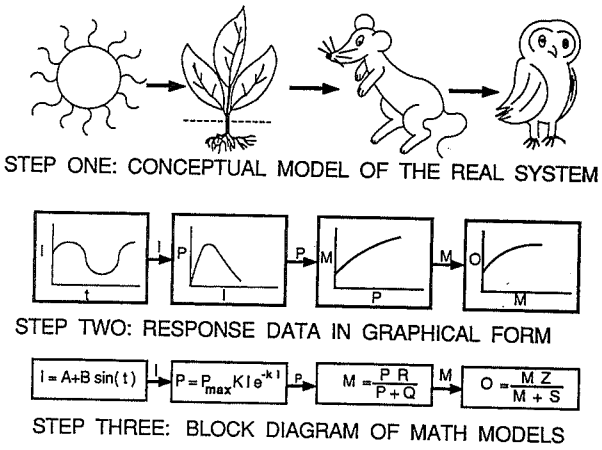

Figure P2.1. A simple illustration of stepwise replacement. Conceptual components of a hypothetical system are replaced by equations to form a multicomponent model of the system.

1) Learning how to solve several differential equations simultaneously using techniques of numerical integration.
2) Understanding the place of deterministic simulation in the scheme of biological investigation.
3) Learning to use the concepts of compartmental models.
4) Learning to use matrix algebra in implementing biological simulations.

Each chapter of this section includes several introductory simulations as exercises at the beginning of each chapter. More complex simulations are included in most chapters to provide follow-up practice for modeling systems. The chapters cover a range of biological subject material, from molecular interactions through ecosystems. It is good practice to select an area of particular interest and to try some of the more complex simulations.

CHAPTER 6

KINETICS OF BIOCHEMICAL REACTIONS

Some fundamental principles of simulation are introduced in this chapter, based on examples taken from the study of biochemical reactions. The kinetics of chemical reactions have been studied for a very long time, and the elementary descriptive mathematics is well established. The concepts are not specialized, and the models derived from them are straightforward. The exercises of this chapter will provide practice in techniques of numerical integration that will be useful in most of the following chapters involving deterministic models.

It would be a mistake to skip this chapter even if you usually prefer to avoid thinking about molecules. Models in all fields of biology are based on rate processes and the law of mass action. Many current models in physiology, population ecology, and ecosystem analysis, for example, are founded in some of the reaction models discussed below.

6.1 Kinetics of Bimolecular Reactions

In Chapters 1 and 2 you had opportunity to look at reactions with rather restricted models based on integration or on assumptions of stable states. The direct approach of numerical integration that you studied in Chapter 5 will let you perform simulations without these restrictions.

We will deal first with a simple reaction between two reactants forming a product:

$$A + B \xrightarrow{k} P$$

The rate of formation of the reaction's product, P, will depend upon the concentrations of the reactants, $[A]$ and $[B]$, and may be expressed as:

$$\frac{d[P]}{dt} = k[A][B] \tag{6.1}$$

As before, the rate at which A and B are used up will depend on their concentrations:

$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k[A][B] \tag{6.2}$$

Whenever several differential equations like these are used simultaneously they form a system. It becomes essential to use the two-stage approach to numerical integration described in Chapter 5. To model the reaction with the simple Euler method, the changes in concentration would be found using these equations as the first stage:

$$\Delta[B] = \Delta[A] = -k[A][B]\Delta t \tag{6.3}$$
$$\Delta[P] = +k[A][B]\Delta t \tag{6.4}$$

The second stage involves updating the variables with these equations:

$$[A] \leftarrow [A] + \Delta[A] \tag{6.5}$$
$$[B] \leftarrow [B] + \Delta[B] \tag{6.6}$$
$$[P] \leftarrow [P] + \Delta[P] \tag{6.7}$$

It is important to put all the variables through the first stage before going on to the second stage.

Implementing the bimolecular reaction model on the computer would typically involve entering initial concentrations of A and B, with output consisting of concentrations of A and B over subsequent time intervals. As discussed in Section 1.8, the order of the reactions depends on the relative concentrations of A and B. If reactant A greatly exceeds reactant B, the reaction will follow first-order kinetics, and a straight line will be observed when $\ln[B]$ is plotted against time. If the two reactants are equal in concentration, they will follow second-order kinetics, and $1/[B]$ will plot as a straight line vs. time (Figure 6.1). Where concentrations are unequal but not extremely different, both $\ln[B]$ and $1/[B]$ will be curved.

Exercise 6-1: Write a program using simple Euler integration (Equations 6.3-6.7) to simulate bimolecular reactions. Set $\Delta t = 0.1$ and test your simulation with two sets of values:

Set 1 :	$[A] = 100$	$[B] = 100$	$k = 0.0003$
Set 2 :	$[A] = 1000$	$[B] = 10$	$k = 0.0003$

As the output for each set of values, obtain graphs of $1/[B]$ and $\ln[B]$ plotted against time to determine whether the data sets produce first- or second-order kinetics. You may write your program to produce two lines on the same graph. (Be aware that all the exercises in this chapter employ arbitrary units of concentration and time. They are models of types of reactions, and are not based on real examples.)

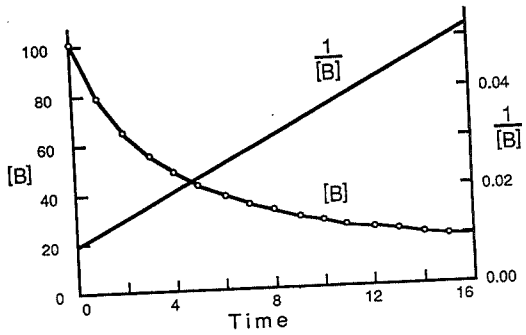

Figure 6.1. Simulation results from a bimolecular reaction showing second-order kinetics. In this example, $[A]_o = [B]_o = 100$ and $k = 0.002$.

6.2 Chemical Equilibrium Model

The model of the approach to chemical equilibrium is described here to demonstrate the use of flux rates in simulation. We will work with a simple model of this type:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C + D$$

We could use the same two-stage Euler technique as in Section 6.1 above to find changes in concentration of A, B, C, and D. (The changes in the concentrations would be calculated as in Equations 6.3-6.4, and then would be updated as in Equations 6.5-6.7.) An alternate approach uses flux rates to simplify programming by dividing the first stage of the two-stage Euler process into two parts. In Part 1, the instantaneous flux rates for the forward and reverse reactions are defined:

$$F_1 = k_1[A][B] \tag{6.8}$$
$$F_2 = k_2[C][D] \tag{6.9}$$

In Part 2 of the first stage, the estimates of change in concentration are calculated, using the flux rates:

$$\Delta[A] = \Delta[B] = (-F_1 + F_2)\Delta t \tag{6.10}$$
$$\Delta[C] = \Delta[D] = (+F_1 - F_2)\Delta t \tag{6.11}$$

The second stage of the Euler process is not changed. Concentrations are updated with:

$$[A] \leftarrow A + \Delta[A] \tag{6.12}$$
$$[B] \leftarrow B + \Delta[B] \tag{6.13}$$
$$[C] \leftarrow C + \Delta[C] \tag{6.14}$$
$$[D] \leftarrow D + \Delta[D] \tag{6.15}$$

This extension of the two-stage procedure will be valuable in later models also, and is readily adapted to the Improved Euler technique. As in the previous section, all the variables are taken through Part 1 of the first stage, before going on to Part 2 of the first stage, and then to the second stage. The equilibrium constant for this general model is defined by

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

where the concentrations of A, B, C, and D are measured at equilibrium.

Exercise 6-2: Write and implement a computer program to simulate the approach to chemical equilibrium using the model above. Use simple Euler methods with $\Delta t = 0.1$. Set $k_1 = 0.0025$ and $k_2 = 0.0015$. Test your simulation with these values for initial concentrations:

$$[A] = [B] = 100 \qquad [C] = [D] = 0$$

Output should be in tabular form showing concentrations of each of the four reactants for times 0, 1, 2, 3, ..., 30, by which time your simulation should have approached a reasonable equilibrium. Have your program find and print the equilibrium constant after 30 time units. Compare this value with the calculated value of k_1/k_2.

Exercise 6-3: Expand your program from Exercise 6-2 to print columns for each of eight variables: elapsed time, $[A]$, $[B]$, $[C]$, $[D]$, the Net Forward Reaction Rate ($F_1 - F_2$), the ratio $[C][D]/[A][B]$, and $\ln([C][D]/[A][B])$. Again, print the results for times 0 to 30.

Also set up your program to produce graphs of $[C][D]/[A][B]$ and $\ln([C][D]/[A][B])$ as functions of Net Forward Reaction Rate (NFRR). These graphs are useful in estimating the equilibrium constant when NFRR = 0, by extrapolating to zero net rate; see Figure 6.2.

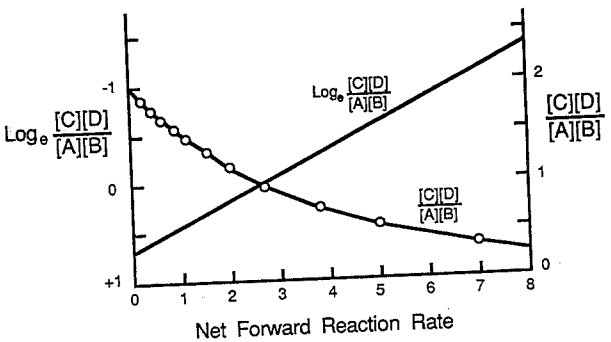

Figure 6.2. Simulation results showing the approach to chemical equilibrium. $K_{eq} = 2.0$.

When you think your program is working successfully, alter the initial concentration of reactants and products, and produce another set of output data. You can confirm the success of the simulation by checking your output against the following properties of chemical equilibria:

1) Computed and extrapolated K_{eq} values should equal k_1/k_2.

2) NFRR should approach zero as concentrations of reactants and products approach equilibrium.

3) Output should show the same K_{eq} regardless of initial concentrations of reactants and products.

6.3 Kinetics of a Sequential Reaction

A sequential reaction is one in which the product of one reaction is a reactant of a second reaction. We will assume a simple reaction in which B reacts with C to yield an intermediate D which then breaks down to yield a product E, as follows:

$$B + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D \xrightarrow{k_3} E$$

The kinetics are described by the following differential equations:

$$\frac{d[B]}{dt} = \frac{d[C]}{dt} = -k_1[B][C] + k_2[D] \tag{6.16}$$

$$\frac{d[D]}{dt} = +k_1[B][C] - k_2[D] - k_3[D] \tag{6.17}$$

$$\frac{d[E]}{dt} = +k_3[D] \tag{6.18}$$

These equations can be numerically integrated following the procedure described in Section 6.2 above, using flux rates with the first stage of the two-stage Euler process divided into two parts. First, the reaction fluxes are defined for each rate-governed process:

$$F_1 = k_1[B][C] \tag{6.19}$$

$$F_2 = k_2[D] \tag{6.20}$$

$$F_3 = k_3[D] \tag{6.21}$$

Then, the changes in concentration are defined using the flux rates:

$$\Delta[B] = (-F_1 + F_2)\Delta t \tag{6.22}$$

$$\Delta[C] = (-F_1 + F_2)\Delta t \tag{6.23}$$

$$\Delta[D] = (+F_1 - F_2 - F_3)\Delta t \tag{6.24}$$

$$\Delta[E] = (+F_3)\Delta t \tag{6.25}$$

The updating process then proceeds as usual: $[B] \leftarrow [B] + \Delta[B]$, etc.

Exercise 6-4: Write a program to simulate the kinetics of sequential reactions. Test your simulation with these coefficients:

$$k_1 = 0.002 \qquad k_2 = 0.04 \qquad k_3 = 0.15$$

For initial conditions, set concentrations as follows:

$$[B] = 100 \qquad [C] = 100 \qquad [D] = 0 \qquad [E] = 0$$

Use simple Euler methods, with $\Delta t = 0.1$. Your output should be in the form of a graph showing $[B]$, $[C]$, $[D]$, and $[E]$ plotted against time, for at least 50 time units.

6.4 The Chance-Cleland Model for Enzyme-Substrate Interaction

The numerical solution of the classical Michaelis-Menten enzyme model (see Section 2.2) was first reported by Chance (1960), and has also been attributed to Cleland by several authors including Mahler and Cordes (1966). The Chance-Cleland model uses techniques similar to those of the previous sections to simulate the interaction of enzyme and substrate to yield a product. The reaction assumed in this model is:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_3} E + P$$

where S is the substrate, E is the enzyme, C is the enzyme-substrate compound, and P is the product. The rate constants are k_1, k_2, and k_3. The differential equations for the kinetics of the reactions are:

$$\frac{d[S]}{dt} = -k_1[S][E] + k_2[C] \tag{6.26}$$

$$\frac{d[C]}{dt} = \frac{-d[E]}{dt} = k_1[S][E] - (k_2 + k_3)[C] \tag{6.27}$$

$$\frac{d[P]}{dt} = k_3[C] \tag{6.28}$$

Exercise 6-5: Program the Chance-Cleland model to simulate enzyme-substrate interactions. Use the two-stage approach described in Section 6.3, based on the simple Euler method with $\Delta t = 1$. Use the following coefficients:

$$k_1 = 0.005 \qquad k_2 = 0.005 \qquad k_3 = 0.1$$

For initial conditions, set

$$[S] = 100 \qquad [E] = 10 \qquad [C] = 0 \qquad [P] = 0$$

Your output should consist of a graph showing $[S]$, $[E]$, $[C]$, and $[P]$ vs. time, for 200 time units.

6.5 Autocatalytic Reactions

Autocatalytic reactions are those which catalyze the formation of the catalyst itself. This property causes autocatalytic reactions to show positive feedback characteristics that resemble the growth of organisms or

populations of organisms. Autocatalytic reactions have been used frequently as models for growth of living organisms.

Classically, these reactions are exemplified by the activation of trypsin in the gastrointestinal tract. Trypsin is formed in the intestine from trypsinogen, a product of the pancreatic cells. This proenzyme or zymogen is converted to the active form by enterokinase of the small intestine. Trypsin, however, is itself capable of activating trypsinogen in the same way. The activation of the enzyme is therefore autocatalytic.

The process may be described by the following:

$$G + E \rightleftharpoons C \longrightarrow T + E$$

where G represents trypsinogen, E is enterokinase, the activating enzyme, T is trypsin, and C is the enzyme-substrate complex.

Assuming steady-state conditions, this will simplify to

$$G \xrightarrow{E+T} T$$

Assuming further that the reaction is catalyzed equally by E and T, we can adapt the Michaelis-Menten model (Equation 2.18) to find the rate of formation of trypsin:

$$F = V\frac{[G]([E]+[T])}{K+[G]} \tag{6.29}$$

where V is the maximum rate of formation for a given unit of enzyme, K is the Michaelis-Menten constant, and $[E]+[T]$ is the total concentration of activating enzyme.

In this system, defining the flux F is Part 1 of the first stage of the simple Euler integration process. Part 2 is a definition of changes in concentration with

$$\Delta[G] = (-F)\Delta t \tag{6.30}$$
$$\Delta[T] = (+F)\Delta t \tag{6.31}$$

The second stage of numerical integration follows as usual:

$$[G] \leftarrow [G] + \Delta[G] \tag{6.32}$$
$$[T] \leftarrow [T] + \Delta[T] \tag{6.33}$$

This is an example of a simulation in which an analytical model (Equation 6.29) is used in conjunction with numerical integration.

Exercise 6-6: Write and implement a program which simulates the effect of an autocatalytic reaction. Use $K = 10$, $V = 0.1$, and employ simple Euler methods with $\Delta t = 0.1$. For initial conditions, use the following:

$$[G] = 100 \qquad [E] = 1.0 \qquad [T] = 0$$

As output, plot $[G]$ and $[T]$ simultaneously over a period of at least 60 time units.

Exercise 6-7: Modify the Chance-Cleland model (see Exercise 6-5) to include rate constants k_4, k_5, and k_6. These involve the interaction of product with enzyme to produce an enzyme-product compound which then converts to an enzyme-substrate compound as follows:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \underset{k_6}{\overset{k_5}{\rightleftharpoons}} D \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E$$

where C is the enzyme-substrate compound and D is the enzyme-product compound. Assume the following coefficients in addition to those listed in Exercise 6-5:

$$k_4 = 0.0013 \qquad k_5 = 0.11 \qquad k_6 = 0.1$$

Begin with $[D] = 0$ at $t = 0$. Graphical output for your simulation should show $[S]$, $[E]$, $[C]$, $[D]$, and $[P]$ over 40 units of time.

Exercise 6-8: The Chance-Cleland model may be modified further to simulate catalysis of bimolecular reactions. Consider a possible sequence of reactions in which $S + E$ produces an intermediate C_1, which reacts in an ordered fashion with a second substrate B, to produce intermediate C_2. This intermediate breaks down sequentially to product F and an intermediate C_3, which breaks down further to product D. The reactions are:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_1$$

$$C_1 + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} C_3 + F$$

$$C_3 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} D + E$$

Set up your simulation with these initial concentrations of reactants:

$$[S] = 100 \qquad [B] = 90 \qquad [E] = 10$$

Let other starting concentrations = 0. For the rate constants k_i:

$$k_1 = 0.005 \qquad k_2 = 0.005 \qquad k_3 = 0.01 \qquad k_4 = 0.001$$
$$k_5 = 0.1 \qquad k_6 = 0.1 \qquad k_7 = 0.01 \qquad k_8 = 0.1$$

Graphical output should show the concentrations of the eight reactants over at least 40 units of time. Use simple Euler methods with $\Delta t = 0.1$.

Conclusion

This chapter has presented examples of systems which lend themselves to deterministic simulations involving numerical integration. Only Exercise 6-1 could have been solved analytically. The other exercises involved sets of equations that would have been very difficult, if not impossible to solve by this method. They provided good examples of the power and simplicity of numerical integration.

CHAPTER 7

MODELS OF HOMOGENEOUS POPULATIONS OF ORGANISMS

In Chapter 6 we simulated chemical reactions among populations of molecules using deterministic models. We assumed that all the molecules of a given type were the same (homogeneous), and ignored the differences that exist among individual molecules. Our reaction rates were really average rates, and would not necessarily apply to any given molecule. Average rate is a reasonable predictor of overall reaction rate, given the large numbers of molecules involved in most chemical reactions.

When the first attempts were made to construct models of homogenous populations of organisms, it was logical to use some of the same principles as in models of homogeneous molecules. Particularly important was the "law of mass action" which states that the rate of interaction depends directly on the product of the concentrations of the interacting types of molecules. The pioneering work of Lotka (1925) frequently made use of this law. (This law is probably impossible to prove in a formal sense; its validity is based upon a very large number of observations, made without finding an exception.)

Homogeneous populations of organisms are assumed to be composed of a single type and described by a single variable, density. Differences in age, sex, genotype, phenotype, etc., are ignored or assumed to be irrelevant for the model. The density variable for organisms is identical to the concentration variable for molecules. Density is typically expressed as number per unit area (e.g. wolves ha^{-1}) or number per unit volume (e.g. *Paramecium* ml^{-1}). The models for homogeneous populations assume that interactions (predation, parasitism, competition) between populations will proceed at rates directly proportional to the product of the densities of the populations.

Most populations of most organisms are not homogeneous, and the unreality of the models in this chapter is admitted at the outset. However, they are constructed from principles that make elementary biological