,S_{A_0}}{k_2 - k_1}(e^{(k_2 - k_1)t} - 1)$$

Dividing both sides by the exponent on the left finally leaves us with

[1.5.A3.16] $$S_B = \frac{k_2}{k_2 - k_1} S_{A_0}(e^{-k_1\,t} - e^{-k_2 t})$$

We have now derived equations for S_A and S_B for the conditions shown in Figure 1.5.A3.1. The graph of $\ln S_A$ versus time will give $\ln S_{A0}$ as the intercept and $-k_1$ as the slope. Eqn [1.5.A3.6] shows that the maximum of the graph of $\ln S_B$ will occur with $S_A = S_B$, because at this point $dB^*/dt = 0$, which means $dS_B/dt = 0$ and therefore $d \ln S_B/dt = 0$. P may be obtained from A and k_1. Figure 1.5.A3.2 shows the results of calculations for Eqns [1.5.A3.5] and [1.5.A3.16] for assumed values of A, A^*, k_1 and k_2. The plot of $\ln S_A$ gives an intercept of 9.2103. Thus $S_{A0} = 10{,}000$ cpm μmol^{-1}. Since the amount of injected radioactivity was 1×10^8 cpm, we can calculate the pool size, A, as

$$A = A^*/S_{A0} = 1 \times 10^8\ \text{cpm}/10^4\text{cpm}\ \mu\text{mol}^{-1}$$
$$= 10^4\ \mu\text{mol} = 0.02\ \text{mol};\ \text{thus}$$
$$\boldsymbol{A = 0.02\ \text{mol}}$$

The fractional turnover rate is given as the negative of the slope: $\boldsymbol{k_1 = 0.02\ \text{min}^{-1}}$. The turnover of the pool at steady state can be calculated as

$P = k_1\,A = 4 \times 10^{-4}$ mol min^{-1}; $\boldsymbol{P = 4 \times 10^{-4}\ \text{mol min}^{-1}}$

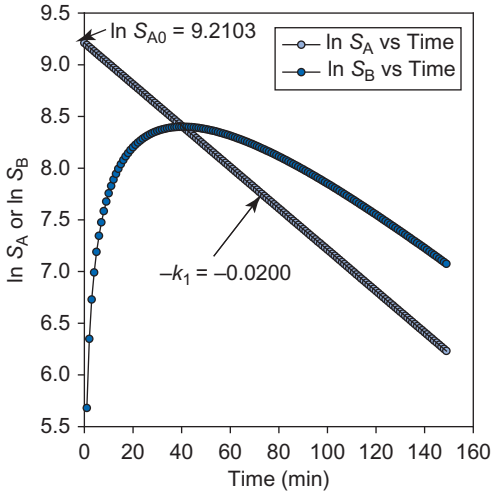

FIGURE 1.5.A3.2 Plot of $\ln S_A$ and $\ln S_B$ against time. The plot of $\ln S_A$ is linear with a Y-intercept of 9.2103 and a slope of -0.02. The plot of $\ln S_B$ intersects that of $\ln S_A$ at the point where $S_A = S_B$.

Note that the plot of $\ln S_B$ peaks at the intersection of the $\ln S_A$ and $\ln S_B$ curve. This occurs at 40.55 minutes and $S_A = S_B = 4444.2$ cpm μmol^{-1}. Eqn [1.5.A3.16] can be reorganized to attempt to solve for k_2. It can be rewritten as

[1.5.A3.17]

$$\frac{(k_2 - k_1)}{k_2} S_B = S_{A_0} e^{-k_1 t} - S_{A_0} e^{-k_2 t}$$

$$\left(1 - \frac{k_1}{k_2}\right) S_B = S_A - S_{A_0} e^{-k_2 t}$$

$$\left(1 - \frac{k_1}{k_2}\right) S_B = S_B - S_B\, e^{k_1 t}\, e^{-k_2 t}$$

$$k_1\, e^{-k_1 t} = k_2\, e^{-k_2 t}$$

This result predicts that the point of intersection of the two curves depends only on the two fractional turnover rates and not on the amount of radioactivity injected. This makes intuitive sense. We know k_1 and the time of intersection $t = 40.55$ min. Thus $k_1\, e^{-k1t} = 0.008888$ min^{-1} and we search for k_2 such that $k_2\, e^{-k2\ t} = 0.008888$ min^{-1}. The solution is

$$\boldsymbol{k_2 = 0.03\ \text{min}^{-1}}$$

which can be verified by substitution. This solution can be obtained graphically by the intersection of the line $y = \ln k_2$ with the line $y = 40.55k_2 - 4.723$.

The pool size B can be calculated from $k_2 = P/B$; we have $k_2 = 0.03$ min^{-1} and $P = 4 \times 10^{-4}$ mol min^{-1}, giving B as

$B = 4 \times 10^{-4}$ mol min^{-1}/0.03 min^{-1} = 0.0133 mol;

$\boldsymbol{B = 0.0133\ \text{mol}}$