CHAPTER

8

Biochemical Reactions and Enzyme Kinetics

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OUTLINE

AT THE CONCLUSION OF THIS CHAPTER, STUDENTS WILL BE ABLE TO:

- Using the law of mass action, quantitatively describe a chemical reaction using differential equations.
- Using the law of mass action, quantitatively describe enzyme kinetics using differential equations.
- Describe and calculate the quasi-steady-state approximation for chemical reactions.
- Simulate chemical reactions and enzyme kinetics using SIMULINK.
- Use the quasi-steady-state approximation to describe metabolism in a compartmental model.
- Model biochemical reactions, enzyme kinetics, diffusion, carrier-mediated transport and active transport in a physiological system.
- Quantitatively describe the Na-K pump.
- Qunatitatively describe cellular respiration.
- Explain and model activators and inhibitors in a chemical reaction.

This chapter focuses on chemical reactions that occur inside and outside a cell following the law of mass action—that is, the rate of accumulation is proportional to the product of the reactants. These chemical reactions support all functions needed to support life and involve such activities as the synthesis of hormones and proteins, muscle contraction, respiration, reproduction, neural signaling, and many other reactions. While the law of mass action is useful, it is not appropriate for all chemical reactions. In some cases, the exact chemical reaction mechanism is not known, and the law of mass action doesn't work.

The law of mass action creates models that are nonlinear and possibly time-varying. In addition, the rate of accumulation is profoundly impacted by temperature, such that an increase in temperature increases the reaction rate. The reactants are assumed to be uniformly distributed in the compartment, the probability of a collision depends on the concentration of the reactants, and such collisions are sufficient to create the products. Since the models here are nonlinear, we typically use SIMULINK to solve these problems. However, we will present analytical solutions to some special nonlinear cases in this chapter.

Catalysts are substances that dramatically change reaction rates. These substances are generally present in small amounts and are not consumed in the reaction. Their function is to decrease the amount of time to reach steady state in a chemical reaction. Consider two reactants that spontaneously create a product at room temperature but do so at a very low rate. In the presence of an appropriate catalyst, the speed of the reaction is dramatically increased, and the time to reach steady state is decreased. Enzymes are protein catalysts used in biological reactions that regulate and control most processes in the body. Enzymes increase the reaction rate by thousands or even trillions and are reactant specific. Typically, the enzyme concentration is rather small in relation to the reactant. While the reaction rate is profoundly increased in the presence of an enzyme, the overall energy used to form the product does not change.

In this chapter, we first examine simple chemical reactions and then enzyme kinetics. Next, we look at quasi-steady-state approximations, the Michaelis-Menten elimination, and enzyme regulation. We finish by examining important processes, such as the transport of oxygen and carbon dioxide through the circulatory system to the cell, the Na-K pump, and cellular respiration.

8.1 CHEMICAL REACTIONS

Consider the following single-stage chemical reaction

$$A + B \xrightarrow{K} P \tag{8.1}$$

in which chemicals A and B react to form the product P, with reaction rate constant K. Equation (8.1) is known as a stoichiometric equation that lists the number of reactants on the left side necessary to form the product on the right side. Conservation of mass requires that the total quantity of reactant A must equal the quantity of A in the reactant and the product, and likewise, the same is true with reactant B. The stoichiometric equation does not describe the dynamics or kinetics of the chemical reaction—that is, the time course of the reaction that may be very fast or very slow. The kinetics of the reaction is written

according to the law of mass action. The arrow in Eq. (8.1) shows the direction of the reaction that occurs spontaneously, and the reaction rate constant describes how quickly the reaction occurs. The reaction rate constant is a function of temperature, whereby an increase in temperature generally increases K. For our purposes, temperature is constant and so is K.

8.1.1 Chemical Bonds

Individual atoms rarely appear in nature, but they appear within a molecule. Molecules are collections of atoms that are held together by strong chemical bonds in which electron(s) are shared or electron(s) are transferred from one atom to another. Forces between molecules are relatively weak, allowing molecules to act independently of one another. A compound is a molecule that contains at least two different atoms, whereas a molecule can contain just one type of atom. Molecules such as hydrogen, H_2, and oxygen, O_2, are not compounds because they consist of a single type of atom. For simplicity in presentation, we will use the term *molecules* to describe both molecules and compounds.

An atom is an electrically neutral particle that consists of an equal number of protons and electrons. An atom with an unbalanced number of electrons or protons is called an ion, which makes it a positively or negatively charged particle. Examples of positively charged ions, called cations, include Na^+, K^+, and Ca^{+2}; Na^+ and K^+ have each lost one electron, and Ca^{+2} has lost two electrons. An example of a negatively charged ion, called an anion, is Cl^-, which has gained an extra electron.

There are many types of chemical bonds that join atoms and molecules together. They vary in strength, with ionic and covalent bonds displaying the strongest bonds, and the hydrogen bond displaying the weakest bond.

Many molecules are composed of positively and negatively charged ions that are bound by an ionic bond. These bonds are extremely strong due to the electrostatic attraction between the oppositely charged ions. Ionic bonds usually involve an atom that has few electrons in the outer shell, with another atom that has an almost complete set of electrons in the outer shell. Ionic bonds involve a transfer of electrons from one atom to another atom. Consider sodium chloride, where sodium has one electron in the outer shell and chloride has seven electrons in its outer shell. The bond is formed by sodium transferring its electron to chloride, resulting in a molecule consisting of Na^+Cl^- (usually written as $NaCl$). Consider magnesium and oxygen atoms forming an ionic bond. Magnesium has two electrons in its outer field that are transferred to oxygen, forming $Mg^{+2}O^{-2}$.

Covalent bonds involve two atoms sharing an electron pair that increases the stability of the molecule. The atoms do not have to be the same type, but they must be the same electronegativity. For instance, H_2 is formed by a covalent bond. Consider carbon dioxide, a molecule that consists of one carbon and two oxygen atoms. These atoms share the electrons, with carbon having four electrons in its outer shell, and oxygen having six electrons in its outer shell. The four electrons in carbon are used in the outer shell of the two oxygen atoms.

A hydrogen bond involves the force between a hydrogen atom in one molecule and an electronegative atom in another molecule, typically with oxygen in H_2O, nitrogen in NH_3, and fluorine in HF. Note that the force experienced in a hydrogen bond is not within a molecule but between molecules. The small size of hydrogen allows it to become very close to another molecule with a small atomic radii like the ones previously mentioned.

An example of a hydrogen bond is found among water molecules. The oxygen in water, which is negatively charged, binds to two positively charged hydrogens from two other water molecules. These bonds connect the water molecules together and give it some unusual properties, such as high surface tension and viscosity. Keep in mind that this bond is a relatively weak bond compared to that holding the water molecule together. As water is heated to the boiling point, all of the hydrogen bonds are dissolved as it becomes a gas. When water freezes, the hydrogen bonds form a structured, less dense orientation. Between freezing and boiling, water is oriented in very dense, random configuration. Hydrogen also plays an an important role within proteins and nucleic acids, which allows bonding within the molecule to achieve different shapes.

A chemical reaction in which a molecule loses electrons is called oxidation, and a gain of electrons is called reduction. Some multistage chemical reactions involve both oxidation and reduction. Energy is released when a chemical bond is formed, which is the same amount of energy necessary to break that chemical bond. Thus, chemical bonds store energy. The first step in a chemical reaction requires energy to break the bonds holding the reactant molecules together so they can be rearranged to form an activated complex, an unstable high-energy intermediate. From the activated complex, the molecules join together to form the product and typically release energy. In chemical reactions, if the energy stored in the chemical bonds of the product is less than the total energy stored in the reactants, then net energy is released. The amount of energy required in a chemical reaction is inversely proportional to the reaction rate constant.

8.1.2 Kinetics of a Single-Stage Chemical Reaction

To understand the law of mass action, a compartment model visualization of Eq. (8.1) is shown in Figure 8.1, with the understanding that the volumes for the two compartments are identical (i.e., the reaction occurs in a single container of constant volume). The differential equation describing Eq. (8.1) or Figure 8.1 is

$$\dot{q}_A = -Kq_Aq_B \tag{8.2}$$

where q_A and q_B are the quantities of reactants A and B, and K is the transfer rate or reaction rate constant. The transfer rate includes the volume in converting the concentrations to quantities in Eq. (8.2). Note that the right-hand side of Eq. (8.2) involves the product q_Aq_B, instead of a single variable as before. In addition, we could have written Eq. (8.2) in terms of q_B, $\dot{q}_B = -Kq_Aq_B$ or in terms of q_P, $\dot{q}_P = Kq_Aq_B$. Since drawing the compartmental model is not essential in writing the differential equation that describes the kinetics of the

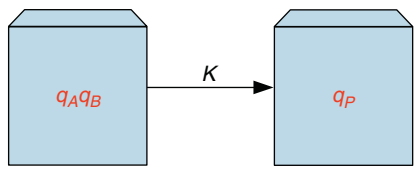

FIGURE 8.1 Compartmental model of the single-stage chemical reaction in Eq. (8.1). Another way to describe this system is to remove one of the reactants in the left box and include it as part of the reaction rate—that is, Kq_B.

chemical reaction, we will write the differential equations directly from the stoichiometric equation. Of course, Eq. (8.2) can be written in terms of concentrations by substituting (concentration×volume) for quantity.

Eq. (8.2) is a nonlinear differential equation. In general, nonlinear equations cannot be solved directly, but they must be simulated using a program like SIMULINK. Keep in mind that there are some special nonlinear differential equations that can be solved, but these are few in number, with a few presented in this chapter. Since $\dot{q}_A = \dot{q}_B$, we can integrate both sides of $\int_0^t dq_A = \int_0^t dq_b$ and have $q_A - q_A(0) = q_B - q_B(0)$, which, when substituted into Eq. (8.2), gives

$$\dot{q}_A = -Kq_Aq_B = -Kq_A(q_A - q_A(0) + q_B(0)) \tag{8.3}$$

Rearranging Eq. (8.3) yields

$$\frac{dq_A}{q_A(q_A - q_A(0) + q_B(0))} = -Kdt \tag{8.4}$$

The left-hand side of Eq. (8.4) is rewritten using a technique called partial fraction expansion (details are briefly described here for the unique roots case)[1] as

$$\begin{gathered}\left(\frac{B_1 dq_A}{(q_A - q_A(0) + q_B(0))} + \frac{B_2 dq_A}{q_A}\right) = -Kdt \\ \left(\frac{1}{q_A(0) - q_B(0)}\right)\left(\frac{dq_A}{(q_A - q_A(0) + q_B(0))} - \frac{dq_A}{q_A}\right) = -Kdt\end{gathered} \tag{8.5}$$

where

$$B_1 = \left[(q_A - q_A(0) + q_B(0))\frac{1}{q_A(q_A - q_A(0) + q_B(0))}\right]\Bigg|_{q_A = q_A(0) - q_B(0)} = \left[\frac{1}{q_A}\right]\Bigg|_{q_A = q_A(0) - q_B(0)} = \frac{1}{q_A(0) - q_B(0)}$$

[1] In general, if

$$\frac{1}{s^n + a_{n-1}s^{n-1} + \cdots a_1 s + a_0} = \frac{1}{(s - s_1)\cdots(s - s_n)}$$

and the roots are real, then

$$\frac{1}{(s - s_1)\cdots(s - s_n)} = \frac{B_1}{s - s_1} + \cdots \frac{B_n}{s - s_n}$$

where

$$B_i = \left[(s - s_i)\frac{1}{(s - s_1)\cdots(s - s_n)}\right]\Bigg|_{s = s_i}$$

It should be clear when evaluating the coefficient B_i in the previous equation that the factor $(s - s_i)$ always cancels with the same term in the denominator before calculating B_i.

$$B_2 = \left[q_A \frac{1}{q_A(q_A - q_A(0) + q_B(0))} \right] \Big|_{q_A=0} = \left[\frac{1}{(q_A - q_A(0) + q_B(0))} \right] \Big|_{q_A=0} = -\frac{1}{q_A(0) - q_B(0)}$$

Integrating both sides of Eq. (8.5) gives

$$\left(\frac{1}{q_A(0) - q_B(0)} \right) \left(\ln \left(\frac{q_A - q_A(0) + q_B(0)}{q_B(0)} \right) - \ln \left(\frac{q_A}{q_A(0)} \right) \right) = -Kt \tag{8.6}$$

and after algebraically manipulating, we have

$$q_A = \frac{(q_A(0) - q_B(0))}{\left(1 - \left(\frac{q_B(0) e^{-K(q_A(0) - q_B(0))t}}{q_A(0)} \right) \right)} \tag{8.7}$$

One final note regarding the solution of Eq. (8.2) is to consider the case in which $q_B(0) \gg q_A(0)$. Here, the change in q_B is small compared to $q_B(0)$, and q_B can be treated essentially as a constant—that is, $q_B = q_B(0)$. Thus, an approximation to Eq. (8.2) is

$$\dot{q}_A = -K q_B q_A = -K q_B(0) q_A \tag{8.8}$$

which can be straightforwardly solved as

$$q_A = q_A(0) e^{-K q_B(0) t} u(t) \tag{8.9}$$

Substituting Eq. (8.9) into $\dot{q}_P = K q_B q_A = K q_B(0) q_A = K q_B(0) q_A(0) e^{-K q_B(0) t}$ and integrating gives

$$q_P = q_A(0) \left(1 - e^{-K q_B(0) t} \right) u(t) \tag{8.10}$$

It should be clear that the steady-state value of q_P equals $q_A(0)$.

Let us take another look at the solution in Eq. (8.7) for q_A, and compare it to that of Eq. (8.9) by assuming that $q_B(0) \gg q_A(0)$, which gives the approximation $q_B = q_B(0)$, and thus $q_A - q_A(0) = q_B - q_B(0) = 0$. Hence, we have $\ln \left(\frac{q_A - q_A(0) + q_B(0)}{q_B(0)} \right) = \ln \left(\frac{q_B(0)}{q_B(0)} \right) = 0$, and therefore Eq. (8.7) becomes

$$\left(\frac{1}{\cancel{q_B(0) - q_A(0)}^{\,q_B(0)}} \right) \left(\ln \left(\frac{q_A}{q_A(0)} \right) \right) = \frac{1}{q_B(0)} \ln \left(\frac{q_A}{q_A(0)} \right) = -Kt \tag{8.11}$$

Rearranging and taking the exponential of both sides gives

$$q_A = q_A(0) e^{-K q_B(0) t} u(t) \tag{8.12}$$

which is the result we found in Eq. (8.9). In terms of the body, many small molecules like glucose and other nutrients are available in large quantities as compared with other reactants. Thus, this simplification is often quite appropriate.

8.1.3 Single-Stage Reversible Chemical Reaction

Next, consider the single-stage reversible chemical reaction as given in Eq. (8.13):

$$A + B \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} P \tag{8.13}$$

Here, the chemicals A and B react to form the product P, and P has a reverse reaction to form A and B. The law of mass action describing this system is given by

$$\begin{aligned} \dot{q}_P &= K_1 q_A q_B - K_{-1} q_P \\ \dot{q}_A &= -K_1 q_A q_B + K_{-1} q_P \\ \dot{q}_B &= -K_1 q_A q_B + K_{-1} q_P \end{aligned} \tag{8.14}$$

Equation (8.14) is nonlinear, which can be solved using SIMULINK or also mathematically, as shown in the following example.

EXAMPLE PROBLEM 8.1

Consider the reaction given in Eq. (8.14), with $q_A(0) = 10, q_B(0) = 15$, and $q_P(0) = 0$, $K_1 = 2$, and $K_{-1} = 3$. Solve for q_P.

Solution

From Eq. (8.14), it is clear that $\dot{q}_P = -\dot{q}_A = -\dot{q}_B$ and after integrating, we have

$$q_P - q_P(0) = q_A(0) - q_A = q_B(0) - q_B \tag{8.15}$$

To solve for q_P, we eliminate q_a and q_B using Eq. (8.15) by substituting $q_A = q_A(0) + q_P(0) - q_P$ and $q_B = q_B(0) + q_P(0) - q_P$ into Eq. (8.14), giving

$$\dot{q}_P = K_1 q_A q_B - K_{-1} q_P = K_1 (q_P(0) + q_A(0) - q_P)(q_P(0) + q_B(0) - q_P) - K_{-1} q_P \tag{8.16}$$

Since $q_P(0) = 0$, we have

$$\begin{aligned} \dot{q}_P &= K_1 (q_A(0) - q_P)(q_B(0) - q_P) - K_{-1} q_P \\ &= 2(10 - q_P)(15 - q_P) - 3q_P \\ &= 2\left(q_P^2 - \frac{53}{2} q_P + 150 \right) \end{aligned} \tag{8.17}$$

and after rearranging terms

$$\frac{dq_P}{q_P^2 - \frac{53}{2} q_P + 150} = \frac{dq_P}{(q_P - 18.3)(q_P - 8.2)} = 2dt \tag{8.18}$$

Once again, partial fraction expansion is used to rewrite Eq. (8.18) as

$$0.0989 \left(\frac{dq_P}{(q_P - 18.3)} - \frac{dq_P}{(q_P - 8.2)} \right) = 2dt \tag{8.19}$$

Continued

where

$$B_1 = \left[(q_P - 18.3)\frac{1}{(q_P - 18.3)(q_P - 8.2)}\right]\Big|_{q_P=18.3} = \left[\frac{1}{(q_P - 8.2)}\right]\Big|_{q_P=18.3} = 0.0989$$

and

$$B_2 = \left[(q_P - 8.2)\frac{1}{(q_P - 18.3)(q_P - 8.2)}\right]\Big|_{q_P=8.2} = \left[\frac{1}{(q_P - 18.3)}\right]\Big|_{q_P=8.2} = -0.0989$$

Integrating Eq. (8.19) and rearranging yields

$$\ln\left(2.2\frac{(q_P - 8.2)}{(q_P - 18.3)}\right) = -20.2t$$

and after solving for q_P, we have

$$q_P = \left(\frac{8.2(1 - e^{-20.2t})}{1 - 0.45e^{-20.2t}}\right)u(t) \tag{8.20}$$

The solution for the reactants are found from $q_A = q_A(0) + q_P(0) - q_P$ and $q_B = q_B(0) + q_P(0) - q_P$, yielding

$$q_A = \left(\frac{1.8059 + 3.7e^{-20.2t}}{1 - 0.45e^{-20.2t}}\right)u(t)$$

and

$$q_B = \left(\frac{6.8059 + 1.4798e^{-20.2t}}{1 - 0.4476e^{-20.2t}}\right)u(t)$$

These results are shown in Figure 8.2. Note that conservation of mass is still maintained, since q_P contains both q_A and q_B, so $q_A + q_B + 2 \times q_P = 25$.

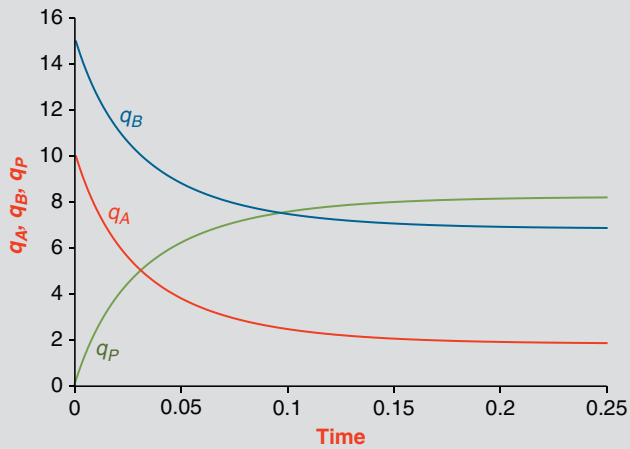

FIGURE 8.2 Illustration of the amount of product, q_P, in Example 8.1.

In general, it can be shown that the solution for q_P in Eq. (8.14) is

$$q_P = \left(\frac{\beta\left(1 - e^{-K_1(\alpha-\beta)t}\right)}{1 - \frac{\beta}{\alpha} e^{-K_1(\alpha-\beta)t}} \right) u(t) \tag{8.21}$$

where

$$\lambda = \sqrt{\left((q_A(0) + q_B(0) + 2q_P(0)) + \frac{K_2}{K_1} \right)^2 - 4(q_A(0) + q_P(0))(q_B(0) + q_P(0))}$$

$$\alpha = \frac{q_A(0) + q_B(0) + 2q_P(0) + \frac{K_2}{K_1} + \lambda}{2}$$

$$\beta = \frac{q_A(0) + q_B(0) + 2q_P(0) + \frac{K_2}{K_1} - \lambda}{2}$$

Next, consider the case in which $q_B(0) \gg q_A(0)$, where the change in q_B is small and can be treated as a constant $q_B(0)$. Thus, an approximation for Eq. (8.14) is

$$\dot{q}_P = K_1 q_B(0) q_A - K_{-1} q_P \tag{8.22}$$

Next, we remove q_A in Eq. (8.22) by letting $q_A = q_P(0) + q_A(0) - q_P$, giving

$$\dot{q}_P = K_1 q_B(0)(q_A(0) + q_P(0)) - (K_1 q_B(0) + K_{-1}) q_P \tag{8.23}$$

which can be straightforwardly solved as

$$q_P = \left(\frac{(K_{-1} q_P(0) - K_1 q_B(0) q_A(0))}{(K_1 q_B(0) + K_{-1})} e^{-(K_1 q_B(0) + K_{-1})t} + \frac{(K_1 q_B(0)(q_A(0) + q_P(0)))}{(K_1 q_B(0) + K_{-1})} \right) u(t) \tag{8.24}$$

8.1.4 Higher-Order Chemical Reactions and Sequential Reactions

Consider the higher-order chemical reaction in Eq. (8.25):

$$\alpha A + \beta B \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} P \tag{8.25}$$

in which αA of reactant A and βB of reactant B form product P, and P has a reverse reaction to form $\alpha A + \beta B$. The law of mass action for Eq. (8.25) is

$$\begin{aligned} \dot{q}_P &= K_1 q_A^\alpha q_B^\beta - K_{-1} q_P \\ \dot{q}_A &= -K_1 q_A^\alpha q_B^\beta + K_{-1} q_P \\ \dot{q}_B &= -K_1 q_A^\alpha q_B^\beta + K_{-1} q_P \end{aligned} \tag{8.26}$$

Note that when there are α molecules of A reacting, the q_A term in Eq. (8.26) is raised to the α power, and similarly, when β molecules of B react, then the q_B term in Eq. (8.26) is raised to the β power. In problems like this, we resort to using SIMULINK for the solution.

Now consider the sequential reaction in Eq. (8.27)

$$A \xrightarrow{K_1} B \xrightarrow{K_2} P \tag{8.27}$$

in which reactant A produces reactant B, which then produces reactant P. The law of mass action for Eq. (8.27) is

$$\begin{aligned} \dot{q}_A &= -K_1 q_A \\ \dot{q}_B &= K_1 q_A - K_2 q_B \\ \dot{q}_P &= K_2 q_B \end{aligned} \tag{8.28}$$

Equation (8.28) is easily solved, assuming nonzero initial condition for reactant A, $q_A(0)$, and zero for the other two reactants, as

$$\begin{aligned} q_A &= q_A(0) e^{-K_1 t} u(t) \\ q_B &= \frac{K_1 q_A(0)}{K_2 - K_1} \left(e^{-K_1 t} - e^{-K_2 t}\right) u(t) \\ q_P &= \frac{K_1 K_2 q_A(0)}{K_2 - K_1} \left(\frac{1 - e^{-K_1 t}}{K_1} - \frac{1 - e^{-K_2 t}}{K_2} \right) u(t) \end{aligned} \tag{8.29}$$

Sequential reactions are typical of those occurring in the body and can be quite complex, as described in Sections 8.4–8.6. As we will see, enzymes play a role in sequential reactions, and reactants can be of higher orders. Some sequential reactions involve many sequences in which some branch backward to previous reactions.

When there are profound differences in the transfer rates in a sequential reaction, simplifications occur that make analysis much easier. For instance, suppose $K_2 \gg K_1$. Here, the second reaction is much faster than the first reaction, and it appears that reactant A immediately produces reactant P. The term *rate limiting* is often used to describe this behavior—that is, the first reaction slows the creation of the product.

For a rate limiting reaction, an approximation to Eq. (8.29) for q_B and q_P is to eliminate the $e^{-K_2 t}$ term, since it goes to zero almost immediately, as compared to the $e^{-K_1 t}$ term, giving

$$\begin{aligned} q_B &\approx \frac{K_1 q_A(0)}{\cancel{K_2 - K_1}^{\,K_2}} \left(e^{-K_1 t} - \cancel{e^{-K_2 t}}^{\,0}\right) = \frac{K_1 q_A(0) e^{-K_1 t}}{K_2} = \frac{K_1}{K_2} q_A \\ q_P &\approx \frac{K_1 K_2 q_A(0)}{\cancel{K_2 - K_1}^{\,K_2}} \left(\frac{1 - e^{-K_1 t}}{K_1} - \frac{1 - \cancel{e^{-K_2 t}}^{\,0}}{K_2} \right) = q_A(0) \left(1 - e^{-K_1 t}\right) \end{aligned} \tag{8.30}$$

8.1.5 Quasi-Steady-State

Another way to look at a rate limiting reaction is to assume that reactant B is in a quasi-steady-state mode—that is $\dot{q}_B = 0$. From $\dot{q}_B = 0$ and Eq. (8.28), we have that $0 = K_1 q_A - K_2 q_B$,

and therefore $q_B = \frac{K_1}{K_2} q_A$. Since $\dot{q}_P = K_1 q_B$, we eliminate q_B by substituting $q_B = \frac{K_1}{K_2} q_A$, which gives $\dot{q}_P = K_1 q_A$ and $q_P = q_A(0)\left(1 - e^{-K_1 t}\right)$.

While quasi-steady-state assumes that reactant B is immediately in steady state and reactant A creates product P directly, there is a period of time, $\left(\frac{5}{K_2}\right)$, in which q_B moves from 0 to $\frac{K_1}{K_2} q_A$. Note also that steady state for reactant B is quite small and equals $\left(\frac{K_1}{K_2} q_A\right)$.

Figure 8.3 illustrates the approximation in Eq. (8.30), with the true solution for Eq. (8.29) given with $q_A(0) = 10, q_B(0) = 0$ and $q_P(0) = 0$, $K_1 = 2$, and $K_2 = 500$. For q_B, the approximation is quite accurate after it reaches quasi-steady-state, $t = \frac{5}{K_2} = 0.01$. For q_P, the approximation is quite accurate for the entire duration.

Now suppose $K_1 \gg K_2$. By a similar rational, the second reaction is now slower as compared to the first reaction and is rate limiting. For the rate limiting second reaction, an approximation to Eq. (8.29) for q_B and q_P is to eliminate the $e^{-K_1 t}$ term, since it goes to zero almost immediately, as compared to the $e^{-K_2 t}$ term, giving

$$
\begin{aligned}
q_A &= q_A(0) e^{-K_1 t} \\
q_B &\approx \frac{K_1 q_A(0)}{K_2 - K_1}\left(e^{-K_1 t} - e^{-K_2 t}\right) = q_A(0) e^{-K_2 t} \\
q_P &\approx \frac{K_1 K_2 q_A(0)}{K_2 - K_1}\left(\frac{1 - e^{-K_1 t}}{K_1} - \frac{1 - e^{-K_2 t}}{K_2}\right) = q_A(0)\left(1 - e^{-K_2 t}\right)
\end{aligned}
\tag{8.31}
$$

Here, reactant A disappears almost immediately and reactant B increases almost immediately to $q_A(0)$.

Another way to look at this rate limiting reaction is to assume that reactant A is in a quasi-steady-state mode—that is $\dot{q}_A = 0$. From $\dot{q}_A = 0$ and Eq. (8.28), we have $q_A = 0$ and

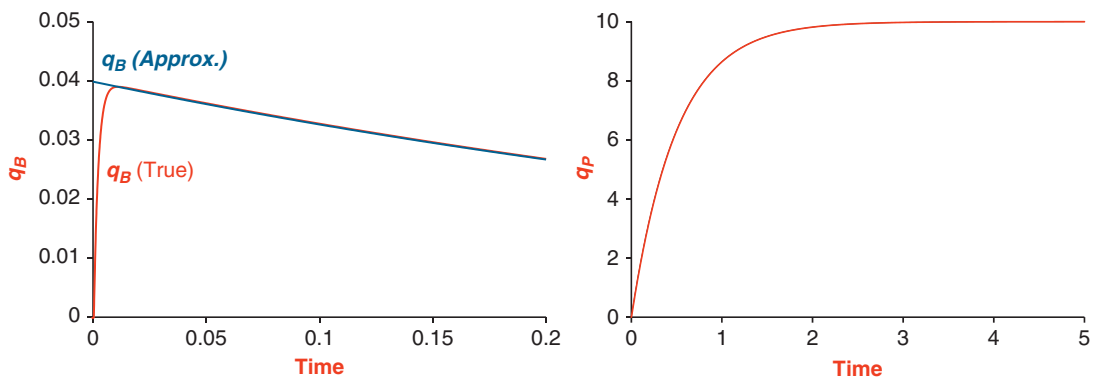

FIGURE 8.3 A rate limiting sequential reaction for K_1. Note that both the approximation and true solution for q_P are drawn in the figure on the right.

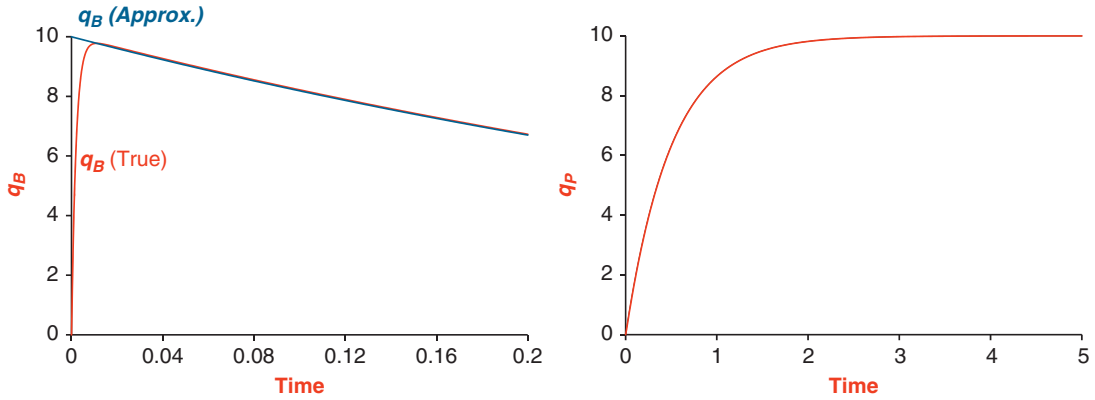

FIGURE 8.4 A rate limiting sequential reaction for K_2. Note that both the approximation and true solution for q_P are drawn in the figure on the right.

$\dot{q}_B = -K_2 q_B$, which has the solution $q_B = q_A(0)e^{-K_2 t}$. Next, $\dot{q}_P = K_2 q_B = q_A(0)K_2 e^{-K_2 t}$, which has the solution $q_P = q_A(0)\left(1 - e^{-K_2 t}\right)$. While quasi-steady-state assumes that reactant A is immediately in steady state at time 0 and reactant B rises to $q_A(0)$ immediately, there is a period of time, $\left(\frac{5}{K_1}\right)$, in which q_A falls to 0 and q_B rises.

Figure 8.4 illustrates the approximation in Eq. (8.31), with the true solution in Eq. (8.29) given with $q_A(0) = 10, q_B(0) = 0$ and $q_P(0) = 0$, $K_1 = 500$, and $K_2 = 2$. For q_B, the approximation is quite accurate after it reaches quasi-steady-state, $t = \frac{5}{K_1} = 0.01$. For q_P, the approximation is quite accurate the entire duration.

8.2 ENZYME KINETICS

As described earlier, catalysts are substances that accelerate reactions but are not consumed or changed by the reaction. Most chemical reactions in the body can occur without the presence of catalysts, but they occur at a very low rate. An enzyme is a large protein that catalyzes biochemical reactions in the body. These reactions convert a reactant, now called a substrate because it involves an enzyme, into a product by lowering the free energy of activation. Enzymes can increase the rate of the reaction by an order of thousands to trillions. Each enzyme is highly specific and only allows a particular substrate to bind to its active site. Many enzymes are used in the control and regulation of functions of the body.

In general, an enzyme reaction involves a series of reactions. Binding the enzyme with the substrate is the first step in creating an intermediate complex, which increases the ability of the substrate to react with other molecules. The next step is when the substrate-enzyme complex breaks down to form the free enzyme and product. Both the first step and the second step are reversible, but in the second step, the reverse reaction is so small that it is often omitted.

The overall rate of the enzyme catalyzed reaction with a single substrate is a function of the amount of enzyme and substrate and is given as

$$\text{Reaction Rate} = \frac{K_2 \times \text{Enzyme} \times \text{Substrate}}{K_M + \text{Substrate}} \tag{8.32}$$

where K_2 and K_M are reaction rate constants (K_M is called the Michaelis constant). We will derive and discuss this equation in more detail later in this section.

In an enzyme catalyzed reaction with much more substrate than enzyme, the reaction rate depends linearly on enzyme concentration according to Eq. (8.32), since the substrate concentration is essentially a constant. When there is much more enzyme than substrate, only a small portion of the enzyme is combined with the substrate, and the reaction rate is determined by both the enzyme and substrate levels. The typical enzyme catalyzed reaction consists of a series of reactions, each step with its own reaction rate. As we will see, the overall reaction rate is determined by the slowest reaction in the chain of reactions. The slowest reaction is called the capacity-limited reaction.

Enzyme catalyzed reactions serve a regulatory role, as well as accelerating biochemical reactions. Consider the relationship between adenosine diphosphate (ADP) and ATP inside the cell. ATP is created in the mitochondria where oxidation of nutrients (carbohydrates, proteins, and fats) produces carbon dioxide, water, and energy. The energy from the oxidation of nutrients converts ADP into ATP. ATP is used as fuel for almost all activities of the body, such as the $Na - K$ pump, action potentials, synthesis of molecules, creation of hormones, and contractions of muscles. At steady state, the concentration of ADP is very low in the cell, and thus the creation of ATP in the mitochondria is at a low rate. During periods of high cell activity, ATP is consumed, releasing energy through the loss of one phosphate radical, leaving ADP. The increased concentration of ADP causes an increase in the oxidation of nutrients in the mitochondria, producing more ATP. Thus, the ADP-ATP cycle is balanced and based on the needs of the cell. The reactions necessary to synthesize ATP are described in Section 8.5.

Enzyme reactions do not appear to follow the law of mass action; that is, as the substrate increases, the reaction rate does not increase without bound but reaches a saturation level (that is, it is capacity-limited). Capacity-limited reactions are quite prevalent and describe most metabolic reactions and functions of the body, such as the movement of molecules across the cell membrane and how substrates are removed from the body through the kidneys.

8.2.1 Michaelis-Menten Kinetics and the Quasi-Steady-State Approximation

In 1903, Victor Henri first described the relationship between a substrate and an enzyme, followed by Michaelis and Menten in 1913, and Briggs and Haldane in 1925, with a capacity-limited elimination rate for the chemical reaction. The first assumption is that the enzyme and substrate quickly reach steady state in the formation of the complex, and then the complex more slowly dissociates into the product and enzyme. We also assume that the amount of enzyme is much smaller than the substrate. We refer to the combined work here

as Michaelis-Menten kinetics. The models presented here assume that temperature and other conditions remain constant unless otherwise indicated.

This section introduces a standard approximation that greatly simplifies the analysis of biochemical reactions and predictions of how fast a reaction will occur. As shown, the quasi-steady-state approximation provides an excellent representation of the system's differential equations, which are problematic, since they involve stiff differential equations. By assuming a quasi-steady-state approximation, the set of differential equations is reduced to a set of algebraic equations. When the early pioneers developed the theory of enzyme reactions, the solution of the differential equations was not possible by either simulation or direct solution. Thus, the quasi-steady-state approximation allowed a rather complete description of the enzyme reaction except for the initial stage involving the formation of the complex. The set of algebraic equations also provides a mechanism to measure the parameters of the reaction.

Stiff differential equations require simulation solutions with an extremely small step size using the standard integrators. Consider Figure 8.4 and the graph for q_B. From 0 to .02 s, q_B changes quickly, and after that, it changes slowly. The step size needs to be very small, after which a small step size is not needed, since q_B is slowly changing. Thus, simulations take a very long time to run using the standard integrator. If the step size is too large, the simulation solution is incorrect because small errors amplify as it reaches steady state. The default integrator `ode45` in SIMULINK is not a good choice for stiff problems because it is inefficient. The integrator `ode23tb` is a better choice for stiff problems, which manipulates the step size using an efficient algorithm. We will explore this issue later in this section.

The capacity-limited reaction model uses a two-step process given by Eq. (8.33):

$$S + E \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} ES \xrightarrow{K_2} E + P \tag{8.33}$$

The enzyme mediated reaction first has substrate S combining with enzyme E to form the unstable complex ES. Then the complex ES breaks down into the product P and E.

The law of mass action equation for Eq. (8.33) is

$$\begin{aligned} \dot{q}_S &= -K_1 q_S q_E + K_{-1} q_{ES} \\ \dot{q}_{ES} &= K_1 q_S q_E - (K_{-1} + K_2) q_{ES} \\ \dot{q}_P &= K_2 q_{ES} \end{aligned} \tag{8.34}$$

Since $q_E(0) = q_E + q_{ES}$ or $q_E = q_E(0) - q_{ES}$, we have $\dot{q}_E = -\dot{q}_{ES}$. Thus,

$$\dot{q}_E = -K_1 q_S q_E + (K_{-1} + K_2) q_{ES} \tag{8.35}$$

To begin with the classical description, we assume that the system is closed—that is, $q_S(0) = q_S + q_{ES} + q_P$ and $q_E(0) = q_E + q_{ES}$. Note that E is not consumed in the reaction. Moreover, it is assumed that the complex ES increases quickly to a maximum, $q_{ES_{\max}}$ and then changes very slowly after that. Therefore, we assume complex ES is in a quasi-steady-state mode shortly after the reaction starts—that is, $\dot{q}_{ES} = 0$. We also assume that

the amount of enzyme is very small in comparison to the substrate and product and that $K_2 \gg K_{-1}$. From $\dot{q}_{ES} = 0$ and Eq. (8.34), we have

$$0 = K_1 q_S q_E - (K_{-1} + K_2) q_{ES} \tag{8.36}$$

Since $q_E(0) = q_E + q_{ES}$, we substitute $q_E = q_E(0) - q_{ES}$ into Eq. (8.36) to give

$$0 = K_1 q_S (q_E(0) - q_{ES}) - (K_{-1} + K_2) q_{ES} \tag{8.37}$$

and after rearranging,

$$q_{ES} = \frac{K_1 q_S q_E(0)}{(K_1 q_S + K_{-1} + K_2)} = \frac{q_S q_E(0)}{\left(q_S + \frac{K_{-1}+K_2}{K_1}\right)} = \frac{q_S q_E(0)}{(q_S + K_M)} = \frac{q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)} \tag{8.38}$$

where $K_M = \frac{K_{-1}+K_2}{K_1}$. The constant, K_M, is called the Michaelis constant, as mentioned in the beginning of this chapter.

The maximum complex ES from Eq. (8.38), with $q_s = q_s(0)$, is approximately

$$q_{ES_{\max}} \approx \left.\frac{q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)}\right|_{q_S = q_S(0)} = \frac{q_E(0)}{\left(1 + \frac{K_M}{q_S(0)}\right)} \tag{8.39}$$

Further, since $q_E = q_E(0) - q_{ES}$, we have

$$q_E = q_E(0) - \frac{q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)} = \frac{q_E(0)}{\left(1 + \frac{q_S}{K_M}\right)} \tag{8.40}$$

To find a quasi-steady-state approximation for q_S, we use Eq. (8.34); $\dot{q}_S = -K_1 q_S q_E + K_{-1} q_{ES} = -\left(\dot{q}_{ES} + K_2 q_{ES}\right)$. With $\dot{q}_{ES} = 0$ and q_{ES} from Eq. (8.38), we have

$$\dot{q}_S = -K_2 q_{ES} = -\frac{K_2 q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)} \tag{8.41}$$

Now, we have only two parameters that describe the change in substrate in Eq. (8.41), which takes the place of the set of differential equations in Eq. (8.34). Moreover, we will show that these two parameters can be estimated directly from data. Rearranging Eq. (8.41) gives

$$\left(1 + \frac{K_M}{q_S}\right) dq_S = -K_2 q_E(0) dt \tag{8.42}$$

and after integrating both sides of Eq. (8.42) yields

$$(q_S(0) - q_S) + K_M \ln\left(\frac{q_S(0)}{q_S}\right) = K_2 q_E(0) t$$

or

$$t = \frac{1}{K_2 q_E(0)} \left((q_S(0) - q_S) + K_M \ln\left(\frac{q_S(0)}{q_S}\right) \right) \tag{8.43}$$

Note that Eq. (8.43) provides a nonlinear expression for t as a function of q_S and that, until recently, it was thought impossible to write an expression of q_S as a function of t. In 1997, Schnell and Mendoza used computer algebra to solve Eq. (8.43) for q_S as a function of t using the Lambert W function. While Eq. (8.43) can be used to solve for t by substituting values of q_S from $q_S(0)$ to zero, it is far easier to simulate Eq. (8.41) for q_S.

The product is given by

$$\dot{q}_P = K_2 q_{ES} = \frac{K_2 q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)} \tag{8.44}$$

To eliminate q_S, we have $q_S = q_S(0) - q_{ES} - q_P$. Since q_{ES} is small compared to the other quantities after the initial phase of the response, we have $q_S = q_S(0) - q_P$ and

$$\dot{q}_P = \frac{K_2 q_E(0)}{\left(1 + \frac{K_M}{q_S}\right)} = \frac{K_2 q_E(0)}{\left(1 + \frac{K_M}{q_S(0) - q_P}\right)} \tag{8.45}$$

Solving Eq. (8.45) using the same approach to find Eq. (8.43), we have

$$t = \frac{1}{K_2 q_E(0)} \left(q_P - K_M \ln\left(\frac{q_S(0) - q_P}{q_S(0)} \right) \right) \tag{8.46}$$

This approximation works well for the reaction, except during the initial quick phase when both q_{ES} and q_P are small.

Biochemists define the reaction rate as either the rate of disappearance of the substrate or the appearance of the product. Using the Michaelis-Menten approximation, the reaction rates are equal and are given by $V = -\dot{q}_S = \dot{q}_P$. However, based on the true differential equations in Eq. (8.34), these two definitions are not equal. Using the approximation in Eq. (8.41), we have

$$V = -\dot{q}_S = \frac{K_2 q_S q_E(0)}{(q_S + K_M)} = \frac{V_{\max} q_S}{(q_S + K_M)} = \frac{V_{\max}}{\left(1 + \frac{K_M}{q_S}\right)} \tag{8.47}$$

where $V_{\max} = K_2 q_E(0)$ is the maximum velocity of the substrate disappearance. Equation (8.47) is plotted in Figure 8.5. Note that at high substrate levels, V approaches $V_{\max}$, since all of the enzyme is engaged and the velocity saturates. In this region, the reaction rate is independent of q_s. At low substrate levels, the reaction rate is approximately linearly dependent on substrate level, $V = \frac{V_{\max}}{K_M} q_s$.

Now consider when $V = \frac{V_{\max}}{2}$. Substituting into Eq. (8.47), we have

$$\frac{V_{\max}}{2} = \frac{V_{\max} q_S}{(q_S + K_M)}$$

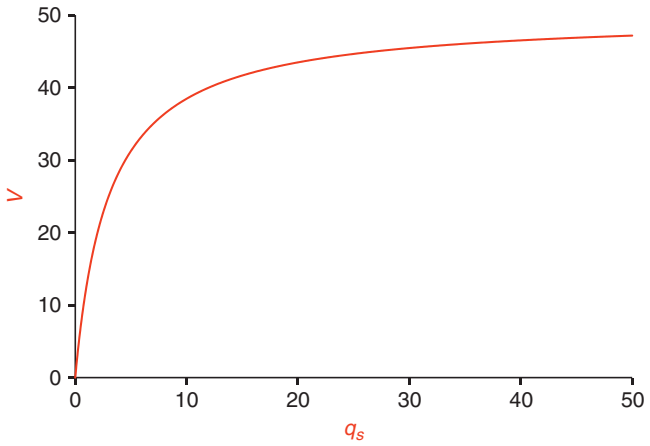

FIGURE 8.5 Velocity of substrate disappearance. Note that the reaction velocity is for $t = 0$, and q_S is the intial quantity of substrate. $V_{\max} = 50$ and $K_M = 3$.

and after dividing both sides by $V_{\max}$, we have

$$\frac{1}{2} = \frac{q_S}{(q_S + K_M)}$$

Simplifying the previous equation gives

$$q_S = K_M$$

when $V = \frac{V_{\max}}{2}$.

Given the lack of computer simulation capability in the early 1900s, the quasi-steady-state approximation gave an excellent and efficient solution to enzyme kinetics. However, with the current computer power and the availability of stiff differential equation simulators, it is far easier to simulate enzyme kinetics using the computer rather than solving a set of algebraic equations.

EXAMPLE PROBLEM 8.2

Simulate the reaction given in Eq. 8.34 and compare with the quasi-steady-state approximation for q_S, q_E, q_{ES}, and q_P. Assume that $K_1 = 8$, $K_{-1} = 0.01$, $K_2 = 5$, $q_S(0) = 1$, $q_E(0) = 0.08, q_{ES}(0) = 0$, and $q_P(0) = 0$.

Solution

The SIMULINK model is shown in Figures 8.6 and 8.7, both executed using the `ode23tb` integrator. Because of the ease in solution, the quasi-steady-state approximation for q_S, q_E, q_{ES}, and q_P is carried out using the differential equation for $\dot{q}_S$, Eq. (8.41), and the algebraic equations,

Continued

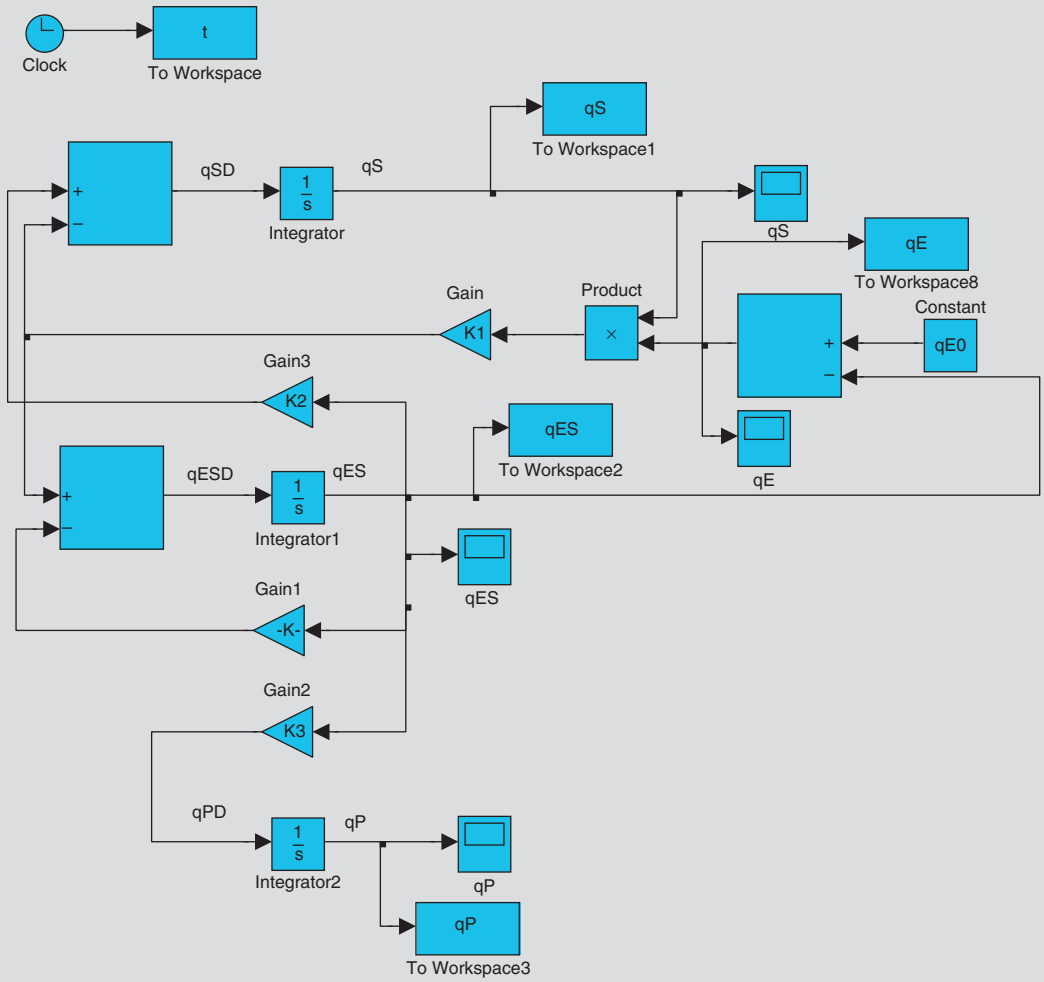

FIGURE 8.6 SIMULINK model for Eq. (8.34) in Example Problem 8.2.

Eqs. (8.38) and (8.40), and $q_P = q_S(0) - q_S - q_{ES}$. Figure 8.8 presents the simulation solution with both models. It is clear that there is an excellent agreement between the two solutions after the quick phase is completed at 0.5 s. Notice the expected error between q_E and q_{EMM}, and q_{ES} and q_{ESMM}. Also note that the initial product predicted by the quasi-steady-state approximation is negative.

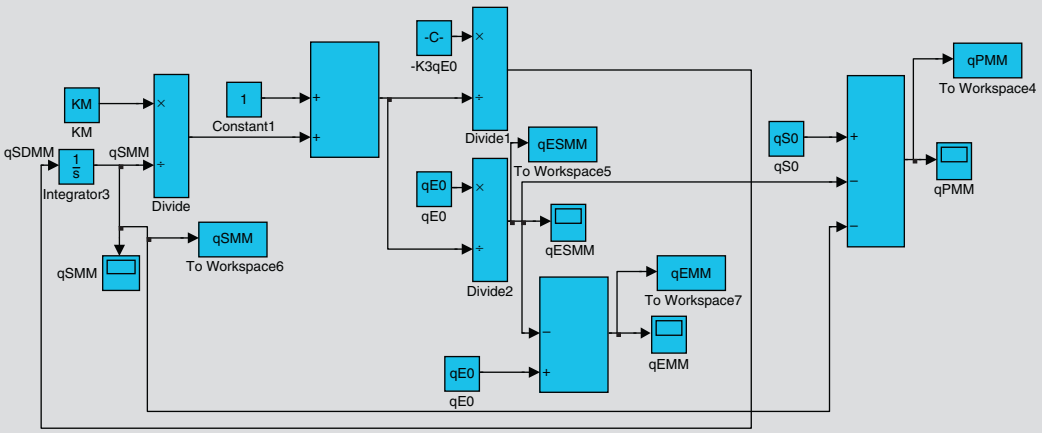

FIGURE 8.7 SIMULINK model in Example Problem 8.2 using the quasi-steady-state approximation.

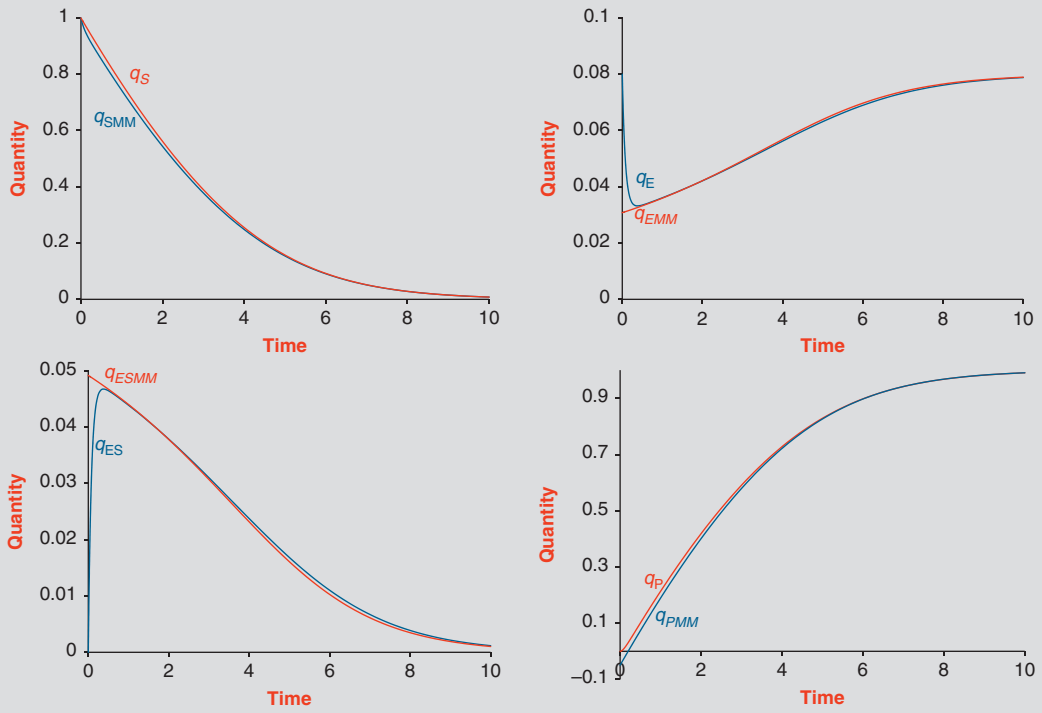

FIGURE 8.8 Simulations for Example Problem 8.2.

8.2.2 Estimation of the Michaelis-Menten Parameters

The reaction rate given by Eq. (8.47) contains two parameters, $V_{\max}$ and K_M, and is given by

$$V = \frac{V_{\max} q_S}{(q_S + K_M)} = \frac{V_{\max}}{\left(1 + \frac{K_M}{q_S}\right)} \tag{8.48}$$

One can estimate $V_{\max}$ and K_M by first taking the reciprocal of Eq. (8.48), giving

$$\left(\frac{1}{V}\right) = \frac{K_M}{V_{\max}}\left(\frac{1}{q_S}\right) + \frac{1}{V_{\max}} \tag{8.49}$$

which is called the Lineweaver-Burk equation. By plotting $\frac{1}{V}$ against $\frac{1}{q_S}$, a straight line results as shown in Figure 8.9, with the ordinate-intercept as $\frac{1}{V_{\max}}$ and the slope as K_M. Since the substrate changes as a function of time, the Lineweaver-Burk equation is usually carried out with the initial value of substrate, $q_S(0)$—that is,

$$\frac{1}{V(0)} = \frac{K_M}{V_{\max}}\left(\frac{1}{q_S(0)}\right) + \frac{1}{V_{\max}} \tag{8.50}$$

The method to generate data for the Lineweaver-Burk equation is to perform a series of experiments that increase the quantity of substrate, while keeping the amount of enzyme constant. Recall at $V = \frac{V_{\max}}{2}$, $q_S = K_M$. If $q_S \gg K_M$, then $V = V_{\max}$. While estimation of the parameters from the Lineweaver-Burk plot looks attractive, any measurement error is amplified by the transformation, thus giving poor estimates of the parameters.

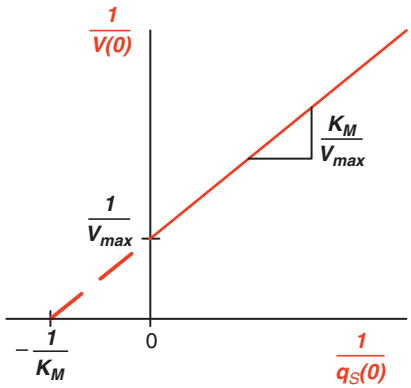

FIGURE 8.9 A Lineweaver-Burk plot.

8.3 ADDITIONAL MODELS USING THE QUASI-STEADY-STATE APPROXIMATION

The quasi-steady-state approximation for Michaelis-Menten kinetics can be used for more than just enzyme reactions.[2] The quasi-steady-state approximation is useful when describing the elimination of substances from the body with capacity-limited rates, such as renal excretion and metabolism, and even for linearized models of muscle using the Hill equation. While the quasi-steady-state approximation was developed for enzyme reactions with variables $q_S, q_E, q_{ES},$ and q_P, we will use it as a nonlinear transfer rate in a compartment model, where the substrate, q_S, in Eq. (8.47) is the quantity in a compartment. Here, we first consider a one-compartment model with a variety of inputs and then a two-compartment model.

8.3.1 One-Compartment Model

Consider a one-compartment model in which the elimination is characterized by the quasi-steady-state approximation

$$\dot{q}_S = -\frac{V_{\max}}{(q_S + K_M)} q_S + f(t) \tag{8.51}$$

Impulse Input

Consider first an impulse input. Previous examples have used $f(t) = \zeta\delta(t)$, where ζ is the strength of the impulse function—a problem that is handled most simply by a change in the initial condition. To solve Eq. (8.51) with an impulse input, we have

$$\dot{q}_S = -\frac{V_{\max}}{(q_S + K_M)} q_S \tag{8.52}$$

with $q_S(0) = \zeta$. As before, Eq. (8.52) is rearranged to give

$$\left(1 + \frac{K_M}{q_S}\right) dq_S = -V_{\max} dt$$

and after integrating, we have

$$t = \frac{1}{V_{\max}} \left(\zeta - q_S + K_M \ln\left(\frac{\zeta}{q_S}\right)\right) \tag{8.53}$$

The same comments about the solution of Eq. (8.43) apply to Eq. (8.53).

[2]Material in this section based on Godfrey.

Step Input

We now draw our attention to the case in which the input to the system in Eq. (8.51) is a step input, $f(t) = \zeta u(t)$. With initial condition $q_S(0)$, we have for $t \geq 0$

$$\dot{q}_S = -\frac{V_{\max}}{(q_S + K_M)} q_S + \zeta = \frac{(\zeta - V_{\max})q_S + \zeta K_M}{(q_S + K_M)} \tag{8.54}$$

Separating q_S and t in Eq. (8.54) gives

$$\begin{aligned} dt &= \frac{K_M dq_S}{(\zeta - V_{\max})q_S + \zeta K_M} + \frac{q_S dq_S}{(\zeta - V_{\max})q_S + \zeta K_M} \\ &= \frac{K_M dq_S}{(\zeta - V_{\max})q_S + \zeta K_M} + \left(\frac{1}{(\zeta - V_{\max})}\right)\left(\frac{q_S dq_S}{q_S + \dfrac{\zeta K_M}{(\zeta - V_{\max})}}\right) \\ &= \frac{K_M dq_S}{(\zeta - V_{\max})q_S + \zeta K_M} + \left(\frac{1}{(\zeta - V_{\max})}\right)\left(1 - \frac{\dfrac{\zeta K_M}{(\zeta - V_{\max})}}{q_S + \dfrac{\zeta K_M}{(\zeta - V_{\max})}}\right) dq_S \end{aligned} \tag{8.55}$$

Then we integrate Eq. (8.55)

$$\int_0^t dt = \int_{q_S(0)}^{q_S} \left(\frac{K_M dq_S}{(\zeta - V_{\max})q_S + \zeta K_M} + \left(\frac{1}{\zeta - V_{\max}}\right)\left(1 - \frac{\dfrac{\zeta K_M}{(\zeta - V_{\max})}}{q_S + \dfrac{\zeta K_M}{(\zeta - V_{\max})}}\right)\right) dq_S \tag{8.56}$$

which gives

$$\begin{aligned} t &= \left(\left(\frac{K_M}{(\zeta - V_{\max})}\right) \ln\left(\frac{(\zeta - V_{\max})q_S + \zeta K_M}{(\zeta - V_{\max})q_S(0) + \zeta K_M}\right) + \left(\frac{q_S - q_S(0)}{\zeta - V_{\max}}\right) \right. \\ &\quad \left. - \frac{\zeta K_M}{(\zeta - V_{\max})^2} \ln\left(\frac{q_S + \dfrac{\zeta K_M}{(\zeta - V_{\max})}}{q_S(0) + \dfrac{\zeta K_M}{(\zeta - V_{\max})}}\right) \right) \\ &= \frac{q_S - q_S(0)}{\zeta - V_{\max}} - \frac{V_{\max} K_M}{(\zeta - V_{\max})^2} \ln\left(\frac{(\zeta - V_{\max})q_S + \zeta K_M}{(\zeta - V_{\max})q_S(0) + \zeta K_M}\right) \end{aligned} \tag{8.57}$$

To determine q_S at steady state for this one-compartment system, we set $\dot{q}_S = 0$ in Eq. (8.54), which gives

$$q_S(\infty) = \frac{\zeta K_M}{V_{\max} - \zeta} \tag{8.58}$$

Since q_S needs to be positive, this requires $V_{\max} > \zeta$. Thus, the maximum removal rate must be larger than the input for a bound solution for q_S.

To examine the case when $V_{\max} = \zeta$, we substitute these values into Eq. (8.54), giving

$$\dot{q}_S = \frac{\zeta K_M}{(q_S + K_M)} \tag{8.59}$$

Separating q_S and t in Eq. (8.59) gives

$$dt = \frac{1}{\zeta K_M}(q_S + K_M)dq_S$$

and after integrating

$$t = \frac{1}{2\zeta K_M}\left(q_S^2 - q_S^2(0)\right) + \frac{1}{\zeta}\left(q_S - q_S(0)\right) \tag{8.60}$$

which increases without bound, $q_S \to \infty$. If $\zeta \geq V_{\max}$, then the substrate is not eliminated quickly enough, continuously increasing, and $q_S \to \infty$.

Exponential Input

Next, consider an exponential input to a one-compartment model of Eq. (8.51), a type of input previously observed in Example Problem 7.8, when a substrate is digested and moves into the plasma. For $t \geq 0$, the model is

$$\dot{q}_S = -\frac{V_{\max}}{(q_S + K_M)}q_S + q_2(0)K_{21}e^{-K_{21}t} \tag{8.61}$$

where $q_2(0)$ is the initial amount of substrate in the digestive system and K_{21} is the transfer rate from the digestive system to the compartment. Simulating Eq. (8.61) is the only way to solve this problem.

Before simulation became an easy solution method, the quasi-steady-state approximation was linearized with a lower and upper bound, with the actual solution falling in between these two bounds. For the lower bound, the quasi-steady-state approximation is $\frac{V_{\max}}{K_M}$, where the quasi-steady-state approximation is a constant. As we will see, the lower bound is a good approximation for small values of q_S. For the upper bound, the quasi-steady-state approximation is $\dfrac{V_{\max}}{q_{S_{\max}} + K_M}$, where $q_{S_{\max}}$ is the maximum q_S. As before with the lower bound, the quasi-steady-state approximation is a constant for the upper bound. The upper bound is a good approximation for large values of q_S. This approach works for all inputs.

For example, consider the exponential input with $q_S(0) = 0$, where the equation for the lower bound is

$$\dot{q}_S = -\frac{V_{\max}}{K_M} q_S + q_2(0) K_{21} e^{-K_{21} t} \tag{8.62}$$

with a solution of

$$q_S = \left(\frac{q_2(0) K_{21}}{\frac{V_{\max}}{K_M} - K_{21}} \right) \left(e^{-K_{21} t} - e^{-\frac{V_{\max}}{K_M} t} \right) \tag{8.63}$$

and the equation for the upper bound is

$$\dot{q}_S = -\frac{V_{\max}}{(q_{S_{\max}} + K_M)} q_S + q_2(0) K_{21} e^{-K_{21} t} \tag{8.64}$$

with a solution of

$$q_S = \left(\frac{q_2(0) K_{21}}{\left(\frac{V_{\max}}{q_{S_{\max}} + K_M} \right) - K_{21}} \right) \left(e^{-K_{21} t} - e^{-\left(\frac{V_{\max}}{q_{S_{\max}} + K_M} \right) t} \right) \tag{8.65}$$

To illustrate the approximations, Figure 8.10 plots the simulation of Eq. (8.61), with the analytic solution of Eqs. (8.63) and (8.65). For small values of q_S, the lower bound

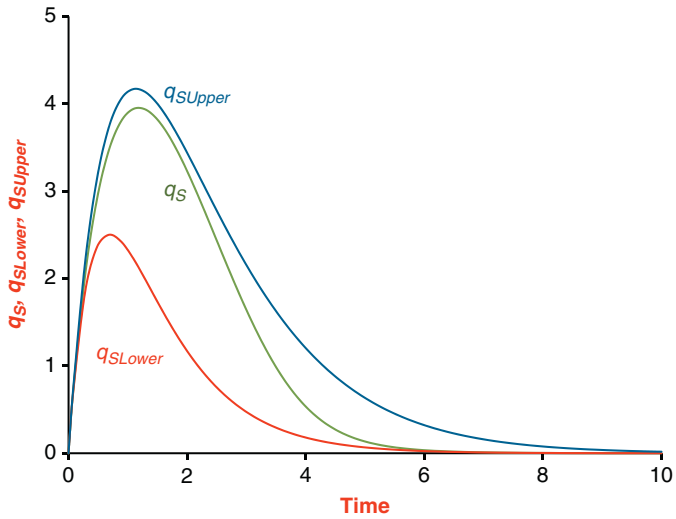

FIGURE 8.10 Responses for a one-compartment model with quasi-steady-state approximation (q_S), lower bound approximation (q_{SLower}), and upper bound approximation (q_{SUpper}), with an exponential input. Parameters are $V_{\max} = 3, K_M = 2.5, K_{21} = 1.0, q_S(0) = 0, q_{S_{\max}} = 4.0$, and $q_2(0) = 10$.

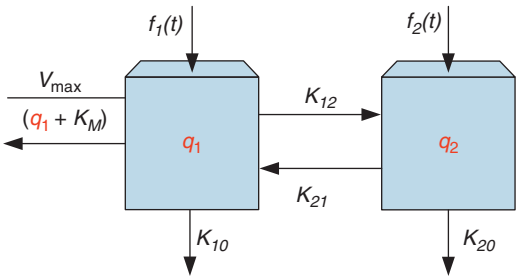

FIGURE 8.11 A two-compartment model with a quasi-steady-state approximation transfer rate.

approximation is quite good, as is the upper bound for large values of q_S. The accuracy of the lower and upper bound approximations depends quite heavily on the closeness of K_M to $q_{S_{\max}}$. The farther apart the two are, the poorer the approximation.

8.3.2 Two-Compartment Model

Next, consider a two-compartment model with a quasi-steady-state approximation transfer rate as shown in Figure 8.11. Such models are useful in describing some capacity-limited biochemical reactions that are more complex than a one-compartment model. Note that the quasi-steady-state approximation is for compartment 1 only, and the other transfer rates are the usual constants. The equations describing the system are

$$\begin{aligned}\dot{q}_1 &= -\left(K_{10} + K_{12} + \frac{V_{\max}}{(q_1 + K_M)}\right) q_1 + K_{21} q_2 + f_1(t) \\ \dot{q}_2 &= K_{12} q_1 - (K_{20} + K_{21}) q_2 + f_2(t)\end{aligned} \tag{8.66}$$

EXAMPLE PROBLEM 8.3

Simulate the model in Eq. (8.66), given that $K_{12} = 1, K_{21} = 2,\ V_{\max} = 3,\ K_M = 0.5,\ q_1(0) = 0,\ q_2(0) = 0,\ K_{10} = 0$, and $K_{20}(0) = 0$. The inputs are $f_1(t) = u(t) - u(t-4)$, and $f_2(t) = 0$.

Solution

The equations describing this system are

$$\dot{q}_1 = -\left(3 + \frac{1}{(q_1 + 0.5)}\right) q_1 + 2q_2 + u(t) - u(t-4)$$

$$\dot{q}_2 = 3q_1 - 2q_2$$

The SIMULINK model shown in Figure 8.12 was executed using the `ode23tb` integrator. The solution is shown in Figure 8.13.

Continued

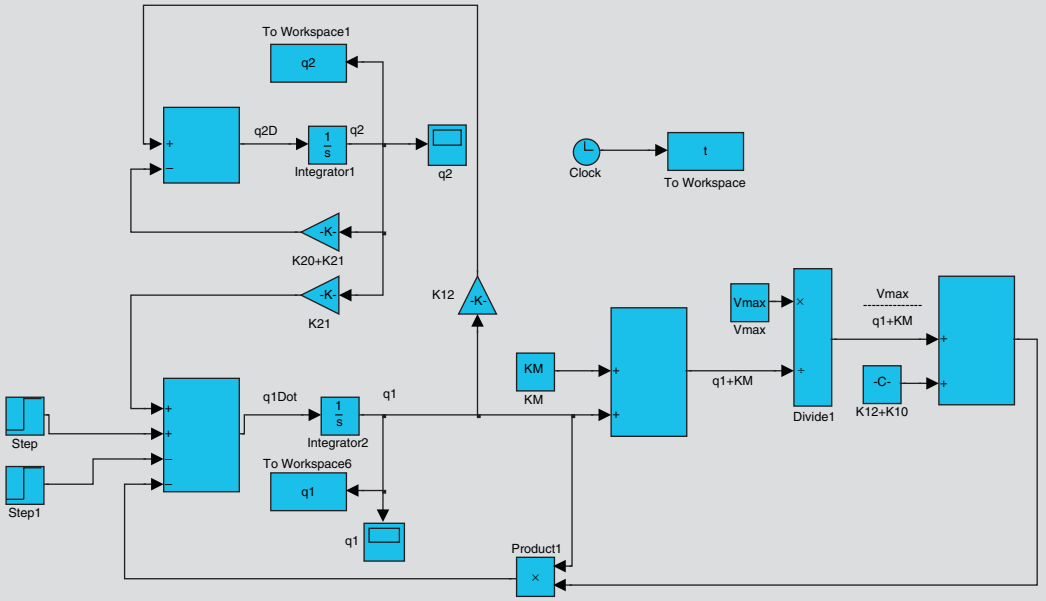

FIGURE 8.12 SIMULINK model for Example Problem 8.3.

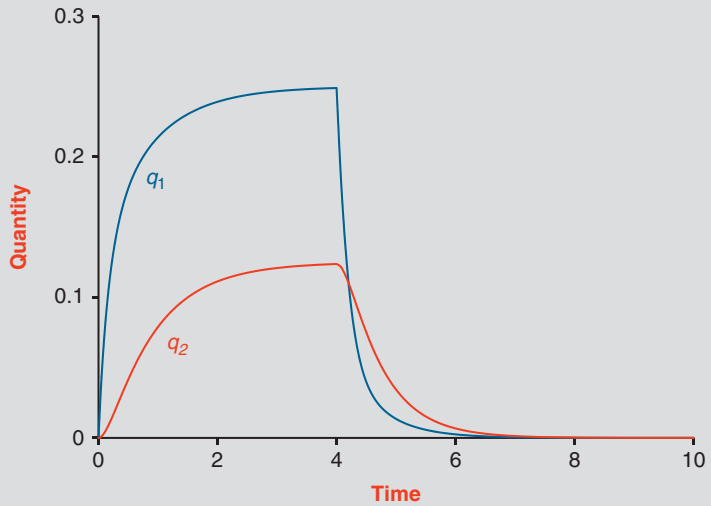

FIGURE 8.13 Response for Example Problem 8.3.

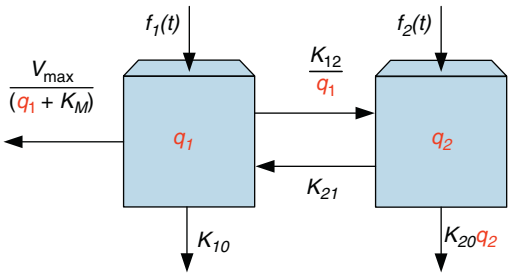

FIGURE 8.14 A two-compartment model with a quasi-steady-state approximation and biochemical reaction transfer rates. In addition, a constant input from compartment 1 into compartment 2 is used, with $\frac{K_{12}}{q_1}$ as the transfer rate.

Consider the model shown in Figure 8.14 that contains a quasi-steady-state approximation and a biochemical reaction as transfer rates. We also have a constant transfer of substrate from compartment 1 into compartment 2 by using $\frac{K_{12}}{q_1}$ as the transfer rate. The equations that describe this system are

$$\begin{aligned} \dot{q}_1 &= -K_{12} - \left(K_{10} + \frac{V_{\max}}{(q_1 + K_M)} \right) q_1 + K_{21} q_2 + f_1(t) \\ \dot{q}_2 &= K_{12} - K_{20} q_2^2 + f_2(t) \end{aligned} \tag{8.67}$$

Note that the constant loss from compartment 1 and input to compartment 2 could describe an active pump. Also note that a chemical reaction in compartment 2 consumes the substrate with rate K_{20}. We will expand on these concepts with multicompartment models in the next section that includes diffusion.

8.4 DIFFUSION, BIOCHEMICAL REACTIONS, AND ENZYME KINETICS

In Chapter 7, we discussed diffusion as a flow of ions down the concentration gradient. Up to now, we have approached biochemical reactions and enzyme kinetics occurring in a homogeneous volume. Now, we include diffusion from another compartment, biochemical reactions, and enzyme kinetics in our analysis. As we will see, the movement of a substrate or an enzyme into the cell by diffusion allows a product to be created. This product then can be used inside the cell or diffused out of the cell to be used by another cell or tissue. Additionally, the same situation occurs within the organelles of the cell. These reactions can serve a regulatory role as well as accelerating biochemical reactions; recall the reaction involving ADP and ATP in the mitochondria, where the availability of ADP regulates the production of ATP.

8.4.1 Diffusion and Biochemical Reactions

Consider the movement of a substrate A into a cell by diffusion, which then reacts with B to form product P, as shown in Figure 8.15. Product P then leaves the cell by diffusion. Assume that the quantity of B_i is regulated by another system. Let subscript i denote inside the cell, and let o denote outside the cell. The chemical reaction is

$$\begin{aligned} A_i + B_i &\underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} P_i \\ \dot{q}_{A_i} &= -K_1 q_{A_i} q_{B_i} + K_{-1} q_{P_i} \\ \dot{q}_{P_i} &= K_1 q_{A_i} q_{B_i} - K_{-1} q_{P_i} \end{aligned} \tag{8.68}$$

where K_1 and K_{-1} are the reaction rates, q_{A_o} and q_{A_i} are the quantities of substrate A, q_{B_i} is the quantity of substrate B_i, and q_{P_o} and q_{P_i} are the quantities of product P. Diffusion across the membrane is given by

$$\begin{aligned} \dot{q}_{A_i} &= C_{oi} q_{Ao} - C_{io} q_{A_i} \\ \dot{q}_{P_i} &= D_{oi} q_{P_o} - D_{io} q_{P_i} \end{aligned} \tag{8.69}$$

where C_{oi}, C_{io}, D_{oi}, and D_{io} are the diffusion transfer rates that depend on the volume, as described in Example Problem 7.5. The equations describing the complete system (biochemical reaction and diffusion) are

$$\begin{aligned} \dot{q}_{A_o} &= -C_{oi} q_{A_o} + C_{io} q_{A_i} \\ \dot{q}_{A_i} &= -K_1 q_{A_i} q_{B_i} + K_{-1} q_{P_i} + C_{oi} q_{A_o} - C_{io} q_{A_i} \\ \dot{q}_{P_o} &= -D_{oi} q_{P_o} + D_{io} q_{P_i} \\ \dot{q}_{P_i} &= K_1 q_{A_i} q_{B_i} - K_{-1} q_{P_i} + D_{oi} q_{P_o} - D_{io} q_{P_i} \end{aligned} \tag{8.70}$$

Transport of Oxygen into a Slow Muscle Fiber

Consider the delivery of oxygen into a slow muscle fiber. Let's begin at the lung where oxygen first diffuses through the alveoli membrane into the capillaries and from the capillaries into the red blood cell, as shown in Figure 8.16. Let q_{O_A} be the quantity of O_2 in the

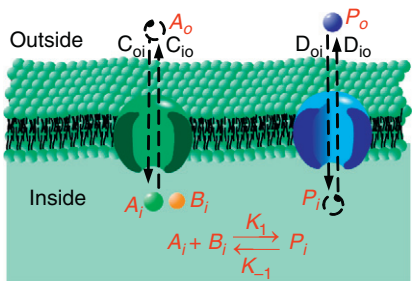

FIGURE 8.15 Diffusion and a biochemical reaction.

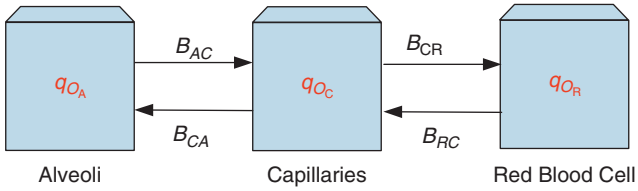

FIGURE 8.16 The diffusion of oxygen from the alveoli into the red blood cell.

alveoli, q_{O_C} be the quantity of O_2 in the capillaries, and q_{O_R} be the quantity of O_2 in the red blood cells. The equation that describes the movement of oxygen is given by

$$\begin{aligned}
\dot{q}_{O_A} &= B_{CA}q_{O_C} - B_{AC}q_{O_A} \\
\dot{q}_{O_C} &= B_{AC}q_{O_A} + B_{RC}q_{O_R} - B_{CA}q_{O_C} - B_{CR}q_{O_C} \\
\dot{q}_{O_R} &= B_{CR}q_{O_C} - B_{RC}q_{O_R}
\end{aligned} \tag{8.71}$$

Once inside the red blood cell, oxygen then binds with hemoglobin (Hb), forming oxyhemoglobin (HbO_8). This is a reversible reaction that allows oxygen to be taken up by the red blood cell and released into the tissues. The binding of O_2 with hemoglobin allows 100 times more oxygen in the blood than if it had just dissociated into the blood. The overall chemical reaction is

$$Hb + 4O_2 \rightleftharpoons HbO_8$$

Hemoglobin has four polypeptide subunits (proteins), with each polypeptide subunit attached to a heme group. Each heme group can bind with a molecule of O_2. The four molecules of O_2 that bind to Hb do not simultaneously react with heme groups but occur in four steps, with each step facilitating the next step. Figure 8.17 illustrates the five states of hemoglobin based on the number of O_2 molecules bound to it, ranging from 0 to 4. Let q_{H_0} be the quantity of Hb, q_{H_1} be the quantity of HbO_2, and so on, up to q_{H_4} be the quantity of HbO_8. Equation (8.72) describes the chemical reactions that take place to create the oxyhemoglobin:

$$\begin{aligned}
\dot{q}_{H_0} &= K_{10}q_{H_1} - K_{01}q_{H_0}q_{O_R} \\
\dot{q}_{H_1} &= K_{01}q_{H_0}q_{O_R} + K_{21}q_{H_2} - K_{10}q_{H_1} - K_{12}q_{H_1}q_{O_R} \\
\dot{q}_{H_2} &= K_{12}q_{H_1}q_{O_R} + K_{32}q_{H_3} - K_{21}q_{H_2} - K_{23}q_{H_2}q_{O_R} \\
\dot{q}_{H_3} &= K_{23}q_{H_2}q_{O_R} + K_{43}q_{H_4} - K_{32}q_{H_3} - K_{34}q_{H_3}q_{O_R} \\
\dot{q}_{H_4} &= K_{34}q_{H_3}q_{O_R} - K_{43}q_{H_4}
\end{aligned} \tag{8.72}$$

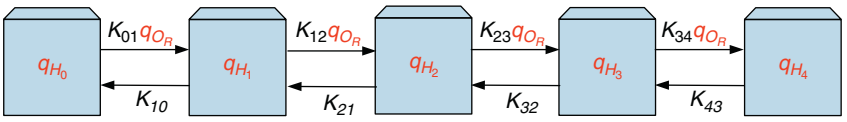

FIGURE 8.17 The five states of hemoglobin.

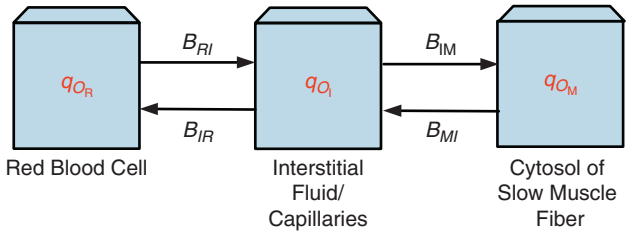

FIGURE 8.18 The diffusion of oxygen from the red blood cell into the cytosol of the slow muscle fiber.

Note that we have assumed that the reverse reactions do not involve oxygen and that oxygen is treated as a molecule and not two oxygen atoms, which would have introduced a q_O^2 term in Eq. (8.72).

Oxygen is transported through the arterial system to the capillaries, where it diffuses out of the red blood cell into the interstitial fluid. It then moves into the cytosol (the liquid part of the cytoplasm that does not contain any organelles) of slow muscle fibers, as shown in Figure 8.18, where q_{O_I} is the quantity of O_2 in the interstitial fluid, q_{O_M} is the quantity of O_2 in the cytosol of the slow muscle fiber, and the other quantities are defined as before. The equation describing the diffusion process and reactions with Hb is given by

$$\begin{aligned}
\dot{q}_{O_R} &= B_{IR}q_{O_C} - B_{RI}q_{O_R} + K_{10}q_{H_1} - K_{01}q_{H_0}q_{O_R} + K_{21}q_{H_2} - K_{12}q_{H_1}q_{O_R} \\
&\quad + K_{32}q_{H_3} - K_{23}q_{H_2}q_{O_R} + K_{43}q_{H_4} - K_{34}q_{H_3}q_{O_R} \\
\dot{q}_{O_I} &= B_{RI}q_{O_R} + B_{MI}q_{O_M} - B_{IR}q_{O_I} - B_{IM}q_{O_I} \\
\dot{q}_{O_M} &= B_{IM}q_{O_I} - B_{MI}q_{O_M}
\end{aligned} \tag{8.73}$$

On the arterioles side of the capillary membrane, PO_2 is approximately 100 mm Hg and 98 percent saturated. On the venule side of the capillary membrane, PO_2 is approximately 40 mm Hg and 75 percent saturated. When PO_2 is high, oxygen quickly binds with hemoglobin, and when PO_2 is low, oxygen is quickly released from hemoglobin. As the red blood cell moves through the capillary, the PO_2 gradient causes oxygen to be released into the interstitial fluid.

Once inside the slow muscle fiber, oxygen is moved to the mitochondria using a different mechanism than that used in other cells. After oxygen diffuses across the cell membrane, it quickly binds to myoglobin (Mb), a protein-like hemoglobin whose function is to transport and store oxygen, and forms oxymyoglobin (MbO_2). By storing oxygen in oxymyoglobin, oxygen is driven into the cell by a large concentration gradient until it binds with all available myoglobin. At this point, the oxygen concentration on either side of the membrane equilibrates. Slow muscle fibers also have many more mitochondria than other cells, which allows higher levels of oxidative metabolism. Thus, the muscle fiber is able to store a large quantity of oxygen in oxymyoglobin, and when needed, it is readily available to the mitochondria to create ATP. This greatly increases oxygen transport to the mitochondria than if the cell just depended on oxygen diffusion in the absence of myoglobin.

When oxygen is bound to myoglobin to create oxymyoglobin in the cytosol, oxymyoglobin then diffuses from the cytosol into the mitochondria, and once in the mitochondria, a

reverse reaction occurs, releasing O_2 and *Mb*. Oxygen in the mitochondria is consumed with sugar to create ATP during cell respiration, as described in the next section. The myoglobin then diffuses back to the cytosol, where the process repeats itself. The first reaction in the cytosol is given by

$$O_2 + Mb \xrightarrow{D_1} MbO_2$$

and in the mitochondria

$$MbO_2 \xrightarrow{D_2} Mb + O_2$$

which is described by the following equations that includes diffusion:

$$\begin{aligned}
\dot{q}_{O_M} &= -D_1 q_{O_M} q_{Mb_M} + B_{IM} q_{O_I} \\
\dot{q}_{MbO_M} &= D_1 q_{O_M} q_{Mb_M} - K_{MT} q_{MbO_M} \\
\dot{q}_{Mb_M} &= -D_1 q_{O_M} q_{Mb_M} + K_{TM} q_{Mb_T} \\
\dot{q}_{Mb_T} &= D_2 q_{MbO_T} - K_{TM} q_{Mb_T} \\
\dot{q}_{MbO_T} &= -D_2 q_{MbO_T} + K_{MT} q_{MbO_M}
\end{aligned} \tag{8.74}$$

where K_{MT} and K_{TM} are the diffusion transfer rates from the cytosol into the mitochondria and the mitochondria into the cytosol, respectively; q_{O_M} is the quantity of O_2 inside the cytosol; q_{Mb_M} is the quantity of *Mb* in the cytosol; q_{MbO_M} is the quantity of MbO_2 in the cytosol; q_{MbO_T} is the quantity of MbO_2 inside the mitochondria; and q_{Mb_T} is the quantity of *Mb* inside the mitochondria. We assume that none of the MbO_2 or *Mb* leaves the cell. We assume that no O_2 leaves the cell and no Mb_i diffuses into the mitochondria.

Carbon dioxide is created in the cells during cell respiration, as discussed shortly. It then diffuses out of the cell into the interstitial fluid and then moves to the capillaries. Once inside the capillaries, 90 percent of the carbon dioxide moves into the red blood cell, and 10 percent dissolves into the fluid of the blood. Carbon dioxide is then transported to the lungs.

When inside the red blood cell, carbon dioxide almost instantaneously goes through the following reactions:

1. Approximately 70 percent of the carbon dioxide reacts with water to form carbonic acid (H_2CO_3), using the enzyme carbonic anhydrase, and then dissociates into hydrogen (H^+) and biocarbonate $\left(HCO_3^-\right)$ ions. These reactions occur within a fraction of a second and are given by

$$CO_2 + H_2O \xrightarrow{carbonic\ anhydrase} H_2CO_3 \longrightarrow HCO_3^- + H^+$$

2. The hydrogen then binds with the hemoglobin in the red blood cell.

$$H^+ + HbH^- \rightarrow (H)Hb$$

3. The biocarbonate diffuses out of the red blood cell, replaced by chloride ions via a biocarbonate-chloride carrier protein in the cell membrane.

4. The remaining 20 percent of the carbon dioxide in the red blood cell combines with hemoglobin to form carbaminohemoglobin. This is a weak bond that is easily broken:

$$Hb + CO_2 \rightarrow HbCO_2$$

After the red blood cell reaches the lungs, the oxygen that diffused across the alveoli membrane displaces the carbon dioxide in the blood and binds with the hemoglobin. Carbon dioxide then diffuses through the alveoli membrane and is then exhaled. The entire process then repeats itself.

8.4.2 Diffusion and Enzyme Kinetics

Consider the movement of a substrate into a cell by diffusion, which then reacts with an enzyme to ultimately form a product that leaves the cell by diffusion, as shown in Figure 8.19. The chemical reaction is

$$S_i + E \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} ES_i \xrightarrow{K_2} E + P_i \tag{8.75}$$

and diffusion by

$$\begin{aligned} \dot{q}_{S_i} &= B_{oi} q_{S_o} - B_{io} q_{S_i} \\ \dot{q}_{P_i} &= D_{oi} q_{P_o} - D_{io} q_{P_i} \end{aligned} \tag{8.76}$$

where B_{oi}, B_{io}, D_{oi}, and D_{io} are the diffusion transfer rates. The equations describing the complete system are

$$\begin{aligned} \dot{q}_{S_i} &= -K_1 q_{S_i} q_E + K_{-1} q_{ES_i} + B_{oi} q_{S_o} - B_{io} q_{S_i} \\ \dot{q}_{ES_i} &= K_1 q_{S_i} q_E - (K_{-1} + K_2) q_{ES_i} \\ \dot{q}_{P_i} &= K_2 q_{ES_i} + D_{oi} q_{P_o} - D_{io} q_{P_i} \end{aligned} \tag{8.77}$$

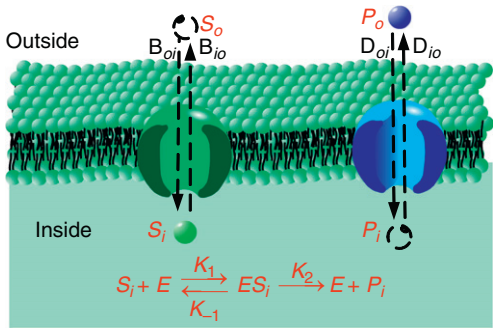

FIGURE 8.19 Diffusion and enzyme kinetics.

To remove q_{S_o} in Eq. (8.77), we assume a constant total substrate $q_{ST} = q_{S_i} + q_{S_o}$, and with $q_{S_o} = q_{ST} - q_{S_i}$, we have

$$\begin{aligned}
\dot{q}_{S_i} &= -K_1 q_{S_i} q_E + K_{-1} q_{ES_i} + B_{oi}(q_{ST} - q_{Si}) - B_{io} q_{S_i} \\
&= -K_1 q_{S_i} q_E + K_{-1} q_{ES_i} - (B_{oi} + B_{io}) q_{S_i} + B_{oi} q_{ST} \\
\dot{q}_{ES_i} &= K_1 q_{S_i} q_E - (K_{-1} + K_2) q_{ES_i} \\
\dot{q}_{P_i} &= K_2 q_{ES_i} + D_{oi} q_{P_o} - D_{io} q_{P_i}
\end{aligned} \tag{8.78}$$

We can substitute the quasi-steady-state approximation, $-K_1 q_{S_i} q_E + K_{-1} q_{ES_i} = -\frac{V_{\max}}{(q_{S_i} + K_M)} q_{S_i}$ (based on Eq. (8.47)), into Eq. (8.78) and get

$$\begin{aligned}
\dot{q}_{S_i} &= -\frac{V_{\max}}{(q_{S_i} + K_M)} q_{S_i} - (B_{oi} + B_{io}) q_{S_i} + B_{oi} q_{ST} \\
&= -\left(\frac{V_{\max}}{(q_{S_i} + K_M)} + B_{oi} + B_{io} \right) q_{S_i} + B_{oi} q_{ST}
\end{aligned} \tag{8.79}$$

where $q_{ES_i} = \frac{q_E(0)}{\left(1 + \frac{K_M}{q_{S_i}}\right)}$ and $q_{P_i} = q_{S_i}(0) - q_{S_i} - q_{ES_i}$.

8.4.3 Carrier-Mediated Transport

Now consider carrier-mediated transport, where an enzyme carrier in the cell membrane has a selective binding site for a substrate, which, when bound, transports the substrate through the membrane to be released inside the cell. Many also refer to this process as facilitated diffusion. Carrier-mediated transport does not use energy to transport the substrate but depends on the concentration gradient. Without carrier-mediated transport, the substrate cannot pass through the membrane.

Carrier-mediated transport differs from diffusion, since it is capacity-limited and diffusion is not. That is, as the quantity of the substrate increases, the carrier-mediated transport reaction rate increases and then saturates, where regular diffusion increases linearly without bound, as shown in Figure 8.20.

Figure 8.21 illustrates carrier-mediated transport, described by

$$\begin{aligned}
&S_o + C_o \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} P_o \underset{K_2}{\overset{K_2}{\rightleftarrows}} P_i \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} C_i + S_i \\
&C_o \underset{K_2}{\overset{K_2}{\rightleftarrows}} C_i
\end{aligned} \tag{8.80}$$

where S_o and S_i are the substrate outside and inside the cell, C_o is the carrier on the outside of the membrane, C_i is the carrier on the inside of the membrane, P_o is the bound substrate and carrier complex on the outside of the membrane, and P_i is the bound substrate and

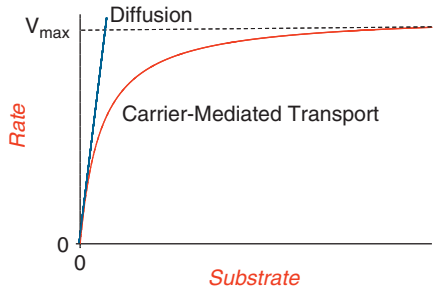

FIGURE 8.20 Comparison of the effect of the amount of substrate and the rate of diffusion and carrier-mediated transport.

carrier complex on the inside of the membrane. As shown in Figure 8.21, the substrate first moves to the binding site on the outside of the membrane and binds with the carrier to form the carrier-substrate complex, P_o (left). Next, the carrier-substrate complex moves from the outside to the inside of the membrane, given by the carrier-substrate complex P_i (right). It is not known how the carrier-substrate complex moves through the membrane, but we are fairly certain it happens.

The last step is when the carrier-substrate complex, P_i, dissociates into the substrate, S_i, and carrier, C_i. We have assumed that the reaction rates are the same for creation and the dissociation of the complex, $\underset{K_2}{\overset{K_2}{\rightleftharpoons}}$. In addition, we assume that the reaction of

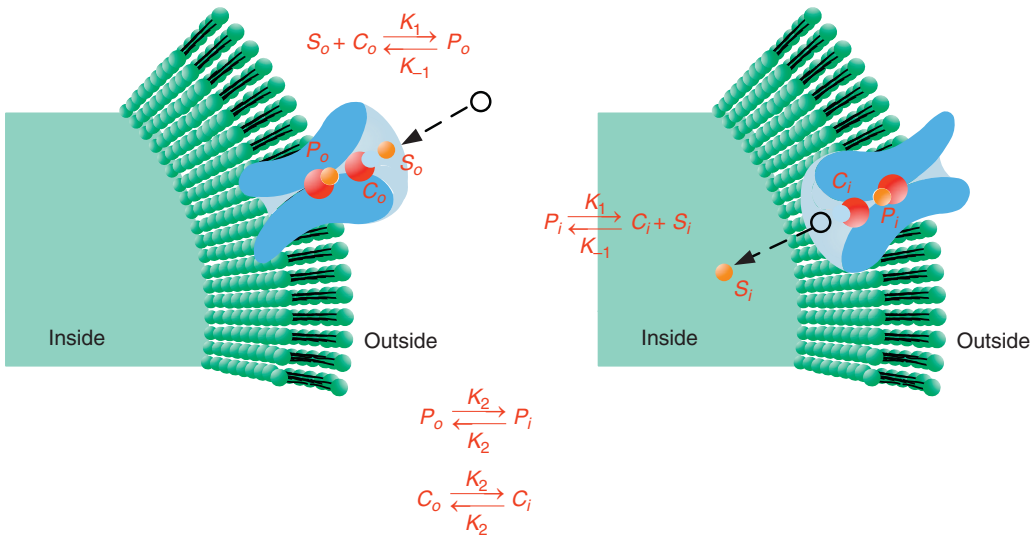

FIGURE 8.21 Carrier-mediated transport, with the carrier within the membrane. On the left side is the binding of the carrier with the substrate. On the right side is the substrate released from the binding site into the cell.

carrier-substrate complex from outside to inside has the same reaction rate, $\underset{K_2}{\overset{K_2}{\rightleftarrows}}$. Using the law of mass action, we get

$$\begin{aligned}
\dot{q}_{S_o} &= -K_1 q_{S_o} q_{C_o} + K_{-1} q_{P_o} \\
\dot{q}_{C_o} &= -K_1 q_{S_o} q_{C_o} + K_{-1} q_{P_o} + K_2 q_{C_i} - K_2 q_{C_o} \\
\dot{q}_{P_o} &= K_1 q_{S_o} q_{C_o} - (K_{-1} + K_2) q_{P_o} + K_2 q_{P_i} \\
\dot{q}_{P_i} &= -(K_1 + K_2) q_{P_i} + K_2 q_{P_o} + K_{-1} q_{S_i} q_{C_i} \\
\dot{q}_{S_i} &= -K_{-1} q_{S_i} q_{C_i} + K_1 q_{P_i} \\
\dot{q}_{C_i} &= -K_{-1} q_{S_i} q_{C_i} + K_1 q_{P_i} + K_2 q_{C_o} - K_2 q_{C_i}
\end{aligned} \tag{8.81}$$

Since the carrier is not consumed in the reaction, then the total carrier is a constant, given as $q_{C_o} + q_{C_i} + q_{P_o} + q_{P_i} = \zeta$.

Naturally, we can add an input to the system in Eq. (8.81) or simplify using the quasi-steady-state approximation as before. In addition, the substrate can be involved in other reactions inside the cell, such as moving into an organelle (mitochondria) via diffusion and then experiencing an enzyme reaction.

Glucose Transport

Consider the transport of glucose across the cell membrane. We know that glucose does not diffuse across the cell membrane but is transported across the cell membrane by a carrier-mediated transport process. Glucose binds to a protein that transports it across the membrane, allowing it to pass into the cytosol. This process does not use any energy. Using the model illustrated in Figure 8.21, we have

$$\begin{gathered}
G_0 + C_0 \xrightarrow{K_1} P_o \underset{K_2}{\overset{K_2}{\rightleftarrows}} P_i \xrightarrow{K_1} G_i + C_i \\
C_o \underset{K_2}{\overset{K_2}{\rightleftarrows}} C_i
\end{gathered} \tag{8.82}$$

where G_o and G_i is glucose outside and inside the cell, respectively; C_o is the carrier on the outside of the membrane; C_i is the carrier on the inside of the membrane; P_o is the bound substrate and carrier complex on the outside of the membrane; and P_i is the bound substrate and carrier complex on the inside of the membrane. Note that there is no reverse reaction for glucose in Eq. (8.82), since glucose does not leave the cell. We assume that glucose is consumed at a constant rate J_i inside the cell during cell respiration (described in the next section) and that glucose is available in the interstitial fluid at a rate of J_o. The equations that describe this system are given by

$$\begin{aligned}
\dot{q}_{G_o} &= -K_1 q_{G_o} q_{C_o} + J_o \\
\dot{q}_{C_o} &= -K_1 q_{G_o} q_{C_o} + K_2 q_{C_i} - K_2 q_{C_o} \\
\dot{q}_{P_o} &= K_1 q_{G_o} q_{C_o} - K_2 q_{P_o} + K_2 q_{P_i} \\
\dot{q}_{G_i} &= K_1 q_{P_i} - J_i \\
\dot{q}_{C_i} &= K_1 q_{P_i} + K_2 q_{C_o} - K_2 q_{C_i} \\
\dot{q}_{P_i} &= K_2 q_{P_o} - K_1 q_{P_i}
\end{aligned} \tag{8.83}$$

As described earlier, transport of glucose through the cell membrane is a capacity-limited reaction because of the enzyme carrier. Glucose concentration in the blood varies from a typical value of $90\frac{\text{mg}}{100\text{ mL}}$, up to $200\frac{\text{mg}}{100\text{ mL}}$ after eating, and down to $40\frac{\text{mg}}{100\text{ mL}}$ three hours after eating. Thus, the input, J_o, is a function of eating. The body uses the following two mechanisms to control glucose concentration:

1. Automatic feedback involving insulin secretion by the pancreas
2. The liver

The pancreas, in addition to digestive functions, secretes insulin directly into the blood. Insulin facilitates diffusion of glucose across the cell membrane. The rate of insulin secretion is regulated so glucose is maintained at a constant level.

The liver acts as a storage vault for glucose. When excess amounts of glucose are present, almost immediately two-thirds is stored in the liver. Conversely, when the glucose level in the blood falls, the stored glucose in the liver replenishes the blood glucose. Insulin has a moderating effect on the function of the liver. The binding of the carrier enzyme with glucose is a function of the transfer rate, K_1, and is a function of the insulin level, which can increase the transport of glucose by as much 20 times the base rate without insulin. We will ignore the aspects of insulin and liver storage in our model.

8.4.4 Active Transport

Now consider active transport, which is similar to carrier-mediated transport but operates against the concentration gradient and uses energy to move the substrate across the cell membrane. Active transport uses an enzyme carrier in the cell membrane that has a selective binding site for a substrate, which, when bound, transports the substrate through the membrane to be released inside the cell.

Active transport uses energy to run, typically by the hydrolysis of ATP. Here, the energy from ATP is used in the transport of the substrate, leaving ADP and an inorganic phosphate $\left(PO_4^{-2}\right)$ in the cytosol. The ADP is then recycled in the mitochondria to create more ATP using glucose as described in the next section. Active transport is capacity-limited like carrier-mediated diffusion: as the quantity of the substrate increases, the transport reaction rate increases and then saturates, as shown in Figure 8.20.

The Na-K pump is the most important active transport process, which pumps Na^+ out of the cell against the concentration gradient and replaces it inside the cell with K^+ against the concentration gradient. This pump is used to maintain the ion gradients and resting membrane potential, as described in Chapter 12, and is also required to maintain cell volume, as described in the previous chapter.

Another important active transport process is the Na-Ca ATP-ase pump that keeps Ca^{+2} levels low inside the cell. It is vitally important that the concentration of Ca^{+2} be kept low inside the cell $\left(\text{approximately } 10^{-7}\text{M}\right)$ as compared to the outside concentration $\left(\text{approximately } 10^{-3}\text{M}\right)$. The concentration gradient drives Ca^{+2} into the cell, and the Na-Ca ATP-ase pump drives Ca^{+2} out of the cell.

Na-K Pump

The Na-K pump is an integral part of the cell membrane that exists in all cells in the body. Approximately 70 percent of all ATP in the neuron and 25 percent of all ATP in all other cells

is used to fuel the Na-K pump at rest. This pump is vital to maintain the cell's resting membrane potential. In this section, we focus on the enzyme reactions. The Na-K pump was discovered by Jens Skou in 1957, who subsequently received a Nobel Prize for his work in 1997. Using radioactive ions, Skou showed that the concentrations of the ions are interdependent, implying the involvement of a common mechanism using an ATP-ase carrier.

The overall reaction for the Na-K pump is given by

$$ATP + 3Na_i^+ + 2K_o^+ \xrightarrow[ATP-ase]{} ADP + P_i + 3Na_o^+ + 2K_i^+$$

where the subscripts i for inside the cell and o for outside the cell are used as before.

The pump has 3 Na^+ binding sites and 2 K^+ binding sites in its two conformations. There is a higher concentration of Na^+ outside the cell than inside and a higher concentration of K^+ inside the cell than outside; left unchecked by the pump, this gradient would drive Na^+ into the cell and K^+ out of the cell, and thus change the resting membrane potential. Any change in the concentration gradient of K^+ and Na^+ is prevented by the Na-K pump. The pump transports a steady stream of Na^+ out of the cell and K^+ into the cell.

The Na-K pump uses six steps to move 3 Na^+ ions out of the cell and 2 K^+ ions into the cell at a total cost of 1 ATP molecule. Figure 8.22 illustrates the six steps that are continually repeated at a rate of 100 s^{-1}:

1. Three Na_i^+ ions in the cytosol move into and bind to the carrier in the pump, $(Na_3C)_i$. Note that the pump has a bound ATP molecule.
2. ATP is hydrolyzed. ADP is released, and the inorganic phosphate, P, binds with $(Na_3C)_i$ to create $(Na_3CP)_i$.
3. Using the energy gained by the hydrolyzation, a conformational change occurs in the pump that moves $(Na_3CP)_o$ to the outside of the cell membrane, exposing Na_o^+ ions to the outside. The 3 Na_o^+ ions exit the pump.
4. On the outside of the cell, 2 K_o^+ ions then bind to the carrier and inorganic phosphate in the pump, creating $(K_2CP)_o$.
5. Dephosphorylation of the pump occurs, releasing the inorganic phosphate. Following this, a conformational change occurs in the pump that moves $(K_2C)_i$, exposing 2 K_i^+ ions to the inside of the cell.
6. ATP binds to the pump, and the 2 K_i^+ ions are released into the cell.

The following equations list the reactions that describe the Na-K pump, where C is the carrier and P is the inorganic phosphate. Note that we have eliminated the reverse reactions as they are relatively small.

$$\xrightarrow{K_1} ATP_i$$
$$3Na_i^+ + C_i \xrightarrow{K_2} (Na_3C)_i$$
$$(Na_3C)_i + ATP_i \xrightarrow{K_3} (Na_3CP)_i + ADP_i$$
$$(Na_3CP)_i \xrightarrow{K_4} (Na_3CP)_o \xrightarrow{K_5} 3Na_o^+ + (CP)_o$$
$$(CP)_o + 2K_o^+ \xrightarrow{K_6} (K_2CP)_o \xrightarrow{K_7} P_i + (K_2C)_i$$
$$(K_2C)_i \xrightarrow{K_8} 2K_i^+ + C_i$$
$$ADP_i \xrightarrow{K_9}$$

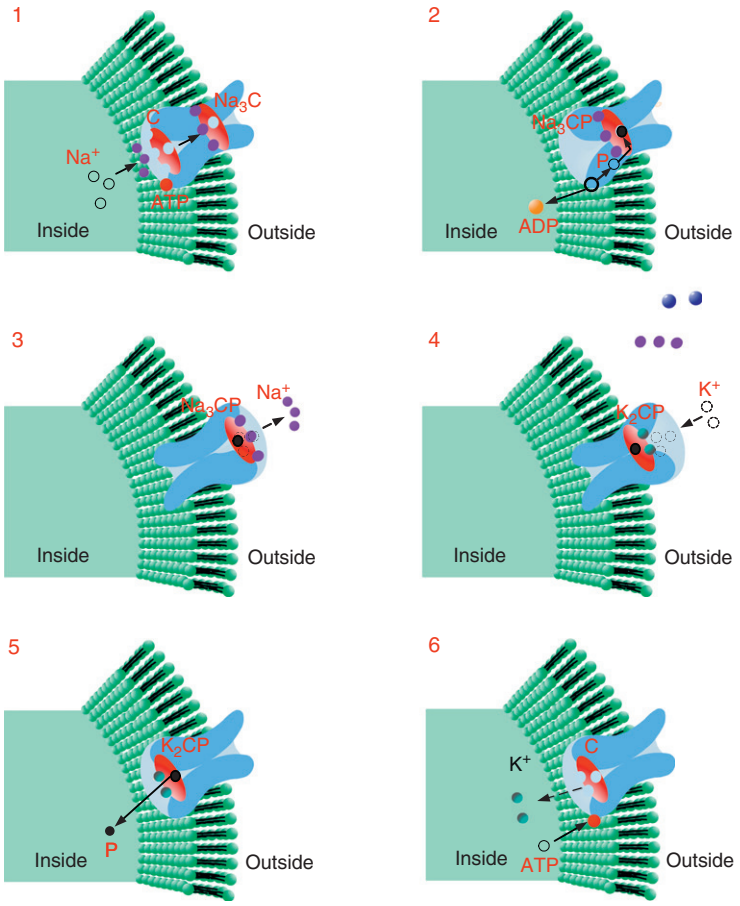

FIGURE 8.22 The six steps that characterize the Na-K pump.

Using the law of mass action, the Na-K pump is characterized by the following set of differential equations:

$$
\begin{aligned}
\dot{q}_{Na_i^+} &= J_{Na_D} - K_2 q_{Na_i^+}^3 q_{C_i} \\
\dot{q}_{(Na_3C)_i} &= K_2 q_{Na_i^+}^3 q_{C_i} - K_3 q_{(Na_3C)_i} q_{ATP_i} \\
\dot{q}_{ATP_i} &= K_1 - K_3 q_{(Na_3C)_i} q_{ATP_i} \\
\dot{q}_{(Na_3CP)_i} &= K_3 q_{(Na_3C)_i} q_{ATP_i} - K_4 q_{(Na_3CP)_i} \\
\dot{q}_{(Na_3CP)_o} &= K_4 q_{(Na_3CP)_i} - K_5 q_{(Na_3CP)_o} \\
\dot{q}_{Na_o^+} &= K_5 q_{(Na_3CP)_o} - J_{Na_D} \\
\dot{q}_{(CP)_o} &= K_5 q_{(Na_3CP)_o} - K_6 q_{K_o^+}^2 q_{(CP)_o} \\
\dot{q}_{K_o^+} &= J_{K_D} - K_6 q_{K_o^+}^2 q_{(CP)_o}
\end{aligned}
\tag{8.84}
$$

$$\begin{aligned}
\dot{q}_{(K_2CP)_o} &= K_7 q_{(K_2CP)_o} \\
\dot{q}_{P_i} &= K_7 q_{(K_2CP)_o} \\
\dot{q}_{(K_2C)_i} &= K_7 q_{(K_2CP)_o} - K_8 q_{(K_2C)_i} \\
\dot{q}_{ADP_i} &= -K_9 q_{ADP_i} \\
\dot{q}_{C_i} &= K_8 q_{(K_2C)_i} - K_2 q_{C_i} q_{Na_i^+}^3 \\
\dot{q}_{K_i^+} &= K_8 q_{(K_2C)_i} - J_{K_D}
\end{aligned}$$

where we assume a flow of K^+ out of the cytosol at a rate of J_{K_D} due to diffusion, a flow of Na^+ into the cell at a rate of J_{Na_D} from diffusion, ATP into the cytosol from the mitochondria at a rate of K_1, and ADP into the mitochondria from the cytosol at a rate of K_9. Note that there are other models that describe the Na-K pump based on different assumptions.

8.5 CELLULAR RESPIRATION: GLUCOSE METABOLISM AND THE CREATION OF ATP

At this time we wish to discuss cellular respiration involving glucose metabolism in more detail by describing the chemical processes that enable the body to create energy in the form of ATP. As previously noted, ATP is the fuel that supports life's processes, where energy is stored in the inorganic phosphate bonds. Energy is required to form these bonds in ATP, and energy is released when the bonds are broken.

Cellular respiration is among the best-known metabolic pathways, with detailed models going back to the 1960s. While much progress has been made, much uncertainty exists, and the system is still under considerable research. Here we will provide sufficient coverage based on the techniques in this chapter and focus on aerobic respiration that uses oxygen to create ATP in the mitochondria.

Cellular respiration consists of three major steps: glycolysis, Krebs cycle, and the electron transport chain, as illustrated in Figure 8.23. The first step occurs in the cytosol, and the two other steps occur in the mitochondria. Each step is very complex, with an overall reaction given by

$$C_6H_{12}O_6 + 6O_2 + 36ADP + 36P \longrightarrow 6CO_2 + 6H_2O + 36ATP \tag{8.85}$$

which consists of the oxidation of glucose $(C_6H_{12}O_6)$ to CO_2 and O_2, and a reduction of O_2 to H_2O. Once inside the cell, 1 M of glucose is transformed into 36 M of ATP by adding an inorganic phosphate P to each ADP. While there is some adenosine monophosphate (AMP) that, when combined with two inorganic phosphates, creates ATP, we will ignore this part of the reaction in this section.

Mitochondria are the powerhouses of the cell that produces the ATP used by the cell. A mitochondrion has two lipid bilayer membranes. The outer membrane is smooth and acts like a typical membrane. The inner membrane has many infoldings that contain the oxidative enzymes. Within the inner membrane is a cavity called the matrix that contains a nucleus with DNA, ribosomes, and dissolved enzymes. The matrix enzymes work with the oxidative enzymes to create ATP. The mitochondria's nucleus allows it to self-replicate

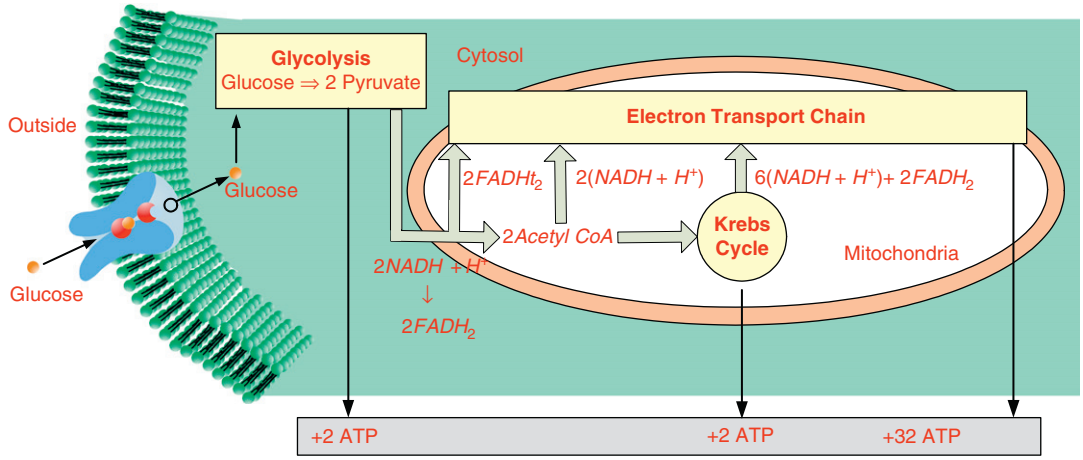

FIGURE 8.23 Cellular respiration consisting of three steps: glycolysis, Krebs cycle, and the electron transport chain. The amount of ATP created or lost in each step is indicated at the bottom of the figure. A total of 36 molecules of ATP are created from 1 molecule of glucose.

based on the needs of the cell for ATP. If there is an increased need for ATP, more mitochondria are produced.

We call the process of adding an inorganic phosphate to a molecule phosphorylation. We call the use of O_2 in the phosphorylation process oxidative phosphorylation. While the overall chemical reaction in Eq. (8.85) looks simple enough, it consists of many enzyme catalyzed reactions.

A carrier-mediated transport process is the mechanism postulated to move glucose into the cell from the interstitial fluid. Ten enzyme catalyzed reactions take place in the cytosol during glycolysis,[3] where a glucose molecule is broken down into two pyruvate $(C_3H_4O_3)$ molecules in which energy is consumed and produced. The overall reaction in glycolysis is given by

$$\begin{aligned} C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P \longrightarrow \\ 2C_3H_4O_3 + 2(NADH + H^+) + 2ATP + 2H_2O \end{aligned} \tag{8.86}$$

where NAD^+ is nicotinamide adenine dinucleotide and when combined with two hydrogen, forms $NADH + H^+$. During the electron transport chain, each molecule of $NADH + H^+$ is used to create three molecules of ATP.

The next two steps occur in the mitochondria. The second step begins with the conversion of pyruvate, $C_3H_4O_3$, into acetyl coenzyme A, followed by eight more major enzyme catalyzed reactions called the Krebs cycle.[4] The overall result of the Krebs cycle is the

[3]Glycolysis is also known as the Embden-Meyerhoff pathway.

[4]The Krebs cycle is also known as the citric acid and tricarboxylic acid cycle.

conversion of pyruvate into CO_2 and $NADH + H^+$. The overall reaction for the Krebs cycle is given by

$$\begin{aligned} &Acetyl\text{-}CoA + 3NAD^+ + FAD + 2H_2O + ADP + P \longrightarrow \\ &\quad CoA - SH + 2CO_2 + FADH_2 + ATP + 3(NADH + H^+) \end{aligned} \tag{8.87}$$

where FAD is flavin adenine dinucleotide, and when combined with two hydrogen forms $FADH_2$. During the electron transport chain, each molecule of $FADH_2$ is used to create two molecules of ATP.

The last step in cellular respiration is the electron transport chain.[5] Each of the large number of enzyme catalyzed reactions leading up to the electron transport chain contributes only two molecules of ATP, four molecules of $FADH_2$, and eight molecules of $NADH + H^+$. During the electron transport chain, $NADH - H^+$ and $FADH_2$ are used to create ATP by the oxidation of hydrogen atoms. Almost all of the energy created in cellular respiration occurs in the electron transport chain in the metabolism of glucose (32 molecules of ATP from one molecule of glucose). The overall reaction for the electron transport chain is given by

$$(NADH + H^+) + \frac{1}{2}O_2 + 3ADP + 3P \longrightarrow NAD^+ + 4H_2O + 3ATP$$

and

$$FADH_2 + \frac{1}{2}O_2 + 2ADP + 2P \longrightarrow FAD + 3H_2O + 2ATP \tag{8.88}$$

The analysis in this section illustrates some of the major steps in converting glucose into ATP. To maximize the amount of energy created from glucose, many reactions occur, rather than a direct reaction of glucose into one ATP, water, and carbon dioxide. Using a series of reactions in the cytosol and the mitochondria, a total of 36 molecules of ATP are created from one molecule of glucose. We focus more detail on glycolysis and the Krebs cycle and a broader treatment of the electron transport chain. We assume that the process of transporting glucose into the cell occurs via a carrier-mediated transport and begins the glycolysis of glucose in the cytosol.

8.5.1 Glycolysis

Glycolysis involves 10 enzyme catalyzed reactions in the cytosol, which transform one glucose molecule into two molecules of pyruvic acid, as shown in Figure 8.24. The intermediate complex reactions of the substrates and enzymes are not shown in Figure 8.24 for simplicity. However, they are included in the differential equations using a reaction rate constant for the intermediate complex given by B_i that corresponds to the reaction rate K_i. As shown in Figure 8.23, a net gain of 2 molecules of ATP and $2(NADH + H+)$ for each glucose molecule is seen during glycolysis. The major output of glycolysis is the creation of 2 pyruvate molecules from one molecule of glucose.

[5]The electron transport chain is also known as the chemiosmotic mechanism.

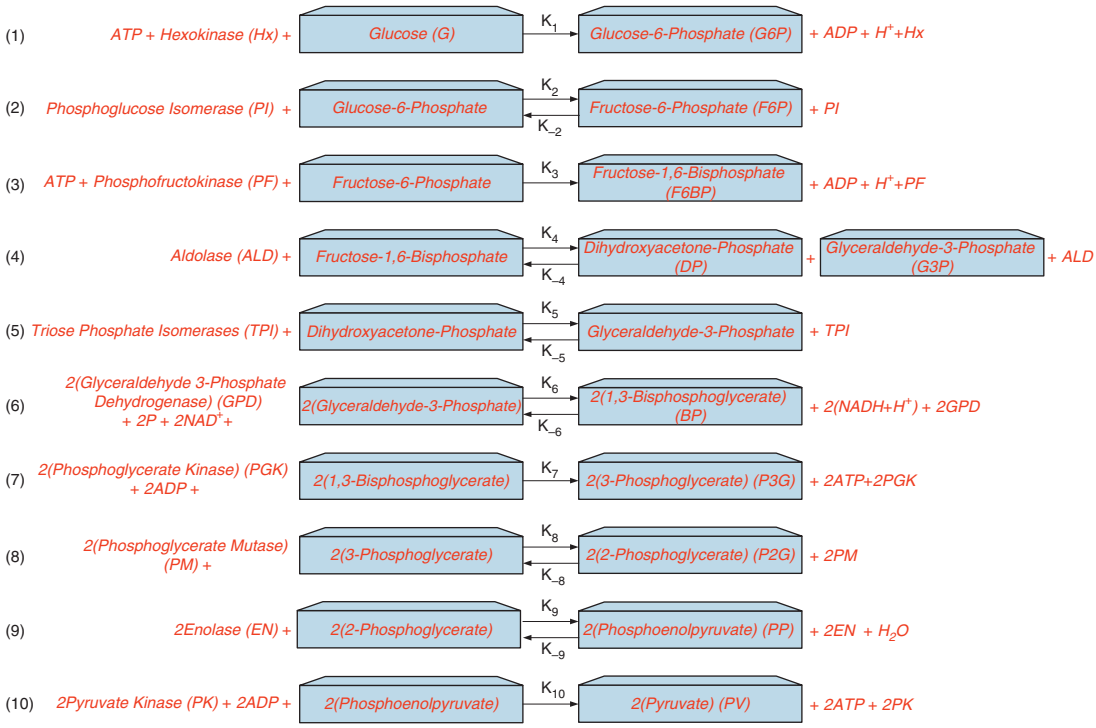

FIGURE 8.24 An overview of the 10 steps of glycolysis. Note that in steps 6–10, there are two molecules of everything, since 2 molecules of glyceraldehyde-3-phosphate are created for each glucose molecule. Also note that the intermediate complex reactions are not included in the illustration but are included in the differential equations describing glycolysis. Abreviations are listed next to each molecule.

Step 1 of glycolysis begins with the binding of glucose to a phosphate enzyme called hexokinase, resulting in glucose-6-phosphate. The immediate phosphorylation of glucose is required so glucose does not leave the cell. This reaction requires ATP and is given by

$$\begin{aligned} &G + Hx \xrightarrow{B_1} GHx \\ &GHx + ATP \xrightarrow{K_1} G6P + ADP + H^+ + Hx \\ &\dot{q}_G = -B_1 q_G q_{Hx} + J_G \\ &\dot{q}_{GHx} = B_1 q_G q_{Hx} - K_1 q_{ATP} q_{GHx} \end{aligned} \tag{8.89}$$

where J_G is the flow of glucose into the cell, as described in Section 8.4.3.

The second step involves the reaction of glucose-6-phosphate with the enzyme phosphoglucose isomerase enzyme to create fructose-6-phosphate. This reaction is given by

$$G6P + PI \underset{B_{-2}}{\overset{B_2}{\rightleftarrows}} G6PPI \underset{K_{-2}}{\overset{K_2}{\rightleftarrows}} FP6 + PI$$

$$\begin{aligned} \dot{q}_{GP6} &= K_1 q_{ATP} q_{GHx} + B_{-2} q_{G6P\,PI} - B_2 q_{GP6} q_{PI} \\ \dot{q}_{G6PPI} &= B_2 q_{GP6} q_{PI} + K_{-2} q_{FP6} q_{PI} - (B_{-2} + K_2) q_{G6PPI} \end{aligned} \tag{8.90}$$

The third step involves the reaction of fructose-6-phosphate and ATP with the enzyme phosphofructokinase to create fructose-1,6-bisphosphate and is given by

$$\begin{aligned} &F6P + PF \xrightarrow{B_3} F6PPF \\ &F6PPF + ATP \xrightarrow{K_3} F6BP + ADP + H^+ + PF \\ &\dot{q}_{F6P} = K_2 q_{G6PPI} - K_{-2} q_{F6P} q_{PI} - B_3 q_{F6P} q_{PF} \\ &\dot{q}_{F6PPF} = B_3 q_{F6P} q_{PF} - K_3 q_{F6PPF} q_{ATP} \end{aligned} \tag{8.91}$$

The fourth step involves the reaction of fructose-1,6-bisphosphate with the enzyme aldolase to create dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate and is given by

$$\begin{aligned} &F6BP + ALD \underset{B_{-4}}{\overset{B_4}{\rightleftharpoons}} F6BPALD \underset{K_{-4}}{\overset{K_4}{\rightleftharpoons}} DP + G3P + ALD \\ &\dot{q}_{F6BP} = K_3 q_{FP6\,PF} q_{ATP} + B_{-4} q_{F6BPALD} - B_4 q_{F6BP} q_{ALD} \\ &\dot{q}_{F6BPALD} = B_4 q_{F6BP} q_{ALD} + K_{-4} q_{DP} q_{G3P} q_{ALD} - (B_{-4} + K_4) q_{F6BPALD} \end{aligned} \tag{8.92}$$

The fifth step involves the reaction of dihydroxyacetone-phosphate with the enzyme triose phosphate isomerases to create glyceraldehyde-3-phosphate and is given by

$$\begin{aligned} &DP + TPI \underset{B_{-5}}{\overset{B_5}{\rightleftharpoons}} DPTPI \underset{K_{-5}}{\overset{K_5}{\rightleftharpoons}} G3P + TPI \\ &\dot{q}_{DP} = K_4 q_{F6BPALD} + B_{-5} q_{DPTPI} - K_{-4} q_{DP} q_{G3P} q_{ALD} - B_5 q_{DP} q_{TPI} \\ &\dot{q}_{DPTPI} = B_5 q_{DP} q_{TPI} + K_{-5} q_{G3P} q_{TPI} - (B_{-5} + K_5) q_{DPTPI} \end{aligned} \tag{8.93}$$

The sixth step involves the reaction of glyceraldehyde-3-phosphate with the enzyme glyceraldehyde-3-phosphate dehydrogenase, an inorganic phosphate, and NAD^+ to create 1,3-bisphosphoglycerate and is given by

$$\begin{aligned} &G3P + GPD \underset{B_{-6}}{\overset{B_6}{\rightleftharpoons}} G3PD \\ &G3PD + P + NAD^+ \underset{K_{-6}}{\overset{K_6}{\rightleftharpoons}} BP + (NADH + H^+) + GPD \\ &\dot{q}_{G3P} = K_4 q_{F6BPALD} + K_5 q_{DPTPI} + B_{-6} q_{G3PD} - K_{-4} q_{DP} q_{G3P} q_{ADL} - K_{-5} q_{G3P} q_{TPI} \\ &\qquad - B_6 q_{G3P} q_{GPD} \\ &\dot{q}_{G3PD} = B_6 q_{G3P} q_{GPD} + K_{-6} q_{BP} q_{NADH+H^+} q_{GPD} - B_{-6} q_{G3PD} - K_6 q_{G3PD} q_P q_{NAD^+} \end{aligned} \tag{8.94}$$

The seventh step involves the reaction of 1,3-bisphosphoglycerate with the enzyme phosphoglycerate kinase and ADP to create 3-phosphoglycerate and ATP and is given by

$$\begin{aligned} &BP + PGK \xrightarrow{B_7} BPPGK \\ &BPPGK + ADP \xrightarrow{K_7} P3G + PGK + ATP \\ &\dot{q}_{BP} = K_6 q_{G3PD} - K_{-6} q_{BP} q_{NADH-H^+} q_{GPD} - B_7 q_{BP} q_{PGK} \\ &\dot{q}_{BPPGK} = B_7 q_{BP} q_{PGK} - K_7 q_{BPPGK} q_{ADP} \end{aligned} \tag{8.95}$$

Note that we have assumed only a forward reaction in this step.

The eighth step involves the reaction of 3-phosphoglycerate with the enzyme phosphoglycerate mutase to create 2-phosphoglycerate and is given by

$$\begin{aligned} &P3G + PM \underset{B_{-8}}{\overset{B_8}{\rightleftarrows}} P3GPM \underset{K_{-8}}{\overset{K_8}{\rightleftarrows}} P2G + PM \\ &\dot{q}_{P3G} = K_7 q_{BPPGK} q_{ADP} + B_{-8} q_{P3GPM} - B_8 q_{P3G} q_{PM} \\ &\dot{q}_{P3GPM} = B_8 q_{P3G} q_{PM} + K_{-8} q_{PGM} q_{PM} - (B_{-8} + K_8) q_{P3GPM} \end{aligned} \tag{8.96}$$

The ninth step involves the reaction of 2-phosphoglycerate with the enzyme enolase to create phosphoenolpyruvate and water, and is given by

$$\begin{aligned} &P2G + EN \underset{B_{-9}}{\overset{B_9}{\rightleftarrows}} P2GEN \underset{K_{-9}}{\overset{K_9}{\rightleftarrows}} PP + EN + H_2O \\ &\dot{q}_{P2G} = K_8 q_{P3GPM} + B_{-9} q_{P2GEN} - K_{-8} q_{P2G} q_{PM} - B_9 q_{P2G} q_{EN} \\ &\dot{q}_{P2GEN} = B_9 q_{P2G} q_{EN} + K_{-9} q_{PP} q_{EN} q_{H_2O} - (B_{-9} + K_9) q_{P2GEN} \end{aligned} \tag{8.97}$$

The last step involves the reaction of phosphoenolpyruvate, ADP, and H^+ with the enzyme pyruvate kinase to create pyruvate and ATP and is given by

$$\begin{aligned} &PP + PK \xrightarrow{B_{10}} PPPK \\ &PPPK + ADP \xrightarrow{K_{10}} PV + ATP + PK \\ &\dot{q}_{PP} = K_9 q_{P2GEN} - K_{-9} q_{PP} q_{EN} q_{H_2O} - B_{10} q_{PP} q_{PK} \\ &\dot{q}_{PPPK} = B_{10} q_{PP} q_{PK} - K_{10} q_{PPPK} q_{ADP} \\ &\dot{q}_{PV=} K_{10} q_{PPPK} q_{ADP} \end{aligned} \tag{8.98}$$

The equations for ATP and ADP in the cytosol are

$$\begin{aligned} \dot{q}_{ATP} &= -K_1 q_{ATP} q_{GHx} - K_3 q_{F6PPF} q_{ATP} + K_7 q_{BPPGK} q_{ADP} + K_{10} q_{PPPK} q_{ADP} + J_{ATP} \\ \dot{q}_{ADP} &= K_1 q_{ATP} q_{GHx} + K_3 q_{F6PPF} q_{ATP} - K_7 q_{BPPGK} q_{ADP} - K_{10} q_{PPPK} q_{ADP} + J_{ADP} \end{aligned} \tag{8.99}$$

where J_{ATP} and J_{ADP} are the net usage of ATP and ADP from other processes, such as the reactions in Sections 8.5.2 and 8.5.3 in the mitochondria and the Na-K pump described in Section 8.4.4.

ADP and ATP Movement in/out of the Mitochondria

Let's assume that the movement of ADP and ATP through the mitochondria's membranes is by diffusion from the cytosol through the outer membrane and carrier-mediated diffusion through the inner membrane. Further, we assume that ADP only enters the mitochondria and that ATP only leaves the mitochondria for simplicity. The equations that describe this transport are given in Eqs. (8.100) and (8.101).

$$
\begin{aligned}
&ADP_c \xrightarrow{D_1} ADP_o \\
&ADP_o + C_o \xrightarrow{B_1} T_o \underset{B_2}{\overset{B_2}{\rightleftarrows}} T_i \xrightarrow{B_1} C_i + ADP_i \\
&C_o \underset{B_2}{\overset{B_2}{\rightleftarrows}} C_i \\
&ATP_i + E_i \xrightarrow{B_3} R_i \underset{B_4}{\overset{B_4}{\rightleftarrows}} R_o \xrightarrow{B_3} E_o + ATP_o \\
&ATP_o \xrightarrow{D_2} ATP_c \\
&E_o \underset{B_5}{\overset{B_5}{\rightleftarrows}} E_i
\end{aligned}
\tag{8.100}
$$

where the subscript c is for the cytosol, o is for the outer membrane, and i is for the matrix; Ds are the diffusivity constants and Bs are the reaction rates; Cs and Es are enzymes; and Rs and Ts are the intermediate complexes. The equations that describe this system are

$$
\begin{aligned}
\dot{q}_{ADP_c} &= -D_1 q_{ADP_c} + J_{ADP_c} \\
\dot{q}_{ADP_o} &= D_1 q_{ADP_c} - B_1 q_{ADP_o} q_{C_o} \\
\dot{q}_{ADP_i} &= B_1 T_i - J_{ADP_i} \\
\dot{q}_{T_o} &= B_1 q_{ADP_o} q_{C_o} - B_2 q_{T_o} + B_2 q_{T_i} \\
\dot{q}_{T_i} &= B_2 q_{T_o} - (B_2 + B_1) q_{T_i} \\
\dot{q}_{C_o} &= -B_1 q_{ADP} q_{C_o} + B_2 q_{C_i} - B_2 q_{C_o} \\
\dot{q}_{C_i} &= B_1 q_{T_i} + B_2 q_{C_o} - B_2 q_{C_i} \\
\dot{q}_{ATP_i} &= -B_3 q_{ATP_i} q_{E_i} + J_{ATP_i} \\
\dot{q}_{R_i} &= B_3 q_{ATP_i} q_{E_i} + B_4 R_o - B_4 R_i \\
\dot{q}_{R_o} &= B_4 q_{R_i} - (B_4 + B_3) q_{R_o} \\
\dot{q}_{ATP_o} &= B_3 q_{R_o} - D_2 q_{ATP_o} \\
\dot{q}_{ATP_c} &= D_2 q_{ATP_o} - J_{ATP_c} \\
\dot{q}_{E_i} &= -B_3 q_{ATP_i} q_{E_i} + B_5 q_{E_o} - B_5 q_{E_i} \\
\dot{q}_{E_o} &= B_3 q_{R_o} + B_5 q_{E_i} - B_5 q_{E_o}
\end{aligned}
\tag{8.101}
$$

where J_{ATP_c} and J_{ATP_i} are the consumption/production of ATP in the cytosol and matrix, and J_{ADP_c} and J_{ADP_i} are the consumption/production of ADP in the cytosol and matrix.

Conversion of Pyruvate to Acetyl CoA

Pyruvate moves into the mitochondria by diffusion. Once inside the mitochondria, an enzyme catalyzed reaction occurs that converts pyruvate (PV) into acetyl coenzyme A (AC) as follows:

$$\begin{aligned} &PV + CoA - SH \xrightarrow{B_0} PVCoA \\ &PVCoA + NAD^+ \xrightarrow{K_0} AC + CoA - SH + CO_2 + (NADH + H^+) \end{aligned} \tag{8.102}$$

$$\begin{aligned} \dot{q}_{PV} &= J_{PV} - B_0 q_{PV} q_{CoA} \\ \dot{q}_{CoA} &= -B_0 q_{PV} q_{CoA} + K_0 q_{PVCoA} q_{NAD^+} \\ \dot{q}_{PVCoA} &= B_0 q_{PV} q_{CoA} - K_0 q_{PVCoA} q_{NAD^+} \\ \dot{q}_{AC} &= K_0 q_{PVCoA} q_{NAD^+} \end{aligned}$$

where CoA-SH (CoA) is coenzyme A, $PVCoA$ is the complex pyruvate dehydrogenase, and J_{PV} is the production of pyruvate given by Eq. (8.98). Keep in mind that from one glucose molecule, two pyruvate molecules are created that pass into the mitochondria. Also note that $2(NADH + H^+)$ are converted into $2FADH_2$ to transfer acetyl coenzyme A across the mitochondrial membrane, thus costing 2 ATP.

8.5.2 Krebs Cycle

The Krebs cycle involves a series of enzyme catalyzed reactions that reduce the acetyl portion of acetyl coenzyme A in the mitochondrial matrix, as shown in Figure 8.25. The Krebs cycle continuously recycles, reusing the substrates and enzymes with an overall reaction given by

$$\begin{aligned} &Acetyl\text{-}CoA + 3NAD^+ + FAD + 2H_2O + ADP + P \longrightarrow \\ &\quad CoA - SH + 2CO_2 + FADH_2 + ATP + 3(NADH + H^+) \end{aligned} \tag{8.103}$$

The reaction begins with the joining of *acetyl-coenzyme A* with *oxaloacetate* and water to form citrate and is given by

$$\begin{aligned} &AC + H_2O + CS + O \xrightarrow{B_1} ACOCS \xrightarrow{K_1} CT + CoA - SH + CS \\ &\dot{q}_{AC} = -B_1 q_{AC} q_{H_2O} q_{CS} q_O + J_{AC} \\ &\dot{q}_{ACOCS} = B_1 q_{AC} q_{H_2O} q_{CS} q_O - K_1 q_{ACOCS} \end{aligned} \tag{8.104}$$

where J_{AC} is the flow *of* acetyl coenzyme A based on Eq. (8.102).

The next step involves the reaction of *citrate* with the enzyme *aconitase* to create *isocitrate*, which is given by

$$\begin{aligned} &CT + AT \underset{B_{-2}}{\overset{B_2}{\rightleftarrows}} CTAT \underset{K_{-2}}{\overset{K_2}{\rightleftarrows}} IT + AT \\ &\dot{q}_{CT} = K_1 q_{ACOCS} + B_{-2} q_{CTAT} - B_2 q_{CT} q_{AT} \\ &\dot{q}_{CTAT} = B_2 q_{CT} q_{AT} + K_{-2} q_{IT} q_{AT} - (B_{-2} + K_2) q_{CTAT} \end{aligned} \tag{8.105}$$

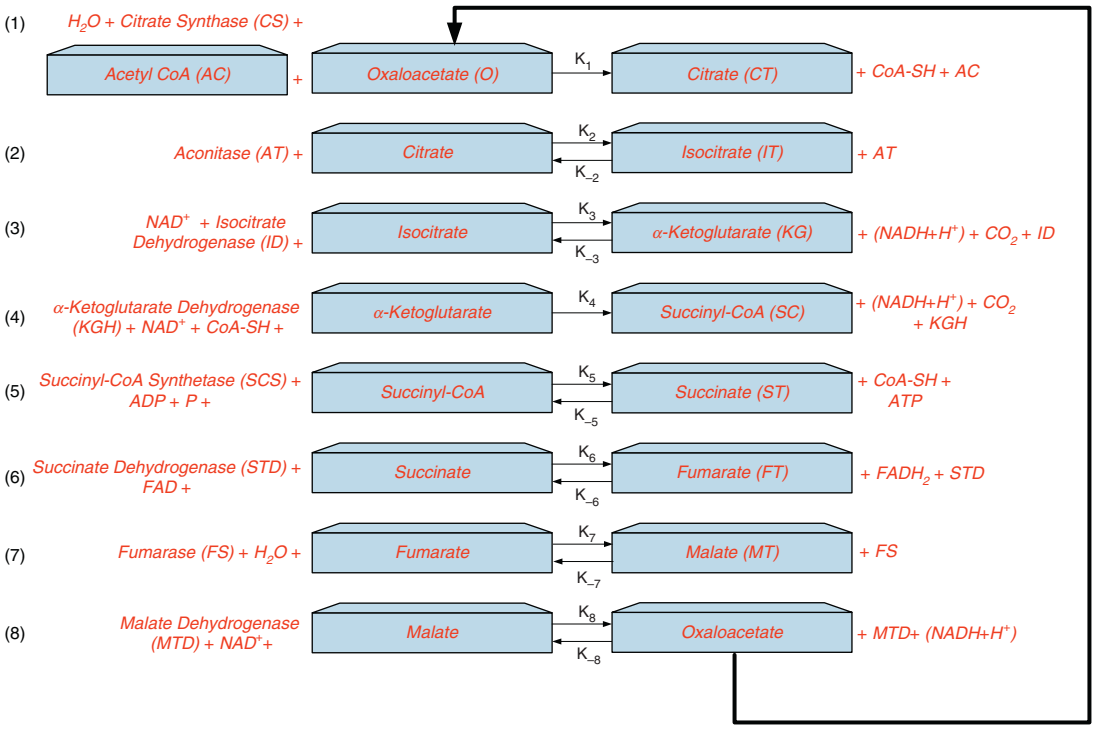

FIGURE 8.25 Overview of the Krebs cycle.

The third step involves the reaction of isocitrate and NAD^+ with the enzyme isocitrate dehydrogenase to create $\alpha-$ ketoglutarate, $(NADH+H^+)$ and CO_2, which is given by

$$\begin{aligned} &IT+ID \underset{B_{-3}}{\overset{B_3}{\rightleftharpoons}} ITID \\ &ITID+NAD^+ \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} KG+ID+(NADH+H^+)+CO_2 \end{aligned} \tag{8.106}$$

$$\begin{aligned} \dot{q}_{IT} &= K_2 q_{CTAT} + B_{-3} q_{ITID} - K_{-2} q_{IT} q_{AT} - B_3 q_{IT} q_{ID} \\ \dot{q}_{ITID} &= B_3 q_{IT} q_{ID} + K_{-3} q_{KG} q_{ID} q_{NADH+H^+} q_{CO_2} - B_{-3} q_{ITID} - K_3 q_{ITID} q_{NAD^+} \end{aligned}$$

The fourth step involves the reaction of $\alpha-$ *ketoglutarate*, NAD^+ and *CoA* with the enzyme $\alpha-$ ketoglutarate dehydrogenase to create succinyl $-$ CoA, $(NADH+H^+)$, and CO_2. This reaction is given by

$$\begin{aligned} &KG+KGH \xrightarrow{B_4} KGKGH \\ &KGKGH+NAD^+ + CoA-SH \xrightarrow{K_4} SC+KGH+(NADH+H^+)+CO_2 \end{aligned} \tag{8.107}$$

$$\begin{aligned} \dot{q}_{KG} &= K_3 q_{ITID} q_{NAD^+} - B_4 q_{KG} q_{KGH} - K_{-3} q_{KG} q_{ID} q_{NADH+H^+} q_{CO_2} \\ \dot{q}_{KGKGH} &= B_4 q_{KG} q_{KGH} - K_4 q_{KGKGH} q_{NAD^+} q_{CoA} \end{aligned}$$

The fifth step involves the reaction of succinyl − CoA, ADP, and P with the enzyme succinyl − CoA synthetase, to create succinate, CoA and ATP. This reaction is given by

$$\begin{aligned} &SC + SCS \underset{B_{-5}}{\overset{B_5}{\rightleftharpoons}} SCSCS \\ &SCSCS + ADP + P \underset{K_{-5}}{\overset{K_5}{\rightleftharpoons}} ST + SCS + CoA - SH + ATP \end{aligned} \tag{8.108}$$

$$\begin{aligned} \dot{q}_{SC} &= K_4 q_{KGKGH} q_{NAD^+} q_{CoA} + B_{-5} q_{SCSCS} - B_5 q_{SC} q_{SCS} \\ \dot{q}_{SCSCS} &= B_5 q_{SC} q_{SCS} + K_{-5} q_{ST} q_{SCS} q_{CoA} q_{ATP} - B_{-5} q_{SCSCS} - K_5 q_{SCSCS} q_{ADP} q_P \end{aligned}$$

The sixth step involves the reaction of succinate and FAD with the enzyme succinate dehydrogenase to create fumarate and $FADH_2$. This reaction is given by

$$\begin{aligned} &ST + STD \underset{B_{-6}}{\overset{B_6}{\rightleftharpoons}} STSTD \\ &STSTD + FAD \underset{K_{-6}}{\overset{K_6}{\rightleftharpoons}} FT + STD + FADH_2 \end{aligned} \tag{8.109}$$

$$\begin{aligned} \dot{q}_{ST} &= K_5 q_{SCSCS} q_{ADP} q_P + B_{-6} q_{STSTD} - K_{-5} q_{ST} q_{SCS} q_{CoA} q_{ATP} - B_6 q_{ST} q_{STD} \\ \dot{q}_{STSTD} &= B_6 q_{ST} q_{STD} + K_{-6} q_{FT} q_{STD} q_{FADH_2} - B_{-6} q_{STSTD} - K_6 q_{STSTD} q_{FAD} \end{aligned}$$

The seventh step involves the reaction of fumarate and H_2O with the enzyme fumarase to create malate. This reaction is given by

$$\begin{aligned} &FT + FS \underset{B_{-7}}{\overset{B_7}{\rightleftharpoons}} FTFS \\ &FTFS + H_2O \underset{K_{-7}}{\overset{K_7}{\rightleftharpoons}} MT + FS \end{aligned} \tag{8.110}$$

$$\begin{aligned} \dot{q}_{FT} &= K_6 q_{STSTD} q_{FAD} + B_{-7} q_{FTFS} - K_{-6} q_{FT} q_{STD} q_{FADH_2} - B_7 q_{FT} q_{FS} \\ \dot{q}_{FTFS} &= B_7 q_{FT} q_{FS} + K_{-7} q_{MT} q_{FS} - B_{-7} q_{STSTD} - K_7 q_{FTFS} q_{H_2O} \end{aligned}$$

The last step involves the reaction of malate and NAD^+ with the enzyme malate dehydrogenase to create oxaloacetate and $(NADH + H^+)$. This reaction is given by

$$\begin{aligned} &MT + MTD \underset{B_{-8}}{\overset{B_8}{\rightleftharpoons}} MTMTD \\ &MTMTD + NAD^+ \underset{K_{-8}}{\overset{K_8}{\rightleftharpoons}} O + MTD + (NADH + H^+). \end{aligned} \tag{8.111}$$

$$\begin{aligned} \dot{q}_{MT} &= K_7 q_{FTFS} q_{H_2O} + B_{-8} q_{FTFS} - K_{-7} q_{MT} q_{FS} - B_8 q_{MT} q_{MTD} \\ \dot{q}_{MTMTD} &= B_8 q_{MT} q_{MTD} + K_{-8} q_O q_{MTD} q_{NADH+H^+} - B_{-8} q_{MTMTD} - K_8 q_{MTMTD} q_{NAD^+} \end{aligned}$$

The final equation combines the oxaloacetate from the first reaction with that in the last reaction:

$$\dot{q}_O = K_8 q_{MTMTD} q_{NAD^+} - K_{-8} q_O q_{MTD} q_{NADH+H^+} - B_1 q_O \tag{8.112}$$

It should be clear that this reaction continually recycles and that it requires two cycles to process each glucose molecule.

8.5.3 Electron Transport Chain

The electron transport chain is the last step in the conversion of glucose into ATP, as illustrated in Figure 8.26. It involves a series of enzyme catalyzed chemical reactions that transfer electrons from $(NADH + H^+)$ and $FADH_2$ (donor molecules) to acceptor molecules. Ultimately the electron transport chain produces 32 molecules of ATP from one molecule of glucose through hydrogen oxidation, and also regenerates NAD and FAD for reuse in glycolysis. The overall reaction is given by

$$(NADH + H^+) + \frac{1}{2}O_2 + 3ADP + 3P \longrightarrow NAD^+ + 4H_2O + 3ATP$$

and

$$FADH_2 + \frac{1}{2}O_2 + 2ADP + 2P \longrightarrow FAD + 3H_2O + 2ATP \tag{8.113}$$

The electron transport chain activity takes place in the inner membrane and the space between the inner and outer membrane, called the intermembrane space. In addition to one molecule of ATP created during each Krebs cycle, three pairs of hydrogen are released and bound to $3NAD^+$ to create $3(NADH + H^+)$, and one pair of hydrogen is bound to FAD to form $FADH_2$ within the mitochondrial matrix. As described before, two cycles through the Krebs cycle are needed to fully oxidize one molecule of glucose, and thus $6(NADH + H^+)$ and $2FADH_2$ molecules are created.

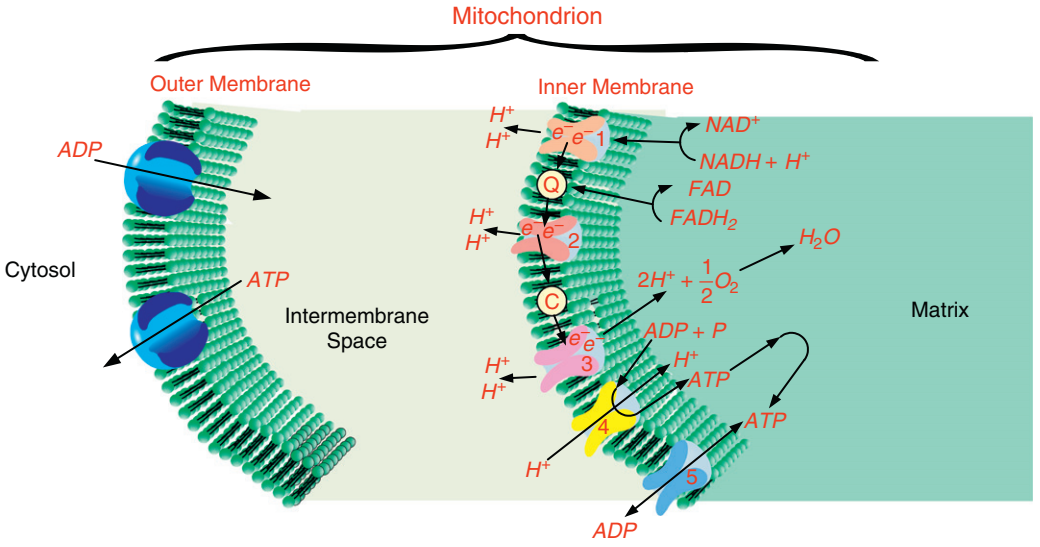

FIGURE 8.26 A simplified illustration of the mitochondrion electric transport chain. Hydrogen pumps are labeled 1 (NADH dehydrogenase), 2 (cytochrome bc_1 complex), and 3 (cytochrome c oxidase complex). Electron carriers are labeled Q (Coenzyme Q) and C (cytochrome c). The conversion of ADP+P to ATP is accomplished in the protein channel 4 (ATP synthetase), which also moves hydrogen ions back into the matrix, where they are used again in sites 1–3. Carrier mediated diffusion exchanges ATP and ADP between the matrix and the intermembrane space. Then ATP and ADP are exchanged between the intermembrane space and the cytosol by diffusion.

The energy stored in these molecules of $(NADH + H^+)$ and $FADH_2$ is used to create ATP by the release of hydrogen ions through the inner membrane and electrons within the inner membrane. The energy released by the transfer of each pair of electrons from $(NADH + H^+)$ and $FADH_2$ is used to pump a pair of hydrogen ions into the intermembrane space. The transfer of a pair of electrons is through a chain of acceptors from one to another, with each transfer providing the energy to move another pair of hydrogen ions through the membrane. At the end of the acceptor chain, the two electrons reduce an oxygen atom to form an oxygen ion, which is then combined with a pair of hydrogen ions to form H_2O. The movement of the hydrogen ions creates a large concentration of positively charged ions in the intermembrane space and a large concentration of negatively charged ions in the matrix, which sets up a large electrical potential. This potential is used by the enzyme ATP synthase to transfer hydrogen ions into the matrix and to create ATP. The ATP produced in this process is transported out of the mitochondrial matrix through the inner membrane using carrier facilitated diffusion and diffusion through the outer membrane. In the following description, we assume all of the hydrogen and electrons are available from these reactions. In reality, some are lost and not used to create ATP. Other descriptions of the electron transport chain have additional sites and are omitted here for simplicity.

We first consider the use of $(NADH + H^+)$ in the electron transport chain. During the first step, a pair of electrons from $NADH + H^+$ are transferred to the electron carrier coenzyme Q by NADH dehydrogenase (site 1 and Q in Figure 8.26), and using the energy released, a pair of hydrogen ions are pumped into the intermembrane space.

Next, the coenzyme Q carries the pair of electrons to the cytochrome bc_1 complex (site 2 in Figure 8.26). When the pair of electrons are transfered from the cytochrome bc_1 complex to cytochrome c (site C in Figure 8.26), the energy released is used to pump another pair of hydrogen ions into the intermembrane space through the cytochrome bc_1 complex.

In the third step, cytochrome c transfers electrons to the cytochrome c oxidase complex (site 3 in Figure 8.26), and another pair of hydrogen ions are pumped through the cytochrome c oxidase complex into the intermembrane space. A total of 6 hydrogen ions have now been pumped into the intermembrane space, which will allow the subseqent creation of 3 molecules of ATP.

Also occuring in this step, the cytochrome oxidase complex transfers the pair of electrons within the inner membrane from the cytochrome c to oxygen in the matrix. Oxygen then combines with a pair of hydrogen ions to form water.

As described previously, the transfer of hydrogen ions into the intermembrane space creates a large concentration of positive charges and a large concentration of negative charges in the matrix, creating a large electrical potential across the inner membrane. The energy from this potential is used in this step by the enzyme ATP synthase (site 4 in Figure 8.26) to move hydrogen ions in the intermembrane space into the matrix and to synthesize ATP from ADP and P.

The ATP in the matrix is then transported into the intermembrane space and ADP is transported into the matrix using a carrier-mediated transport process (site 5 in Figure 8.26). From the intermembrane space, ATP diffuses through the outer membrane into the cytosol, and ADP diffuses from the cytosol into the intermembrane space.

In parallel with $(NADH + H^+)$, $FADH_2$ goes through a similar process but starts at coenzyme Q, where it directly provides a pair of electrons. Thus, $FADH_2$ provides two fewer hydrogen ions than $(NADH + H^+)$.

The focus of this section has been the synthesis of ATP. Glycolysis and the Krebs cycle are also important in the synthesis of small molecules such as amino acids and nucleotides, and large molecules such as proteins, DNA, and RNA. There are other metabolic pathways to store and release energy that were not covered here. The interested reader can learn more about these pathways using the references at the end of this chapter and the website http://www.genome.jp.

8.6 ENZYME INHIBITION, ALLOSTERIC MODIFIERS, AND COOPERATIVE REACTIONS

Up to this point, we have considered the case of an enzyme binding with one substrate to make a product. Here, we examine the case in which the enzyme is free to bind with more than one molecule and form a product. As we will see, these reactions can regulate the amount of product synthesized and change the overall reaction rate in forming the product.

In this section, we begin with enzyme inhibitors that are either competitive or allosteric. A competitive enzyme inhibitor, referred to as the inhibitor, binds to the active site on the enzyme and prevents the substrate from binding with the enzyme. Thus, the inhibitor competes with the substrate to bind with the substrate and reduces the synthesis of the product and its overall reaction rate.

Some enzymes have more than one binding site and are called allosteric enzymes. The site that binds with the substrate is called the active site. The other site, called the allosteric or regulatory site, binds with another molecule called a modifier or effector. A modifier binds to the regulatory site on the enzyme and doesn't directly block the binding of the substrate with the enzyme at the active site. The effector role is to either increase (allosteric activator) or decrease (allosteric inhibitor) the activity of the enzyme. Some refer to allosteric inhibition as noncompetitive inhibition.

Finally, we examine reactions that are cooperative. These reactions are sequential and have a sigmoidal reaction velocity.

8.6.1 Competitive Enzyme Inhibitors

Consider an enzyme catalyzed reaction between a substrate, S, and enzyme, E, synthesizing product, P_1, as

$$S + E \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} C_1 \xrightarrow{K_2} P_1 + E \tag{8.114}$$

and another enzyme reaction, where enzyme inhibitor I reacts with E to form product P_2 as

$$I + E \underset{K_{-3}}{\overset{K_3}{\rightleftarrows}} C_2 \xrightarrow{K_4} P_2 + E \tag{8.115}$$

where C_1 and C_2 are the intermediate complexes. Note that we have eliminated the reverse reaction from the product to the intermediate complex. Another form of a competitive enzyme inhibitor eliminates the formation of product P_2 (i.e., $K_4 = 0$).

The equations that describe this system in Eqs. (8.114) and (8.115) are

$$\begin{aligned}\dot{q}_S &= K_{-1}q_{C_1} - K_1 q_S q_E \\ \dot{q}_{C_1} &= K_1 q_S q_E - (K_{-1} + K_2)q_{C_1} \\ \dot{q}_I &= K_{-3}q_{C_2} - K_2 q_I q_E \\ \dot{q}_{C_2} &= K_3 q_I q_E - (K_{-3} + K_4)q_{C_2}\end{aligned} \tag{8.116}$$

We eliminate q_E from Eq. (8.116) by using $q_E = E_0 - q_{C_1} - q_{C_2}$, giving

$$\begin{aligned}\dot{q}_S &= (K_1 q_S + K_{-1})q_{C_1} + K_1 q_S q_{C_2} - K_1 E_0 q_S \\ \dot{q}_{C_1} &= K_1 E_0 q_S - (K_1 q_S + K_{-1} + K_2)q_{C_1} - K_1 q_S q_{C_2} \\ \dot{q}_I &= (K_3 q_I + K_{-3})q_{C_2} + K_3 q_I q_{C_1} - K_3 E_0 q_I \\ \dot{q}_{C_2} &= K_3 E_0 q_I - (K_3 q_I + K_{-3} + K_4)q_{C_2} - K_3 q_I q_{C_1}\end{aligned} \tag{8.117}$$

with nonzero initial conditions of $q_S(0) = S_0, q_I(0) = I_0$ and $q_E(0) = E_0$.

The quasi-steady-state approximation is found from Eq. (8.117) with $\dot{q}_{C_1} = \dot{q}_{C_2} = 0$, yielding

$$\begin{aligned}0 &= K_1 E_0 q_S - (K_1 q_S + K_{-1} + K_2)q_{C_1} - K_1 q_S q_{C_2} \\ 0 &= K_3 E_0 q_I - (K_3 q_I + K_{-3} + K_4)q_{C_2} - K_3 q_I q_{C_1}\end{aligned} \tag{8.118}$$

Next, we solve for q_{C_2}, which gives

$$q_{C_2} = \frac{K_3 q_I (E_0 - q_{C_1})}{K_3 q_I + K_{-3} + K_4} \tag{8.119}$$

and then we solve for q_{C_1}, giving

$$q_{C_1} = \frac{E_0 q_S}{q_S + K_M^s\left(1 + \frac{q_I}{K_M^i}\right)} \tag{8.120}$$

where $K_M^s = \frac{K_{-1}+K_2}{K_1}$ and $K_M^i = \frac{K_{-3}+K_4}{K_3}$. The velocity of the reaction is given by

$$V = K_2 q_{C_1} = \frac{K_2 E_0 q_S}{q_S + K_M^s\left(1 + \frac{q_I}{K_M^i}\right)} = \frac{V_{\max}}{1 + \frac{K_M^s}{q_S}\left(1 + \frac{q_I}{K_M^i}\right)} \tag{8.121}$$

where $V_{\max} = K_2 E_0$.

Comparing Eq. (8.121) with Eq. (8.47), we see that $V_{\max}$ does not change with the inclusion of an enzyme inhibitor. In this case the term K_M in Eq. (8.47) has been replaced by the term $K_M^s\left(1 + \frac{q_I}{K_M^i}\right)$ in Eq. (8.121), which reduces the reaction rate. The left side of Figure 8.27 shows a plot of the reaction rate versus the substrate with increasing quantities of the

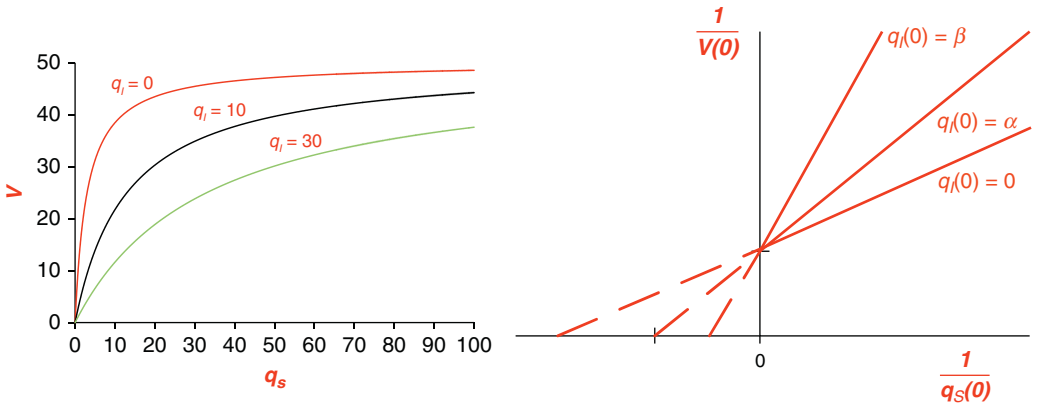

FIGURE 8.27 (Left) Velocity of product appearance for a substrate, enzyme, and enzyme inhibitor using Eq. (8.121). $V_{\max} = 50, K_M^s = 3$, and $K_M^i = 3$. (Right) Lineweaver-Burk plot, where $\beta > \alpha$.

inhibitor enzyme. As shown, increasing the quantity of the enzyme inhibitor shows a slower synthesis of the product, P_1. Keep in mind that all of the substrate will eventually be synthesized into the P_1 but it does so more slowly.

To obtain the Lineweaver-Burk equation for this system, we take the reciprocal of Eq. (8.121), giving

$$\frac{1}{V} = \frac{1}{V_{\max}}\left(1 + \left(\frac{1}{q_S}\right)K_M^s\left(1 + \frac{q_I}{K_M^i}\right)\right) \tag{8.122}$$

and at $t = 0$, we have

$$\frac{1}{V(0)} = \frac{1}{V_{\max}}\left(1 + \left(\frac{1}{q_S(0)}\right)K_M^s\left(1 + \frac{q_I(0)}{K_M^i}\right)\right) \tag{8.123}$$

The plot of the Lineweaver-Burk equation is shown on the right side of Figure 8.27 for three values of the quantity of the enzyme inhibitor. One again, the relationship between $\frac{1}{V(0)}$ vs. $\frac{1}{q_s(0)}$ is a straight line. Further, we note that the intercept of the $\frac{1}{V(0)}$ axis is a constant as the quantity of the enzyme inhibitor is increased. Additionally, the slope of the line increases as the quantity of the enzyme inhibitor is increased, which is indicative of a slowing reaction rate.

8.6.2 Allosteric Activators and Inhibitors

Next, consider the noncompetitive allosteric modifier that binds with an enzyme on a regulatory site and a substrate that binds with the enzyme on the active site. The effect of a modifier on the reaction is to either increase or decrease the activity of the enzyme.

Consider an enzyme catalyzed reaction between a substrate, S, modifier, M, and enzyme, E, synthesizing product, P, as

$$\begin{aligned} S + E &\underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} C_1 \xrightarrow{K_2} P + E \\ M + E &\underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} C_2 \\ S + C_2 &\underset{K_{-4}}{\overset{K_4}{\rightleftharpoons}} C_3 \xrightarrow{K_5} P + C_2 \\ M + C_1 &\underset{K_{-6}}{\overset{K_6}{\rightleftharpoons}} C_3 \xrightarrow{K_5} P + C_2 \end{aligned} \tag{8.124}$$

where $C_1 = SE, C_2 = ME$, and $C_3 = SME$ are the intermediate complexes.[6] Note that we have eliminated the reverse reaction from the product to the intermediate complex. Another variation of allosteric reaction eliminates the product from forming through C_3.

The equations that describe the system in Eq. (8.124) are

$$\begin{aligned} \dot{q}_S &= K_{-1}q_{C_1} + K_{-4}q_{C_3} - K_1 q_S q_E - K_4 q_S q_{C_2} \\ \dot{q}_M &= K_{-3}q_{C_2} + K_{-6}q_{C_3} - K_3 q_M q_E - K_6 q_M q_{C_1} \\ \dot{q}_{C_1} &= K_1 q_S q_E + K_{-6}q_{C_3} - (K_{-1} + K_2)q_{C_1} - K_6 q_M q_{C_1} \\ \dot{q}_{C_2} &= K_3 q_M q_E + (K_{-4} + K_5)q_{C_3} - K_{-3}q_{C_2} - K_4 q_S q_{C_2} \\ \dot{q}_{C_3} &= K_4 q_S q_{C_2} + K_6 q_M q_{C_1} - (K_{-4} + K_5 + K_{-6})q_{C_3} \\ \dot{q}_P &= K_2 q_{C_1} + K_5 q_{C_3} \end{aligned} \tag{8.125}$$

We eliminate q_E from Eq. (8.125) by using $q_E = E_0 - q_{C_1} - q_{C_2} - q_{C_3}$, giving

$$\begin{aligned} \dot{q}_S &= (K_{-1} + K_1 q_S)q_{C_1} + (K_{-4} + K_1 q_S)q_{C_3} - K_1 q_S E_0 - (K_4 - K_1)q_S q_{C_2} \\ \dot{q}_M &= (K_{-3} + K_3 q_M)q_{C_2} + (K_{-6} + K_3 q_M)q_{C_3} - K_3 q_M E_0 - (K_6 - K_3)q_M q_{C_1} \\ \dot{q}_{C_1} &= K_1 q_S E_0 - K_1 q_S q_{C_2} + (K_{-6} - K_1 q_S)q_{C_3} - (K_{-1} + K_2 + K_6 q_M + K_1 q_S)q_{C_1} \\ \dot{q}_{C_2} &= K_3 q_M E_0 - K_3 q_M q_{C_1} + (K_{-4} + K_5 - K_3 q_M)q_{C_3} - (K_3 q_M + K_{-3} + K_4 q_S)q_{C_2} \\ \dot{q}_{C_3} &= K_4 q_S q_{C_2} + K_6 q_M q_{C_1} - (K_{-4} + K_5 + K_{-6})q_{C_3} \\ \dot{q}_P &= K_2 q_{C_1} + K_5 q_{C_3} \end{aligned} \tag{8.126}$$

with nonzero initial conditions of $q_S(0) = S_0, q_M(0) = M_0$, and $q_E(0) = E_0$.

[6] This model is adapted from Rubinow, page 89.

Quasi-Steady-State Approximation to the Reaction Rate

The quasi-steady-state approximation is found from Eq. (8.126) with $\dot{q}_{C_1} = \dot{q}_{C_2} = \dot{q}_{C_3} = 0$, yielding

$$\begin{aligned} 0 &= K_1 q_S E_0 - K_1 q_S q_{C_2} + (K_{-6} - K_1 q_S) q_{C_3} - (K_{-1} + K_2 + K_6 q_M + K_1 q_S) q_{C_1} \\ 0 &= K_3 q_M E_0 - K_3 q_M q_{C_1} + (K_{-4} + K_5 - K_3 q_M) q_{C_3} - (K_3 q_M + K_{-3} + K_4 q_S) q_{C_2} \\ 0 &= K_4 q_S q_{C_2} + K_6 q_M q_{C_1} - (K_{-4} + K_5 + K_{-6}) q_{C_3} \end{aligned} \tag{8.127}$$

Equation (8.127) can be solved for q_{C_1} and q_{C_3} using the D-Operator method or the graph theory method from Rubinow, giving

$$\begin{aligned} q_{C_1} &= \frac{q_S E_0 \left\{ \frac{K_1}{K_3} K_6^i q_S + K_3^i \left(\frac{K_1}{K_4} K_6^i + \frac{K_1}{K_6} K_M^i \right) + K_6^i q_M \right\}}{D} \\ q_{C_3} &= \frac{E_0 q_M q_S \left\{ \frac{K_1}{K_3} q_S + \frac{K_1}{K_4} K_3^i + \frac{K_1}{K_6} K_M^s + q_M \right\}}{D} \end{aligned} \tag{8.128}$$

where

$$\begin{aligned} D = {} & q_S^2 \frac{K_1}{K_3} (K_6^i + q_M) \\ & + q_S \left\{ \begin{array}{l} \frac{K_1}{K_3} K_6^i K_M^s + K_3^i \left(\frac{K_1}{K_4} K_6^i + \frac{K_1}{K_6} K_M^i \right) \\ + q_M \left(K_6^i + \frac{K_1}{K_3} K_M^i + \frac{K_1}{K_4} K_3^i + \frac{K_1}{K_6} K_M^s \right) + q_M^2 \end{array} \right\} \\ & + K_M^s K_3^i \left(\frac{K_1}{K_4} K_6^i + \frac{K_1}{K_6} K_M^i \right) \\ & + q_M \left\{ K_M^s \left(\frac{K_1}{K_4} K_6^i + \frac{K_1}{K_6} K_3^i \right) + K_3^i K_M^i \right\} \\ & + K_M^i q_M^2 \end{aligned}$$

$$K_M^s = \frac{K_{-1} + K_2}{K_1}$$

$$K_M^i = \frac{K_{-4} + K_5}{K_4}$$

$$K_3^i = \frac{K_{-3}}{K_3}$$

$$K_6^i = \frac{K_{-6}}{K_6}$$

The reaction rate is

$$\begin{aligned} V = \dot{q}_P &= K_2 q_{C_1} + K_5 q_{C_3} \\ &= \frac{q_S \left[\begin{array}{l} K_2 E_0 \left\{ \frac{K_1}{K_3} K_6^i q_S + K_3^i \left(\frac{K_1}{K_4} K_6^i + \frac{K_1}{K_6} K_M^i \right) + K_6^i q_M \right\} \\ + K_5 E_0 q_M \left\{ \frac{K_1}{K_3} q_S + \frac{K_1}{K_4} K_3^i + \frac{K_1}{K_6} K_M^s + q_M \right\} \end{array} \right]}{D} \end{aligned} \tag{8.129}$$

This reaction rate is quite complex compared with the others derived previously. The reaction velocity from Eq. (8.129) is plotted in Figure 8.28. As observed, as the quantity of the allosteric modifier increases, it reduces $V_{\max}$ and the reaction rate.

Reaction Rate from the True Steady-State

We will consider a simpler allosteric modifier model[7] to further investigate the velocity of the reaction using the true steady-state rather than the quasi-steady-state approximation in the analysis. Here, the product is synthesized only from the intermediate complex C_1, and a single reaction rate is used for S and M as follows:

$$\begin{aligned} &S + E \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} C_1 \xrightarrow{K_2} P + E \\ &M + E \underset{K_{-3}}{\overset{K_3}{\rightleftarrows}} C_2 \\ &S + C_2 \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} C_3 \\ &M + C_1 \underset{K_{-3}}{\overset{K_3}{\rightleftarrows}} C_3 \end{aligned} \tag{8.130}$$

[7]This model is based on Keener and Sneyd, page 11.

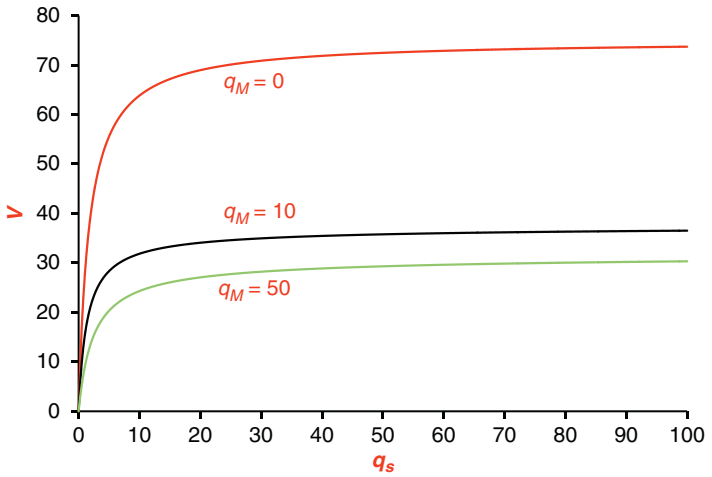

FIGURE 8.28 Velocity of product appearance for a substrate, enzyme, and allosteric modifier using Eq. (8.129). The parameters used are: $K_1 = 4, K_{-1} = 2, K_2 = 5, K_3 = 1, K_{-3} = 10, K_4 = 0.3,\ K_{-4} = 0.03, K_5 = 2, K_6 = 2, K_{-6} = 4$, and $E_0 = 15$.

The equations describing the system are given as

$$\begin{aligned}
\dot{q}_S &= K_{-1}(q_{C_1} + q_{C_3}) - K_1 q_S (q_E + q_{C_2}) \\
\dot{q}_M &= K_{-3}(q_{C_2} + q_{C_3}) - K_3 q_M (q_E + q_{C_1}) \\
\dot{q}_{C_1} &= K_{-3} q_{C_3} + K_1 q_S q_E - (K_{-1} + K_2 + K_3 q_M) q_{C_1} \\
\dot{q}_{C_2} &= K_3 q_M q_E + K_{-1} q_{C_3} - (K_{-3} + K_1 q_S) q_{C_2} \\
\dot{q}_{C_3} &= K_1 q_S q_{C_2} + K_3 q_M q_{C_1} - (K_{-1} + K_{-3}) q_{C_3} \\
\dot{q}_P &= K_2 q_{C_1}
\end{aligned} \tag{8.131}$$

where the variables and initial conditions are given as before. As before, q_E is eliminated from Eq. (8.131) by using $q_E = E_0 - q_{C_1} - q_{C_2} - q_{C_3}$, and a steady-state analysis is used to determine the reaction rate—that is, we let $\dot{q}_S = \dot{q}_M = \dot{q}_{C_1} = \dot{q}_{C_2} = \dot{q}_{C_3} = 0$. Keener and Sneyd give the reaction rate as

$$V = \frac{V_{\max}}{\left(1 + \dfrac{K_3 q_M}{K_{-3}}\right)\left(1 + \dfrac{K_{-1}}{K_1 q_S}\right)} \tag{8.132}$$

8.6.3 Cooperative Reactions

Our last topic in this chapter deals with cooperative reactions involving a sequence of biochemical reactions. The enzyme is able to bind with more than one substrate, and with each successive binding, the reaction of the next reaction is impacted. These reactions can have a sigmoidal reaction rate, allowing for a more precise control of the reaction, either

by enhancing or reducing the overall reaction. Hemoglobin is a prime example of a cooperative reaction involving the storing of oxygen in red blood cells.

A simple cooperative reaction is given by the following model:

$$\begin{gathered} S+E \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} C_1 \xrightarrow{K_2} E+P \\ S+C_1 \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} C_2 \xrightarrow{K_4} C_1+P \end{gathered} \tag{8.133}$$

using the variables previously defined. The equations that describe this system are given by

$$\begin{aligned} \dot{q}_S &= K_{-1}q_{C_1} + K_{-3}q_{C_2} - K_1 q_S q_E - K_3 q_S q_{C_1} \\ \dot{q}_{C_1} &= K_1 q_S q_E + K_{-3}q_{C_2} + K_4 q_{C_2} - (K_{-1}+K_2)q_{c_1} - K_3 q_S q_{C_1} \\ \dot{q}_{C_2} &= K_3 q_S q_{C_1} - K_{-3}q_{C_2} \\ \dot{q}_P &= K_2 q_{C_1} + K_4 q_{C_2} \end{aligned} \tag{8.134}$$

using the quantities previously defined. The quantity q_E is removed using $q_E = E_0 - q_{C_1} - q_{C_2}$, giving

$$\begin{aligned} \dot{q}_S &= (K_{-1}+K_1 q_S)q_{C_1} + (K_{-3}+K_1 q_S)q_{C_2} - K_1 E_0 q_S - K_3 q_S q_{C_1} \\ \dot{q}_{C_1} &= K_1 E_0 q_S + K_{-3}q_{C_2} + (K_4 - K_1 q_S)q_{C_2} - (K_{-1}+K_2 - K_1 q_S)q_{c_1} - K_3 q_S q_{C_1} \\ \dot{q}_{C_2} &= K_3 q_S q_{C_1} - K_{-3}q_{C_2} \\ \dot{q}_P &= K_2 q_{C_1} + K_4 q_{C_2} \end{aligned} \tag{8.135}$$

with nonzero initial conditions of $q_S(0) = S_0$, and $q_E(0) = E_0$. The quasi-steady-state approximation is found from Eq. (8.135) with $\dot{q}_{C_1} = \dot{q}_{C_2} = 0$, yielding

$$\begin{aligned} 0 &= K_1 E_0 q_S + K_{-3}q_{C_2} + (K_4 - K_1 q_S)q_{C_2} - (K_{-1}+K_2 - K_1 q_S)q_{c_1} - K_3 q_S q_{C_1} \\ 0 &= K_3 q_S q_{C_1} - K_{-3}q_{C_2} \end{aligned} \tag{8.136}$$

Equation (8.136) is solved for q_{C_1} and q_{C_2}, giving

$$\begin{aligned} q_{C_1} &= \frac{K_M^i E_0 q_S}{K_M^i K_M^s + K_M^i q_S + q_S^2} \\ q_{C2} &= \frac{E_0 q_S^2}{K_M^i K_M^s + K_M^i q_S + q_S^2} \end{aligned} \tag{8.137}$$

where $K_M^s = \frac{K_{-1}+K_2}{K_1}$ and $K_M^i = \frac{K_{-3}+K_4}{K_3}$. The velocity of the reaction is given by

$$\begin{aligned} V = K_2 q_{C_1} + K_4 q_{C_2} &= \frac{K_2 K_M^i E_0 q_S}{K_M^i K_M^s + K_M^i q_S + q_S^2} + \frac{K_4 E_0 q_S^2}{K_M^i K_M^s + K_M^i q_S + q_S^2} \\ &= \frac{E_0 q_S \left(K_2 K_M^i + K_4 q_S\right)}{K_M^i K_M^s + K_M^i q_S + q_S^2} \end{aligned} \tag{8.138}$$

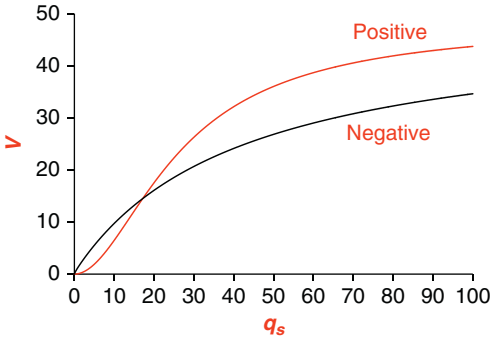

FIGURE 8.29 Velocity of product appearance for cooperative reactions using Eq. (8.138). Positive: The parameters used for the positive cooperativity are $K_1 = 0.001$, $K_{-1} = 0.00001$, $K_2 = 0.1$, $K_3 = 0.05$, $K_{-3} = 0.00002$, $K_4 = 0.3$, and $E_0 = 7$. The parameters used for the negative cooperativity are $K_1 = 1$, $K_{-1} = 0.2$, $K_2 = 0.1$, $K_3 = 0.01$, $K_{-3} = 0.4$, $K_4 = 0.02$, and $E_0 = 7$.

The overall reaction can exhibit positive or negative cooperativity depending on the parameter values. For positive cooperativity, one case is the slow binding of $S + E$ to form C_1 (K_1 is small), and the binding of $S + C_1$ to form C_2 is fast (K_3 is large), which increases the overall reaction. For negative cooperativity, one case is when the binding of $S + E$ to form C_1 (K_1 is large) slows the binding of $S + C_1$ to form C_2 (K_3 is small), which slows the overall reaction. Figure 8.29 illustrates positive and negative cooperativity. Notice the sigmoidal shape for the reaction rate for a positive cooperativity in Figure 8.29, where the negative cooperativity has a hyperbolic shape like the other reaction rates illustrated before.

8.7 EXERCISES

1. For the reaction given in Eq. (8.1) and with $q_A(0) = 15, q_B(0) = 8, q_P(0) = 0,$ and $K = 3,$ solve and simulate the solution for q_P.
2. For the reaction given in Eq. (8.1) and with $q_A(0) = 10, q_B(0) = 20, q_P(0) = 0,$ and $K = 5,$ solve and simulate the solution for q_A.
3. For the reaction given in Eq. (8.13) and with $q_A(0) = 10, q_B(0) = 20, q_P(0) = 0, K_1 = 7,$ and $K_{-1} = 5,$ solve and simulate the solution for q_A.
4. For the reaction given in Eq. (8.13) and with $q_A(0) = 18, q_B(0) = 5, q_P(0) = 0, K_1 = 10,$ and $K_{-1} = 4,$ solve and simulate the solution for q_P.
5. For the reaction given in Eq. (8.13) and with $q_A(0) = 30, q_B(0) = 10, q_P(0) = 0, K_1 = 1,$ and $K_{-1} = 0.1,$ solve and simulate the solution for q_B.
6. Show that Eq. (8.24) follows from Eq. (8.23).
7. For the reaction given in Eq. (8.25) and with $q_A(0) = 10, q_B(0) = 20, q_P(0) = 0, K_1 = 4$ $K_{-1} = 2, \alpha = 2,$ and $\beta = 1,$ simulate the solution for q_A.
8. For the reaction given in Eq. (8.25) and with $q_A(0) = 30, q_B(0) = 10, q_P(0) = 0, K_1 = 8$ $K_{-1} = 3, \alpha = 1,$ and $\beta = 3,$ simulate the solution for q_P.

Continued

9. For the reaction given in Eq. (8.25) and with $q_A(0) = 10, q_B(0) = 10, q_P(0) = 0,\ K_1 = 0.5, K_{-1} = 0.3, \alpha = 3,$ and $\beta = 2,$ simulate the solution for q_P.
10. For the reaction given in Eq. (8.27) and with $q_A(0) = 15, q_B(0) = 0, q_P(0) = 0, K_1 = 8,$ and $K_2 = 3,$ solve and simulate the solution for q_A, q_B and q_P. Compare these results with the quasi-steady-state solutions.
11. For the reaction given in Eq. (8.27) and with $q_A(0) = 25, q_B(0) = 0, q_P(0) = 0, K_1 = 2,$ and $K_2 = 10,$ solve and simulate the solution for q_A, q_B and q_P. Compare these results with the quasi-steady-state solutions.
12. For the reaction given in Eq. (8.27) and with $q_A(0) = 10, q_B(0) = 0, q_P(0) = 0, K_1 = 5,$ and $K_2 = 20,$ solve and simulate the solution for q_A, q_B and q_P. Compare these results with the quasi-steady-state solutions.
13. Generate the solutions for Figures 8.3 and 8.4.
14. Simulate the reaction given in Eq. (8.33) and compare with the quasi-steady-state approximation for q_S, q_E, q_{ES} and q_P. Assume that $K_1 = 5, K_{-1} = 0.3, K_2 = 1,\ q_S(0) = 9, q_E(0) = 0.01, q_{ES}(0) = 0,$ and $q_P(0) = 0$.
15. Simulate the reaction given in Eq. (8.33) and compare with the quasi-steady-state approximation for q_S, q_E, q_{ES} and q_P. Assume that $K_1 = 1, K_{-1} = 0.1, K_2 = 5,$ $q_S(0) = 20, q_E(0) = 0.008, q_{ES}(0) = 0,$ and $q_P(0) = 0$.
16. Simulate the reaction given in Eq. (8.33) and compare with the quasi-steady-state approximation for q_S, q_E, q_{ES} and q_P. Assume that $K_1 = 10, K_{-1} = 1, K_2 = 3,\ q_S(0) = 30, q_E(0) = 1, q_{ES}(0) = 0,$ and $q_P(0) = 0$.
17. Given the model in Eq. (8.51) and with $q_S(0) = 10, V_{\max} = 25, K_M = 5,$ and $f(t) = 5\delta(t-1),$ simulate the solution for q_S.
18. Given the model in Eq. (8.51) and with $q_S(0) = 50, V_{\max} = 5, K_M = 1,$ and $f(t) = u(t) - u(t-1),$ simulate the solution for q_S.
19. Given the model in Eq. (8.51) and with $q_S(0) = 25, V_{\max} = 10, K_M = 3,$ and $f(t) = 5e^{-t},$ simulate the solution for q_S.
20. Given the model in Eq. (8.51) and with $q_S(0) = 10, V_{\max} = 25, K_M = 5,$ and $f(t) = 5\delta(t-1),$ simulate the solution for q_S.
21. Simulate the model in Eq. (8.66) given that $K_{12} = 3, K_{21} = 1, V_{\max} = 10, K_M = 1,\ K_{10} = 0,$ and $K_{20} = 0.02$. The inputs are $f_1(t) = 4\delta(t)$ and $f_2(t) = 0$. The initial conditions are zero.
22. Simulate the model in Eq. (8.66) given that $K_{12} = 3, K_{21} = 2, V_{\max} = 20, K_M = 2,\ K_{10} = 0,$ and $K_{20}(0) = 0$. The inputs are $f_1(t) = 4(u(t) - u(t-10))$ and $f_2(t) = 3(u(t-1) - u(t-6))$. The initial conditions are zero.
23. Simulate the model in Eq. (8.67) given that $K_{12} = 0.25, K_{21} = 3, V_{\max} = 30, K_M = 5,\ K_{10} = 0,$ and $K_{20}(0) = 0.2$. The inputs are $f_1(t) = 2u(t)$ and $f_2(t) = 3u(t)$. The initial conditions are zero.
24. Simulate the model in Eq. (8.67) given that $K_{12} = 2, K_{21} = 3, V_{\max} = 40, K_M = 8,\ K_{10} = 0,$ and $K_{20}(0) = 0.2$. The inputs are $f_1(t) = 5u(t)$ and $f_2(t) = 3e^{-.03t}u(t)$. The initial conditions are zero.
25. Simulate the model in Eq. (8.67) given that $K_{12} = 3, K_{21} = 2, V_{\max} = 10, K_M = 0.8,\ K_{10} = 0.1,$ and $K_{20}(0) = 0.3$. The inputs are $f_1(t) = 4u(t)$ and $f_2(t) = 0$. The initial conditions are zero.

26. Given the model in Figure 8.15 with $q_{A_i}(0) = 30, q_{B_i}(0) = 15, q_{A_o}(0) = 20,\ C_{oi} = 3, C_{io} = 0.2,$ $D_{io} = 2, D_{oi} = 0.1, K_1 = 2,$ and $K_{-1} = 0.5,$ assume that there is flow of q_A into the exterior of the cell equal to $3u(t),$ and a production of q_{B_i} inside the cell equal to $u(t).$ All other initial conditions are zero. Simulate the solution for all quantities.

27. Given the model in Figure 8.15 with $q_{A_i}(0) = 10, q_{B_i}(0) = 25, q_{A_o}(0) = 10,\ C_{oi} = 5, C_{io} = 1,$ $D_{io} = 4,\ D_{oi} = 0.1, K_1 = 3,$ and $K_{-1} = 1,$ assume that there is flow of q_A into the exterior of the cell equal to $5u(t),$ and a production of q_{B_i} inside the cell equal to $2u(t).$ All other initial conditions are zero. Simulate the solution for all quantities.

28. Given the model in Figure 8.15 with $q_{A_i}(0) = 20, q_{B_i}(0) = 5, q_{A_o}(0) = 30,\ C_{oi} = 10, C_{io} = 0.2,$ $D_{io} = 6, D_{oi} = 0.1, K_1 = 5,$ and $K_{-1} = 0.2,$ assume that there is flow of q_A into the exterior of the cell equal to $5u(t),$ and a production of q_{B_i} inside the cell equal to $2u(t).$ All other initial conditions are zero. Simulate the solution for all quantities.

29. Given the model in Figure 8.19 with $q_{S_i}(0) = 10, q_E(0) = 0.25, q_{S_o}(0) = 10,\ B_{oi} = 4, B_{io} = 0.5,$ $D_{io} = 5, D_{oi} = 0.05, K_2 = 0.5, K_1 = 5,$ and $K_{-1} = 0.5,$ assume that there is flow of q_S into the exterior of the cell equal to $5u(t).$ All other initial quantities are zero. Simulate the solution for all quantities.

30. Given the model in Figure 8.19 with $q_{S_i}(0) = 50, q_E(0) = 2, q_{S_o}(0) = 70,\ B_{oi} = 2, B_{io} = 0.1, D_{io} = 4,\ D_{oi} = 0.1, K_2 = 5, K_1 = 8,$ and $K_{-1} = 0.1,$ assume that there is flow of q_S into the exterior of the cell equal to $8u(t).$ All other initial quantities are zero. Simulate the solution for all quantities.

31. Given the model in Figure 8.19 with $q_{S_i}(0) = 25, q_E(0) = 0.25, q_{S_o}(0) = 10,\ B_{oi} = 10, B_{io} = 0.5,$ $D_{io} = 10,\ D_{oi} = 1, K_2 = 10, K_1 = 3,$ and $K_{-1} = 0.5,$ assume that there is flow of q_S into the exterior of the cell equal to $10u(t).$ All other initial quantities are zero. Simulate the solution for all quantities.

32. Given the model in Figure 8.21 with $q_{S_i}(0) = 15, q_{C_o}(0) = q_{C_i}(0) = 5, q_{S_o}(0) = 10,\ K_2 = 4,$ $K_1 = 2,$ and $K_{-1} = 0.5,$ assume that there is flow of q_S into the exterior of the cell equal to $4u(t).$ All other initial quantities are zero. Simulate the solution for all quantities.

33. Given the model in Figure 8.21 with $q_{S_i}(0) = 25, q_{C_o}(0) = q_{C_i}(0) = 8, q_{S_o}(0) = 20,\ K_2 = 7,$ $K_1 = 5,$ and $K_{-1} = 0.1,$ assume that there is flow of q_S into the exterior of the cell equal to $10u(t).$ All other initial quantities are zero. Simulate the solution for all quantities.

34. Given the model in Figure 8.21 with $q_{S_i}(0) = 35, q_{C_o}(0) = q_{C_i}(0) = 4, q_{S_o}(0) = 50,\ q_{P_o}(0) = 0,$ $K_2 = 3, K_1 = 1,$ and $K_{-1} = 0.01,$ assume that there is flow of q_S into the exterior of the cell equal to $15u(t).$ Simulate the solution for all quantities.

35. Simulate the model in Eqs. (8.114) and (8.115) given that $q_S(0) = 10, q_E(0) = 0.1, K_1 = 4, K_{-1} = 0.2,\ K_2 = 3, K_3 = 2, K_{-3} = 0.01, K_4 = 1,$ and $q_I(0) = 3.$ All other initial quantities are zero.

36. Simulate the model in Eqs. (8.114) and (8.115) given that $q_S(0) = 20, q_E(0) = 0.01, K_1 = 6,$ $K_{-1} = 0.1, K_2 = 10, K_3 = 5, K_{-3} = 0.05, K_4 = 7,$ and $q_I(0) = 5.$ All other initial quantities are zero.

37. Simulate the model in Eqs. (8.114) and (8.115) given that $q_S(0) = 30,\ q_E(0) = 0.1,\ K_1 = 10,$ $K_{-1} = 0.1, K_2 = 3, K_3 = 7, K_{-3} = 0.09, K_4 = 9,$ and $q_I(0) = 8.$ All other initial quantities are zero.

Continued

38. Simulate the model in Eq. (8.124) given that $q_S(0) = 30$, $q_E(0) = 0.1$, $q_M = 10$, $K_1 = 10$, $K_{-1} = 0.1$, $K_2 = 3, K_3 = 3, K_{-3} = 0.1, K_4 = 2, K_{-4} = 0.1, K_5 = 5$, $K_6 = 1$, and $K_{-6} = 5$. All other initial quantities are zero.

39. Simulate the model in Eq. (8.124) given that $q_S(0) = 20$, $q_E(0) = 0.01$, $q_M = 20$, $K_1 = 5$, $K_{-1} = 1$, $K_2 = 7, K_3 = 3, K_{-3} = 10, K_4 = 0.2, K_{-4} = 0.001, K_5 = 1$, $K_6 = 6$, and $K_{-6} = 0.5$. All other initial quantities are zero.

40. Simulate the model in Eq. (8.124) given that $q_S(0) = 10$, $q_E(0) = 0.1$, $q_M = 40$, $K_1 = 7$, $K_{-1} = 1$, $K_2 = 5, K_3 = 1, K_{-3} = 5, K_4 = 2, K_{-4} = 0.1, K_5 = 2$, $K_6 = 2$, and $K_{-6} = 5$. All other initial quantities are zero.

41. Simulate the model in Eq. (8.133) given that $q_S(0) = 50$, $q_E(0) = 7$, $K_1 = 0.001$, $K_{-1} = 0.00001$, $K_2 = 0.1, K_3 = 0.05, K_{-3} = 0.00002$, and $K_4 = 0.3$. All other initial quantities are zero.

42. Simulate the model in Eq. (8.133) given that $q_S(0) = 50$, $q_E(0) = 7$, $K_1 = 1$, $K_{-1} = 0.2$, $K_2 = 0.1$, $K_3 = 0.01, K_{-3} = 0.4$, and $K_4 = 0.02$. All other initial quantities are zero.

Suggested Readings

E. Ackerman, L.C. Gatewood, Mathematical Models in the Health Sciences, University of Minnesota Press, Minneapolis, 1979.

E.S. Allman, J.A. Rhodes, Mathematical Models in Biology, An Introduction, Cambridge University Press, Cambridge, UK, 2004.

J.E. Bailey, D.F. Ollis, Biochemical Engineering Fundamentals, McGraw-Hill, Singapore, 1986.

G.E. Briggs, J.B.S. Haldane, A note on the kinetics of enzyme action, Biochem. J. 19 (1925) 338–339.

N.F. Britton, Essential Mathematical Biology, Springer, London, 2003.

M.B. Cutlip, M. Shacham, Problem Solving in Chemical and Biochemical Engineering with POLYMATH, Excel, and MATLAB, second ed., Prentice Hall, Upper Saddle River, NJ, 2008.

L. Edelstein-Keshet, Mathematical Models in Biology, SIAM, New York, 2005.

K. Godfrey, Compartmental Models and Their Applications, Academic Press, San Diego, California, 1983.

A.C. Guyton, Textbook of Medical Physiology, eighth ed., W.B. Saunders Company, Philadelphia, 1991.

V. Henri, Lois Générales de l'Action des Diastases, Hermann, Paris, 1903.

J. Keener, J. Sneyd, Mathematical Physiology, Springer, New York, 1998.

L. Michaelis, M. Menten, Die Kinetik der Invertinwirkung, Biochem. Z. 49 (1913) 333–369.

J.D. Murray, Mathematical Biology, third ed., Springer, New York, 2001.

R.B. Northrop, A.N. Connor, Introduction to Molecular Biology, Genomics and Proteomics for Biomedical Engineers, CRC Press, Boca Raton, FL, 2009.

R.B. Northrop, Endogenous and Exogenous Regulation and Control of Physiological Systems, CRC Press, Boca Raton, FL, 1999.

S.I. Rubinow, Introduction to Mathematical Biology, Dover Publications, Mineola, NY, 2002.

W.M. Saltzman, Biomedical Engineering, Bridging Medicine and Technology, Cambridge University Press, NY, 2009.

S. Schnell, C. Mendoza, Closed form solution for time-dependent enzyme kinetics, J. Theor. Biol. 187 (1997) 207–212.

M.L. Shuler, F. Kargi, Bioprocess Engineering, Basic Concepts, second ed., Prentice Hall, Upper Saddle River, NJ, 2008.

G.A. Truskey, F. Yuan, D.F. Katz, Transport Phenomena in Biological Systems, Pearson, Prentice Hall, Upper Saddle River, NJ, 2004.