7 Models for Molecular Events

All the effects of nature are only the mathematical consequences of a small number of immutable laws.
-P. S. Laplace (1749–1827) quoted in E. T. Bell (1937) *Men of Mathematics* p. 172 Simon & Schuster, N.Y.

The realm of molecular biology lies at the outermost limits of resolution of our best microscopes. Just beyond is a world that is both fascinating and mysterious. This is the world of *macromolecules:* versatile entities that give cells their structure, store or transmit information, recognize and respond to other macromolecules, build or synthesize each other, and regulate all the other chemical events in the living cell.

It is sometimes easy to forget that our familiarity with the subcellular realm is very recent. Until 1838, when Schleiden and Schwann proposed their *cell theory,* scientists scarcely appreciated that living things were made up of smaller units called cells. Only by the mid 1900s had the electron microscope extended our visual frontier into the finer structures of the cell. Numerous contemporary techniques eventually led to the important discovery by J. Watson and F. Crick in 1953 of the structure of DNA (deoxyribonucleic acid), the fundamental genetic material. Since that time, and especially within the last two decades, the field of molecular biology has undergone rapid, explosive growth. It is now universally recognized as a field of nearly unlimited promise in medicine, industry, agriculture, and many other areas of application.

Despite this recent surge of knowledge and fervent ongoing exploration of the molecular world, much is still unknown about the microcosm inside the living cell. How do all of these complicated entities work so well as a unit? How are a multitude of simultaneous processes controlled, each with split-second accuracy? What makes

it all a cohesive living unit that can respond to its environment while maintaining an identity despite potentially destructive influences? Answers to these questions are not within our immediate grasp and must await further discoveries on the experimental frontier. They are, broadly speaking, related to similar questions one could pose about any complex system that consists of myriad interrelated units working in concert.

While molecular biology owes much, in its advances, to the technological breakthroughs that stem from the "hard" sciences, it may be considered presumptuous to overplay the role of mathematics. Nevertheless, mathematics does indeed have a contribution to make, at least in helping us understand the very basic building blocks of behavior exhibited by the cell, the gene, and the enzyme. A traditionally mathematical approach underlies *enzymology,* the study of dynamic interactions between *enzymes* (large protein molecules that catalyze reactions in the living cell) and their *substrates*. It is not always clear whether mathematical modeling can help illuminate fundamental questions about "how things work." Yet, it is our gradual perception that such approaches have borne fruit in disciplines of parallel complexity such as ecology and physiology; it is to be hoped that similar combinations of theory and experiment will lead to progress in molecular biology as well.

This chapter could be viewed as an analog of Chapter 6 dealing with the "population dynamics" of molecules rather than whole organisms. Perhaps the key ideas presented here are that some parallels exist between such disparate realms and that certain *dynamic properties* are shared by unrelated entities. To begin this excursion, we delve into a familiar macroscopic observation and study its molecular foundation. Looking more closely at events on a bacterial cell's membrane, we show that Michaelis-Menten kinetics (used in Section 4.4) correspond to saturating nutrient-conveying carrier molecules. Mathematical methods applied to this problem are used again for studying two related situations (Sections 7.3 and 7.4) in which sigmoidally saturating kinetics are implicated.

As a departure from the somewhat technical enzyme kinetics, we explore how two simple molecular events lead to an aspect of behavior that mimics certain properties of the cell. Here a somewhat abstract mathematical approach leads to insights that, although oversimplified, are nevertheless useful.

An extension of the geometric and graphical analysis of Chapter 5 is applied to two chemical situations (Sections 7.7 and 7.8) simply for further practice in abstract reasoning. Finally, we conclude with a brief exposé of mathematical theories that could be applied to certain biochemical and molecular systems too complicated to be analyzed by standard modeling techniques.

For a condensed coverage of this chapter, the following material could be covered: Section 7.1 and part of Section 7.2, Sections 7.3, 7.5, and 7.6; the material in Sections 7.7 and 7.8 can be assigned to more advanced students or omitted.

7.1 MICHAELIS-MENTEN KINETICS

In describing bacterial growth within a chemostat (Chapter 4) we assumed an expression for the nutrient-dependent growth rate that had the property of saturation; for low levels of the nutrient concentration c, bacterial growth rate given by equation

(15) in Chapter 4 is roughly proportional to c. At high c levels, though, this rate approaches a constant value, K_{max}. (See Figure 4.3.) Numerous biological phenomena exhibit saturating kinetics. The expression

$$K(C) = \frac{K_{max}\, C}{K_n + C}, \tag{1}$$

which depicts such a property, is called the *Michaelis-Menten kinetics*. This expression actually stems from a particular set of assumptions about what may be occurring at the molecular level on the surface of the bacterial cell membrane. We explore this mechanism in some detail in this and the following section.[1]

Figure 7.1 gives a schematic version of how bacteria consume organic substances such as glucose. Most water-soluble molecules are unable to pass through the hydrophobic environment of the cell membrane directly and must be carried across by special means. Typically, molecular receptors embedded in the bacterial cell membrane are involved in "capturing" these polar molecules in a loose complex, conveying them across the membrane barrier, and releasing them to the interior of the cell. *Saturation* results from the limited number of receptors and the limited rate at which their "conveyor-belt" mechanism can operate.

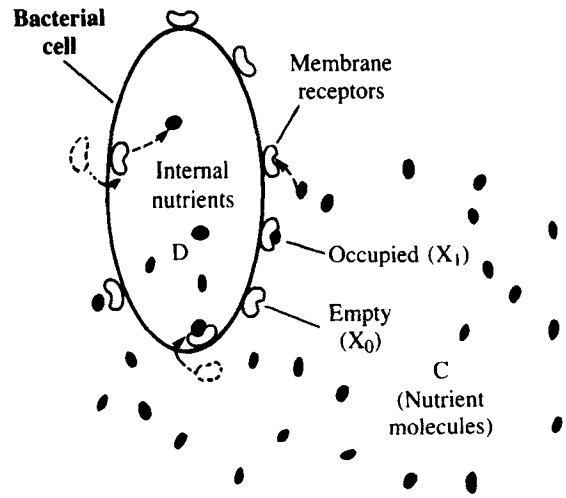


Figure 7.1 *The passage of nutrient molecules into a cell may be mediated by membrane-bound receptors. This saturating mechanism for nutrient uptake can be described by Michaelis-Menten kinetics.*

Let us write the molecular scheme of this event in the form of chemical equations. We will denote an external nutrient molecule by C, an unoccupied receptor by X_0, a nutrient-receptor complex by X_1, and a nutrient molecule successfully captured by the cell by P. The constants k_1, k_{-1}, and k_2 depict the various rates with which these reactions proceed. The following equations summarize the directions and rates of reactions:

1. The approach in this material is partly based on a lecture given by L. A. Segel at the Weizmann Institute, where the author was a graduate student.

$$C + X_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1, \tag{2a}$$

$$X_1 \xrightarrow{k_2} P + X_0. \tag{2b}$$

That is, C and X_0 can combine to form the complex X_1, which either breaks down into the former constituents, or else produces P and X_0. The fact that reaction (2a) is reversible means that the carrier receptor sometimes fails to transport its nutrient load into the cell interior, dumping it instead outside the cell.

A reaction diagram such as the one given by equations (2a,b) can be translated into a set of differential equations that describe rates of change of concentrations of the participating reactants. The diagram encodes both the sequence of steps and the rates with which these steps occur. To write corresponding equations, we must use the law of mass action (encountered previously), which states that when two or more reactants are involved in a reaction step, the rate of reaction is proportional to the product of their concentrations. By convention, the rate constants k_i in the reaction diagram are the proportionality constants. We define the *volumetric receptor concentration* by averaging over a population of bacteria, as follows:

$$\begin{array}{l}\text{number of receptors} \\ \text{per unit volume}\end{array} = \begin{array}{l}\text{average number of} \\ \text{receptors per cell}\end{array} \times \begin{array}{l}\text{number of cells} \\ \text{per unit volume}\end{array}.$$

The lower-case letters c, x_0, x_1, and p will be used to denote concentrations of C, X_0, X_1, and P. Keeping track of each chemical participant allows us to derive the set of equations (3). (It is a good idea to attempt to write these equations independently before proceeding.)

$$\frac{dc}{dt} = -k_1 c x_0 + k_{-1} x_1, \tag{3a}$$

$$\frac{dx_0}{dt} = -k_1 c x_0 + k_{-1} x_1 + k_2 x_1, \tag{3b}$$

$$\frac{dx_1}{dt} = k_1 c x_0 - k_{-1} x_1 - k_2 x_1, \tag{3c}$$

$$\frac{dp}{dt} = k_2 x_1. \tag{3d}$$

Adding equations (3b,c) reveals a feature common to many *enzymatic reactions:*

$$\frac{dx_0}{dt} + \frac{dx_1}{dt} = 0, \tag{4}$$

which simply means that $x_0 + x_1$, the total of occupied and unoccupied receptors, is a constant. This is not at all surprising, since receptors are neither formed nor destroyed in the process of conveying their cargo into the cell. Suppose we started out with an initial concentration of receptors r. Then the total concentration would remain r:

$$x_1 + x_0 = r. \tag{5}$$

It is generally true that the enzymes are conserved in the reactions in which they participate.

Equation (5) permits us to simplify the system of equations (3a,b,c) by eliminating either x_1 or x_0. We arbitrarily choose to eliminate x_0. Furthermore, because equations (3a,b,c) are not dependent on concentration p, we shall set aside (3d), which can always be solved independently once solutions for the other variables are known. In problem 1 you are asked to verify that these steps lead to the following:

$$\frac{dc}{dt} = -k_1 rc + (k_{-1} + k_1 c)x_1, \tag{6a}$$

$$\frac{dx_1}{dt} = k_1 rc - (k_{-1} + k_2 + k_1 c)x_1. \tag{6b}$$

7.2 THE QUASI-STEADY-STATE ASSUMPTION

At this point we could proceed with a full analysis of equations (6a,b) using the phase-plane methods described in Chapter 6 (see problem 7). However, in this section we concentrate on an assumption that leads directly to the Michaelis-Menten rate law and then examine the restrictions to which this approximation is subject.

Typically, small molecules such as glucose or other nutrients are found in concentrations much higher than those of the receptors (in the sense of our previous definition of receptor concentration). We could argue, therefore, that receptors are always working at maximal capacity, so that their occupancy rate is virtually constant. This assumption, which leads us to write

$$\frac{dx_1}{dt} \simeq 0, \tag{7a}$$

or equivalently,

$$k_1 rc - (k_{-1} + k_2 + k_1 c)x_1 = 0 \tag{7b}$$

is called the *quasi-steady-state* hypothesis and permits further simplification by allowing x_1 to be eliminated from the system of equations (6a,b).

In problem 2 you are asked to detail the algebraic steps showing that this assumption results in

$$\frac{dc}{dt} = -\frac{K_{\max} c}{k_n + c}, \tag{8}$$

where

$$K_{\max} = k_2 r, \qquad k_n = \frac{k_{-1} + k_2}{k_1}.$$

There is one serious problem with this reasoning. Setting $dx_1/dt = 0$ in equation (6b) changes the character of the mathematical problem from a system of two ODEs to a simple ODE coupled with the algebraic equation (7b). This change in the problem can have drastic consequences and should not be taken lightly. In order to more fully understand when this approximation can be used, it seems prudent to make a more careful comparison of magnitudes of terms in these equations.

Because these magnitudes really depend on the system of units adopted to measure concentrations and time, a prerequisite step in making such comparisons is

to reduce the equations to a dimensionless form. (Detailed steps are left as an exercise in problem 3.) First we choose the following scales:

$$\tau = 1/k_1 r, \text{ for units of time,} \tag{9a}$$

$$\hat{x}_1 = r = \text{initial receptor concentration, for units of } x, \tag{9b}$$

$$\hat{c} = c_0 = \text{initial nutrient concentration, for units of } c. \tag{9c}$$

The equations can then be written in the dimensionless form,

$$\frac{dc^*}{dt^*} = -c^* + \left(\frac{k_{-1}}{k_1 c_0} + c^*\right)x_1^*, \tag{10a}$$

$$\frac{dx_1^*}{dt^*} = \frac{c_0}{r}c^* - \frac{c_0}{r}\left(\frac{k_{-1} + k_2}{k_1 c_0} + c^*\right)x_1^*. \tag{10b}$$

Next, drop the asterisks and define

$$\epsilon = \frac{r}{c_0}, \qquad K = \frac{k_{-1} + k_2}{k_1 c_0}, \qquad \lambda = \frac{k_2}{k_1 c_0}.$$

Notice that ϵ is the ratio of concentrations of receptors and nutrient molecules. The equations become

$$\frac{dc}{dt} = -c + (K - \lambda + c)x_1, \tag{11a}$$

$$\epsilon\frac{dx_1}{dt} = c - (K + c)x_1. \tag{11b}$$

We now realize that neglecting the LHS of equation (6b) is equivalent to assuming that $\epsilon\, dx_1/dt$ is small. This would be fine provided ϵ is small, that is, the receptor concentration is lower than nutrient concentration. Notice that dimensional analysis has given a much more precise meaning to the quasi-steady-state assumption.

To summarize results, it has been concluded that for time scales on the order of $\tau = 1/(k_1 r)$ the process of receptor-mediated nutrient uptake is, to first-order approximation, given by the equations

$$\frac{dc}{dt} = -c + (K - \lambda + c)x_1,$$

$$0 = c - (K + c)x_1.$$

More simply stated, this means that

$$\frac{dc}{dt} = \frac{-\lambda c}{K + c}, \tag{12a}$$

$$x_1 = \frac{c}{K + c}. \tag{12b}$$

We recognize this as another version of the Michaelis-Menten rate law. From equation (12a) observe that whenever $c > 0$, $dc/dt < 0$ so that c is a decreasing function of time. [This equation can be integrated to obtain an implicit solution for $c(t)$:

See problem 4a.] From (12b) we also observe that x_1 decreases as c decreases. [See problem 4b.] Thus on the time scale τ, the concentrations of both the nutrient and the nutrient-receptor complex will be decreasing with time. This is one approximation of the nutrient-receptor kinetics.[2]

We now repeat a step in our analysis by examining the same process again but with a different choice of time scale. Let us now choose

$$\tilde{\tau} = \frac{1}{k_1 c_0}, \tag{13}$$

and retain the previous choices $\hat{x}_1 = r$ and $\hat{c} = c_0$. In problem 5 it is shown that resulting dimensionless equations are

$$\frac{dc^*}{dt} = -\epsilon c^* + \epsilon(K - \lambda + c^*)x_1, \tag{14a}$$

$$\frac{dx_1^*}{dt} = c^* - (K + c^*)x_1^*, \tag{14b}$$

where ϵ, K, and λ have their previous meaning.

How do the two time scales τ and $\tilde{\tau}$ compare? According to our assumption, ϵ is small; that is, $c_0 \gg r$. Thus

$$\tau \gg \tilde{\tau}.$$

With our second choice of time scales we are studying the behavior for *short times*, close to $t = 0$. For example, in a situation in which substrate at concentration c_0 is abruptly added to the solution at $t = 0$, this second time scale would be appropriate for understanding the way initially free receptor sites fill up with their ligands.

Again exploiting the fact that ϵ is small now leads to the conclusion that the RHS of equation (14a) can be neglected to first-order approximation, so that for time scales on the order of $\tilde{\tau}$ we can say that

$$\frac{dc^*}{dt^*} \simeq 0 \qquad (c^* = 1,\ c = c_0), \tag{15a}$$

$$\frac{dx_1^*}{dt^*} \simeq 1 - (K + 1)x_1^*. \tag{15b}$$

The equation for x_1^* can then be integrated (this is left as an exercise), and we then observe that the receptors that at $t = 0$ are unoccupied $[x_1(0) = 0]$ quickly fill up, approaching a fixed fractional occupancy rate. By our previous analysis, x_1 eventually decreases as c is depleted from the environment of the cells.

2. To achieve greater accuracy, we can refine this approximation by assuming that the functions x_1 and c are made up of sums of terms that are proportional to ϵ^0, ϵ^1, ϵ^2, . . . , ϵ^n. These are called *asymptotic expansions*, and the procedure for then getting successive approximations for x_1 and c is called a *singular perturbation method*. This important method has rather broad application to problems in applied mathematics. However, the numerous technical details involved are beyond the scope of this book.

A thorough exposition of the method of asymptotic expansions and its application to enzyme kinetics is given by Murray (1977) and Lin and Segel (1974).

Summary of Steps Leading to the Michaelis-Menten Rate Equation

1. Draw the following reaction diagram:

$$C + X_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1 \xrightarrow{k_2} X_0 + P.$$

2. Write equations for changes in concentrations c, x_0, x_1, and p using the law of mass action.
3. Use the fact that the total number of receptor molecules $x_0 + x_1 = r$ is fixed and eliminate one variable.
4. Assume receptors are at quasi steady state so that $dx_1/dt = 0$ to get a relationship between x_1 and c.
5. Eliminate x_1 from the equation for dc/dt, and obtain[3]

$$\frac{dc}{dt} = -K_{\max}\frac{c}{k_n + c},$$

where

$$K_{\max} = k_2 r, \qquad k_n = \frac{k_{-1} + k_2}{k_1}.$$

We have seen previously that on two different time scales one can ascertain the behavior by solving different approximate versions of the equations. The final step, that of matching these short and long time solutions, is accomplished by the technique of *matched asymptotic analysis* and will not be discussed here. However, the results enable us to establish a complete time sequence of events, as illustrated in Figure 7.2.

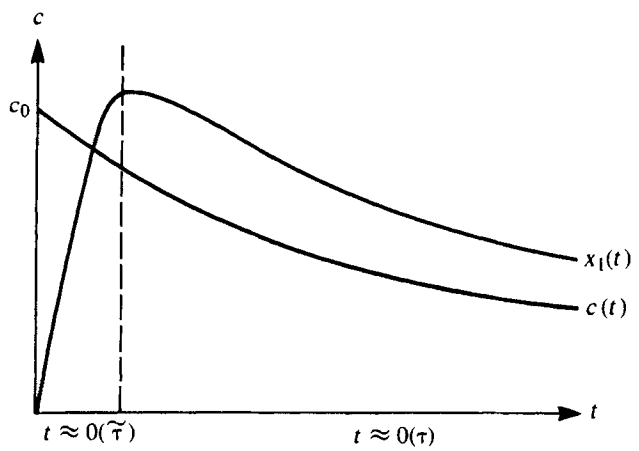


***Figure 7.2** A reversible reaction such as equation (2a) has distinctly different kinetics at different time scales: On a short time scale* ($t \simeq 0(\tilde{\tau})$) *receptors fill up quickly, and* $c \simeq c_0$. *On longer time scales* ($t \simeq 0(\tau)$) *the receptor occupancy* x_1 *decreases as c is progressively depleted. (Most of the nutrient has been transported into the cell interior.)*

3. dc/dt is negative since the concentration of substance outside of the cell is decreasing.

In problem 7 it is shown that these results are consistent with a phase-plane analysis of equations (6a,b) in which no quasi-steady-state approximation is made.

7.3 A QUICK, EASY DERIVATION OF SIGMOIDAL KINETICS

One of the features of Michaelis-Menten kinetics not shared by more complex molecular pathways is that for low precursor concentrations it yields an approximately linear rate of reaction. (The graph of $-dc/dt$ versus c is almost a straight line close to $c = 0$.) Stated another way, for moderately low precursor levels (those that do not oversaturate the receptors), increasing the precursor concentration by 50% tends to increase the reaction rate by 50% simply because the chances of encounter between receptors and precursors increase proportionately.

We shall see that this simple proportionality changes when more than one precursor molecule is implicated in forming a complex. Instead we typically observe a sigmoidally saturating graph of the rate kinetics. A simple but naive way of demonstrating this is to consider the following double-substrate complexing reaction:

$$2C + X_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_2 \xrightarrow{k_2} X_0 + 2P. \tag{16}$$

Here two molecules of the substance C are required for forming the complex X_2, which then yields products X_0 and P. With the preparation given in Sections 7.1 and 7.2, it is a straightforward matter to draw the necessary conclusions. Indeed, we need only insert a single change in the previous steps to see the result. According to the law of mass action the reaction that feeds on two molecules of C and one of X_0 proceeds at a rate $k_1c^2x_0$. Thus the first two equations describing the reaction are

$$\frac{dc}{dt} = -k_1c^2x_0 + k_{-1}x_2, \tag{17a}$$

$$\frac{dx_0}{dt} = -k_1c^2x_0 + k_{-1}x_2 + k_2x_2, \tag{17b}$$

Others in the series are equally easy to write down. We recognize (17) as a thinly disguised version of the previous rate laws but with the following changes:

c is replaced by c^2,

x_1 is replaced by x_2.

For practice, you may want to reconstruct the steps (identical to those of the previous sections) that lead to the rate equation for c given a quasi-steady-state assumption for x_2. Because the only substantive change is replacing c with c^2, it should come as no surprise that the result is

$$\frac{dc}{dt} = -\frac{K_{\max}c^2}{k_n + c^2}, \tag{18}$$

where $K_{\max} = k_2r$ and $k_n = (k_{-1} + k_2)/k_1$ as before. The graph of this function, shown in Figure 7.3, is sigmoidal, where $\sqrt{k_n}$ the concentration required for half-maximal response. $c = \sqrt{k_n}$ gives $dc/dt = K_{\max}/2$. For small c the graph is approx-

imately quadratic; that is,

$$\frac{dc}{dt} \simeq -K_{\max} \frac{c^2}{k_n},$$

where $c^2 \ll k_n$.

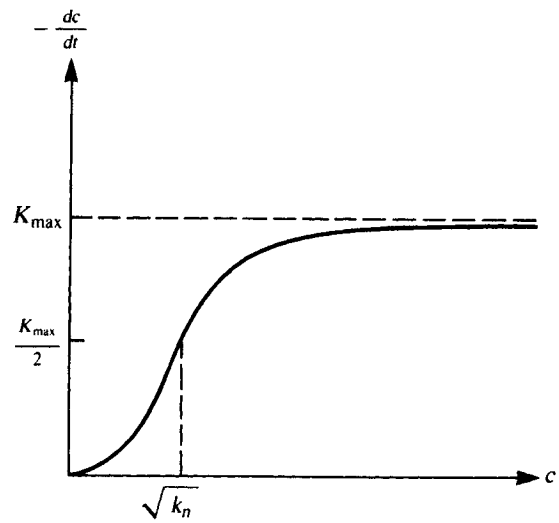


***Figure 7.3** A sigmoidal reaction rate for a reaction involving two molecules of precursor.*

7.4 COOPERATIVE REACTIONS AND THE SIGMOIDAL RESPONSE

In most biochemical systems, trimolecular reactions are considered unlikely, as they involve a collision between three molecules. For this reason, simultaneous complexing of two molecules with a receptor is generally unrealistic. In this section we examine a more plausible version that involves only sequential bimolecular steps. The connection between these distinct mechanisms will then be established by comparing results.

Let us suppose that a double complex forms in the following way. First a single molecule attaches to the receptor, then a second. Products might be formed at an intermediate stage (with rate κ_1) or at the end (rate κ_2). A diagram typical for this reaction would be as follows:

$$C + X_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_1 \xrightarrow{\kappa_1} X_0 + P, \qquad (19a)$$

$$C + X_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} X_2 \xrightarrow{\kappa_2} X_1 + P. \qquad (19b)$$

Corresponding equations for c and x_1 must now include all reactions in which these appear as products or reactants, as follows:

$$\frac{dc}{dt} = -k_1 c x_0 + x_1(k_{-1} - k_2 c) + k_{-2} x_2, \tag{20a}$$

$$\frac{dx_0}{dt} = -k_1 c x_0 + x_1(\kappa_1 + k_{-1}), \tag{20b}$$

$$\frac{dx_1}{dt} = k_1 c x_0 + x_2(\kappa_2 + k_{-2}) - x_1(\kappa_1 + k_{-1} + k_2 c), \tag{20c}$$

$$\frac{dx_2}{dt} = k_2 c x_1 - x_2(k_{-2} + \kappa_2), \tag{20d}$$

$$\frac{dp}{dt} = \kappa_1 x_1 + \kappa_2 x_2. \tag{20e}$$

The conservation of receptors in the reaction now implies that

$$x_0 + x_1 + x_2 = r \tag{20f}$$

where r is a fixed constant. This equation can be used to eliminate any x from equations (20a–e) for example, x_0.

We now make a quasi-steady-state assumption for each receptor occupancy state and set $dx_1/dt = dx_2/dt = 0$ after eliminating x_0). The resulting relations involve certain ratios of rate constants that we shall define, following Rubinow (1975), as follows:

$$K_m = \frac{\kappa_1 + k_{-1}}{k_1}, \tag{21a}$$

$$K'_m = \frac{\kappa_2 + k_{-2}}{k_2}. \tag{21b}$$

In problem 9 you are asked to demonstrate that the quasi-steady-state assumptions lead to the relations

$$K_m x_1 = c x_0, \tag{22a}$$

$$K_m K'_m x_2 = c^2 x_0. \tag{22b}$$

Consequently, using equation (20f), it is possible to express x_0 in terms of c; when this is done, the following relation is obtained:

$$x_0 = \frac{r}{K_m K'_m}(K_m K'_m + K'_m c + c^2). \tag{23}$$

As a last step, we rewrite the equation for dc/dt using equations (21) to (23). The result is

$$\frac{dc}{dt} = \frac{-rc(\kappa_1 K'_m + \kappa_2 c)}{K_m K'_m + K'_m c + c^2}. \tag{24}$$

This equation bears an apparent connection with the simpler sigmoidal kinetics in equation (18), but it contains several terms that were absent before. It is somewhat revealing to examine when these terms can be ignored so as to establish a connection between the two mechanisms shown in equations (16) and (19).

We notice in the numerator that the term linear in c vanishes if $\kappa_1 = 0$, that is, if products are not formed in the intermediate steps of the reaction. When is the term $K'_m c$ in the denominator of equation (24) small enough to neglect? This term is small relative to c^2 and to $K_m K'_m$ provided

$$K_m K'_m \gg K'_m c \qquad \text{and} \qquad c^2 \gg K'_m c.$$

Combining inequalities leads to

$$K_m \gg c \gg K'_m, \tag{25}$$

which indicates that the constant K_m must be larger than K'_m. Furthermore, the term $K'_m c$ can only be neglected at intermediate levels of concentrations c; that is, at lower or higher levels the presence of this term tends to distort somewhat the graph of the function shown in Figure 7.3 (see problem 11).

Rewriting the above inequalities in terms of original parameters leads to the following:

$$\begin{aligned} &\frac{1}{K'_m} > \frac{1}{K_m}, \\ &\frac{k_2}{\kappa_2 + k_{-2}} > \frac{k_1}{\kappa_1 + k_{-1}}. \end{aligned} \tag{26}$$

This indicates that the tendency of the first reaction step in (19b) to proceed in the forward direction is greater than that of the first step in (19a). Stated another way, once a single molecule of C has complexed with a receptor, a second molecule complexes more readily. Thus the intermediate complex X_1 is short-lived and can almost be neglected, as it has been in the simplified scheme of the trimolecular mechanism, equation (16).

Many biologically important reactions have the characteristic that once a first step is complete, others follow rapidly. A notable example is that of *hemoglobin* (a macromolecule in red blood cells which conveys oxygen). Hemoglobin has four *polypeptide* (protein) components, each of which contains a heme group that can bind with an oxygen molecule. After a single oxygen molecule is attached, the binding of others is enhanced. This reaction and others like it are termed *positively cooperative*. Mechanisms for cooperativity may include *conformational changes* (changes in shape) of the macromolecule that enhance exposure of active sites. Further details about these fascinating topics can be obtained from any current text on biochemistry or molecular biology.

Based on the investigation in this section we conclude that for highly cooperative bimolecular reactions involving a complex between one macromolecule and two substrate molecules, equation (18) is a reasonable approximation for the reaction rate (subject to all the appropriate conditions outlined earlier). A generalization of this rate law to *n-substrate complexes* is

$$\frac{dc}{dt} = \frac{-K_{\max} c^n}{(k_n + c^n)}. \tag{27}$$

7.5 A MOLECULAR MODEL FOR THRESHOLD-GOVERNED CELLULAR DEVELOPMENT

Over the years our understanding of molecular processes within the cell has increased. This knowledge has led to much greater insight into the way cells acquire a commitment to specific developmental pathways. The quest to probe these complex processes further is at the forefront of science, technology, and perhaps even our philosophical view of living things.

All cells in our body have one ancestral cell: the fertilized egg created at conception. Since the advent of molecular biology of the gene we know that all these progeny cells, no matter how diverse their functions, have identical "blueprints" encoded in genetic material in the nucleus. Somehow during the life history of the cell these blueprints are selectively transcribed and used in building the unique character of the cell, be it neuron, epithelial cell, hepatic cell, or one of thousands of other cell types in our bodies. How this developmental process occurs is still largely a mystery.

Mathematics has played an admittedly modest role in solving the mysteries of molecular biology. Nevertheless, mathematical reasoning can illuminate specific questions that may then be clues to a tremendously complex puzzle. We shall see two examples in this and the following sections.

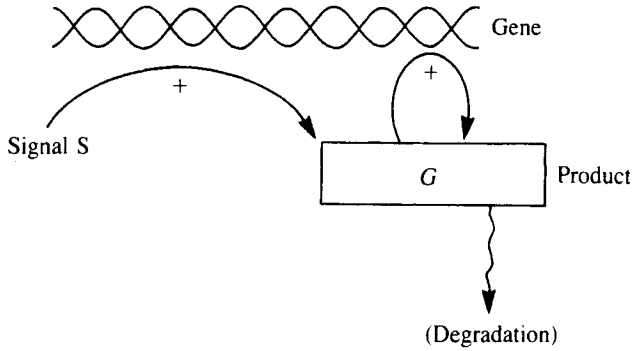
We first discuss a model by Lewis et al. (1977) that illustrates the idea that chemical reactions can act as logical elements, helping to make decisions about the developmental processes that occur in a cell. To discover how this works, a rather simple idealized example serves as the focal point of our discussion.

Consider a row of cells connected to each other in a one-dimensional filament. Originally the cells are identical; after a process of differentiation the row consists of two distinct cell types (say, pigmented and unpigmented cells). A well-defined border between these types appears in a predictable and controlled position. How is this achieved?

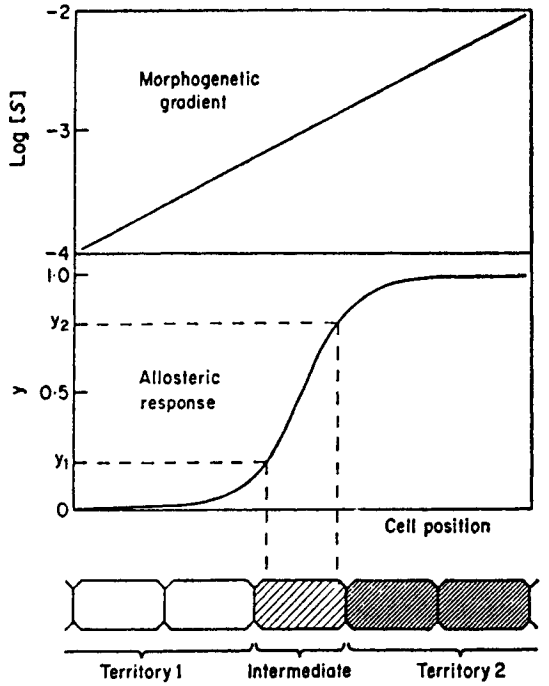
One theory, by no means the only one, is that cells have *positional information,* cues by which they assess their locations relative to particular points of demarcation. (The ends of a filament and the boundary of a two-dimensional tissue are examples of such demarcation points.) These cues, which may be carried by chemical messages, then have to be interpreted by the cell to arrive at a set of instructions that determine the course of differentiation.

We can imagine how positional information might be created and maintained. For example, in our example of filament of cells, a chemical source at one end (say the "head") and a sink at the other (the "tail" end) could result in a permanent and continuous gradient of a chemical signal S across the tissue. Cells closest to the head would be exposed to high levels of S; those closest to the tail would sense low S concentrations; and those at intermediate positions would detect moderate levels [see Figure 7.4(*b*)]. Each cell could then "feel its position" by assessing the concentrations of S about it. S could be called a *morphogenetic* substance since it controls the differentiation and development of form in the tissue.

It still remains to determine how a continuous spatially varying signal is inter-



(a)



(b)

Figure 7.4 *(a) In this model,* S *is the signal for gene activation and* G *is the product synthesized by the gene that contributes to further activation. (b) A continuous morphogenetic gradient of* S *thus results in a response that undergoes a rapid transition from* 0 *to* 1. *[y represents the magnitude of the sigmoidal term in equation (28).] This means that sharp transitions in developmental processes can occur in a row of cells that experience a continuous signal gradient. (Reprinted with permission of the authors and publisher from Lewis, J., Slack, J.M.W., and Wolpert, L. (1977). Thresholds in development. J. Theor. Biol., 65, 579–590.)*

preted in a discontinuous developmental response. Clearly what is needed is a cellular on-off switch, a mechanism that governs whether pigment is synthesized or not, depending on the concentration of S. It happens that simple biochemical processes can provide such decision elements.

While many possible hypothetical molecular systems could work in principle, we examine now a simple example due to Lewis et al. (1977). What is attractive about this example is that it is relatively elementary, uses chemical kinetics discussed in a previous section, and has rather interesting dynamical behavior that we will ascertain by methods given in Section 5.1.

It is an acceptable assumption that production of a pigment (or, for that matter, any other protein product of the cell) requires activation of a gene that may normally be quiescent. Suppose gene G produces its product (whose concentration is g) at a rate that depends linearly on the level s of signal S. Furthermore, suppose G exerts a sigmoidal positive feedback effect on its formation and is degraded at a rate proportional to its concentration [see Figure 7.4(a)]. The level of the product of G within the cell could then be governed by the equation

$$\frac{dg}{dt} = k_1 s - k_2 g + \frac{Kg^2}{k_n + g^2}. \tag{28}$$

To understand the implications of (28), we study a graph of dg/dt versus g. A simple way of arriving at such a graph is to superimpose graphs of the two functions $k_1 s - k_2 g$ and $Kg^2/(k_n + g^2)$ and add these. The first is a straight line of slope $-k_2$ that intercepts the g axis at $k_1 s$. The second is a sigmoidal function like the one in Figure 7.3. The sum of the two leads to one of the graphs shown in Figure 7.5 depending on the size of $k_1 s$.

This model consists of a single nonlinear differential equation (28), whose form is $dg/dt = f(g)$. (s is assumed to be a known parameter, so g is the only variable.) We have encountered such equations in Section 5.1. Figure 7.5 identifies the steady states of (28) as points of intersection of $y = f(g)$ with the g axis. It can be seen that the number of such intersections can vary from one to three, depending on the height of the dip in the curve. Moreover, the stability properties of these states can also change in the transition from Figure 7.5(a) to (c). Recall that stability of the steady states can be inferred from the direction in which changes take place close to these points. This in turn depends only on the sign of dg/dt, which can be read directly from the graphs.

For example, in Figures 7.5(a,b), dg/dt is positive for all g values to the left of $\bar{g}_1$. In Figure 7.5(c) the fact that the valley in the graph dips below the g axis means that dg/dt is negative for $\bar{g}_3 < g < \bar{g}_2$. As shown in the graphs, these observations lead us to deduce a "flow" along the g axis. In cases (a) and (b) this flow is towards $\bar{g}_1$ (which is then a *globally stable steady state*). In case (c) both $\bar{g}_3$ and $\bar{g}_1$ are stable; if g is initially smaller than $\bar{g}_2$ it will approach $\bar{g}_3$ with time, whereas if g is initially higher than $\bar{g}_2$, it will be attracted to $\bar{g}_1$. Using the methods given in Chapter 5 we have determined qualitative properties of the chemical kinetics depicted by equation (28) without explicitly calculating anything.

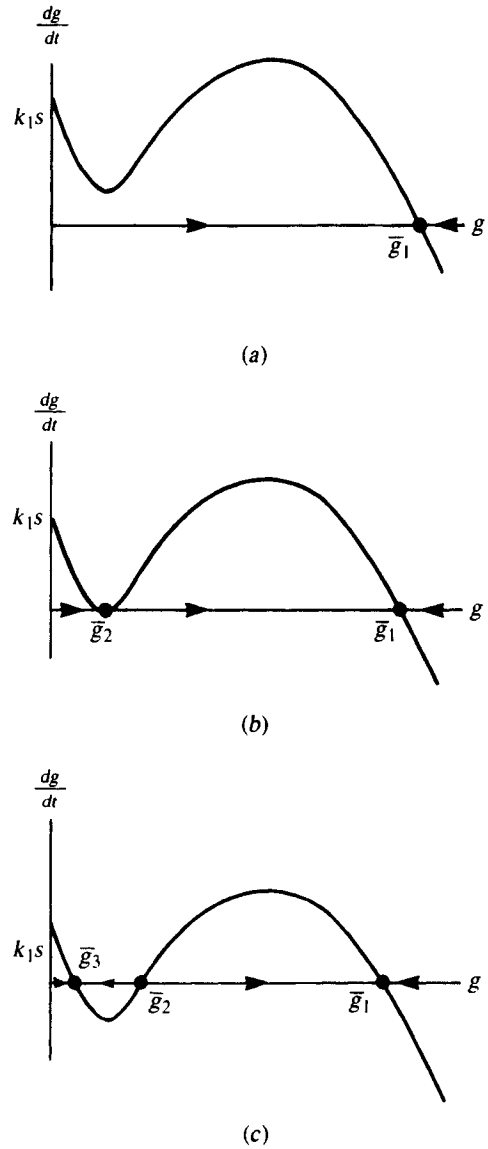


Figure 7.5 *Equation (28) describes two possible qualitative behaviors depending on the size of* $k_1 s$ *(on the level of signal). In (a) there is a single steady state* $\bar{g}_1$. *In (c) there are three steady states, two of which are stable. (b) marks the transition between these two regimes.*

Now we consider what this model implies about cellular differentiation. Suppose that initially all cells have no gene product, so $g = 0$. As the chemical gradient of S is established in the row of cells, there will be a continuous spatial transition from cases (*a*) to (*b*) to (*c*) (and all intermediate cases) across the length of the tissue. Close to the tail end of the row of cells, where s is very low, the kinetics of the gene product is given by Figure 7.5(*c*): the intercept $k_1 s$ is close to zero. Thus, since initially $g < \bar{g}_2$, the cell is led into steady state $\bar{g}_3$; that is, very low levels of gene product are formed. Somewhere along the row of cells a threshold level of s is present; there Figure 7.5(*b*) applies. The steady state $\bar{g}_3$ has disappeared. $\bar{g}_2$ and $\bar{g}_1$

are the only remaining steady states; the former is an ephemeral one, vanishing when s increases by the slightest amount. Thenceforth, in all cells beyond the transition point, gene product is synthesized up to a concentration $g = \bar{g}_1$, which is the unique steady state.

We see that the mechanism indeed captures the essence of a threshold switch. Another interesting feature noted by Lewis et al. (1977) is the memory built into the scheme: Once s is raised above threshold, the state of the cell changes permanently to $\bar{g}_1$. Even if s subsequently decreases, so that Figure 7.5(c) is obtained, the cell is "trapped" in $\bar{g}_1$ and will not return to its former state. The authors point out that a transient signal can thus be used to control discontinuous transitions in this developmental model as well as in other cellular processes.

7.6 SPECIES COMPETITION IN A CHEMICAL SETTING

A second example of biochemical control is discussed in this section. The model is presented here more to provoke your imagination and amuse you than to make a serious claim about cellular development. Perhaps as important as the message is the approach that differs from previous modeling in that a mechanism is *inferred* from an abstract set of equations.

Consider the following hypothetical situation. A cell can produce two types of chemical products X and Y. Under some circumstances it is advantageous to produce only one of the two products, while under other circumstances it is requisite to form both in a predetermined ratio. How is this to be achieved?

Suppose X and Y are components of some structural macromolecules. Figure 7.6 illustrates an artist's conception of how the *axis of polarity* of a two-dimensional cell could be determined by combining monomers of two types into longer structures. The scheme only works if the cell is "glued" into position with a permanently affixed immobile cornerstone on which macromolecular structures are built like scaffolds. The idea is then to combine appropriate ratios of X-type and Y-type bricks, thereby obtaining a structure whose orientation is given approximately by the vector (percent X type, percent Y type). Thus a way of controlling the polarity of the cell (whether it elongates horizontally, vertically, or in some other direction) is by controlling the relative percentages of X- and Y-type monomers that are synthesized inside the cell.

How cellular polarity is actually controlled is still an unsolved problem. Beautiful and exciting examples of the effects of polarity differentiation are often demonstrated in plant cells (which, incidentally are largely immobile, unlike the fluid-like cells of animals). A particularly striking pattern of horizontal-versus-vertical cellular orientation is exhibited in tree trunks and other woody parts of plants in the tissue called the *xylem* (which consists of cells that have died after differentiating). There one sees "islands" of horizontal radially directed cells (called *ray cells*) embedded in a sea of vertical cells (*tracheids*). (See Figure 7.7.) The remarkable point is that both cell types arise from rather similar precursors in the actively growing tissue called the *cambium*. There are virtually no cells that do not fall into this dichotomy, indicating that the control mechanism governing polarity commitment is rather strict.

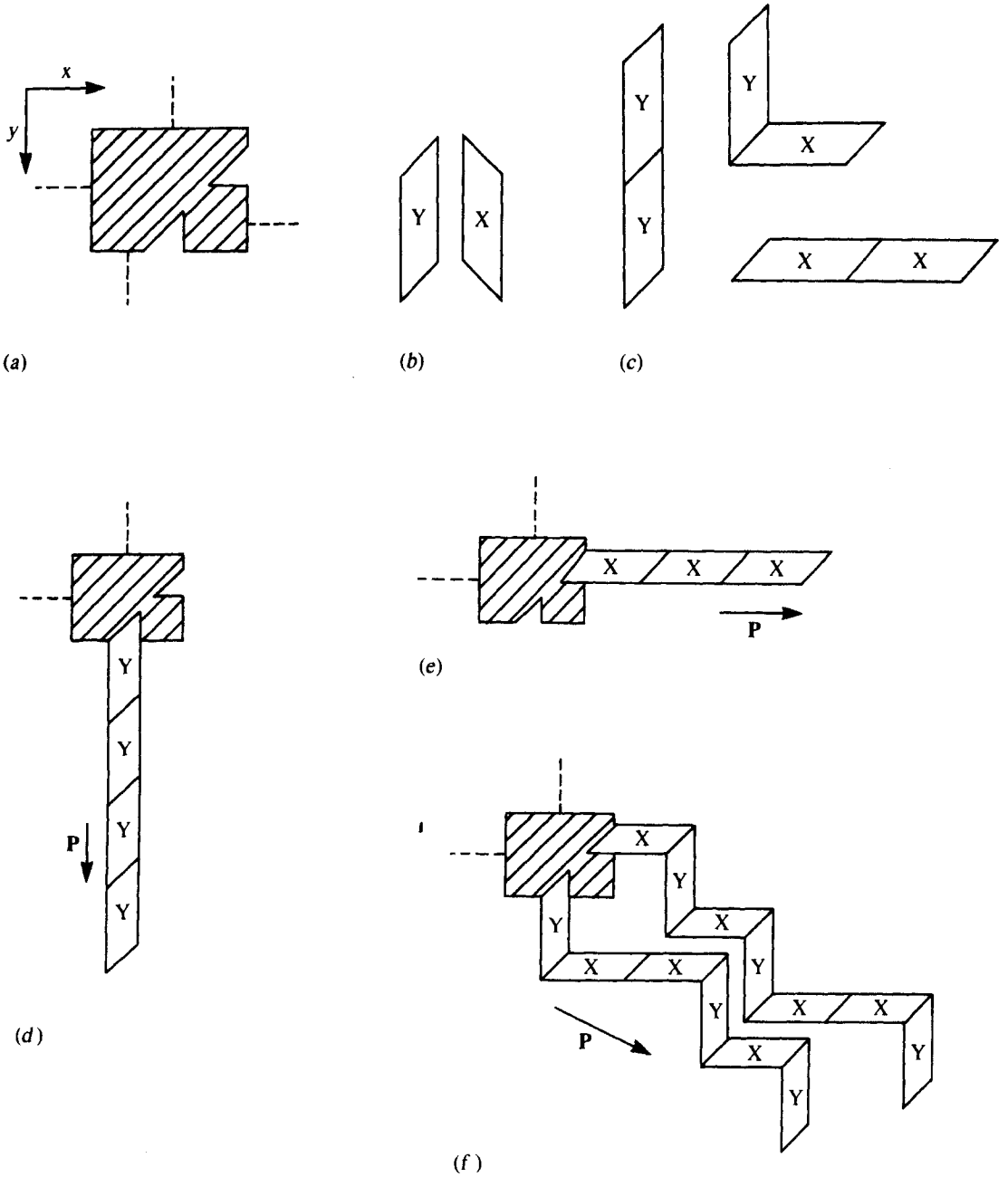


Figure 7.6 *The polarity of structures within the cell and hence the orientation of the cell could perhaps be governed by the relative proportions of monomers* X *and* Y *that are made. The cell must have (a) a fixed "cornerstone" and (b) synthesize* X *and* Y*, which can form complexes such as (c) dimers or larger polymers. (d) If only* Y *is made or (e) only* X*, the cell will have a horizontal or vertical polarity. (f) Intermediate orientations occur if both products are made in some relative proportion.*

Looking at examples like that of *rays* and *tracheids* in the xylem of plants leads us to feel that some sort of competition takes place within the precursor cell between the tendencies to promote horizontal and vertical characteristics. This intuition leads to the model that follows, though clearly many other approaches to the problem are possible.[4]

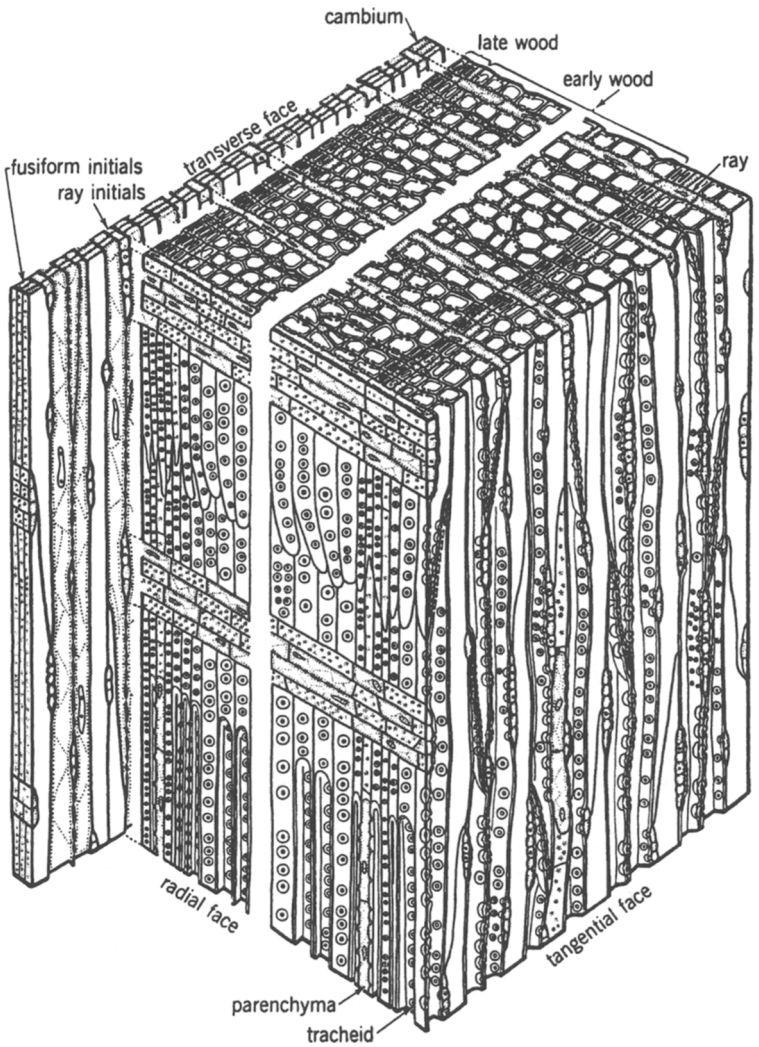


Figure 7.7 *Cells that are oriented precisely in the horizontal or vertical directions can be observed in the woody parts of higher plants. The question of how such polarity is determined led to the model given by equations (29a,b). This diagram shows the xylem of white cedar with its ray cells and tracheids. [From Esau, K. (1965). Plant Anatomy, Wiley, New York. Copyright © 1965 by K. Esau. Reprinted by permission of John Wiley & Sons, Inc.]*

4. The models of plant cell polarity were inspired through conversations with Tsvi Sachs of the Hebrew University, Israel.

At this point we pause for a pitch on the advantages of modeling. We are about to witness the fact that a mathematical abstraction permits us to make a connection between two seemingly unrelated phenomena that share similar dynamical properties. In this case the suspicion that competition between two forces or two chemical species might be involved leads us to recollect that a competition model in another context is already known to us. From Section 6.3 we borrow equations (9a,b):

$$\frac{dN_1}{dt} = r_1 N_1 \frac{\kappa_1 - N_1 - \beta_{12} N_2}{\kappa_1}, \qquad \textit{(9a, Chap. 6)}$$

$$\frac{dN_2}{dt} = r_2 N_2 \frac{\kappa_2 - N_2 - \beta_{21} N_1}{\kappa_2}. \qquad \textit{(9b, Chap. 6)}$$

These equations describe the populations of two animal species. One wonders to what extent they could pertain to chemical species, where reproduction, survivorship, or mortality have vague meanings if any. If we are to proceed with a reinterpretation, we must use a more general restatement of the equations. Accordingly, we replace variable names by x and y (concentrations of the two molecular species X and Y), use new parameters μ, α, γ and multiply the RHS expressions to get the following rejuvenated model:

$$\frac{dx}{dt} = \mu_1 x - \alpha_1 x^2 - \gamma_{12} xy, \qquad \textit{(29a)}$$

$$\frac{dy}{dt} = \mu_2 y - \alpha_2 y^2 - \gamma_{21} xy. \qquad \textit{(29b)}$$

Note that the new parameters are related to the old as follows: $\kappa_i = \mu_i/\alpha_i$ and $\kappa_i/\beta_{ij} = \mu_i/\gamma_{ij}$. The behavior of solutions to these equations falls into one of the four categories shown in Figure 6.6:

1. species Y always predominates.
2. species X always predominates.
3. X or Y predominates depending on initial conditions.
4. Stable coexistence of both species at some ratio.

Which case is obtained depends on the relative magnitudes of certain combinations of the parameters:

1. $\mu_2/\mu_1 > \gamma_{21}/\alpha_1$ and $\mu_2/\mu_1 > \alpha_2/\gamma_{12}$.
2. $\mu_2/\mu_1 < \gamma_{21}/\alpha_1$ and $\mu_2/\mu_1 < \alpha_2/\gamma_{12}$.
3. $\mu_2/\mu_1 < \gamma_{21}/\alpha_1$ and $\mu_2/\mu_1 > \alpha_2/\gamma_{12}$.
4. $\mu_2/\mu_1 > \gamma_{21}/\alpha_1$ and $\mu_2/\mu_1 < \alpha_2/\gamma_{12}$.

On the face of it, the equations can potentially describe the very phenomenon that we are attempting to understand in this section, namely a mechanism of controlling synthesis of species at some relative proportions. However, in order to reap some benefit from this conclusion we might wish for some kind of molecular interpretation

for terms in equations (29a,b). Since the control of synthesis of large molecules ultimately resides within the genome, a suitable interpretation would be to view these terms as effects on the genetic material that codes for species X and Y.

A positive feature of equations (29a,b) that was not readily apparent in their previous form is that the quadratic terms they contain are rather familiar mass-action terms for interactions of pairs of molecules (X with X, Y with Y, or X with Y). This suggests the following intriguing hypothesis.

Suppose the X and Y molecules can form dimers such as X—X, X—Y, and Y—Y in some rapidly equilibrating reversible reaction. In this case, the cellular concentration of such dimers would be approximately proportional to the products of concentrations of the participating monomers (see problem 12). Precisely such terms appear in equations (29a,b) accompanied by minus signs. This suggests that these dimers tend to inhibit the production of the substances X and/or Y (or possibly activate enzymes that degrade these chemicals).

We can go further in putting together the puzzle by interpreting a complete molecular mechanism as follows:

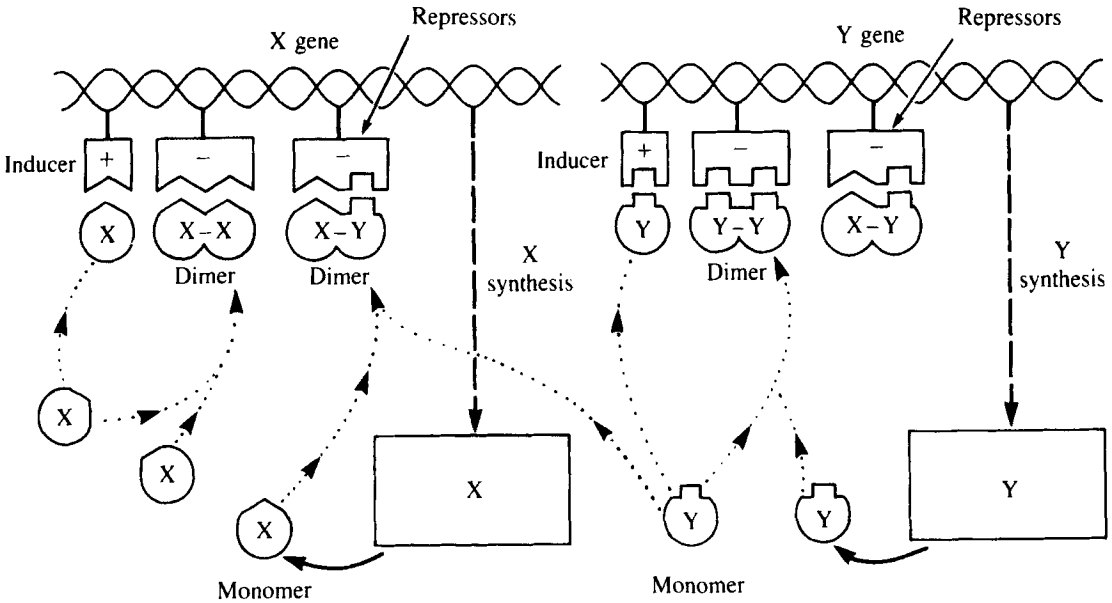
1. Each monomer activates its own gene. (Witness the positive contributions of terms $\mu_1 x$ and $\mu_2 y$ in the equations.)
2. Dimers made up of identical monomers (X—X and Y—Y) repress only the gene that codes for that particular molecule.
3. Mixed dimers (X—Y) repress both the X gene and the Y gene.

See Figure 7.8(*a*) for a schematic view of these events.

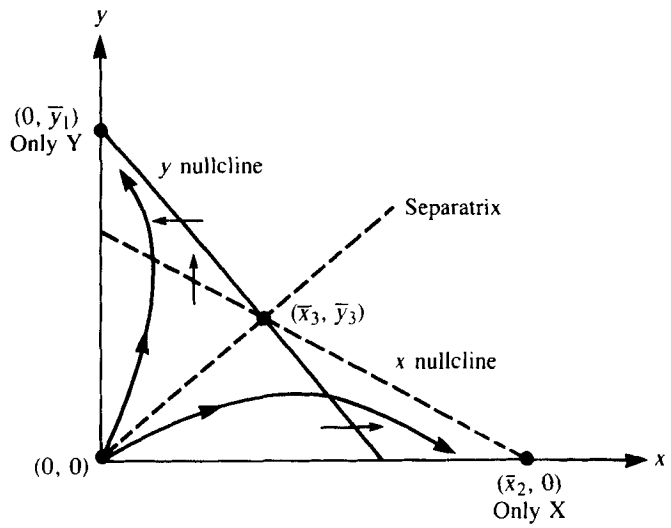
We have seen earlier that with the appropriate relations between the various rate constants this regulatory mechanism would select for the synthesis of a single product or some proportion of both products. What do such rate constants represent? Previous analysis in this chapter demonstrates that rate constants are often ratios or more complicated combinations of parameters that depict forward or reverse reaction rates. Loosely speaking, the constants appearing in equations (29a,b) may depict affinities of molecules for each other (as for dimers) or for regions of the genome that control synthesis of the products X and Y.

Since it is known that slight changes in molecular conformations can alter such affinities, it is reasonable to think of cells as having a whole range of permissible values of $(\mu_i, \alpha_i, \gamma_{ij})$. Some values would lead to all-or-none behavior, while others would govern the relative frequency of X and Y synthesis. What makes this fact intriguing is that we can envision a *developmental pathway*, in which a cell changes its character throughout various stages of its cycle to meet various needs. This could be accomplished by a gradual variation of one or several rate constants. (See problem 13.)

For example, if $\mu_2/\mu_1 < \gamma_{21}/\alpha_1$ and $\mu_2/\mu_1 > \alpha_2/\gamma_{12}$ (case 3) the cell would have the dynamical behavior shown in Figure 7.8(*b*): given any initial concentrations (x_0, y_0) the final outcome would be synthesis of only X or only Y depending on which gene gets more strongly repressed. This in turn depends on whether (x_0, y_0) falls above or below a *separatrix* in the xy plane, a curve that subdivides the positive



(*a*)



(*b*)

Figure 7.8 *(a) The model given by equations (29a,b) could be interpreted in terms of a molecular mechanism for genetic control. Shown are the two genes coding for* X *and* Y *molecules. Repressors on these genes are sensitive to concentrations of the dimers* X—X, Y—Y, *and* X—Y. *Inducers are sensitive to monomer concentrations of* X *or* Y. *(b) Provided condition (3) is satisfied by the parameters (see text), the dynamic behavior of equations (29a,b) resembles that of a switch. There are two possible outcomes (only* X *or only* Y *synthesized), depending on the initial concentrations* (x, y). *Note that the phase plane is divided into two domains by a curve called a separatrix. Points in a given domain are attracted to one of the two steady states on the axes. The steady state in the positive xy quadrant is a saddle point.*

xy quadrant into two separate *basins of attraction* [see Figure 7.8(b)]. Should the parameters change so that one or both of the above inequalities is reversed, the outcome would be independent of the initial concentrations.

Within the context of cellular polarity, we see that depending on molecular affinities and initial conditions, the (percent X type, percent Y type) structural molecules in the cell could so evolve that the cell is eventually polarized entirely in the x or in the y direction. (Alternately, in case 4 the cell could attain some intermediate orientation governed by the coordinates $(\bar{x}_3, \bar{y}_3)$ of the steady state in the positive quadrant.)

The problem of control of relative proportions of biosynthesis is clearly of much wider applicability. To give but one other related example, consider the events underlying synthesis of an enzyme, lactate dehydrogenase (LDH). It is known that a single enzyme molecule is comprised of four subunits. Subunits come in two varieties, A and B, and any of the following five combinations can occur:

LDH-1: BBBB, LDH-4: AAAB,
LDH-2: ABBB, LDH-5: AAAA.
LDH-3: AABB,

It is interesting to learn that in certain cells in the body (for example, mouse kidney cells) there is a progressive shift from production of LDH-5 to LDH-1 from birth to adulthood. This may imply that biosynthesis gradually changes from production of all B subunits to certain proportions of B to A and finally to all A subunits. There is no indication that the genetic mechanism is related to the simple scheme discussed in this section. However, we nevertheless observe that such developmental transitions could stem from gradual parameter changes in some underlying system of molecular interactions.

What do we gain from this modeling exercise? First and foremost is an appreciation of the fact that mathematical equations are abstract statements that may have applications to more than one area. But can we really believe that the simple mechanism proposed on the basis of the species-competition equations could be at work inside a cell? Here we must take some care, for even appealing analogies such as the one used here may be false or inaccurate. Ultimately the test lies in empirical evidence for or against a given hypothesis. You may wish to consult Edelstein (1982) for indications of possible empirical tests of the model just discussed.

The two models examined in these sections were obtained in a way different from previous examples; here a dynamical behavior was known *a priori*. The behavior was reminiscent of solutions to previous equations that derived from quite different contexts. These equations were then rewritten and reinterpreted, leading to new suggestions for underlying mechanisms. This approach cannot be expected to work in every case, but it is of surprising value when it does. One is led to feel that some control mechanisms are universal, reappearing in the contexts of ecology, engineering, molecular biology, and other systems. This observation underscores the power and generality of mathematical modeling that directly illuminates the connection between such diverse problems.

In the next section we regard one of the four cases discussed here somewhat more abstractly and identify the particular attributes that generate switch-like behavior. For further examples of this abstract modeling approach, a good source is Rosen (1970, 1972). For more about the versatility of biochemical control, consult Savageau (1976).

7.7 A BIMOLECULAR SWITCH

In this section we examine case 3 of equations (29a,b) and explore what general assumptions suffice to produce similar dynamic behavior in other systems. Recall in case 3 that whether x or y predominates depends only on initial concentrations of X and Y. In the xy-plane shown in Figure 7.8(b), the first quadrant is divided into two regimes of influence; in one, all starting points head towards $(0, \bar{y}_1)$, whereas in the other the attractor is $(\bar{x}_2, 0)$. ($\bar{x}_2$ and $\bar{y}_1$ stand for the steady state values on the two axes). A system that behaves in this way can be described as a *switch*, a means for sorting an initial situation into one of two possible outcomes.

Since such mechanisms can have potential aplications to other physical phenomena, we shall extract the features of equations (29a,b) that lead to this behavior.

From Figure 7.8(b) it is evident that a rather general property of the switch is that the positions of steady states meet the following conditions:

1. There are four steady states: one at (0, 0), two on the axes, and one in the first quadrant at $(\bar{x}_3, \bar{y}_3)$.
2. (0, 0) is an unstable node.
3. $(\bar{x}_3, \bar{y}_3)$ is a saddle point.

Below we restrict attention to equations of the form

$$\dot{x} = xf(x, y) \tag{30a}$$

$$\dot{y} = yg(x, y). \tag{30b}$$

These will satisfy condition 1 provided there are values $\bar{x}_3$, and $\bar{y}_3$ such that

$$f(\bar{x}_3, \bar{y}_3) = g(\bar{x}_3, \bar{y}_3) = 0 \qquad (x^*, y^* > 0). \tag{31}$$

The proof of this assertion is left as a problem. We note that the fact that $(\bar{x}_3, \bar{y}_3)$ is in the first quadrant is equivalent to assuming that the nullclines $f = 0$ and $g = 0$ intersect in this quadrant.

To satisfy condition 2 we need to assume that $f(0, 0)$ and $g(0, 0)$ are both positive. (See problem 14.)

To satisfy condition 3 it is necessary that $(f_x/f_y)|_{ss} < (g_x/g_y)|_{ss}$ where f_x, f_y, g_x, and g_y are partial derivatives of f and g evaluated at $(\bar{x}_3, \bar{y}_3)$. This condition is rather interesting. It can be obtained by either one of two reasoning processes. A straightforward calculation of the Jacobian of (30) at $(\bar{x}_3, \bar{y}_3)$ reveals that

$$\det \mathbf{J} = \bar{x}\bar{y}(f_x g_y - f_y g_x). \tag{32}$$

(See problem 14.) Requiring that $\det \mathbf{J} < 0$ leads to the above inequality.

A more novel approach is to use geometric reasoning. To obtain case 3 the nullclines have to be so situated that the y nullcline $g(x, y) = 0$ is more steeply inclined than the x nullcline $f(x, y) = 0$ at their intersection $(\bar{x}_3, \bar{y}_3)$ such that both have *negative* slopes.

Using *implicit differentiation* below, we arrive at the following results:

$$x \text{ nullcline:} \qquad f(x, y) = 0, \tag{33a}$$

$$f_x + f_y \frac{dy}{dx} = 0, \tag{33b}$$

$$\text{slope of } x \text{ nullcline:} \qquad \frac{dy}{dx} = -\frac{f_x}{f_y} < 0, \tag{33c}$$

$$y \text{ nullcline:} \qquad g(x, y) = 0, \tag{34a}$$

$$g_x + g_y \frac{dy}{dx} = 0, \tag{34b}$$

$$\text{slope of } y \text{ nullcline:} \qquad \frac{dy}{dx} = -\frac{g_x}{g_y} < 0. \tag{34c}$$

The slopes must satisfy the relation $f_x/f_y < g_x/g_y$, establishing the result. In the alternate form $(f_x g_y < f_y g_x)$, we can interpret this result as follows. The product of the effect of each species on its own growth rate should be smaller than the product of the cross effects of species i on species j.

We see now that more general kinetic expressions can also be used to construct a switching mechanism. [See problem 14(d).] Another rather nice example of a switching mechanism is given by Thornley (1976) for the biochemistry of flower initiation.

The abstract way in which we studied properties of the nullclines of equations (30) can be used for other general questions. In the next section a similar approach will be used to derive geometric conditions for stability of interacting chemical species.

7.8 STABILITY IN ACTIVATOR-INHIBITOR AND POSITIVE FEEDBACK SYSTEMS

Systems in which only qualitative properties of the interactions are known were discussed in Section 6.5. We now investigate a pair of chemical systems that have particular qualitative sign patterns (and associated graphs shown in Figure 7.9). The analysis is of interest for two reasons. First, it illustrates a method for understanding stability in a geometric way. Second, the results will be of relevance to material discussed in Chapter 11, where activator-inhibitor and positive-feedback systems are of special importance.

The systems considered here each consist of two chemicals, with mutual effects depicted by the following sign patterns:

1. Activator-inhibitor system: $\mathbf{Q}_1 = \begin{bmatrix} + & - \\ + & - \end{bmatrix}$, *(35a)*

2. Positive feedback system: $\mathbf{Q}_2 = \begin{bmatrix} - & - \\ + & + \end{bmatrix}$. *(35b)*

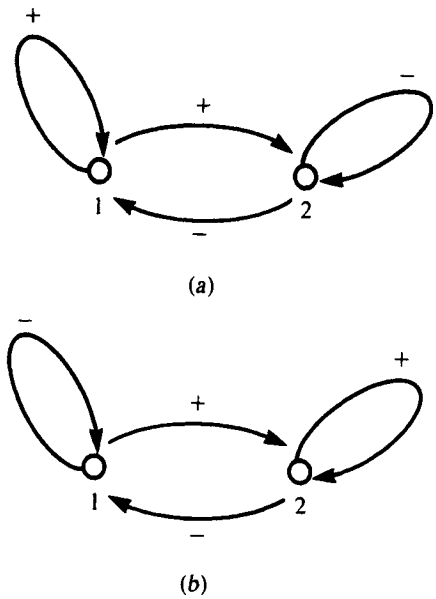


Figure 7.9 *Signed directed graphs for (a) the activator-inhibitor and (b) positive feedback systems discussed in Section 7.8.*

To be more explicit, $\mathbf{Q}_1$ and $\mathbf{Q}_2$ are sign patterns of elements in the Jacobian of each system, evaluated at some steady state. In equation (35a) the distribution of signs implies that chemical 1 has a positive effect on its own synthesis and on the synthesis of chemical 2, whereas chemical 2 inhibits the formation of both substances. For this reason, chemical 1 is termed the *activator* and chemical 2 the *inhibitor*. (Notice that this is a molecular analog of an ecological predator-prey pair.) In equation (35b) either participant promotes increase in the second chemical and decrease in the first. The term *positive feedback system* has a historical source and should not be taken too literally since, in fact, both (35a) and (35b) have positive as well as negative feedback loops.

Indeed from Figure 7.9 it is evident that neither system is qualitatively stable in the sense discussed in Section 6.5 because of the positive feedback loops on one of the participating species. Stability thus depends on other constraints, to be reviewed presently. Rather than merely restating these in terms of the coefficients in the Jacobian, we will derive analogous conditions on the intersection properties of the nullclines. Thus, let us momentarily suppose that we have functional expressions for the kinetic terms and work backwards. We deal first with (35a) and then with (35b).

The Activator-Inhibitor System

Suppose the kinetics of chemical interactions are governed by the rate laws

$$\frac{dx}{dt} = f_1(x, y), \tag{36a}$$

$$\frac{dy}{dt} = f_2(x, y), \tag{36b}$$

and that $(\bar{x}, \bar{y})$ is a nontrivial steady state of this system. In the xy phase plane, nullclines for x and y would then be those curves for which

$$f_1(x, y) = 0 \qquad (x \text{ nullcline}), \tag{37a}$$

$$f_2(x, y) = 0 \qquad (y \text{ nullcline}), \tag{37b}$$

and $(\bar{x}, \bar{y})$ would be a point of intersection of these curves. We now resort to implicit differentiation to draw conclusions about the slope of the nullclines at $(\bar{x}, \bar{y})$.

First note that, after differentiating both sides of the equations with respect to x, we arrive at

$$\frac{\partial f_1}{\partial x} + \frac{\partial f_1}{\partial y}\left(\frac{dy}{dx}\right)_1 = 0, \tag{38a}$$

$$\frac{\partial f_2}{\partial x} + \frac{\partial f_2}{\partial y}\left(\frac{dy}{dx}\right)_2 = 0. \tag{38b}$$

Here $(dy/dx)_1$ means "slope of the nullcline $f_1 = 0$ at some point P." Similarly $(dy/dx)_2$ is the slope of $f_2 = 0$ at some point P.

We must now use information specified in the problem, namely that the sign pattern in equation (35a) determines the signs of elements of the Jacobian. Since these elements are precisely partial derivatives evaluated at $(\bar{x}, \bar{y})$, we use this fact in deducing that

$$\left.\frac{\partial f_1}{\partial x}\right|_{ss} = a, \qquad \left.\frac{\partial f_1}{\partial y}\right|_{ss} = -b, \qquad \left.\frac{\partial f_2}{\partial x}\right|_{ss} = c. \qquad \left.\frac{\partial f_2}{\partial y}\right|_{ss} = -d \tag{39}$$

where a, b, c, and d are some positive constants.

Define the quantities

$$s_1 = \left.\left(\frac{dy}{dx}\right)_1\right|_{(\bar{x},\bar{y})}, \tag{40a}$$

$$s_2 = \left.\left(\frac{dy}{dx}\right)_2\right|_{(\bar{x},\bar{y})}. \tag{40b}$$

Then, as mentioned above, s_1 and s_2 are slopes of the two nullclines at their intersection, $(\bar{x}, \bar{y})$. Equations (38a,b) can now be written in terms of the new quantities as follows:

$$a - bs_1 = 0 \tag{41a}$$

$$c - ds_2 = 0 \tag{41b}$$

This implies that $a = bs_1$ and $c = ds_2$. Thus the Jacobian of equations (36a,b) can be written in two ways:

$$\mathbf{J} = \begin{pmatrix} a & -b \\ c & -d \end{pmatrix} = \begin{pmatrix} bs_1 & -b \\ ds_2 & -d \end{pmatrix}. \tag{42}$$

Now we may determine when $(\bar{x}, \bar{y})$ is stable. The conditions are that

$$\beta = \operatorname{Tr} \mathbf{J} = bs_1 - d < 0 \qquad \Rightarrow s_1 < d/b, \tag{43a}$$

and

$$\gamma = \det \mathbf{J} = -dbs_1 + dbs_2 > 0 \Rightarrow s_2 > s_1. \tag{43b}$$

We note from equations (41a,b) that s_1 and s_2 must both be positive since a, b, c, and d are assumed to be positive. Thus stability implies that at $(\bar{x}, \bar{y})$ the y nullcine will be more steeply sloped than the x nullcline. Figure 7.10 illustrates how these conclusions affect the *local* geometry of the phase plane. Further discussion of this case is suggested in problem 15.

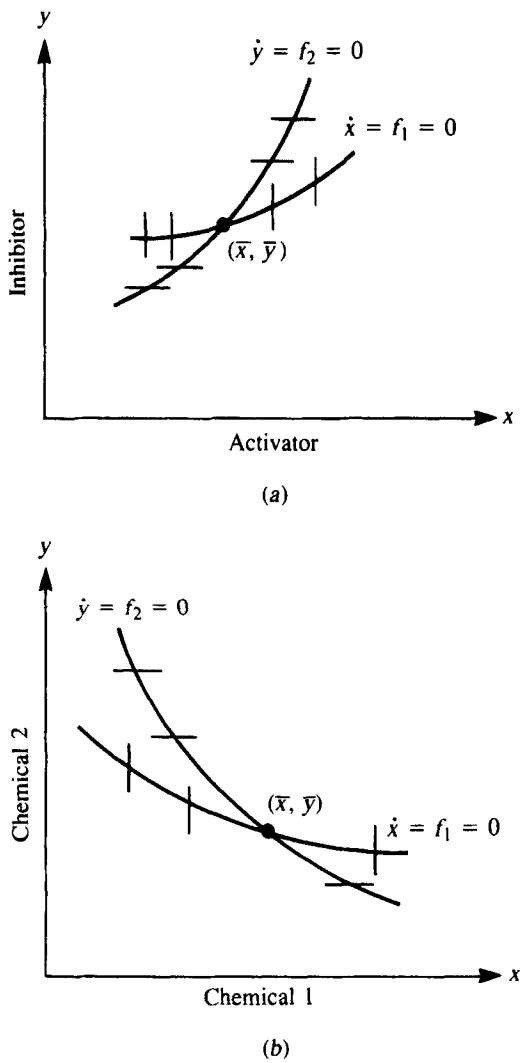


Figure 7.10 *(a) In an activator-inhibitor system, a steady state* $(\bar{x}, \bar{y})$ *is stable only if the inhibitor nullcline* $(\dot{y} = 0)$ *is steeper than the activator nullcline* $(\dot{x} = 0)$ *with both curves having positive slopes. It is further necessary that the slope of* $\dot{x} = 0$ *at the steady state be not too steep, that is, less than* d/b*; see equation (43a). (b) In a positive-feedback system the two nullclines must have negative slopes such that* $\dot{y} = 0$ *is steeper than* $\dot{x} = 0$ *for stability of* $(\bar{x}, \bar{y})$.

Positive Feedback

We proceed in a similar way in dealing with the second case, which is left largely as an exercise. Now, however, we define

$$\left.\frac{\partial f_1}{\partial x}\right|_{ss} = -a, \quad \left.\frac{\partial f_1}{\partial y}\right|_{ss} = -b, \quad \left.\frac{\partial f_2}{\partial x}\right|_{ss} = c, \quad \left.\frac{\partial f_2}{\partial y}\right|_{ss} = \mathrm{d}, \qquad (44)$$

where a, b, c and d are again positive constants. In problem 16 you are asked to verify that this method, with s_1, and s_2 defined as before, leads to the conclusion that both s_1 and s_2 are negative, such that

$$s_2 < s_1 < \frac{-d}{b}. \tag{45}$$

That is, s_2 is "more negative" than s_1, so that the y nullcline is again steeper than the x nullcline, but now both have negative slopes. Figure 7.10(b) shows what this implies about the local geometry of the nullclines in the positive feedback case.

Based on these properties, it was proved by Kadas (1982) that *a two-species reaction mechanism with a monotonic nullcline cannot have steady states of both types* (*35a*) and (*35b*). This allows one to classify the mechanisms as activator-inhibitor or positive-feedback in a broader sense, even though their properties are only known locally (close to their steady-state levels).

7.9 SOME EXTENSIONS AND SUGGESTIONS FOR FURTHER STUDY

In this section we outline several alternate approaches to molecular systems, some of which are longstanding and others more recent. References for independent exploration are suggested.

1. Summaries of enzyme action and of the pertinent mathematical methods appear in the encyclopedic book by Dixon and Webb (1979). Reiner (1969), and Boyer (1970) are much shorter. Numerous special cases, such as *multivalent enzymes, product inhibition, allosteric effects,* and *endogenous activators* are discussed and accompanied by standard kinetic analysis. The chief visual device used in studying such systems is graphs of the reaction rate v plotted against concentration c of one of the chemical participants. (The reaction rate is a measure of the disappearance of substrate or appearance of product; for example, $v = dc/dt$; see Figure 7.3 for example.) Alternative graphical constructions include the Lineweaver-Burk plot, which is simply a graph of $1/v$ versus $1/c$. Such graphs have conventionally been used to identify rate constants in chemical kinetic studies and as a convenient way of summarizing and comparing enzyme systems.
2. *Understanding large chemical systems from network structure.* Most biochemical pathways contain a large number of intermediates that react and affect each other in complex chemical networks. Mathematical analysis of such chemical systems by standard methods is impractical, yet one is often interested in addressing fairly general and important questions, such as:
   (a) Does the system admit steady-state solutions? (Are there *multiple* steady states?)
   (b) Are such steady states stable?
   (c) Can such systems admit temporal oscillations of chemical concentrations?

   In a series of papers, Feinberg (1977, 1980, in press) has addressed such questions by methods that utilize the structure of the chemical network rather

than the differential equations that correspond to the chemical kinetics. The approach consists of defining three integers, n, l, and s, which represent respectively the number of entities appearing in the network, the extent of linkage of the network, and the span of a vectorspace defined by assigning a vector to each of the reactions. The integer $\delta = n - \ell - s$, called the *deficiency* of the network, is then indicative of the expected dynamics. For example, in a network in which all reactions are reversible, if $\delta = 0$, then there is always a single (strictly positive) steady state that is stable and *no* oscillatory solutions exist. (See references for details of the definitions and stronger statements of these results.) The Feinberg network method is unfortunately not yet general enough to lead to strong conclusions in every case. However, where applicable it is a valuable and computationally inexpensive technique.

Papers given in the references are expository and would be accessible to students who have a minimal background in linear algebra. (It is, for example, necessary to be able to find the *rank* of a matrix in computing the integer s.)

3. A brief but thorough summary of enzyme kinetics that gives most of the technical highlights is to be found in Rubinow (1975). This source demonstrates a somewhat different application of graph theory (based on Volkenshtein, 1969); here the goal is to derive expressions for the reaction velocity of a chemical system. Using the quasi-steady-state assumption, this problem is essentially one of solving a system of linear algebraic equations. When the network is large, the corresponding system of algebraic equations can be rather cumbersome. However, by invoking certain rules, it is possible to simplify the network (for example, by adding parallel branches and merging nodes in a particular way) and so deduce a relation between the reaction velocity v and the concentrations and kinetic rate constants in the pathway without solving a complicated system of algebraic equations. You should recognize that this method does not address questions regarding the dynamic behavior of a chemical reaction scheme under general conditions since the quasi-steady-state assumption underlies the method. Rubinow (1975) gives details and several worked-out examples.

4. A contemporary approach to the analysis of biochemical systems has been described by Rosen (1970, 1972) and Savageau (1976) and comes under the general heading "biochemical systems analysis." An important point their work addresses is that enzymes are not only catalysts of reactions but also the control elements that can be modulated by a variety of influences. It is commonplace in biochemical pathways to encounter examples of feedback control. End products of the reaction may directly affect the catalysis of a key enzyme by attaching to it and changing its physical conformation. This leads to changes in the biological function of the enzyme and may result in total inhibition of further product synthesis.

Savageau (1976) compares the design of a number of biochemical and genetic control pathways, summarized by reaction diagrams that convey the sequence of products and their feedback control. (This systems approach appears in Rosen, 1970, 1972.) An interesting feature Savageau then explores

is comparison of alternative control patterns, in which control is exerted at a variety of nodes and by a variety of intermediates. In the cases where the number of intermediates is small (for example, $n = 3$), explicit stability analysis is carried out. Certain networks lead to more stable interactions than others. (For example, a simple end-product inhibition in which the last product inhibits the first reaction step has a steady state that can more readily be destabilized by parameter variations than can a system of the same size with another pattern of feedback interactions.) Using such analysis, Savageau addresses the question of optimality of design in assessing whether real biochemical networks have advantages over other possible networks less commonly encountered. Detailed discussions of stability, Routh-Hurwitz tests, and many interesting examples make Savageau's book a good source for further study.

Rapp (1979) gives a control-theory approach to metabolic regulation. His paper, suitable for advanced students, contains numerous interesting examples, a clear discussion of techniques, and a thorough bibliography.

PROBLEMS*

The following questions pertain to Michaelis-Menten kinetics.

1. Verify that equations (6a,b) are obtained by eliminating x_0 from equations (3a,c).

2. Show that when the quasi-steady-state assumption is made for x_1 in equations (6a,b) one can algebraically simplify the model with the following procedure:
   (a) First write x_1 in terms of an expression involving only c.
   (b) Substitute this expression into equation (6a) and simplify to obtain equation (8).

3. Show that equations (6a,b) can be reduced to the dimensionless equations (10a,b) by the choice of reference scales given by equations (9a–c). Interpret the meanings of τ, ϵ, κ, and λ. Verify that the equations can be written in the form (11a,b).

4. **(a)** Integrate equation (12a) to obtain an implicit solution (an expression linking the variables c and t.) Use the fact that at $t = 0$, $c = c_0$ to eliminate the constant of integration.
   (b) Use equation (12b) and the fact that [by (12a)] $c(t) \to 0$ to reason that $x(t) \to 0$. (*Hint:* Consider $\lim_{c \to 0} c/[k + c]$.)

5. Show that equations (6a,b) can be reduced to the second dimensionless equations (14a,b) by choosing the reference time scale of equation (13).

*Problems preceded by an asterisk are especially challenging.

6. Integrate equation (15b). Is $x_1(t)$ an increasing or a decreasing function of time on this time scale? (Note: You should use the initial conditions described in the text to get a meaningful solution.)

***7.** Equations (6a,b) can be studied by phase-plane methods.

(a) Show that c and x_1 nullclines have the form

$$\dot{c} = 0 \qquad \text{when} \qquad x_1 = \frac{Kc}{a + c},$$

$$\dot{x}_1 = 0 \qquad \text{when} \qquad x_1 = \frac{Kc}{b + c}.$$

Identify K, a, and b in terms of the original parameters, r_0, k_1, k_{-1}, and k_2. Which is larger, a or b?

(b) Sketch the nullclines in an x_1c phase plane. One point of intersection is $x_1 = c = 0$. Is there another? Determine directions of flow along these curves and along the c and x_1 axes.

(c) Draw a trajectory beginning at the state in which all receptors are unoccupied and the initial nutrient concentration is c_0. What is the eventual outcome? Explain your result in terms of the original cellular process.

(d) Which portions of your trajectory correspond to the initial fast transition and which to the gradual slow decline shown in Figure 7.2?

In problems 8 – 11 we discuss details of the derivation and implications of sigmoidal kinetics.

8. Write down a complete set of equations for the reaction diagram (16). [The first two equations are given in (17a,b).] Show that equation (18) is obtained by making a quasi-steady-state assumption.

9. **(a)** Verify that the relations (22a,b) are obtained when we assume that $dx_2/dt \simeq 0$ and $dx_0/dt \simeq 0$.

(b) Demonstrate that equation (23) is obtained by eliminating all variables except c and x_0 in equation (20f).

(c) Show that this leads to equation (24) for c.

10. Find examples of other positively cooperative biochemical reactions and describe their kinetics.

11. Determine how a graph of the kinetics given by equation (24) compares with that for equation (18).

12. Suppose A and B are monomers that undergo dimerization in a rapidly equilibrating reaction:

$$\text{A} + \text{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{A—B}]$$

Show that the concentration of dimers is proportional to the product of the monomer concentrations.

13. Discuss what gradual changes in the rate constants appearing in equations (29a,b) would lead to the following developmental process:

(a) A cell that initially produces only product x will eventually produce only y.

(b) A cell that initially produces some fixed ratio of product x to product y eventually produces either x or y but not both.

14. **(a)** Suggest why it is reasonable to assume equations of the form (30) in Section 7.7.

(b) Show that to satisfy condition 2 we must assume that $f(0, 0)$ and $g(0, 0)$ are both positive.

(c) Show that the determinant of the Jacobian of equations (30a,b) at the steady state $(\bar{x}, \bar{y})$ is given by equation (32).

(d) Suggest other reaction mechanisms that would give dynamic behavior like that of the biochemical switch discussed in Section 7.6. Interpret your model(s) biochemically.

***15.** *Stability in an activator-inhibitor system.* From the information given in Section 7.8 can one deduce the directions of arrows on the nullclines shown in Figure (10a,b)? Is the result unique, or are there several possibilities?

16. *Stability in a positive-feedback system*

(a) With the definitions given in equation (44), use implicit differentiation along the nullclines to show that

$$a = -bs_1 \qquad \text{and} \qquad c = -ds_2.$$

(b) What is the Jacobian matrix in terms of b, d, s_1, and s_2?

(c) Use stability conditions to verify equation (45).

17. The following chemical reaction mechanism was studied by Lotka in 1920 and later in 1956:

$$\begin{aligned} A + X &\xrightarrow{k_1} 2X \\ X + Y &\xrightarrow{k_2} 2Y \\ Y &\xrightarrow{k_3} B \end{aligned}$$

Assume that A and B are kept at a constant concentration.

(a) Write a set of equations for the concentrations of X and Y using the law of mass action. Suggest a dimensionless form of the equations.

(b) Show that there are two steady states, and use the methods of Chapter 5 to demonstrate that Lotka's system has oscillatory solutions. Compare with the Lotka-Volterra predator-prey system.

18. A system of three chemical species has a steady state $\bar{x}, \bar{y}, \bar{z}$. The Jacobian of the system at steady state is

$$\mathbf{J} = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & 0 & 0 \\ a_{31} & 0 & a_{33} \end{pmatrix}.$$

Magnitudes of a_{ij} are not known. It is known that a_{11}, a_{33}, a_{21}, and a_{13} are negative, while a_{12}, and a_{31} are positive. Is the steady state stable or unstable?

19. *The glycolytic oscillator.* A biochemical reaction that is ubiquitous in metabolic systems contains the following sequence of steps:

$$\text{glucose} \rightarrow \text{GGP} \rightarrow \text{FGP} \xrightarrow[\text{ATP}\ \curvearrowright\ \text{ADP}]{\text{PFK}} \text{FDP} \rightarrow \text{products},$$

where

GGP = glucose-6-phosphate,
FGP = fructose-6-phosphate,
FDP = fructose-1,6-diphosphate,
ATP = adenosine triphosphate,
ADP = adenosine diphosphate,
PFK = phosphofructokinase.

An assumption generally made is that the enzyme phosphofructokinase has two states, one of which has a higher activity. ADP stimulates this allosteric regulatory enzyme and produces the more active form. Thus a product of the reaction step mediated by PFK *enhances* the rate of reaction. A schematic version of the kinetics is

$$\text{substrates} \rightarrow \text{FGP} \overset{+}{\dashrightarrow} \text{ADP} \rightarrow \text{products}$$

Equations for this system, where x stands for FGP and y for ADP, are as follows:

$$\frac{dx}{dt} = \delta - kx - xy^2,$$

$$\frac{dy}{dt} = kx + xy^2 - y.$$

These equations, derived in many sources (see references), are known to have stable oscillations as well as other interesting properties.

(a) Show that the steady state of these equations is

$$(\bar{x}, \bar{y}) = \left(\frac{\delta}{(k + \delta^2)}, \delta\right).$$

(b) Find the Jacobian of the glycolytic oscillator equations at the above steady state.

(c) Find conditions on the parameters so that the system is a positive-feedback system as described in Section 7.8.

20. *The Brusselator.* This hypothetical system was first proposed by a group working in Brussels [see Prigogine and Lefever (1968)] in connection with spatially nonuniform chemical patterns. Because certain steps involve trimolecular reactions, it is not a model of any real chemical system but rather a prototype that has been studied extensively. The reaction steps are

$$A \rightarrow X,$$
$$B + X \rightarrow Y + D,$$
$$2X + Y \rightarrow 3X,$$
$$X \rightarrow E.$$

It is assumed that concentrations of A, B, D, and E are kept artificially constant so that only X and Y vary with time.

(a) Show that if all rate constants are chosen appropriately, the equations describing a Brusselator are:

$$\frac{dx}{dt} = A - (B + 1)x + x^2y,$$
$$\frac{dy}{dt} = Bx - x^2y.$$

(b) Find the steady state.

(c) Calculate the Jacobian and show that if $B > 1$, the Brusselator is a positive-feedback system as described in Section 7.8.

21. *An activator-inhibitor system (Gierer-Meinhardt).* A system also studied in connection with spatial patterns (see Chapter 10) consists of two substances. The activator enhances its own synthesis as well as that of the inhibitor. The inhibitor causes the formation of both substances to decline. Several versions have been studied, among them is the following system:

$$\frac{dx}{dt} = \rho + \frac{x^2}{y} - x,$$
$$\frac{dy}{dt} = x^2 - \gamma y.$$

(a) Find the steady state for this system.

(b) Show that if the input of activator is sufficiently small compared to the decay rate of inhibitor, the system is an activator-inhibitor system as described in Section 7.8.

(c) In a modified version of these equations the term x^2/y is replaced by $x^2/y(1 + kx^2)$. Suggest what sort of chemical interactions may be occurring in this and in the original system.

(d) Why is it not possible to solve for the steady state of the modified Gierer-Meinhardt equations?

(e) Find the Jacobian of the modified system. When does the Jacobian have the sign pattern of an activator-inhibitor system?

22. Consider the following hypothetical chemical system:

$$M_0(\text{a catalyst}) + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M_1 \qquad \text{(active complex)},$$
$$M_1 + X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} M_2 \qquad \text{(inactive complex)},$$
$$M_1 + Y \xrightarrow{k_3} P + Q + M_0 \qquad \text{(products plus catalyst)}.$$

This system is called a *substrate-inhibited reaction* since the chemical X can deactivate the complex M_1 which is required in forming the products.

(a) Write equations for the chemical components.

(b) Assume $M_0 + M_1 + M_2 = C$ (where m_0, m_1, and m_2 are concentrations of M_0, M_1, and M_2, and C is a constant). Make a quasi-steady-state assumption for M_0, M_1, and M_2 and show that

$$m_0 = m_1 \frac{k_{-1} + k_3 y}{k_1 x}, \qquad m_2 = k_2 x \frac{m_1}{k_{-2}}.$$

(c) Use these results to show that

$$m_1 = \frac{C k_1 x}{k_{-1} + k_3 y + k_1 x[1 + (k_2/k_{-2})x]}$$

(d) Now show that x will satisfy an equation whose dimensionless form is

$$\frac{dx}{dt} = \gamma - x - \frac{\beta x y}{1 + x + y + (\alpha/\delta)x^2}$$

Identify the various combinations of parameters. When can the term y in the denominator be neglected?

23. The following model was proposed by Othmer and Aldridge (1978): A cell can produce two chemical species x and y from a substrate according to the reaction

$$\text{substrate} \rightarrow x \rightarrow y \rightarrow \text{products}.$$

Species x can diffuse across the cell membrane at a rate that depends linearly on its concentration gradient. The ratio of the volume of cells to the volume of external medium is given by a parameter ϵ; x and y are intracellular concentrations of X and Y and x^0 is the extracellular concentration of X. The equations they studied were

$$\frac{dx}{dt} = \delta - F(x, y) + P(x^0 - x)m,$$

$$\frac{dy}{dt} = \alpha[F(x, y) - G(y)],$$

$$\frac{dx^0}{dt} = \epsilon P(x - x^0).$$

(a) Explain the equations. Determine the values of $\bar{x}$, $F(\bar{x}, \bar{y})$ and $G(\bar{y})$ at the steady state $(\bar{x}, \bar{y}, \bar{x}^0)$.

(b) The matrix of linearization of these equations about this steady state is

$$\mathbf{J} = \begin{pmatrix} k_{11} - P & k_{12} & P \\ k_{21} & k_{22} & 0 \\ \epsilon P & 0 & -\epsilon P \end{pmatrix}.$$

What are the constants k_{ij}?

(c) For the characteristic equation

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0,$$

find a_1, a_2, and a_3 in terms of k_{ij} and in terms of partial derivatives of F and G.

24. A model for control of synthesis of a gene product that activates mitosis was suggested by Tyson and Sachsenmaier (1979), based on repression and derepression (the reversal of repression) of a genetic *operon* (a sequence of genes that are controlled as a unit by a single gene called the *operator*.) They assumed that the gene consists of two portions, one replicating earlier than the second, with the following control system.

Protein R (coded by 1) binds to the operator region O of gene 2, repressing transcription of genes G_P and G_A. Protein P (product of G_P) inactivates the repressor and thus has a positive influence on its own synthesis, as well as on synthesis of A (see figure).

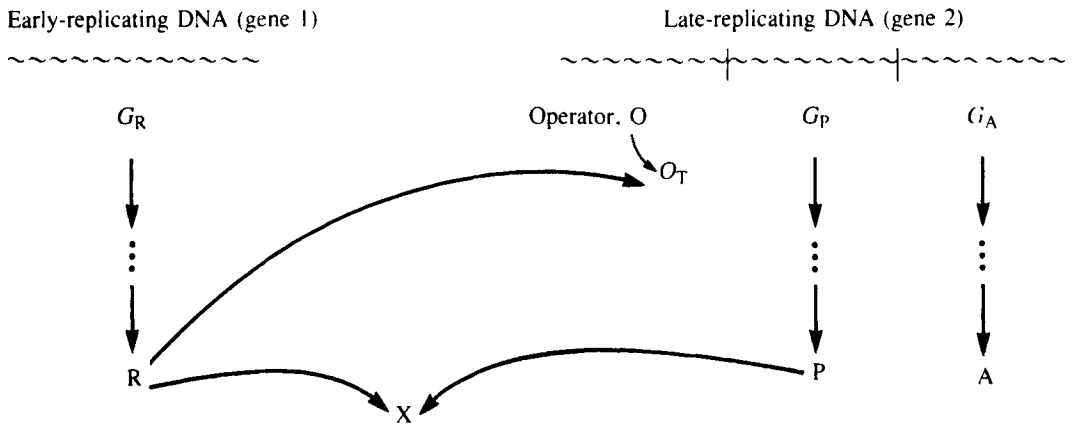


Figure for problem 24. *[After Tyson and Sachsenmaier (1979).]*

Assume that R, P, and A have removal rates ℓ_1, ℓ_2, and ℓ_3. Let G_R, G_P, and G_A be the *number* of genes coding for R, P, and A, at rates K_1, K_2, and K_3 (when actively transcribing). Let f be the fraction of operons of the late-replicating DNA that are active at a given time.

(a) Give equations governing the concentrations of R, P, and A in terms of R_is, G_is, and f.

(b) Following are the operator and repressor binding reactions:

$$\mathrm{P} + \mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{X}$$

$$\mathrm{R} + \mathrm{O} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{O_R}$$

where X is an inducer-repressor complex and O_R is a repressed operator. Write down equations for P, R, and O based on these kinetics.

(c) Now assume that these reactions are always in equilibrium and that the total number of operator molecules is O_T, a constant. Let

$$K_2 = \frac{k_2}{k_{-2}} \quad \text{and} \quad K_1 = \frac{k_1}{k_{-1}}.$$

Find an expression relating the fraction f of repressed operators ($f = O/O_T$) (1) to these rate constants and to the concentration of R.

(d) For the specific situation in which more than one repressor molecule can bind the operator,

$$n\mathrm{R} + \mathrm{O} \rightleftharpoons \mathrm{OR}_n,$$

(again where $K_2 = k_2/k_{-2}$ is the equilibrium constant), Tyson and Sachsenmaier showed that

$$f = (\epsilon + x)^n[1 + (\epsilon + x)^n]^{-1}, \qquad \text{for} \qquad x = \frac{K_1 P}{K_2 X}$$

$$\epsilon = (K_2 R_T)^{-1}.$$

provided ($O_T \ll X$).

Sketch f as a function of x for $n = 2$.

25. *Positive feedback to one gene.* The following model and analysis appears in Griffith (1971, pp 118 – 122). Parts d–f of this problem depend on the solution to problem 22 in Chapter 5. Consider a gene that is directly induced by m copies of the protein E for which it codes. Suppose

$$\mathrm{G} + m\mathrm{E} \rightarrow \mathrm{X}$$

where G is the gene and X is a complex composed of m molecules of E and one molecule of G. Let M be the concentration of *messenger RNA* (mRNA) that conveys the code for synthesis of the protein to the *ribosomes* (where the protein is assembled from amino acids).

(a) Griffith assumes that the fraction of time p for which the gene G is active given by

$$p = \frac{KE^m}{1 + KE^m},$$

where E is the concentration of E and m the number of E molecules that participate in forming a complex X. Explain this assumption.

(b) Explain the following equations:

$$\dot{M} = \frac{aKE^m}{1 + KE^m} - bM$$

$$\dot{E} = cM - dE.$$

(c) What are the meanings of the constants K, b, c, and d?

(d) In problem 22 of Chapter 5 it was shown that the behavior of this system depends on m and on a dimensionless multiple where

$$\alpha = b\tau, \qquad \beta = d\tau, \qquad \tau = \frac{K^{-m}}{c}.$$

Interpret the meanings of each of these quantities.

(e) Use the results of phase-plane analysis in each of the cases given in problem 22 of Chapter 5 to draw biological conclusions about this system.

(f) Suggest situations in which the dynamic behavior may be of biological relevance.

REFERENCES

Enzyme and Biochemical Kinetics (General)

Boyer, P. D. (1970). *Kinetics and Mechanisms*. (*The Enzymes*, vol. 2.) Academic Press, New York.

Dixon, M., and Webb, E. C. (1979). *Enzymes*. 3d ed. Academic Press, New York.

Reiner, J. M. (1969). *Behavior of Enzyme Systems*. 2d ed. Van Nostrand, New York.

Rubinow, S. I. (1975). *Introduction to Mathematical Biology*. Wiley, New York.

Savageau, M. A. (1976). *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology*. Addison-Wesley, London.

Volkenshtein, M. V. (1969). *Enzyme Physics*. Plenum Press, New York.

Molecular Biology and Biochemistry (Nonmathematical References)

Lehninger, A. L. (1982). *Principles of Biochemistry*. Worth Publishing, New York.

Price, F. N. (1979). *Basic Molecular Biology*. Wiley, New York.

Yudkin, M., and Offord, R. (1980). *A Guidebook to Biochemistry*. 4th ed. Cambridge University Press, Cambridge.

Genetic Control

Edelstein, L. (1982). A molecular switching mechanism in differentiation: Induction of polarity in cambial cells. *Differentiation*, *23*, 1–9.

Griffith, J. S. (1971). *Mathematical Neurobiology*. Academic Press, New York.

Lewis, J.; Slack, J. M. W.; and Wolpert, L. (1977). Thresholds in development. *J. Theor. Biol.*, *65*, 579–590.

Rosen, R. (1970). *Dynamical System Theory in Biology*. Wiley-Interscience, New York.

Rosen, R. (1972). Mechanics of Epigenetic Control. In R. Rosen, ed. *Foundations of Mathematical Biology, II*. Academic Press, New York, pp. 79–140.

Thornley, J. H. M. (1976). *Mathematical Models in Plant Physiology*. Academic Press, New York.

Network Analysis

Feinberg, M. (1977). Mathematical aspects of mass action kinetics. In L. Lapidus and N. Amundson, eds., *Chemical Reactor Theory: A Review*. Prentice-Hall, Englewood Cliffs, N.J., pp. 59–130.

Feinberg, M. (1980). Chemical oscillations, multiple equilibria, and reaction network structure. In W. E. Stewart, W. H. Ray, and C. C. Conley, eds., *Dynamics and Modelling of Reactive Systems*. Academic Press, New York.

Feinberg, M. (in press). Chemical reaction network structure and the stability of complex isothermal reactors. I. The deficiency zero and deficiency one theorems; II. Multiple steady states for networks of deficiency one. *Chemical Engineering Science*.

Other References:

Banks, H. T., and Mahaffy, J. M. (1979). *Mathematical Models for Protein Biosynthesis*. LCDS Report. Brown University, Providence, Rhode Island.

Banks, H. T.; Miech, R. P.; and Zinberg, D. S. (1975). Nonlinear systems in models for enzyme cascades. In A. Ruberti and R. Mohler, eds. *Variable Structure Systems with Applications to Economy and Biology*. (*Lecture Notes in Economy and Mathematical Systems*, vol. 3.) Springer-Verlag, New York.

Kadas, Z. M. (1982). A piecewise linear activator-inhibitor model. (preprint of unpublished paper).

Lin, C. C., and Segel, L. A. (1974). *Mathematics Applied to Deterministic Problems in the Natural Sciences*. Macmillan, New York.

Murray, J. D. (1977). *Lectures on Nonlinear Differential Equation Models in Biology*. Clarendon Press, Oxford.

Othmer, H., and Aldridge, J. (1978). The effects of cell density and metabolite flux on cellular dynamics. *J. Math. Biol.*, *5*, 169–200.

Prigogine, I. and Lefever, R. (1968). Symmetry-breaking instabilities in dissipative systems. II *J. Chem. Phys.*, *48*, 1695–1700.

Prigogine, I., and Nicolis, G. (1967). On symmetry-breaking instabilities in dissipative systems. *J. Chem. Phys.*, *46*, 3542–3550.

Rapp, P. E. (1979): Bifurcation theory, control theory, and metabolic regulation. in D. A. Linkens, ed. *Biological Systems, Modelling and Control*. Peregrinus, New York.

Segel, L. A., ed. (1980). *Mathematical Models in Molecular and Cellular Biology*. Cambridge University Press, Cambridge.

Tyson, J., and Sachsenmaier, W. (1979). Derepression as a model for control of the DNA-division cycle in eukaryotes. *J. Theor. Biol.*, *79*, 275–280.