6. Reaction Kinetics

6.1 Enzyme Kinetics: Basic Enzyme Reaction

Biochemical reactions are continually taking place in all living organisms and most of them involve proteins called *enzymes*, which act as remarkably efficient catalysts. Enzymes react selectively on definite compounds called *substrates*. For example, haemoglobin in red blood cells is an enzyme and oxygen, with which it combines, is a substrate. Enzymes are important in regulating biological processes, for example, as activators or inhibitors in a reaction. To understand their role we have to study enzyme kinetics which is mainly the study of rates of reactions, the temporal behaviour of the various reactants and the conditions which influence them. Introductions with a mathematical bent are given in the books by Rubinow (1975), Murray (1977) and the one edited by Segel (1980). Biochemically oriented books, such as Laidler and Bunting (1977) and Roberts (1977), go into the subject in more depth.

The complexity of biological and biochemical processes is such that the development of a simplifying model is often essential in trying to understand the phenomenon under consideration. For such models we should use reaction mechanisms which are plausible biochemically. Frequently the first model to be studied may itself be a model of a more realistic, but still too complicated, biochemical model. Models of models are often first steps since it is a qualitative understanding that we want initially. In this chapter we discuss some model reaction mechanisms, which mirror a large number of real reactions, and some general types of reaction phenomena and their corresponding mathematical realisations; a knowledge of these is essential when constructing models to reflect specific known biochemical properties of a mechanism.

Basic Enzyme Reaction

One of the most basic enzymatic reactions, first proposed by Michaelis and Menten (1913), involves a substrate S reacting with an enzyme E to form a complex SE which in turn is converted into a product P and the enzyme. We represent this schematically by

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE \overset{k_2}{\rightarrow} P + E. \tag{6.1}$$

Here k_1, k_{-1} and k_2 are constant parameters associated with the rates of reaction; they are defined below. The double arrow symbol $\rightleftharpoons$ indicates that the reaction is reversible

while the single arrow $\rightarrow$ indicates that the reaction can go only one way. The overall mechanism is a conversion of the substrate S, via the enzyme catalyst E, into a product P. In detail it says that one molecule of S combines with one molecule of E to form one of SE, which eventually produces one molecule of P and one molecule of E again.

The *Law of Mass Action* says that the rate of a reaction is proportional to the product of the concentrations of the reactants. We denote the concentrations of the reactants in (6.1) by lowercase letters

$$s = [S], \quad e = [E], \quad c = [SE], \quad p = [P], \tag{6.2}$$

where [ ] traditionally denotes concentration. Then the Law of Mass Action applied to (6.1) leads to one equation for each reactant and hence the system of nonlinear reaction equations

$$\begin{aligned} \frac{ds}{dt} &= -k_1 es + k_{-1} c, \quad \frac{de}{dt} = -k_1 es + (k_{-1} + k_2) c \\ \frac{dc}{dt} &= k_1 es - (k_{-1} + k_2) c, \quad \frac{dp}{dt} = k_2 c. \end{aligned} \tag{6.3}$$

The k's, called *rate constants*, are constants of proportionality in the application of the Law of Mass Action. For example, the first equation for s is simply the statement that the rate of change of the concentration $[S]$ is made up of a loss rate proportional to $[S][E]$ and a gain rate proportional to $[SE]$.

To complete the mathematical formulation we require initial conditions which we take here as those at the start of the process which converts S to P, so

$$s(0) = s_0, \quad e(0) = e_0, \quad c(0) = 0, \quad p(0) = 0. \tag{6.4}$$

The solutions of (6.3) with (6.4) then give the concentrations, and hence the rates of the reactions, as functions of time. Of course in any reaction kinetics problem we are only concerned with nonnegative concentrations.

The last equation in (6.3) is uncoupled from the first three; it gives the product

$$p(t) = k_2 \int_0^t c(t')\, dt', \tag{6.5}$$

once $c(t)$ has been determined, so we need only be concerned (analytically) with the first three equations in (6.3).

In the mechanism (6.1) the enzyme E is a catalyst, which only facilitates the reaction, so its total concentration, free plus combined, is a constant. This conservation law for the enzyme also comes immediately from (6.3) on adding the 2nd and 3rd equations, those for the free (e) and combined (c) enzyme concentrations respectively, to get

$$\frac{de}{dt} + \frac{dc}{dt} = 0 \quad \Rightarrow \quad e(t) + c(t) = e_0 \tag{6.6}$$

on using the initial conditions (6.4). With this, the system of ordinary differential equations reduces to only two, for s and c, namely,

$$\begin{aligned} \frac{ds}{dt} &= -k_1 e_0 s + (k_1 s + k_{-1})c, \\ \frac{dc}{dt} &= k_1 e_0 s - (k_1 s + k_{-1} + k_2)c, \end{aligned} \tag{6.7}$$

with initial conditions

$$s(0) = s_0, \quad c(0) = 0. \tag{6.8}$$

The usual approach to these equations is to assume that the initial stage of the complex, c, formation is very fast after which it is essentially at equilibrium, that is, $dc/dt \approx 0$ in which case from the second of (6.7) we get c in terms of s,

$$c(t) = \frac{e_0 s(t)}{s(t) + K_m}, \quad K_m = \frac{k_{-1} + k_2}{k_1} \tag{6.9}$$

which on substituting into the first of (6.7) gives

$$\frac{ds}{dt} = -\frac{k_2 e_0 s}{s + K_m}, \tag{6.10}$$

where K_m is called the *Michaelis constant*. Since the enzyme is traditionally considered to be present in small amounts compared with the substrate the assumption is that the substrate concentration effectively does not change during this initial transient stage. In this case the (approximate) dynamics is governed by (6.10) with the initial condition $s = s_0$. This is known as the pseudo- or quasi-steady state approximation. Solving (6.10) with the initial condition on $s(t)$ we obtain an implicit solution; namely,

$$s(t) + K_m \ln s(t) = s_0 + K_m \ln s_0. \tag{6.11}$$

If we now substitute this into (6.9) we get an expression for the complex $c(t)$. But this does not satisfy the initial condition on $c(t)$ in (6.8). However, perhaps it is a reasonable approximation for most of the time; this is the belief in the usual application of this approach. In fact for many experimental situations it is sufficient, but crucially not always. There are in fact two timescales involved in this system: one is the initial transient timescale near $t = 0$ and the other is the longer timescale when the substrate changes significantly during which the enzyme complex is reasonably approximated by (6.9) with $s(t)$ from (6.11). This basic reasoning raises several important questions such as (i) how fast is the initial transient; (ii) for what range of the parameters is the approximation (6.9) and (6.11) a sufficiently good one; (iii) if the enzyme concentration is not small compared with the substrate concentration, how do we deal with it?

Other questions arise, and are also dealt with, later. As a first step we must clearly nondimensionalise the system. There are several ways this can be done, of course. A key

dimensionless quantity is the time since the basic assumptions above depend on how short the transient period is. The standard way of doing the quasi-steady state analysis is to introduce dimensionless quantitites

$$\begin{aligned} &\tau = k_1 e_0 t, \quad u(\tau) = \frac{s(t)}{s_0}, \quad v(\tau) = \frac{c(t)}{e_0}, \\ &\lambda = \frac{k_2}{k_1 s_0}, \quad K = \frac{k_{-1} + k_2}{k_1 s_0} = \frac{K_m}{s_0}, \quad \varepsilon = \frac{e_0}{s_0} \end{aligned} \tag{6.12}$$

which is a reasonable nondimensionalisation if $\varepsilon \ll 1$. Substituting these into (6.7) together with (6.8) gives the dimensionless system for the traditional quasi-steady state approximation

$$\begin{gathered} \frac{du}{d\tau} = -u + (u + K - \lambda)v, \quad \varepsilon \frac{dv}{d\tau} = u - (u + K)v \\ u(0) = 1, \quad v(0) = 0. \end{gathered} \tag{6.13}$$

Note that $K - \lambda > 0$ from (6.12). With the solutions $u(\tau)$, $v(\tau)$ we then immediately get e and p from (6.6) and (6.5) respectively.

From the original reaction (6.1), which converts S into a product P, we clearly have the final steady state $u = 0$ and $v = 0$; that is, both the substrate and the substrate–enzyme complex concentrations are zero. We are interested here in the time evolution of the reaction so we need the solutions of the nonlinear system (6.13), which we cannot solve analytically in a simple closed form. However, we can see what $u(\tau)$ and $v(\tau)$ look like qualitatively. Near $\tau = 0$, $du/d\tau < 0$ so u decreases from $u = 1$ and since there $dv/d\tau > 0$, v increases from $v = 0$ and continues to do so until $v = u/(u + K)$, where $dv/d\tau = 0$ at which point, from the first of (6.13), u is still decreasing. After v has reached a maximum it then decreases ultimately to zero as does u, which does so monotonically for all t. The dimensional enzyme concentration $e(t)$ first decreases from e_0 and then increases again to e_0 as $t \to \infty$. Typical solutions are illustrated in Figure 6.1 below. Quite often a qualitative feel for the solution behaviour can be obtained from just looking at the equations; it is always profitable to try.

6.2 Transient Time Estimates and Nondimensionalisation

It is widespread in biology that the remarkable catalytic effectiveness of enzymes is reflected in the small concentrations needed in their reactions as compared with the concentrations of the substrates involved. In the Michaelis–Menten model in dimensionless form (6.13) this means $\varepsilon = e_0/s_0 \ll 1$. However, as mentioned above, it is not always the case that $e_0/s_0 \ll 1$. Segel (1988) and Segel and Slemrod (1989) extended the traditional analysis with a new nondimensionalisation which includes this case but which also covers the situation where $e_0/s_0 = O(1)$. It is their analysis which we now describe.

We first need estimates for the two timescales, the fast transient, t_c, and the longer, or slow, time, t_s, during which $s(t)$ changes significantly. During the initial transient the

complex $c(t)$ increases rapidly while $s(t)$ does not change appreciably so an estimate of this fast timescale is obtained from the second of (6.7) with $s(t) = s_0$, that is,

$$\frac{dc}{dt} = k_1 e_0 s_0 - k_1(s_0 + K_m)c. \tag{6.14}$$

The solution involves an exponential, the timescale of which is

$$t_c = \frac{1}{k_1(s_0 + K_m)}. \tag{6.15}$$

To estimate the long timescale, t_s, in which $s(t)$ changes significantly we take the maximum change possible in the substrate, namely, s_0, divided by the size of the maximum rate of change of $s(t)$ given by setting $s = s_0$ in (6.10). So,

$$t_s \approx \frac{s_0}{\left|\frac{ds}{dt}\right|_{\max}} \approx \frac{s_0 + K_m}{k_2 s_0}. \tag{6.16}$$

One assumption on which the quasi-steady state approximation is valid is that the fast initial transient time is much smaller than the long timescale when $s(t)$ changes noticeably which means that necessarily $t_c \ll t_s$. With the expressions (6.15) and (6.16), this requires the parameters to satisfy

$$\frac{k_2 e_0}{k_1(s_0 + K_m)^2} \ll 1. \tag{6.17}$$

Another requirement of the quasi-steady state approximation is that the initial condition for $s(t)$ can be taken as the first of (6.8). This means that the substrate depletion $\Delta s(t)$ during the fast transient is only a small fraction of s_0; that is, $|\,\Delta s/s_0\,| \ll 1$. An overestimate of $\Delta s(t)$ is given by the maximum rate of depletion possible from the first of (6.7), which is $k_1 e_0 s_0$ multiplied by t_c. So, dividing this by s_0 gives the following requirement on the parameters,

$$\varepsilon = \frac{e_0}{s_0 + K_m} \ll 1. \tag{6.18}$$

But condition (6.17), with K_m from (6.9), can be written as

$$\frac{e_0}{s_0 + K_m} \cdot \frac{1}{1 + (k_{-1}/k_2) + (s_0 k_1/k_2)} \ll 1 \tag{6.19}$$

so the condition in (6.18) is more restrictive than (6.19) which is therefore the condition that guarantees the quasi-steady state approximation. With this condition we see that even if $e_0/s_0 = O(1)$, condition (6.19) can still be satisfied if K_m is large as is actually the case in many reactions.

Since the nondimensionalisation depends crucially on the timescale we are focusing on, we have two timescales, t_c and t_s, from which we can choose. Which we use depends on where we want the solution: with t_c we are looking at the region near $t = 0$

while with t_s we are interested in the long timescale during which $s(t)$ changes significantly. A problem which involves two such timescales is generally a *singular pertubation* problem for which there are standard techniques (see, for example, the small book by Murray 1984). We carry out, in detail, the singular pertubation analysis for such a problem in the following section.

If we use the fast transient timescale t_c from (6.15) we introduce the following nondimensional variables and parameters,

$$\begin{aligned} u(\tau) &= \frac{s(t)}{s_0}, \quad v(\tau) = \frac{(s_0 + K_m)c(t)}{e_0 s_0}, \quad \tau = \frac{t}{t_c} = k_1(s_0 + K_m)t, \\ K_m &= \frac{k_{-1} + k_2}{k_1}, \quad \rho = \frac{k_{-1}}{k_2}, \quad \sigma = \frac{s_0}{K_m}, \quad \varepsilon = \frac{e_0}{s_0 + K_m} \end{aligned} \tag{6.20}$$

which on substituting into (6.7) and (6.8) give

$$\begin{aligned} \frac{du}{d\tau} &= \varepsilon\left[-u + \frac{\sigma}{1+\sigma}uv + \frac{\rho}{(1+\sigma)(1+\rho)}v\right] \\ \frac{dv}{d\tau} &= u - \frac{\sigma}{1+\sigma}uv - \frac{v}{1+\sigma} \\ u(0) &= 1, \quad v(0) = 0. \end{aligned} \tag{6.21}$$

With the long or slow timescale, t_s, we nondimensionalise the time by writing

$$T = (1+\rho)t/t_s = \frac{(1+\rho)k_2 e_0}{s_0 + K_m}t = \varepsilon(1+\rho)k_2 t. \tag{6.22}$$

The reason for the scale factor $(1+\rho)$ is simply for algebraic simplicity. With the dimensionless forms in (6.20) but with the dimensionless time T from the last equation, the model equations (6.7) become

$$\begin{aligned} \frac{du}{dT} &= -(1+\sigma)u + \sigma uv + \frac{\rho}{1+\rho}v, \\ \varepsilon\frac{dv}{dT} &= (1+\sigma)u - \sigma uv - v. \end{aligned} \tag{6.23}$$

We should keep in mind that the system we are investigating is (6.7). The three equation systems (6.13), (6.21) and (6.23) are exactly the same; they only differ in the way we nondimensionalised them, important though that is. They both have the small parameter, ε, but it appears in the equations in a different place. Where a small parameter appears determines the analytical procedure we use. We discuss a specific example in the next section and introduce asymptotic, or singular perturbation, techniques. These very powerful techniques provide a uniformly valid approximate solution for all time which is a remarkably good approximation to the exact solution of the system.

Before leaving the topic of nondimensionalisation it is relevant to ask what we must do if the enzyme is in excess such that ε in (6.20) is not small. This occurs in various enzyme reactions but also arises in a quite different situation involving T-cell proliferation in response to an antigen. This was studied by De Boer and Perelson (1994).

Here the 'substrate' is a replicating cell, the 'enzyme' the site on the antigen-presenting cell and the 'complex' is the bound T-cell and antigen-presenting cell. The kinetics is represented by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \overset{k_2}{\to} E + 2S.$$

Borghans et al. (1996) investigated this reaction system in which the enzyme is in excess and extended the above analysis to obtain a uniformly valid asymptotic solution. They did this by replacing the substrate, s-equation in (6.7) by the equation for the total substrate,

$$\bar{s}(t) = s(t) + c(t)$$

which is given by adding the first and third equations in (6.3). The system they studied is this equation for $\bar{s}(t)$ and the third of (6.3) written in terms of c and $\bar{s}$, together with the boundary conditions. It is

$$\frac{d\bar{s}}{dt} = -k_2 c, \quad \frac{dc}{dt} = k_1 \left[(e_0 - c)(\bar{s} - c) - K_m c\right], \quad \bar{s}(0) = s_0, \quad c(0) = 0.$$

The analysis is a little more involved but the concepts are similar. They derive conditions for an equivalent quasi-steady state approximation and discuss several examples including a general class of predator–prey models.

6.3 Michaelis–Menten Quasi-Steady State Analysis

Here we carry out a singular perturbation analysis on one of the above possible dimensionless equation systems. The technique can be used on any of them but to be specific we carry out the detailed pedagogical analysis on (6.13) to explain the background reasoning for the technique and show how to use it. We thereby obtain a very accurate approximate, or rather asymptotic, solution to (6.13) for $0 < \varepsilon \ll 1$. Before doing this we should reiterate that the specific nondimensionalisation (6.12) is only one of several we could choose. In the following section we analyse a system, a somewhat more complex one, which arises in a class of practical enzyme reactions using the more general formulation, since there, e_0/s_0 is not small but K_m is sufficiently large that ε as defined in (6.20) is small. Another practical reaction in which it is the large Michaelis constant K_m which makes $0 < \varepsilon \ll 1$ was studied by Frenzen and Maini (1988), who used the same type of analysis we discuss in the case study in Section 6.4.

Let us consider then the system (6.13). Suppose we simply look for a regular Taylor expansion solution to u and v in the form

$$u(\tau; \varepsilon) = \sum_{n=0} \varepsilon^n u_n(\tau), \quad v(\tau; \varepsilon) = \sum_{n=0} \varepsilon^n v_n(\tau), \tag{6.24}$$

which, on substituting into (6.13) and equating powers of ε, gives a sequence of differential equations for the $u_n(\tau)$ and $v_n(\tau)$. In other words we assume that $u(\tau; \varepsilon)$ and

$v(\tau;\varepsilon)$ are analytic functions of ε as $\varepsilon \to 0$. The $O(1)$ equations are

$$\frac{du_0}{d\tau} = -u_0 + (u_0 + K - \lambda)v_0, \quad 0 = u_0 - (u_0 + K)v_0, \qquad u_0(0) = 1, \quad v_0(0) = 0. \tag{6.25}$$

We can already see a difficulty with this approach since the second equation is simply algebraic and does not satisfy the initial condition; in fact if $u_0 = 1$, $v_0 = 1/(1+K) \neq 0$. If we solve (6.25)

$$v_0 = \frac{u_0}{u_0 + K} \quad \Rightarrow \quad \frac{du_0}{d\tau} = -u_0 + (u_0 + K - \lambda)\frac{u_0}{u_0 + K} = -\lambda\frac{u_0}{u_0 + K}$$

and so

$$u_0(\tau) + K \ln u_0(\tau) = A - \lambda\tau,$$

which is the same as (6.11). If we require $u_0(0) = 1$ then $A = 1$. Thus we have a solution $u_0(\tau)$, given implicitly, and the corresponding $v_0(\tau)$,

$$u_0(\tau) + K \ln u_0(\tau) = 1 - \lambda\tau, \quad v_0(\tau) = \frac{u_0(\tau)}{u_0(\tau) + K}, \tag{6.26}$$

which is the same as the solution (6.9). However, this solution is not a uniformly valid approximate solution for all $\tau \geq 0$ since $v_0(0) \neq 0$. This is not surprising since (6.25) involves only one derivative; it was obtained on setting $\varepsilon = 0$ in (6.13). The system of equations (6.25) has only one constant of integration from the u-equation so it is not surprising that we cannot satisfy initial conditions on *both* u_0 and v_0.

The fact that a small parameter $0 < \varepsilon \ll 1$ multiplies a derivative in (6.13) indicates that it is a *singular perturbation* problem. One class of such problems is immediately recognised if, on setting $\varepsilon = 0$, the order of the system of differential equations is reduced; such a reduced system cannot in general satisfy all the initial conditions. Singular perturbation techniques are very important and powerful methods for determining asymptotic solutions of such systems of equations for small ε. Asymptotic solutions are usually remarkably accurate approximations to the exact solutions. A practical and elementary discussion of some of the key techniques is given in Murray's (1984) book on asymptotic analysis. In the following, the philosophy and actual technique of the singular perturbation method is described in detail and the asymptotic solution to (6.13) for $0 < \varepsilon \ll 1$ derived. The main reason for doing this is to indicate when we can neglect the ε-terms in practical situations.

Since the solution (6.26), specifically $v_0(\tau)$, does not satisfy the initial conditions (and inclusion of higher-order terms in ε cannot remedy the problem) we must conclude that at least one of the solutions $u(\tau;\varepsilon)$ and $v(\tau;\varepsilon)$ is *not* an analytic function of ε as $\varepsilon \to 0$. By assuming $\varepsilon dv/d\tau$ is $O(e)$ to get (6.25) we tacitly assumed $v(\tau;\varepsilon)$ to be analytic; (6.24) also requires analyticity of course. Since the initial condition $v(0) = 0$ could not be satisfied because we neglected $\varepsilon dv/d\tau$ we must therefore retain this term in our analysis, at least near $\tau = 0$. So, a more appropriate timescale *near* $\tau = 0$ is

$\sigma = \tau/\varepsilon$ rather than τ; this makes $\varepsilon dv/d\tau = dv/d\sigma$. The effect of the transformation $\sigma = \tau/\varepsilon$ is to magnify the neighbourhood of $\tau = 0$ and let us look at this region more closely since, for a fixed $0 < \tau \ll 1$, we have $\sigma \gg 1$ as $\varepsilon \to 0$. That is, a very small neighbourhood near $\tau = 0$ corresponds to a very large domain in σ. We now use this to analyse (6.13) near $\tau = 0$, after which we shall get the solution away from $\tau = 0$ and finally show how to get a uniformly valid solution for all $\tau \geq 0$.

With the transformations

$$\sigma = \frac{\tau}{\varepsilon}, \quad u(\tau;\varepsilon) = U(\sigma;\varepsilon), \quad v(\tau;\varepsilon) = V(\sigma;\varepsilon) \tag{6.27}$$

the equations in (6.13) become

$$\begin{gathered} \frac{dU}{d\sigma} = -\varepsilon U + \varepsilon(U + K - \lambda)V, \quad \frac{dV}{d\sigma} = U - (U + K)V, \\ U(0) = 1, \quad V(0) = 0. \end{gathered} \tag{6.28}$$

If we now set $\varepsilon = 0$ to get the $O(1)$ system in a regular perturbation solution

$$U(\sigma;\varepsilon) = \sum_{n=0} \varepsilon^n U_n(\sigma), \quad V(\sigma;\varepsilon) = \sum_{n=0} \varepsilon^n V_n(\sigma), \tag{6.29}$$

we get

$$\begin{gathered} \frac{dU_0}{d\sigma} = 0, \quad \frac{dV_0}{d\sigma} = U_0 - (U_0 + K)V_0, \\ U_0(0) = 1, \quad V_0(0) = 0 \end{gathered} \tag{6.30}$$

which is *not* of lower order than the original system (6.28). The solution of (6.30) is

$$U_0(\sigma) = 1, \quad V_0(\sigma) = \frac{1}{1+K}(1 - \exp[-(1+K)\sigma]). \tag{6.31}$$

The last solution cannot be expected to hold for all $\tau \geq 0$, since if it did it would mean that $dv/d\sigma = \varepsilon dv/d\tau$ is $O(1)$ for all τ. The part of the solution given by (6.31) is the *singular* or *inner* solution for u and v and is valid for $0 \leq \tau \ll 1$, while (6.26) is the *nonsingular* or *outer* solution valid for all τ not in the immediate neighbourhood of $\tau = 0$. If we now let $\varepsilon \to 0$ we have for a fixed $0 < \tau \ll 1$, however small, $\sigma \to \infty$. Thus in the limit of $\varepsilon \to 0$ we expect the solution (6.26) as $\tau \to 0$ to be equal to the solution (6.31) as $\sigma \to \infty$; that is, the singular solution as $\sigma \to \infty$ matches the nonsingular solution as $\tau \to 0$. This is the essence of *matching* in singular perturbation theory. From (6.31) and (6.26) we see in fact that

$$\lim_{\sigma\to\infty}[U_0(\sigma), V_0(\sigma)] = \left[1, \frac{1}{1+K}\right] = \lim_{\tau\to 0}[u_0(\tau), v_0(\tau)].$$

Figure 6.1 illustrates the solution $u(\tau)$ and $v(\tau)$, together with the dimensionless enzyme concentration e/e_0 given by the dimensionless form of (6.6); namely, $e/e_0 =$

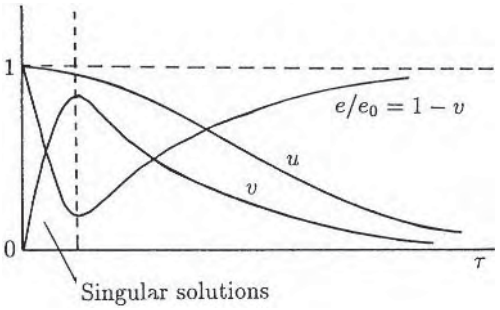

Figure 6.1. Schematic behaviour of the solutions of (6.13) for the dimensionless substrate (u), substrate–enzyme complex (v) and free enzyme ($e/e_0 = 1 - v$) concentrations as functions of the time τ.

$1 - v(\tau)$. The thin $O(\varepsilon)$ layer near $\tau = 0$ is sometimes called the *boundary layer* and is the τ-domain where there are very rapid changes in the solution. Here, from (6.31),

$$\left.\frac{dV}{d\tau}\right]_{\tau=0} \sim \varepsilon^{-1}\left.\frac{dV_0}{d\sigma}\right]_{\sigma=0} = \varepsilon^{-1} \gg 1.$$

Of course from the original system (6.13) we can see this from the second equation and the boundary conditions.

To proceed in a systematic singular perturbation way, we first look for the outer solution of the full system (6.13) in the form of a regular series expansion (6.24). The sequence of equations is then

$$\begin{aligned} O(1): \quad & \frac{du_0}{d\tau} = -u_0 + (u_0 + K - \lambda)v_0, \quad 0 = u_0 - (u_0 + K)v_0, \\ O(\varepsilon): \quad & \frac{du_1}{d\tau} = u_1(v_0 - 1) + (u_0 + K - \lambda)v_1, \\ & \frac{dv_0}{d\tau} = u_1(1 - v_0) - (u_0 + K)v_1, \end{aligned} \tag{6.32}$$

which are valid for $\tau > 0$. The solutions involve undetermined constants of integration, one at each order, which have to be determined by matching these solutions as $\tau \to 0$ with the singular solutions as $\sigma \to \infty$.

The sequence of equations for the singular part of the solution, valid for $0 \leq \tau \ll 1$, is given on substituting (6.29) into (6.28) and equating powers of ε; namely,

$$\begin{aligned} O(1): \quad & \frac{dU_0}{d\sigma} = 0 \quad \frac{dV_0}{d\sigma} = U_0 - (U_0 + K)V_0, \\ O(\varepsilon): \quad & \frac{dU_1}{d\sigma} = -U_0 + (V_0 + K - \lambda)V_0, \\ & \frac{dV_1}{d\sigma} = (1 - V_0)U_1 - (V_0 + K)V_1, \end{aligned} \tag{6.33}$$

and so on. The solutions of these must satisfy the initial conditions at $\sigma = 0$; that is, $\tau = 0$,

$$\begin{aligned} 1 = U(0;\varepsilon) &= \sum_{n=0} \varepsilon^n U_n(0) \quad \Rightarrow \quad U_0(0) = 1, \quad U_{n\geq 1}(0) = 0, \\ 0 = V(0;\varepsilon) &= \sum_{n=0} \varepsilon^n V_n(0) \quad \Rightarrow \quad V_{n\geq 0}(0) = 0. \end{aligned} \tag{6.34}$$

In this case the singular solutions of (6.33) are determined completely. This is not generally the case in singular perturbation problems (see, for example, Murray 1984). Matching of the inner and outer solutions requires choosing the undetermined constants of integration in the solutions of (6.32) so that to all orders of ε,

$$\lim_{\sigma\to\infty} [U(\sigma;\varepsilon), V(\sigma;\varepsilon)] = \lim_{\tau\to 0} [u(\tau;\varepsilon), v(\tau;\varepsilon)]. \tag{6.35}$$

Formally from (6.32), but as we had before,

$$u_0(\tau) + K \ln u_0(\tau) = A - \lambda\tau, \quad v_0(\tau) = \frac{u_0(\tau)}{u_0(\tau) + K},$$

where A is the constant of integration we must determine by matching. The solution of the first of (6.33) with (6.34) has, of course, been given before in (6.31). We get it now by applying the limiting process (6.35) to (6.31) and the last equations

$$\begin{aligned} &\lim_{\sigma\to\infty} V_0(\sigma) = \frac{1}{1+K} = \lim_{\tau\to 0} v_0(\tau) \\ \Rightarrow \quad & v_0(0) = \frac{1}{1+K} = \frac{u_0(0)}{u_0(0)+K} \\ \Rightarrow \quad & u_0(0) = 1 \quad \Rightarrow \quad A = 1. \end{aligned}$$

We thus get the uniformly valid asymptotic solution for $0 < \varepsilon \ll 1$ to $O(1)$, derived heuristically before and given by (6.26) for $\tau > 0$ and (6.31) for $0 < \tau \ll 1$, although the singular part of the solution is more naturally expressed in terms of $0 \leq \tau/\varepsilon < \infty$.

We can now proceed to calculate $U_1(\sigma)$ and $V_1(\sigma)$ from (6.33) and $u_1(\tau)$ and $v_1(\tau)$ from (6.32) and so on to any order in ε; the solutions become progressively more complicated even though all the equations are linear. In this way we get a uniformly valid asymptotic solution for $0 < \varepsilon \ll 1$ for all $\tau \geq 0$ of the nonlinear kinetics represented by (6.13). In summary, to $O(1)$ for small ε,

$$\begin{aligned} u(\tau;\varepsilon) &= u_0(\tau) + O(\varepsilon), \quad u_0(\tau) + K \ln u_0(\tau) = 1 - \lambda\tau, \\ v(\tau;\varepsilon) &= V_0(\sigma) + O(\varepsilon), \quad V_0(\sigma) = \frac{1}{1+K}\left(1 - \exp\left[-(1+K)\frac{\tau}{\varepsilon}\right]\right), \\ & \qquad\qquad\qquad\qquad\qquad\qquad 0 < \tau \ll 1; \\ &= v_0(\tau) + O(\varepsilon), \quad v_0(\tau) = \frac{u_0(\tau)}{u_0(\tau)+K}, \quad 0 < \varepsilon \ll \tau. \end{aligned} \tag{6.36}$$

Since in most biological applications $0 < \varepsilon \ll 1$, we need only evaluate the $O(1)$ terms: the $O(\varepsilon)$ terms' contributions are negligible.

To complete the analysis of the original kinetics problem (6.3) with (6.4), if we write the dimensionless product and free enzyme concentrations as

$$z(\tau) = \frac{p(t)}{s_0}, \quad w(\tau) = \frac{e(t)}{e_0}$$

then, using (6.36) for u and v, (6.5) and (6.6) give

$$z(\tau) = \lambda \int_0^\tau v(\tau')\, d\tau', \quad w(\tau) = 1 - v(\tau).$$

The rapid change in the substrate–enzyme complex $v(\tau; \varepsilon)$ takes place in dimensionless times $\tau = O(\varepsilon)$ which is very small. The equivalent dimensional time t is also very short, $O(1/k_1 s_0)$ in fact, and for many experimental situations is not measurable. Thus in many experiments the singular solution for $u(\tau)$ and $v(\tau)$ is never observed. The relevant solution is then the $O(1)$ outer solution $u_0(\tau)$, $v_0(\tau)$ in (6.26), obtained from the kinetics system (6.13) on setting $\varepsilon = 0$ and satisfying only the initial condition on $u(\tau)$, the substrate concentration. In other words we say that the reaction for the complex $v(\tau)$ is essentially in a steady state, or mathematically that $\varepsilon dv/d\tau \approx 0$. That is, the v-reaction is so fast it is more or less in equilibrium at all times. This is the usual Michaelis and Menten's *pseudo-* or *quasi-steady state hypothesis*.

The form of (6.13) is generally like

$$\frac{du}{d\tau} = f(u, v), \quad \frac{dv}{d\tau} = \varepsilon^{-1} g(u, v), \quad 0 < \varepsilon \ll 1, \tag{6.37}$$

which immediately shows that $dv/d\tau \gg 1$ if $g(u, v)$ is not approximately equal to zero. So the v-reaction is very fast compared with the u-reaction. The v-reaction reaches a quasi-steady state very quickly, which means that for times $\tau = O(1)$ it is essentially at equilibrium and the model mechanism is then approximated by

$$\frac{du}{d\tau} = f(u, v), \quad g(u, v) = 0, \quad u(0) = 1. \tag{6.38}$$

If we solve the algebraic equation $g(u, v) = 0$ to get $v = h(u)$ then

$$\frac{du}{d\tau} = f(u, h(u)), \tag{6.39}$$

which is the rate or *uptake* equation for the substrate concentration. Much modelling of biological processes hinges on qualitative assumptions for the uptake function $f(u, h(u))$.

What is of interest biologically is the *rate of reaction*, or the rate of uptake; that is, $du/d\tau$ when $u(\tau)$ has been found. It is usually determined experimentally by measuring the dimensional substrate concentration $s(t)$ at various times, then extrapolating back to $t = 0$, and the magnitude r of the initial rate $[ds/dt]_{t=0}$ calculated. Since the time

measurements are almost always for $\tau \gg \varepsilon$, that is, $t \gg 1/k_1 s_0$, which is usually of the order of seconds, the equivalent analytical rate is given by the *nonsingular* or *outer solution*. Thus, from the first of (6.36) the $O(1)$ solution with $0 < \varepsilon \ll 1$ for the rate r, r_0 say, is

$$r_0 = \left[\frac{du_0(\tau)}{d\tau}\right]_{\tau=0} = \lambda \frac{u_0(0)}{u_0(0) + K_m} = \frac{\lambda}{1 + K}. \tag{6.40}$$

In dimensional terms, using (6.12), the $O(1)$ rate of reaction R_0 is

$$R_0 = \frac{k_2 e_0 s_0}{s_0 + K_m} = \frac{Q s_0}{s_0 + K_m}, \quad K_m = \frac{k_{-1} + k_2}{k_1}, \quad Q = [R_0]_{\max} = k_2 e_0, \tag{6.41}$$

where Q is the maximum velocity, or rate, of the reaction and K_m is the *Michaelis constant* of (6.9). This rate, based on the pseudo-steady state hypothesis, is what is usually wanted from a biological point of view. From (6.13), the exact initial rate for the substrate is $[du/d\tau]_{\tau=0} = -1$ while for the complex it is $[dv/d\tau]_{\tau=0} = 1/\varepsilon$.

When the uptake of a substrate, or whatever, is described as a Michaelis–Menten uptake, what is understood is a rate of reaction like (6.41) and which is illustrated in Figure 6.2. The rate of reaction, which in fact varies with time, is the magnitude of ds/dt from the outer solution $du_0/d\tau$ and written in dimensional form. Thus the (Michaelis–Menten) uptake of S is governed by the equation

$$\frac{ds}{dt} = -\frac{Qs}{K_m + s}. \tag{6.42}$$

This is simply the dimensional form of (6.39) (and the same as (6.10)) on carrying out the algebra for $f(u, v)$, $g(u, v)$ in (6.38), with (6.13) defining them. For $s \ll K_m$ the uptake is linear in s; the right-hand side of (6.42) is approximately $-Qs/K_m$. The maximum rate $Q = k_2 e_0$, from (6.41), depends on the rate constant k_2 of the product reaction $SE \to P + E$; this is called the *rate limiting* step in the reaction mechanism (6.1).

Useful and important as the quasi-steady state hypothesis is, something is lost by assuming $\varepsilon dv/dt$ is negligible in (6.13) and by applying experimental results to a theory which cannot satisfy all the initial conditions. What can be determined, using experi-

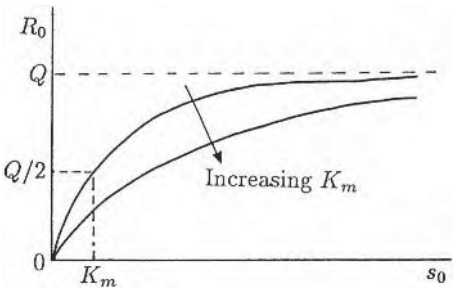

Figure 6.2. Michaelis–Menten rate of uptake $R_0 = Qs_0/(K_m + s_0)$ as a function of the substrate concentration s_0: Q is the maximum rate and K_m is the Michaelis constant.

mental results with a Michaelis–Menten theory, is a curve such as in Figure 6.2, which gives values for the maximum rate Q and the Michaelis constant K_m. This does not determine all three rate constants k_1, k_{-1} and k_2, only k_2 and a relationship between them all. To determine all of them, measurements for $\tau = O(\varepsilon)$ would be required. Usually, however, the rate of uptake from the quasi-steady state hypothesis, that is, a Michaelis–Menten theory, is all that is required.

6.4 Suicide Substrate Kinetics

An enzyme system of considerable experimental interest (see, for example, Seiler et al. 1978, Walsh 1984) is the mechanism-based inhibitor, or suicide substrate system, represented by Walsh et al. (1978),

$$\begin{array}{c} E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X \overset{k_2}{\to} Y \overset{k_3}{\to} E + P, \\ \qquad\qquad\quad \downarrow k_4 \\ \qquad\qquad\quad E_i \end{array} \tag{6.43}$$

where E, S and P denote enzyme, substrate and product, respectively, X and Y enzyme–substrate intermediates, E_i inactivated enzyme, and the k's are positive rate constants. In this system, Y can follow one of two pathways, namely, to $E + P$ with rate k_3 or to E_i with rate k_4. The ratio of these rates, k_3/k_4, is called the *partition ratio* and is denoted by r. Both of these pathways are considered to be irreversible over the timescale of the reaction (Waley 1980). S is known as a *suicide substrate* because it binds to the active site of an enzyme—like a substrate—but the enzyme converts it into an inhibitor which irreversibly inactivates the enzyme. Thus, the enzyme 'commits suicide.'

Suicide substrates are important because they provide a way to target a specific enzyme for inactivation. They are especially useful in drug administration, since they are not harmful in their common form and only the designated enzyme can convert them to their inhibitor form. For example, suicide substrates have been investigated for use in the treatment of depression (monoamine oxidase inhibitors, Seiler et al. 1978), epilepsy (brain GABA transaminase inhibitors, Walsh 1984), and some tumors (ornithine decarboxylase inhibitors, Seiler et al. 1978).

Suicide substrate kinetics have been considered by Waley (1980) and by Tatsunami et al. (1981), who were interested in the factor which determined whether the substrate was exhausted before all the enzyme was inactivated. Waley suggested it was $r\mu$, where μ is the ratio of the initial concentration of enzyme to that of substrate, namely, e_0/s_0, our ε in (6.12). Tatsunami et al. (1981), on the other hand, found the determining factor to be $(1+r)\mu$. When $(1+r)\mu > 1$ the substrate is exhausted, while for $(1+r)\mu < 1$, all the enzyme is inactivated. When $(1 + r)\mu = 1$, both occur. An in depth analysis using singular perturbation analysis is given by Burke et al. (1990). It is their analysis we follow below. The interest is when e_0/s_0 is not small, which was in effect assumed since both Waley (1980) and Tatsunami et al. (1981) used a quasi-steady state approximation. From our experience above, the validity decreases for increasing values of e_0/s_0.

Duggleby (1986) pointed out that in fact e_0/s_0 is not small. So, we must use a singular perturbation technique but in this case we must use an equivalent nondimensionalisation to (6.20) rather than (6.12), since here it is $e_0/(s_0 + K_m)$ which is small, while e_0/s_0 is $O(1)$.

The rate equations obtained from (6.43) using the Law of Mass Action are:

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[X] \tag{6.44}$$

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[X] + k_3[Y] \tag{6.45}$$

$$\frac{d[X]}{dt} = k_1[E][S] - k_{-1}[X] - k_2[X] \tag{6.46}$$

$$\frac{d[Y]}{dt} = k_2[X] - k_3[Y] - k_4[Y] \tag{6.47}$$

$$\frac{d[E_i]}{dt} = k_4[Y] \tag{6.48}$$

$$\frac{d[P]}{dt} = k_3[Y], \tag{6.49}$$

where, as before, [ ] denotes concentration, and t time. Typical experimental initial conditions which complete the mathematical formulation are

$$\begin{aligned} &E(0) = e_0, \quad S(0) = s_0, \\ &X(0) = Y(0) = E_i(0) = P(0) = 0. \end{aligned} \tag{6.50}$$

Again, (6.49) is uncoupled and $[P]$ can be evaluated by integration after $[Y]$ has been found.

The order of the system can be further reduced using conservation of enzyme, since adding (6.45)–(6.48) gives

$$\frac{d}{dt}\{[E] + [X] + [Y] + [E_i]\} = 0 \tag{6.51}$$

$$\Rightarrow [E] + [X] + [Y] + [E_i] = e_0. \tag{6.52}$$

Using (6.52) to eliminate $[E]$, we obtain the reduced system

$$\frac{d[S]}{dt} = -k_1\left(e_0 - [X] - [Y] - [E_i]\right)[S] + k_{-1}[X] \tag{6.53}$$

$$\frac{d[X]}{dt} = k_1\left(e_0 - [X] - [Y] - [E_i]\right)[S] - (k_{-1} + k_2)[X] \tag{6.54}$$

$$\frac{d[Y]}{dt} = k_2[X] - (k_3 + k_4)[Y] \tag{6.55}$$

$$\frac{d[E_i]}{dt} = k_4[Y]. \tag{6.56}$$

Nondimensional Form

There are several ways to nondimensionalise the system. Since $e_0/s_0 = O(1)$, we follow the appropriate procedure in Section 6.2, equivalent to (6.20) for the outer region and (6.22) for the inner region.

We nondimensionalise the variables by setting

$$\begin{aligned} [S] &= s_0 s, \quad [X] = \frac{e_0 s_0}{s_0 + K_m} x, \\ [Y] &= e_0 y, \quad [E_i] = e_0 e_i, \end{aligned} \tag{6.57}$$

where

$$K_m = \frac{k_{-1} + k_2}{k_1}. \tag{6.58}$$

The fast-transient timescale is (cf. (6.20)) taken as

$$\tau = t/t_c = t k_1 (s_0 + K_m) \tag{6.59}$$

and the quasi-steady state timescale as

$$T = (1 + \rho) t/t_s = t\varepsilon (k_{-1} + k_2)(1 + \rho) \tag{6.60}$$

with ρ as in (6.66) below and

$$\varepsilon = \frac{e_0}{e_0 + K_m}. \tag{6.61}$$

Using the scalings in (6.57) with τ as the timescale, equations (6.53) to (6.56) for the fast-transient phase are

$$\frac{ds}{d\tau} = \varepsilon \left[-s + \frac{\sigma}{1+\sigma} sx + sy + se_i + \frac{\rho}{(1+\rho)(1+\sigma)} x \right] \tag{6.62}$$

$$\frac{dx}{d\tau} = s - \left(\frac{\sigma}{1+\sigma} \right) sx - sy - se_i - \frac{x}{1+\sigma} \tag{6.63}$$

$$\frac{dy}{d\tau} = \left(\frac{\sigma}{(1+\sigma)^2 (1+\rho)} \right) x - \left(\frac{\psi}{(1+\sigma)} \right) y \tag{6.64}$$

$$\frac{de_i}{d\tau} = \left(\frac{\phi}{1+\sigma} \right) y, \tag{6.65}$$

where

$$\sigma = \frac{s_0}{K_m}, \quad \rho = \frac{k_{-1}}{k_2}, \quad \psi = \frac{k_3 + k_4}{k_{-1} + k_2}, \quad \phi = \frac{k_4}{k_{-1} + k_2}. \tag{6.66}$$

The initial conditions (6.50) become, on using (6.57),

$$s(0) = 1, \quad x(0) = 0, \quad y(0) = 0, \quad e_i(0) = 0. \tag{6.67}$$

Equations (6.62)–(6.65) are the equivalent of (6.21); they give the singular or inner solution.

With T as the timescale, the rate equations for the nonsingular, or outer, quasi-steady state phase are

$$\frac{ds}{dT} = -s\,[(\sigma+1) - \sigma x - (\sigma+1)y - (\sigma+1)e_i] + \frac{\rho}{1+\rho}x \tag{6.68}$$

$$\varepsilon\frac{dx}{dT} = s\,[(\sigma+1) - \sigma x - (\sigma+1)y - (\sigma+1)e_i] - x \tag{6.69}$$

$$\varepsilon\frac{dy}{dT} = \left(\frac{\sigma}{(1+\sigma)(1+\rho)}\right)x - \psi y \tag{6.70}$$

$$\varepsilon\frac{de_i}{dT} = \phi y, \tag{6.71}$$

where ε, σ, ρ, ψ and ϕ are given by (6.66). These are the equivalent here of (6.23).

Asymptotic Technique and Solutions

We now exploit the fact that $0 < \varepsilon \ll 1$ for ε in (6.61) and solve for the equations by the singular perturbation technique discussed in detail in the last section. There are some significant differences in the analysis, however, other than just being more complicated algebraically.

Inner or Singular Solutions

We begin with the fast-transient phase equations, (6.62)–(6.65), with initial conditions (6.67), and because ε is small we look for a Taylor series solution in the form

$$s(\tau) = s^{(0)}(\tau) + \varepsilon s^{(1)}(\tau) + \varepsilon^2 s^{(2)}(\tau) + \ldots \tag{6.72}$$

for each of the variables s, x, y and e_i. Substituting these into (6.62)–(6.65) and equating like powers of ε, we find

$$\frac{ds^{(0)}}{d\tau} = 0 \quad , \quad \frac{dy^{(0)}}{d\tau} = -\frac{\psi}{1+\sigma}y^{(0)}, \tag{6.73}$$

which with (6.67) give as the unique solutions $s^{(0)}(\tau) \equiv 1$, $y^{(0)}(\tau) \equiv 0$. In the same way, (6.65) yields, to $O(1)$,

$$\frac{de_i^{(0)}}{d\tau} = -\frac{\phi}{1+\sigma}y^{(0)} = 0 \tag{6.74}$$

which implies that $e_i^{(0)}(\tau) \equiv 0$ since $e_i(0) = 0$. Finally, substituting the series solutions

into (6.65), we obtain

$$\frac{dx^{(0)}}{d\tau} = s^{(0)} - s^{(0)}y^{(0)} - s^{(0)}e_i^{(0)} - \frac{x^{(0)}}{1+\sigma} - \frac{\sigma s^{(0)}x^{(0)}}{1+\sigma}. \tag{6.75}$$

With the above solutions for $s^{(0)}$, $y^{(0)}$ and $e_i^{(0)}$, this becomes

$$\frac{dx^{(0)}}{d\tau} = 1 - x^{(0)} \tag{6.76}$$

which, with $x(0) = 0$, gives $x^{(0)}(\tau) = 1 - e^{-\tau}$.

To obtain nonzero solutions for y and e_i, we need to determine at least the $O(\varepsilon)$ terms, $y^{(1)}(\tau)$ and $e_i^{(1)}(\tau)$. This involves matching the coefficients of $O(\varepsilon)$ terms.

Note that, from (6.61), with (6.66),

$$\varepsilon = \frac{e_0}{s_0(1 + K_m/s_0)} = \frac{e_0}{s_0}\frac{\sigma}{1+\sigma} \tag{6.77}$$

which implies that

$$\sigma = \left(\frac{s_0}{e_0}\right)\varepsilon + O(\varepsilon^2). \tag{6.78}$$

Since $s_0/e_0 = O(1)$, this implies that $\sigma = O(\varepsilon)$. Here we introduce a *similarity variable* for σ,

$$\sigma = \varepsilon p, \tag{6.79}$$

where p is a constant of $O(1)$. We show the ε factor explicitly so that we can match it with the $O(\varepsilon)$ terms. Substituting (6.79) for σ in (6.64), we equate terms of $O(\varepsilon)$:

$$\frac{dy^{(1)}}{d\tau} = \frac{p}{(1+\rho)}x^{(0)} - \psi y^{(1)}. \tag{6.80}$$

Since we already know $x^{(0)}(\tau) = 1 - e^{-\tau}$, we can solve this linear equation for $y^{(1)}(\tau)$:

$$y^{(1)}(\tau) = \frac{p}{\psi(1+\rho)}\left(\frac{1 - e^{-\psi\tau}}{\psi} + \frac{e^{-\psi\tau} - e^{-\tau}}{\psi - 1}\right). \tag{6.81}$$

Now, matching coefficients in (6.65) to $O(\varepsilon)$ gives an equation for $de_i/d\tau$ in terms of $y^{(1)}$. A little algebra gives the solution as

$$e_i^{(1)}(\tau) = \frac{\phi p}{(1+\rho)}\left(\frac{\tau}{\psi} + \frac{e^{-\tau} - 1}{\psi - 1} + \frac{1 - e^{-\psi\tau}}{\psi^2(\psi - 1)}\right). \tag{6.82}$$

In obtaining $e_i^{(1)}(\tau)$, we assumed $\phi = O(1)$. If it were the case that $\phi = O(\varepsilon)$, we would have used another similarity variable, $q = \varepsilon\phi$, and found that $e_i^{(1)}(\tau)$, but that $e_i^{(2)}(\tau)$ gives the same result as $e_i^{(1)}(\tau)$ above.

In a similar manner, we can find the coefficients of higher-order terms in the series. For example, the $O(\varepsilon)$ terms of (6.62) give

$$s^{(1)}(\tau) = -\frac{\tau}{1+\rho} + \frac{\rho}{1+\rho}(e^{-\tau} - 1). \tag{6.83}$$

All of these solutions satisfy the initial conditions, (6.67).

Outer or Quasi-Steady State Solutions

We now proceed to look for solutions in the long timescale which gives the quasi-steady state approximation. We then want to match the two time period solutions. Recall that these long timescale solutions will not in general satisfy the initial conditions. Undetermined constants of integration are evaluated by matching the solution domains as we did in Section 6.3.

Here we look for solutions to (6.68)–(6.71) in the form

$$s(T) = s_{(0)}(T) + \varepsilon s_{(1)}(T) + \varepsilon^2 s_{(2)}(T) + \ldots \tag{6.84}$$

for each of the variables s, x, y and e_i. Substituting these into (6.68)–(6.71), we again equate coefficients of powers of ε. Here, however, we must solve for the undetermined constants of integration, which we do by the method of matched asymptotic expansions; that is, the inner solution as $\tau \to \infty$ must match the outer solution as $T \to 0$.

Taking the $O(1)$ terms, we get, remembering that $\sigma = \varepsilon p = O(\varepsilon)$ from (6.79),

$$0 = s_{(0)} - s_{(0)}y_{(0)} - x_{(0)} - s_{(0)}e_{i(0)} \tag{6.85}$$

from (6.69), and, assuming $\psi = O(1)$, $y_{(0)} = 0$ from (6.71). Together, these give

$$x_{(0)} = s_{(0)}\left(1 - e_{i(0)}\right). \tag{6.86}$$

Similarly, we obtain

$$y_{(1)} = \frac{p}{\psi(1+\rho)}x_{(0)}. \tag{6.87}$$

To equate coefficients further, we need to determine the order of magnitude of each of the terms. Experimentally, we know that there are two fundamentally different outcomes: either all of the substrate is exhausted, or all of the enzyme is inactivated. These correspond to $\phi = O(1)$ with $\psi = O(1)$, and $\psi = O(1)$ with $\phi = O(\varepsilon)$ (refer to (6.66) for the parameter relations). We must therefore solve the equations for each of these sets of constraints.

Case 1 $\rho = O(1), \quad \psi = O(1), \quad \phi = O(1)$

This is the case when all of the rate constants are of the same order of magnitude. By assuming $\phi = O(1)$, (6.68) with (6.79), (6.84) and (6.86) give

$$\frac{ds_{(0)}}{dT} = -\frac{1}{1+\rho} s_{(0)}(1 - e_{i(0)}). \tag{6.88}$$

From (6.69), with (6.79), (6.84), (6.86) and (6.87), we get

$$\frac{de_{i(0)}}{dT} = \frac{\phi\rho}{\psi(1+\rho)} s_{(0)}(1 - e_{i(0)}). \tag{6.89}$$

The last two equations give, on dividing and integrating,

$$e_{i(0)}(T) = \frac{1}{\beta}\left(B - s_{(0)}(T)\right), \tag{6.90}$$

where B is a constant of integration and

$$\beta = \frac{\psi}{\phi\rho}. \tag{6.91}$$

We determine B by using the *matching condition* discussed in detail in Section 6.3 (or for more detail, see Murray 1984). This is the condition that $s_{(0)}(T)$, $x_{(0)}(T)$, $y_{(0)}(T)$ and $e_{i(0)}(T)$ as $T \to 0$ must match with the values, respectively, of $s^{(0)}(\tau)$, $x^{(0)}(\tau)$, $y^{(0)}(\tau)$ and $e_i^{(0)}(\tau)$ as $\tau \to \infty$. We know that $s^{(0)}(\tau) \equiv 1$, $x^{(0)}(\tau) = 1 - e^{-\tau}$, $y^{(0)}(\tau) \equiv 0$, $e_i^{(0)}(\tau) \equiv 0$ so the conditions on the $O(1)$ outer solution are

$$s_{(0)}(T) \to 1, \quad x_{(0)}(T) \to 1, \quad y_{(0)}(T) \to 0 \quad \text{and} \quad e_{i(0)}(T) \to 0 \text{ as } T \to 0. \tag{6.92}$$

With these we see that $B = 1$ in (6.90) which then, on substituting into (6.88), gives

$$\frac{ds_{(0)}}{dT} = -\frac{(\beta - 1)}{\beta(1+\rho)} s_{(0)}\left[1 - \frac{s_{(0)}}{1-\beta}\right]$$

which on integrating and using the condition as $T \to 0$ from (6.92) gives $s_{(0)}(T)$ and $e_{i(0)}(T)$ as

$$\begin{aligned} s_{(0)}(T) &= \frac{1-\beta}{1 - \beta e^{T[1-(1/\beta)]/(1+\rho)}}, \\ e_{i(0)}(T) &= \frac{1 - s_{(0)}(T)}{\beta}. \end{aligned} \tag{6.93}$$

Case 2 $\rho = O(1), \quad \psi = O(1), \quad \phi = O(\varepsilon)$

Assuming $\phi = O(\varepsilon)$ gives

$$s_{(0)}(T) = e^{-T/(1+\rho)}, \quad e_{i(0)} = 0, \quad \varepsilon e_{i(1)}(T) = \frac{1 - e^{-T/(1+\rho)}}{\beta}, \tag{6.94}$$

where again we have matched with the inner solutions.

In both the inner and outer solutions, we could continue to solve for terms of higher-order of ε in the series, (6.72) and (6.84). The solutions would become progressively more complicated, but in each case the equations are linear. For most practical purposes the first nonzero terms are sufficiently accurate.

Uniformly Valid Solution for All Time

Now that we have solutions for the fast transient and the quasi-steady state time periods, we can obtain *composite solutions* that are valid for all time $t \geq 0$ by a simple method detailed, for example, in Kevorkian and Cole (1996). We add the first term of the inner solutions to the corresponding term of the outer solutions and subtract their common part—the limit of the inner solution as time (τ) goes to infinity, which is the same as the limit of the outer solutions as time (T) tends toward zero. For example, the inner solution for s is $s^{(0)}(\tau) = 1$. The outer solution for Case 2 is $s_{(0)}(T) = \exp(-T/(1+\rho))$. The limits described above are both 1, so the composite solution is:

$$s^0_{\text{comp}} = 1 + e^{-T/(1+\rho)} - 1 = e^{-t/t_s} = e^{-\varepsilon(k_{-1}+k_2)t} \tag{6.95}$$

on using (6.60).

Doing the same for the other solutions, we obtain two sets of composite solutions, one for Case 1 and one for Case 2, which are valid for all time.

Case 1:

$$\begin{aligned}
&s^0_{\text{comp}}(t) = \frac{1-\beta}{1-\beta e^{t(1-1/\beta)/t_s}}, \quad e^0_{i\,\text{comp}}(t) = \frac{1-s^0_{\text{comp}}}{\beta},\\
&x^0_{\text{comp}}(t) = s^0_{\text{comp}}\left(1 - e^0_{i\,\text{comp}}\right) - e^{-t/t_c}, \quad y^0_{\text{comp}}(t) = 0,\\
&\varepsilon y^1_{\text{comp}}(t) = \frac{\sigma}{\psi(1+\rho)}\left(\frac{e^{-\psi t/t_c} - \psi e^{-t/t_c}}{\psi - 1} + s^0_{\text{comp}}\left(1 - e^0_{\text{comp}}\right)\right).
\end{aligned} \tag{6.96}$$

Case 2:

$$\begin{aligned}
&s^0_{\text{comp}}(t) = e^{-t/t_s}, \quad e^0_{i\,\text{comp}}(t) = 0, \quad \varepsilon e^1_{i\,\text{comp}}(t) = \frac{1-s^0_{\text{comp}}}{\beta},\\
&x^0_{\text{comp}}(t) = s^0_{\text{comp}} - e^{-t/t_c}, \quad y^0_{\text{comp}}(t) = 0,\\
&\varepsilon y^1_{\text{comp}}(t) = \frac{\sigma}{\psi(1+\rho)}\left(\frac{e^{-\psi t/t_c} - \psi e^{-t/t_c}}{\psi - 1} + s^0_{\text{comp}}\right),
\end{aligned} \tag{6.97}$$

where $\beta = \psi/\phi p$ and σ, ρ and ψ are as in (6.66).

Note that the important parameter distinguishing Cases 1 and 2 is β. When $\beta < 1$, Case 1 holds, and when $\beta > 1$, Case 2 holds. This β is, in fact, the same parameter that Tatsunami et al. (1981) called $(1+r)\mu$. The above expressions show that for $\beta < 1$, $e_i \to 1$ as $T \to \infty$ (to first-order in ε), while for $\beta > 1$, $s \to 0$ as $T \to \infty$ (to first-order in ε). These directly relate to the amount of inactivated enzyme as we discussed at the beginning of this section.

Numerical Solutions and Comparison with Analytic Solutions

Now that we have approximate asymptotic solutions to our nondimensionalised systems, we compare them to the numerical solutions obtained by Burke et al. (1990) to highlight their accuracy.

They solved the dimensional system, (6.53)–(6.56), numerically. Since the numerical analysis was carried out on the dimensional system, the nondimensional concentrations were multiplied by their scale factors before plotting for ease of comparison. The first two terms of the composite solutions are compared to the numerical solutions in Figure 6.3. These graphs illustrate that the composite solutions are far more accurate than previous solutions in the inner domain.

Figure 6.4 shows the numerical solutions compared to the composite solutions for intermediate concentrations X and Y. The first term of the Case 2 composite solution as previously given was used for each of X and Y. These intermediate results are more ac-

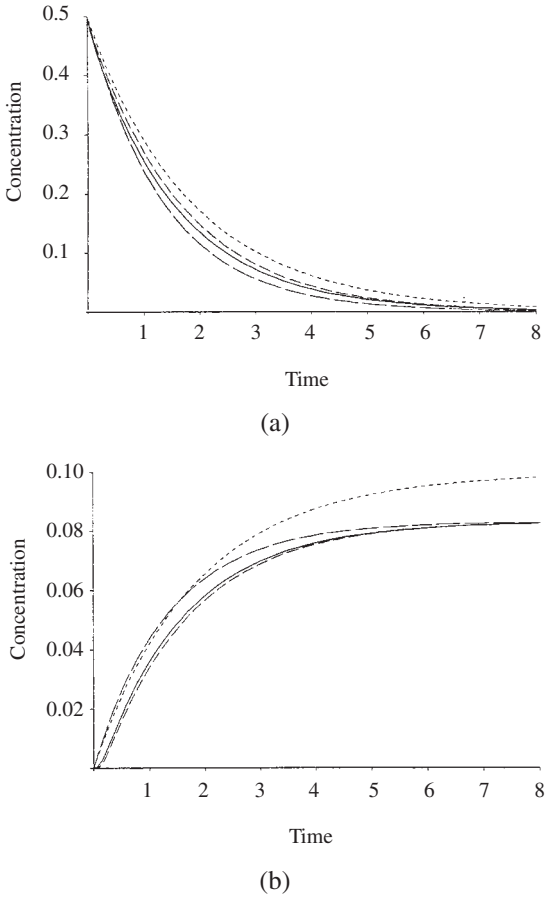

Figure 6.3. First two terms of the Case 2 composite series solutions (– – –) compared to numerical solutions (——) and previous approximations: Waley (1985) (- - -) and Tatsunami et al. (1981) (— — —). (**a**) Substrate concentration; (**b**) inactive enzyme concentration. Parameters: $k_1 = 2$, $k_{-1} = 4$, $k_2 = 12$, $k_3 = 10$, $k_4 = 2$, $e_0 = 0.5$, $s_0 = 0.5$. These give $\varepsilon = 5.88 \times 10^{-2}$, $\rho = 0.333$, $\beta = 5.647$. (From Burke et al. 1990)

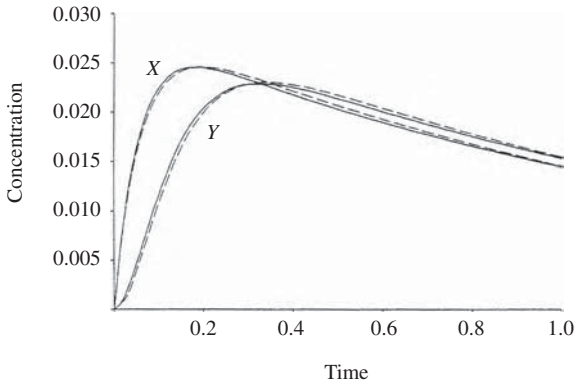

Figure 6.4. Case 2 composite (– – –) solutions compared to numerical solutions (——) for X and Y, the intermediate concentrations. Parameters are the same as in Figure 6.3. (From Burke et al. 1990)

curate than any quasi-steady state method achieves, since the method here incorporates the variation of the intermediate time derivatives prior to the quasi-steady state.

The above results show that the analytical solutions are a very good approximation of the kinetics of the suicide substrate system represented by (6.43). It does not appear much more complex than the basic enzyme reaction (6.1) but, as we have seen, the analysis is much more involved. The method developed here is particularly useful in estimating the intermediate (X and Y) concentrations which no previous analysis had been able to do. Perhaps the most important result of the method described here is that the solutions are obtained analytically in terms of the kinetic parameters. These solutions may be used to estimate the parameters by the methods described by Waley (1985) and Duggleby (1986). Such analytical solutions are especially important when the equations are stiff, that is, when small parameters multiply derivatives in the differential equation system, when numerical solutions can be delicate to compute accurately.

6.5 Cooperative Phenomena

In the model mechanism (6.1) one enzyme molecule combines with one substrate molecule; that is, the enzyme has one binding site. There are many enzymes which have more than one binding site for substrate molecules. For example, haemoglobin (Hb), the oxygen-carrying protein in red blood cells, has 4 binding sites for oxygen (O_2) molecules. A reaction between an enzyme and a substrate is described as *cooperative* if a single enzyme molecule, after binding a substrate molecule at one site can then bind another substrate molecule at another site. Such phenomena are common.

Another important cooperative behaviour is when an enzyme with several binding sites is such that the binding of one substrate molecule at one site can affect the activity of binding other substrate molecules at another site. This indirect interaction between distinct and specific binding sites is called *allostery*, or an *allosteric effect*, and an enzyme exhibiting it, an allosteric enzyme. If a substrate that binds at one site increases the binding activity at another site then the substrate is an *activator*; if it decreases the

activity it is an *inhibitor*. The detailed mathematical analysis for the kinetics of such allosteric reactions is given briefly in the book by Murray (1977) and in more detail in the one by Rubinow (1975). The latter book also gives a graph-theoretic approach to enzyme kinetics.

As an example of a cooperative phenomenon we consider the case where an enzyme has 2 binding sites and calculate an equivalent quasi-steady state approximation and the substrate uptake function. A model for this consists of an enzyme molecule E which binds a substrate molecule S to form a single bound substrate–enzyme complex C_1. This complex C_1 not only breaks down to form a product P and the enzyme E again; it can also combine with another substrate molecule to form a dual bound substrate–enzyme complex C_2. This C_2 complex breaks down to form the product P and the single bound complex C_1. A reaction mechanism for this model is then

$$\begin{aligned} S + E &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \overset{k_2}{\to} E + P, \\ S + C_1 &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \overset{k_4}{\to} C_1 + P, \end{aligned} \tag{6.98}$$

where the k's are the rate constants as indicated.

With lowercase letters denoting concentrations, the mass action law applied to (6.98) gives

$$\begin{aligned} \frac{ds}{dt} &= -k_1 se + (k_{-1} - k_3 s)c_1 + k_{-3}c_2, \\ \frac{dc_1}{dt} &= k_1 se - (k_{-1} + k_2 + k_3 s)c_1 + (k_{-3} + k_4)c_2, \\ \frac{dc_2}{dt} &= k_3 sc_1 - (k_{-3} + k_4)c_2, \\ \frac{de}{dt} &= -k_1 se + (k_{-1} + k_2)c_1, \\ \frac{dp}{dt} &= k_2 c_1 + k_4 c_2. \end{aligned} \tag{6.99}$$

Appropriate initial conditions are

$$s(0) = s_0, \quad e(0) = e_0, \quad c_1(0) = c_2(0) = p(0) = 0. \tag{6.100}$$

The conservation of the enzyme is obtained by adding the 2nd, 3rd and 4th equations in (6.99) and using the initial conditions; it is

$$\frac{dc_1}{dt} + \frac{dc_2}{dt} + \frac{de}{dt} = 0 \quad \Rightarrow \quad e + c_1 + c_2 = e_0. \tag{6.101}$$

The equation for the product $p(t)$ is again uncoupled and given, by integration, once c_1 and c_2 have been found. Thus, using (6.101), the resulting system we have to solve is

$$\begin{aligned}
\frac{ds}{dt} &= -k_1 e_0 s + (k_{-1} + k_1 s - k_3 s)c_1 + (k_1 s + k_{-3})c_2,\\
\frac{dc_1}{dt} &= k_1 e_0 s - (k_{-1} + k_2 + k_1 s + k_3 s)c_1 + (k_{-3} + k_4 - k_1 s)c_2,\\
\frac{dc_2}{dt} &= k_3 s c_1 - (k_{-3} + k_4)c_2,
\end{aligned} \tag{6.102}$$

with initial conditions (6.100).

As always, we nondimensionalise the system. As we saw above, there are several ways we can do this. If $e_0/s_0 \ll 1$, we write

$$\begin{aligned}
&\tau = k_1 e_0 t, \quad u = \frac{s}{s_0}, \quad v_1 = \frac{c_1}{e_0}, \quad v_2 = \frac{c_2}{e_0},\\
&a_1 = \frac{k_{-1}}{k_1 s_0}, \quad a_2 = \frac{k_2}{k_1 s_0}, \quad a_3 = \frac{k_3}{k_1}, \quad a_4 = \frac{k_{-3}}{k_1 s_0},\\
&a_5 = \frac{k_4}{k_1 s_0}, \quad e = \frac{e_0}{s_0},
\end{aligned} \tag{6.103}$$

and (6.102) becomes

$$\frac{du}{d\tau} = -u + (u - a_3 u + a_1)v_1 + (a_4 + u)v_2 = f(u, v_1, v_2), \tag{6.104}$$

$$\varepsilon \frac{dv_1}{d\tau} = u - (u + a_3 u + a_1 + a_2)v_1 + (a_4 + a_5 - u)v_2 = g_1(u, v_1, v_2), \tag{6.105}$$

$$\varepsilon \frac{dv_2}{d\tau} = a_3 u v_1 - (a_4 + a_5)v_2 = g_2(u, v_1, v_2), \tag{6.106}$$

which, with the initial conditions

$$u(0) = 1, \quad v_1(0) = v_2(0) = 0, \tag{6.107}$$

represents a well-posed mathematical problem.

This problem, just as the Michaelis–Menten one (6.13) analyzed in Section 6.5, is a singular perturbation one for $0 < \varepsilon \ll 1$. The complete inner and outer solution can be found in a comparable way using the method set out there so we leave it as an exercise. What is of interest here, however, is the form of the uptake function for the substrate concentration u, for times $\tau \gg \varepsilon$, that is, for times in the experimentally measurable regime. So, we only need the outer, or nonsingular, solution which is given to $O(1)$ for $0 < \varepsilon \ll 1$ by (6.104)–(6.107) on setting the ε-terms to zero. This gives

$$\frac{du}{d\tau} = f(u, v_1, v_2), \quad g_1(u, v_1, v_2) = 0, \quad g_2(u, v_1, v_2) = 0.$$

The last two equations are algebraic, which on solving for v_1 and v_2 give

$$v_2 = \frac{a_3 u v_1}{a_4 + a_5}, \quad v_1 = \frac{u}{a_1 + a_2 + u + a_3 u^2 (a_4 + a_5)^{-1}}.$$

Substituting these into $f(u, v_1(u), v_2(u))$ we get the uptake equation, or rate equation, for u as

$$\begin{aligned}\frac{du}{d\tau} &= f(u, v_1(u), v_2(u)) \\ &= -u\frac{a_2 + a_3a_5u(a_4 + a_5)^{-1}}{a_1 + a_2 + u + a_3u^2(a_4 + a_5)^{-1}} \\ &= -r(u) < 0.\end{aligned} \tag{6.108}$$

The dimensionless velocity of the reaction is thus $r(u)$. In dimensional terms, using (6.103), the Michaelis–Menten velocity of the reaction for $0 < e_0/s_0 \ll 1$, denoted by $R_0(s_0)$ say, is, from (6.108),

$$\begin{aligned}R_0(s_0) = \left|\frac{ds}{dt}\right|_{t=0} &= e_0s_0\frac{k_2K'_m + k_4s_0}{K_mK'_m + K'_ms_0 + s_0^2} \\ K_m = \frac{k_2 + k_{-1}}{k_1}, \quad K'_m &= \frac{k_4 + k_{-3}}{k_3},\end{aligned} \tag{6.109}$$

where K_m and K'_m are the Michaelis constants for the mechanism (6.98), equivalent to the Michaelis constant in (6.41).

The rate of the reaction $R_0(s_0)$ is illustrated in Figure 6.5. If some of the parameters are zero there is a point of inflexion: for example, if $k_2 = 0$ it is clear from (6.109) since then for s_0 small, $R_0 \propto s_0^2$. A good example of such a cooperative behaviour is the binding of oxygen by haemoglobin; the experimental measurements give an uptake curve very like the lower curve in Figure 6.5. Myoglobin (Mb), a protein in abundance in red muscle fibres, on the other hand has only one oxygen binding site and its uptake is of the Michaelis–Menten form also shown in Figure 6.5 for comparison.

When a cooperative phenomenon in an enzymatic reaction is suspected, a *Hill plot* is often made. The underlying assumption is that the reaction velocity or uptake function is of the form

$$R_0(S_0) = \frac{Qs_0^n}{K_m + s_0^n}, \tag{6.110}$$

where $n > 0$ is not usually an integer; this is often called a Hill equation. Solving the

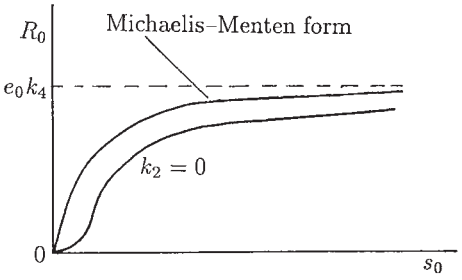

Figure 6.5. Rate of reaction, or substrate uptake, as a function of substrate concentration s_0 for the cooperative reaction (6.98). Note the inflexion in the cooperative uptake curve when $k_2 = 0$.

last equation for s_0^n we have

$$s_0^n = \frac{R_0 K_m}{Q - R_0} \quad \Rightarrow \quad n \ln s_0 = \ln K_m + \ln \frac{R_0}{Q - R_0}.$$

A Hill plot is the graph of $\ln [R_0/(Q - R_0)]$ against $\ln s_0$, the slope of which gives n, and is a constant if the Hill equation is a valid description for the uptake kinetics. If $n < 1$, $n = 1$ or $n > 1$ we say that there is negative, zero or positive cooperativity respectively. Although the Hill equation may be a reasonable quantitative form to describe a reaction's velocity in a Michaelis–Menten sense, the detailed reactions which give rise to it are not too realistic: essentially it is (6.1) but now instead of $E + S$ we require $E + nS$ combining to form the complex in one step. This is somewhat unlikely if n is not an integer although it could be a stoichiometric form. If n is an integer and $n \geq 2$, the reaction is then trimolecular or higher. Such reactions do not occur except possibly through what is in effect a telescoping together of several reactions, because intermediary reactions are very fast.

Even with such drawbacks as regards the implied reaction mechanisms, empirical rate forms like the Hill equation are extremely useful in modelling. After all, what we want from a model is some understanding of the underlying dynamics and mechanisms governing the phenomena. A very positive first step is to find a biologically reasonable model which qualitatively describes the behaviour. Detailed refinements or amendments come later.

6.6 Autocatalysis, Activation and Inhibition

Many biological systems have feedback controls built into them. These are very important and we must know how to model them. In the next chapter on biological oscillators, we shall describe one area where they are essential. A review of theoretical models and the dynamics of metabolic feedback control systems is given by Tyson and Othmer (1978). Here we describe some of the more important types of feedback control. Basically feedback is when the product of one step in a reaction sequence has an effect on other reaction steps in the sequence. The effect is generally nonlinear and may be to activate or inhibit these reactions. The next chapter gives some specific examples with actual reaction mechanisms.

Autocatalysis is the process whereby a chemical is involved in its own production. A very simple pedagogical example is

$$A + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2X, \tag{6.111}$$

where a molecule of X combines with one of A to form two molecules of X. If A is maintained at a constant concentration a, the Law of Mass Action applied to this reaction gives the rate of reaction as

$$\frac{dx}{dt} = k_1 a x - k_{-1} x^2 \quad \Rightarrow \quad x(t) \to x_S = \frac{k_1 a}{k_{-1}}, \tag{6.112}$$

where $x = [X]$ and x_S is the final nonzero steady state as $t \to \infty$. The zero steady state is unstable by inspection. This autocatalytic reaction exhibits a strong feedback with the 'product' inhibiting the reaction rate. It is obvious that some back reaction ($k_{-1} \neq 0$) is necessary. This is the chemical equivalent of logistic growth discussed in Chapter 1.

Suppose, instead of (6.111), the reaction system is

$$A + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2X, \quad B + X \overset{k_2}{\to} C. \tag{6.113}$$

That is, X is used up in the production of C. This mechanism exhibits a simple bifurcation as we show. If B, as well as A, are maintained at constant concentrations, a and b, then

$$\frac{dx}{dt} = k_1 ax - k_{-1}x^2 - k_2 bx = (k_1 a - k_2 b)x - k_{-1}x^2. \tag{6.114}$$

Here $k_1 a$ is the unit production rate of x and $k_2 b$ the unit loss rate. From (6.114) we see that if $k_1 a > k_2 b$ the steady state $x = 0$ is unstable and $x(t) \to x_S = (k_1 a - k_2 b)/k_{-1} > 0$ as $t \to \infty$, which is stable. On the other hand if $k_1 a < k_2 b$ then $x = 0$ is stable, which is not surprising since the inequality implies that the loss rate is greater than the production rate. In this case mathematically there is still, of course, another steady state but it is negative (so unrealistic) and unstable. The simple bifurcation exhibited by this reaction is summarised in Figure 6.6 where the steady states x_S are given in terms of the parameter $k_1 a - k_2 b$. The bifurcation is at $k_1 a - k_2 b = 0$ where the stability changes from one steady state to another.

Anticipating the next chapter on biological oscillators, the classical Lotka (1920) reaction mechanism which he proposed as a hypothetical model oscillator is another example of autocatalysis. It is

$$A + X \overset{k_1}{\to} 2X, \quad X + Y \overset{k_2}{\to} 2Y, \quad Y \overset{k_3}{\to} B, \tag{6.115}$$

where A is maintained at a constant concentration a. The first two reactions are autocatalytic. The Law of Mass Action gives

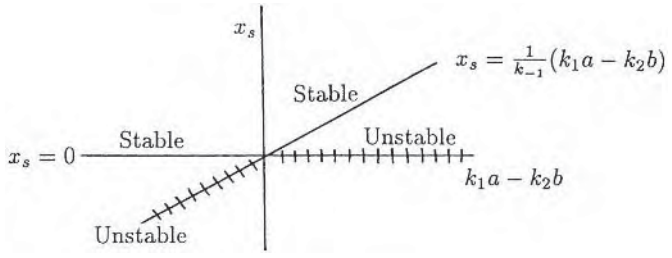

Figure 6.6. Stability of the steady states x_S of the reaction system (6.113) and (6.114). As the parameter $k_1 a - k_2 b$, the difference between the production and loss rates, changes sign, so does the stability, namely, from $x_S = 0$ to $x_S \neq 0$.

$$\frac{dx}{dt} = k_1 ax - k_2 xy, \quad \frac{dy}{dt} = k_2 xy - k_3 y,$$

which, with the nondimensional variables

$$u = \frac{k_2 x}{k_3}, \quad v = \frac{k_2 y}{k_1 a}, \quad \tau = k_1 at, \quad \alpha = k_3/k_1 a,$$

become

$$\frac{du}{d\tau} = u(1 - v), \quad \frac{dv}{d\tau} = \alpha v(u - 1).$$

These are the Lotka–Volterra equations (3.4) discussed in detail in Section 3.1 in Chapter 3; the solutions u and v are periodic in time but, as we saw, are structurally unstable.

In almost all biological processes we do not know the detailed biochemical reactions that are taking place. However, we often do know the qualitative effect of varying a known reactant or of changing the operating conditions in one way or another. So, in modelling such biological processes it is usually much more productive and illuminating to incorporate such known qualitative behaviour in a model mechanism. It is such model mechanisms which have proved so useful in interpreting and unravelling the basic underlying processes involved, and in making useful predictions in a remarkably wide spectrum of biomedical problems. Since we know how to represent a reaction sequence as a differential equation system we can now construct models which incorporate the various qualitative behaviours directly into the differential equations for the concentrations. It is then the differential equation system which constitutes the model.

Suppose we have a differential equation system, the model for which can be reduced, through asymptotic procedures such as we discussed above, to two key elements which are governed by the dimensionless mechanism

$$\begin{aligned} \frac{du}{d\tau} &= \frac{a}{b + v} - cu = f(u, v), \\ \frac{dv}{d\tau} &= du - ev = g(u, v), \end{aligned} \tag{6.116}$$

where a, b, c, d and e are positive constants. The biological interpretation of this model is that u activates v, through the term du, and both u and v are degraded linearly proportional to their concentrations; these are the $-cu$ and $-ev$ terms. This linear degradation is referred to as *first-order kinetics* removal. The term $a/(b + v)$ shows a negative feedback by v on the production of u, since an increase in v decreases the production of u, and hence indirectly a reduction in itself. The larger v, the smaller is the u-production. This is an example of *feedback inhibition*.

We can easily show that there is a stable positive steady state for the mechanism (6.116). The relevant steady state (u_0, v_0) is the positive solution of

$$\begin{aligned} & f(u_0, v_0) = g(u_0, v_0) = 0 \\ \Rightarrow \quad & v_0 = \frac{du_0}{e}, \quad u_0^2 + \frac{ebu_0}{d} - \frac{ae}{cd} = 0. \end{aligned}$$

The differential equation system (6.116) is exactly the same type that we analysed in detail in Chapter 3. The linear stability then is determined by the eigenvalues λ of the linearised Jacobian or *reaction matrix* or stability matrix (equivalent to the community matrix in Chapter 3), and are given by

$$\left| \begin{array}{cc} \dfrac{\partial f}{\partial u} - \lambda & \dfrac{\partial f}{\partial v} \\ \dfrac{\partial g}{\partial u} & \dfrac{\partial g}{\partial v} - \lambda \end{array} \right|_{u_0, v_0} = \left| \begin{array}{cc} -c - \lambda & -c\dfrac{u_0}{v_0 + b} \\ d & -e - \lambda \end{array} \right| = 0.$$

Thus

$$\lambda^2 + (c + e)\lambda + \left[ce + \frac{cdu_0}{b + v_0} \right] = 0 \quad \Rightarrow \quad Re\lambda < 0,$$

and so (u_0, v_0) is linearly stable. It is also a globally attracting steady state: it is straightforward to construct a rectangular confined set in the (u, v) plane on the boundary of which the vector $(du/dt, dv/dt)$ points inwards.

Several specific model systems have been proposed as the mechanisms governing certain basic biological phenomena such as oscillatory behaviour, pattern formation in developing embryos, mammalian coat patterns and so on. We study some of these in detail in subsequent chapters. Here we briefly look at two.

The Thomas (1975) mechanism, is based on a specific reaction involving the substrates oxygen and uric acid which react in the presence of the enzyme uricase. The dimensionless form of the empirical rate equations for the oxygen (v) and the uric acid (u) can be written as

$$\begin{aligned} \frac{du}{dt} &= a - u - \rho R(u, v) = f(u, v), \\ \frac{dv}{dt} &= \alpha(b - v) - \rho R(u, v) = g(u, v), \\ R(u, v) &= \frac{uv}{1 + u + Ku^2}, \end{aligned} \tag{6.117}$$

where a, b, α, ρ and K are positive constants. Basically u and v are supplied at constant rates a and αb, degrade linearly proportional to their concentrations and both are used up in the reaction at a rate $\rho R(u, v)$. The form of $R(u, v)$ exhibits *substrate inhibition*. For a given v, $R(u, v)$ is $O(uv)$ for u small and is thus linear in u, while for u large it is $O(v/Ku)$. So, for u small R increases with u, but for u large it decreases with u. This is what is meant by substrate inhibition. The parameter K is a measure of the severity of the inhibition. From Figure 6.7, giving $R(u, v)$ as a function of u, we see that the uptake rate is like a Michaelis–Menten form for small u, reaches a maximum at $u = 1/\sqrt{K}$ and then decreases with increasing u. The value of the concentration for the maximum $R(u, v)$, and the actual maximum rate, decreases with increasing inhibition, that is, as K increases.

It is always informative to draw the null clines for the reaction kinetics in the (u, v) phase plane in the same way as for the interacting population models in Chapter 3. Here

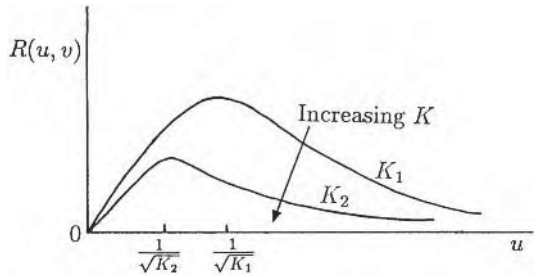

Figure 6.7. Reaction rate $R(u, v)$ in (6.117) for a fixed v. The reduction in R as u increases for $u > 1/\sqrt{K}$ is a typical example of substrate (u) inhibition: the larger the K the greater the inhibition.

the null clines for (6.117) are

$$f(u, v) = 0 \quad \Rightarrow \quad v = (a - u)\frac{1 + u + Ku^2}{\rho u},$$
$$g(u, v) = 0 \quad \Rightarrow \quad v = \alpha b\frac{1 + u + Ku^2}{\rho u + \alpha(1 + u + Ku^2)},$$

which are sketched in Figure 6.8. Depending on the parameters there can be one or three positive steady states. Although these null clines are for a specific substrate–inhibition mechanism they are fairly typical of general substrate–inhibition models, the $f = 0$ null cline in particular; see also Figure 6.9.

The question of the stability of the steady states will be discussed in detail and in some generality in the next chapter. At this stage, however, we can get an intuitive indication of the stability from looking at the null clines in the (u, v) phase plane. Consider the situation in Figure 6.8 when there are three steady states at P_1, P_2 and P_3 and, to be specific, look at $P_1(u_1, v_1)$ first. Now let us move along a line, $v = v_1$ say, through P_1 and note the signs of $f(u, v_1)$ as we cross the $f = 0$ null cline. Let us stay in the neighbourhood of P_1. On the left of the $f = 0$ null cline, $f > 0$ and on the right $f < 0$.

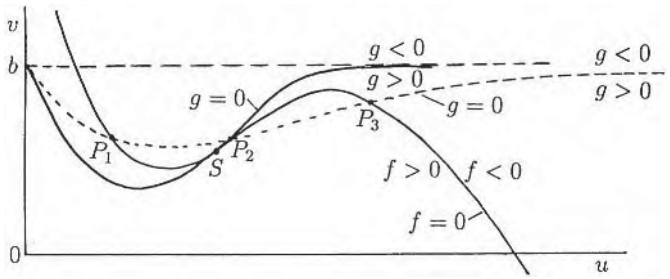

Figure 6.8. Schematic null clines for the substrate–inhibition kinetics (6.117). There may be one, S, or three, P_1, P_2, P_3 (dashed $g = 0$ curve) steady states where $f = 0$ and $g = 0$ intersect. Note the signs of f and g on either side of their null clines.

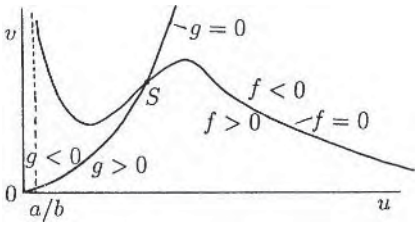

Figure 6.9. Typical null clines for the activator–inhibitor model (6.118).

So with $v = v_1$, a constant, $\partial f/\partial u < 0$ at P_1. If we now consider the kinetics equation for u with $v = v_1$, namely, $du/dt = f(u, v_1)$, we see that locally $\partial f/\partial u < 0$ at P_1 and so, from our discussion in Section 1.1 in Chapter 1, if this were an uncoupled scalar equation for u it would mean that P_1 is a linearly stable steady state. But of course from (6.117) the u-equation is not uncoupled and maybe the coupling has a destabilising affect.

Let us still consider P_1 and use the same kind of argument to move across the $g = 0$ null cline along a line, $u = u_1$ say, through P_1. We now see that $\partial g/\partial v < 0$ so locally $dv/dt = g(u_1, v)$ with $\partial g/\partial v < 0$ at P_1 and by the same argument about scalar equations this would reinforce our intuition that P_1 is linearly stable. So intuitively from both these analyses we would expect P_1 to be linearly stable. These kinds of arguments are developed rigorously in the next chapter where we show that our intuition is indeed correct. In a similar way we can intuitively deduce that P_3 is also stable. If we apply the above sign arguments to P_2 with $v = v_2$ at P_2 we see, from Figure 6.8, that $\partial f(u, v_2)/\partial u > 0$ so we expect P_2 to be unstable. When there is a single steady state at S, the situation needs a careful analysis (see Chapter 7).

Without carrying out any analysis, it is clear that there must be certain parameter ranges where there is a single steady state and where there are three steady states. An informative analysis therefore is to determine the parameter domains for each situation. Although this is simple in principle—you determine the positive steady states from the simultaneous algebraic equations $f(u, v) = g(u, v) = 0$—it is usually hard algebraically and has to be carried out numerically. Such analyses produce some interesting results which we discuss in more detail in Section 6.7.

Another model mechanism, algebraically simpler than the Thomas system (6.117), is the hypothetical but biologically plausible reaction scheme

$$\begin{aligned} \frac{du}{dt} &= a - bu + \frac{u^2}{v(1 + Ku^2)} = f(u, v), \\ \frac{dv}{dt} &= u^2 - v = g(u, v), \end{aligned} \tag{6.118}$$

where a, b and K are constants. This is an *activator* (u)*–inhibitor* (v) *system* and is a dimensionless version of the kinetics of a model proposed by Gierer and Meinhardt (1972). It has been used in a variety of modelling situations which we point out in subsequent chapters. Here there is an autocatalytic production of the activator u via the $u^2/[v(1 + Ku^2)]$ term, but which saturates to $1/(Kv)$ for u large. The inhibitor v is

activated by u according to the second equation, but it inhibits its activator production since $u^2/v(1 + Ku^2)$ decreases as v increases. The null clines $f = 0$ and $g = 0$ from (6.118) are illustrated in Figure 6.9. Note the qualitative similarity between the null clines in Figures 6.8 and 6.9, particularly in the vicinity of the steady state and for large u; we consider the implications of this later. In the next chapter we introduce other reaction systems while in Chapter 8 we discuss in detail a specific system which is of considerable experimental importance and biological relevance.

For a general system

$$\frac{du}{dt} = f(u, v), \quad \frac{dv}{dt} = g(u, v), \tag{6.119}$$

u is an activator of v if $\partial g/\partial u > 0$ while v is an inhibitor of u if $\partial f/\partial v < 0$. Depending on the detailed kinetics a reactant may be an activator, for example, only for a range of concentrations or parameters. There are thus many possibilities of bifurcation phenomena which have biologically important implications as we see later in the book.

With the mathematical parallel between interacting populations and reaction kinetics model systems, we also expect to observe threshold phenomena such as we discussed in Section 3.8 in Chapter 3. This is indeed the case and the model system (6.117) exhibits a similar threshold behaviour if the parameters are such that the steady state is at S, or at S', as in Figure 6.10. The analysis in Section 3.8 is directly applicable here.

We can now start to build model reactions to incorporate a variety of reaction kinetics behaviour such as autocatalysis, activation and inhibition and so on, since we know qualitatively what is required. As an example suppose we have cells which react to the local concentration level of a chemical S by activating a gene so that the cells produce a product G. Suppose that the product is autocatalytically produced in a saturable way and that it degrades linearly with its concentration, that is, according to first-order kinetics. With lowercase letters for the concentrations, a rate equation for the product g which qualitatively incorporates all of these requirements is, for example,

$$\frac{dg}{dt} = k_1 s + \frac{k_2 g^2}{k_3 + g^2} - k_4 g = f(g), \tag{6.120}$$

where the k's are positive constants. This model has some useful biological switch properties which we consider and use later in Chapter 3, Volume II when we discuss models for generating biological spatial patterns.

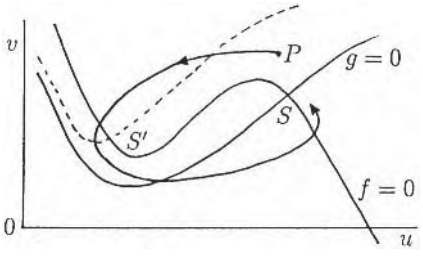

Figure 6.10. Reaction kinetics null clines which illustrate a threshold behaviour. With a perturbation to P, the solution embarks on a large excursion in the phase space before returning to the stable steady state S. A similar threshold behaviour is possible if the null clines intersect at the steady state S'.

It is now clear that the study of the reaction kinetics of n reactions results in an nth order system of first-order differential equations of the form

$$\frac{du_i}{dt} = f_i(u_1, \ldots, u_n), \quad i = 1, \ldots, n. \tag{6.121}$$

This is formally the same type of general system which arose in interacting population models, specifically equations (3.43) in Chapter 3. There we were only concerned with nonnegative solutions and so also here, since $u(t)$ is a vector of concentrations. All of the methods for analysing stability of the steady states, that is, solutions of $f(u_1, \ldots, u_n) = 0$, are applicable. Thus all of the conditions for limit cycles, threshold phenomena and so on also hold here.

The interaction details between reactants and those for interacting populations are of course quite different both in form and motivation. In biological systems there is generally more complexity as regards the necessary order of the differential equation model. As we have seen, however, this is often compensated by the presence of enzyme catalysts and thus a biological justification for reducing the order considerably. For example, a system which results in the dimensionless equations

$$\begin{aligned} \frac{du_i}{dt} &= f_i(u_1, \ldots, u_n), \quad i = 1, 2 \\ \varepsilon_i \frac{du_i}{dt} &= f_i(u_1, \ldots, u_n), \quad i = 3, \ldots, n \\ 0 < \varepsilon_i &\ll 1, \quad i = 3, \ldots, n \end{aligned} \tag{6.122}$$

reduces, for almost all practical purposes, to a second-order system

$$\frac{du_i}{dt} = f_i(u_1, u_2, u_3(u_1, u_2), \ldots, u_n(u_1, u_2)), \quad i = 1, 2$$

for small enough εs. Here $f_i(u_1, \ldots, u_n) = 0$ for $i = 3, \ldots, n$ are algebraic equations which are solved to give $u_{n\geq 3}$ as functions of u_1 and u_2. It is this general extension of the quasi-steady state approximation to higher-order systems which justifies the extensive study of two-reactant kinetics models. Mathematically the last equation is the $O(1)$ asymptotic system, as $\varepsilon_i \to 0$ for all i, for the nonsingular solution of (6.122). Biologically this is all we generally require since it is the relatively long time behaviour of mechanisms which usually dominates biological development.

6.7 Multiple Steady States, Mushrooms and Isolas

We saw in Figure 6.8 that it is possible to have multiple positive steady states. The transition from a situation with one steady state to three occurs when some parameter in the model passes through a bifurcation value. Figure 6.11 illustrates typical scenarios where this occurs. For example, referring to Figure 6.9 and the kinetics in (6.118) the steady state would behave qualitatively like that in Figure 6.11 (a) with the inhibition parameter K playing the role of p.

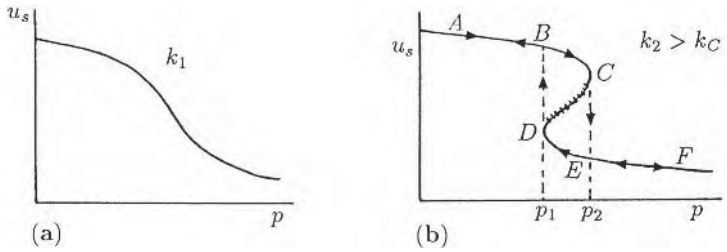

Figure 6.11. (**a**) Typical variation of the steady state u_s as a function of a parameter p in the kinetics for a fixed value k_1 of another kinetics parameter k. (**b**) As the parameter k passes through a bifurcation value k_c, multiple steady states are possible when $p_1 < p < p_2$. The steady state that lies on the branch DC is unstable.

Now suppose that as a parameter, k say, varies the u_s versus p curve changes in such a way that for a range of k the qualitative form of the curve is as in Figure 6.11 (b). For a fixed k and $p_1 < p < p_2$ there are three steady states, one on each branch BC, CD and DE. This is equivalent to the three steady state situation in Figure 6.8. From the discussion in the last section we expect the steady states lying on the CD branch to be linearly unstable; this is proved in the next chapter.

The form of the (u_s, p) graph in Figure 6.11 (b) suggests the possibility of hysteresis (recall Section 1.1) as p varies. Assume, as is the case, that a steady state lying on the branches ABC and DEF is stable. Now suppose we slowly increase the parameter p from a value $p < p_1$ to a value $p > p_2$. Until p reaches p_2, u_s simply increases and is given by the appropriate value on the branch ABC. When p passes through p_2, u_s changes abruptly, moving onto the branch EF; with increasing p it is given by the appropriate value on this branch. Now suppose we slowly decrease p. In this situation u_s stays on the lower branch FED until p reaches p_1 since solutions on this branch are stable. Now the abrupt change takes place at p_1 where u_s jumps up onto the upper BA branch. This is a typical hysteresis loop. For increasing p, the path is along $ABCEF$, while the path through decreasing values of p is $FEDBA$.

Mushrooms

Instead of the (u_s, p) variation in Figure 6.11 (a) another common form simply has u_s increasing with increasing p as in Figure 6.12 (a): the transition to three steady states is then as illustrated. It is not hard to imagine that even more complicated behaviour is possible with the simple curve in Figure 6.12 (a) evolving to form the mushroomlike shape in Figure 6.12 (b) with two regions in p-space where there are multi-steady states.

The mushroomlike (u_s, p) relationship in Figure 6.12 (b) has two distinct p-ranges where there are three steady states. Here the steady states lying on the branches CD and GH are unstable. There are two hysteresis loops equivalent to Figure 6.11 (b), namely, $BCED$ and $IHFG$.

Isolas

The situation shown in Figure 6.12 (c), namely, that of a separate breakaway region, is an obvious extension from Figure 6.12 (b). Such a solution behaviour is called an isola.

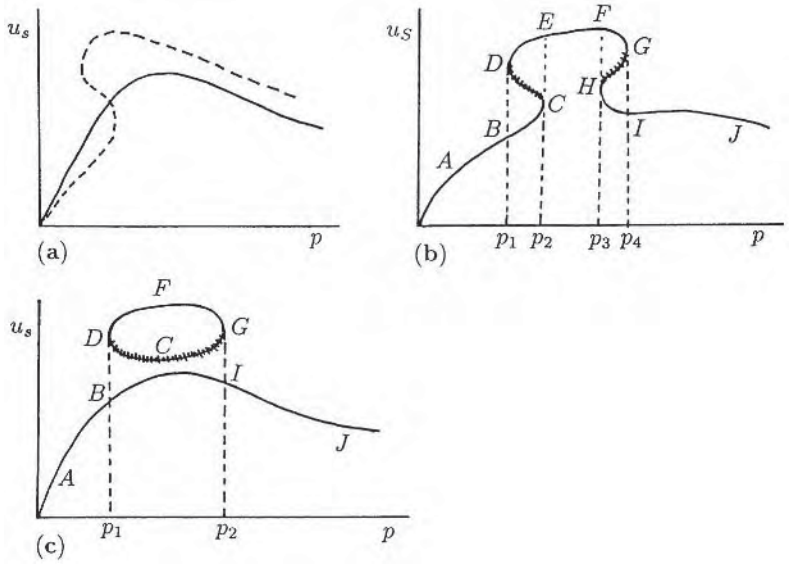

Figure 6.12. (**a**) Another typical example of a steady state dependence on a parameter with transition to multiple steady states; compare with Figure 6.11 (a). (**b**) Typical mushroom dependence of the steady state as a function of a parameter p. (**c**) This shows an example of an isola: it can be a natural evolution from the form in (**b**).

Now we expect the solutions lying on the branch DCG to be unstable. The physical situation represented by this situation is rather different from that which obtains with a mushroom. First there is no hysteresis in the usual way since u_s simply stays on the branch $ABIJ$ as the parameter p increases from a value $p < p_1$ to a value $p > p_2$: it stays on this branch on the return sweep through the multi-steady state region $p_1 < p < p_2$. *Isolas* are isolated closed curves of solution branches and can only arise as solutions of nonlinear equations.

Referring still to Figure 6.12 (c), if u_s lies on the branch BI it is only possible to move onto the other stable branch DFG if u_s is given a finite perturbation so that u moves into the domain of attraction of the stable steady state on the DFG branch. The various possible scenarios are now clear.

It is possible to predict quite complex solution behaviour by simply manipulating the curves, in effect as we have just done. The appearance of multi-steady states is not difficult to imagine with the right kinetics. Dellwo et al. (1982) present a general theory which describes analytically the structure of a class of isolas, namely, those which tend to a point as some parameter tends to a critical value. The question immediately arises as to whether isolas, for example, can exist in the real world. Isolas have been found in a variety of genuine practical situations including chemical reactions; an early review is given by Uppal et al. (1976) with other references in the paper by Gray and Scott (1986).

A simple model kinetics system has been proposed by Gray and Scott (1983, 1986) which exhibits, among other things, multi-steady states with mushrooms and isolas: it

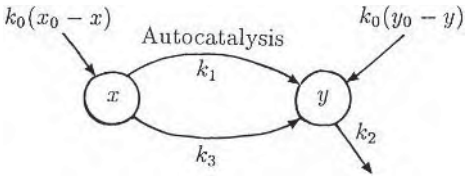

Figure 6.13. Model autocatalytic mechanism which exhibits multi-steady states with mushrooms and isolas. The system is a continuously stirred flow tank reactor (CSTR) mechanism with Y being produced autocatalytically and by a simple uncatalysed process. X and Y are fed into the process and Y degrades with first-order kinetics. The mechanism is described by the differential equation system (6.123). The lowercase letters x and y denote the concentrations of X and Y.

involves autocatalysis in a continuously stirred tank reactor (CSTR). It consists of the following hypothetical reactions involving two reactants X and Y with concentrations x and y respectively. The specific mechanism is represented schematically in Figure 6.13.

The process in the figure involves the trimolecular autocatalytic step $X + 2Y \to 3Y$ and the specific equation system which describes the process is

$$\begin{aligned} \frac{dx}{dt} &= k_0(x_0 - x) - k_1 x y^2 - k_3 x, \\ \frac{dy}{dt} &= k_0(y_0 - y) + k_1 x y^2 + k_3 x - k_2 y, \end{aligned} \tag{6.123}$$

where the k's are the positive rate constants. An appropriate nondimensionalisation is

$$\begin{aligned} &u = \frac{x}{x_0}, \quad v = \frac{y}{x_0}, \quad t^* = t k_1 x_0^2, \quad c = \frac{y_0}{x_0}, \\ &a = \frac{k_0}{k_1 x_0^2}, \quad b = \frac{k_3}{k_1 x_0^2}, \quad d = \frac{k_2}{k_1 x_0^2}, \end{aligned} \tag{6.124}$$

with which (6.123) become, on omitting the asterisk for notational simplicity, the dimensionless system

$$\begin{aligned} \frac{du}{dt} &= a(1 - u) - u v^2 - b u = f(u, v), \\ \frac{dv}{dt} &= a(c - v) + u v^2 + b u - d v = g(u, v), \end{aligned} \tag{6.125}$$

which now involve four dimensionless parameters a, b, c and d.

Here we are only interested in the steady states u_s and v_s which are solutions of $f(u, v) = g(u, v) = 0$. A little algebra shows that

$$u_s(1 + c - u_s)^2 = a\left(1 + \frac{d}{a}\right)^2 (1 - u_s) - b u_s \frac{(a + d)^2}{a^2}, \tag{6.126}$$

which is a cubic, namely,

$$u_s^3 - 2(1+c)u_s^2 + \left[(1+c)^2 + \frac{(a+d)^2}{a} + b\frac{(a+d)^2}{a^2}\right]u_s - \frac{(a+d)^2}{a} = 0. \tag{6.127}$$

Since there are three changes in sign in the cubic there is thus, using Descartes' rule of signs (see Appendix B), the possibility of three positive solutions. Certain analytical solutions for these can be found asymptotically for large and small values of the parameters. The full picture, however, has to be obtained numerically as was done by Gray and Scott (1986). Typical results are illustrated schematically in Figure 6.14. A good review of this reaction and its complex behaviour together with analytical and numerical results is given by Gray (1988).

It is, of course, always possible to construct more and more complex solution behaviours mathematically and to postulate hypothetical reactions which exhibit them. So, the key question at this stage is to ask whether there are any real reaction processes which exhibit these interesting phenomena, such as mushrooms and isolas. The inorganic iodate–arsenous acid reaction under appropriate conditions has been shown experimentally to have the required kinetics. This has been convincingly demonstrated by Ganapathisubramanian and Showalter (1984) whose model and experimental results are described below. Although this is not an enzymatic or biological reaction it nevertheless shows that real reaction mechanisms, which have mushroom and isola solution behaviour, exist. With the richness and complexity of biological processes it would be unbelievable if such reaction systems did not exist within the biomedical sciences. So, it is with this conviction in mind that we describe here the elements of this inorganic reaction and present the relevant experimental results.

Iodate–Arsenous Acid Reaction: Bistability, Mushrooms, Isolas

The iodate–arsenous acid reaction in a continuous flow stirred tank reactor can be described by two composite reactions, namely,

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O, \tag{6.128}$$

$$I_2 + H_3AsO_3 + H_2O \rightarrow 2I^- + H_3AsO_4 + 2H^+. \tag{6.129}$$

The net reaction, given by the (6.128) $+ 3 \times$ (6.129) , is

$$IO_3^- + 3H_3AsO_3 \rightarrow I^- + 3H_3AsO_4. \tag{6.130}$$

The rate of the reaction (6.128) is slow compared with (6.129) and so it is the rate limiting step in the overall process (6.129). If we denote this rate for (6.128) by R, an empirical form has been determined experimentally as

$$R = -\frac{d[IO_3^-]}{dt} = (k_1 + k_2[I^-])[I^-][H^+]^2[IO_3^-]. \tag{6.131}$$

A simple model reaction mechanism, which quantitatively describes the iodate–arsenous acid reaction in a continuous flow stirred tank reactor, consists of rate equations for the

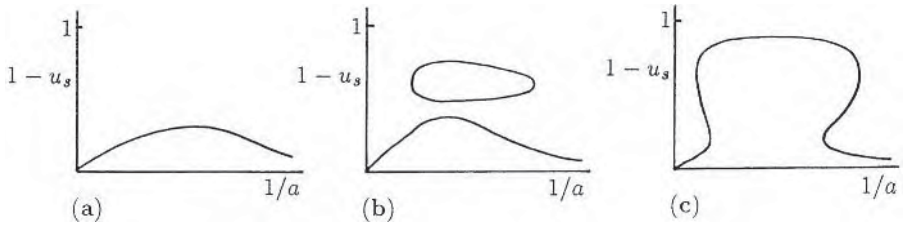

Figure 6.14. The steady states u_s of (6.125) as a function of the parameter a for various values of b, c and d. For a fixed c, less than a critical value, and an increasing d from $d = 0$, the progression of steady state behaviours is from the mushroom situation (**c**), through the isola region (**b**), to the single steady state situation (**a**).

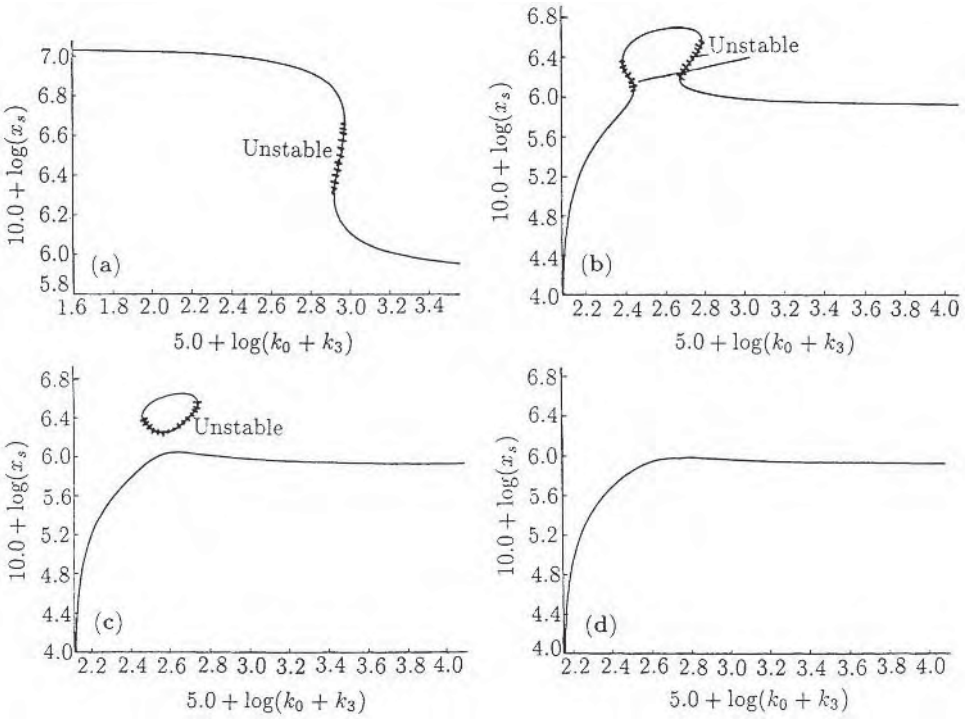

Figure 6.15. Computed steady state iodide concentration X_s from (6.135) as a function of $k_0 + k_3$. The continuous lines represent stable solution branches and the dashed lines unstable branches. Parameter values: $k_1 = 4.5 \times 10^3 M^{-3} s^{-1}$, $k_2 = 4.5 \times 10^8 M^{-4} s^{-1}$, $Y_0 = 1.01 \times 10^{-3} M$, $X_0 = 8.40 \times 10^{-5} M$, $[H^+] = 7.59 \times 10^{-3} M$; (**a**) $k_3 = 0$, (**b**) $k_3 = 1.20 \times 10^{-3} s^{-1}$. (**c**) $k_3 = 1.30 \times 10^{-3} s^{-1}$, (**d**) $k_3 = 1.42 \times 10^{-3} s^{-1}$. Compare (**a**) to (**d**) respectively with the schematic forms in Figure 6.11 (b) and Figures 6.12 (b), (c) and (a). (Redrawn from Ganapathisubramanian and Showalter 1984)

iodide, I^-, and iodate, IO_3^-, in (6.130), with appropriate flow terms and decay terms, given by

$$\frac{d[I^-]}{dt} = R + k_0[I^-]_0 - (k_0 + k_3)[I^-], \tag{6.132}$$

$$\frac{d[IO_3^-]}{dt} = -R + k_0[IO_3^-]_0 - (k_0 + k_3)[IO_3^-], \tag{6.133}$$

where k_0 and k_3 are positive constants, $[I^-]_0$ and $[IO_3^-]_0$ are the concentrations in the inflow and R is given by (6.131).

If we now write

$$\begin{aligned} &X = [I^-], \quad Y = [IO_3^-], \quad X_0 = [I^-]_0, \\ &Y_0 = [IO_3^-]_0, \quad k_1^* = k_1[H^+]^2, \quad k_2^* = k_2[H^+]^2, \end{aligned} \tag{6.134}$$

the steady states X_s and Y_s are given by the solutions of

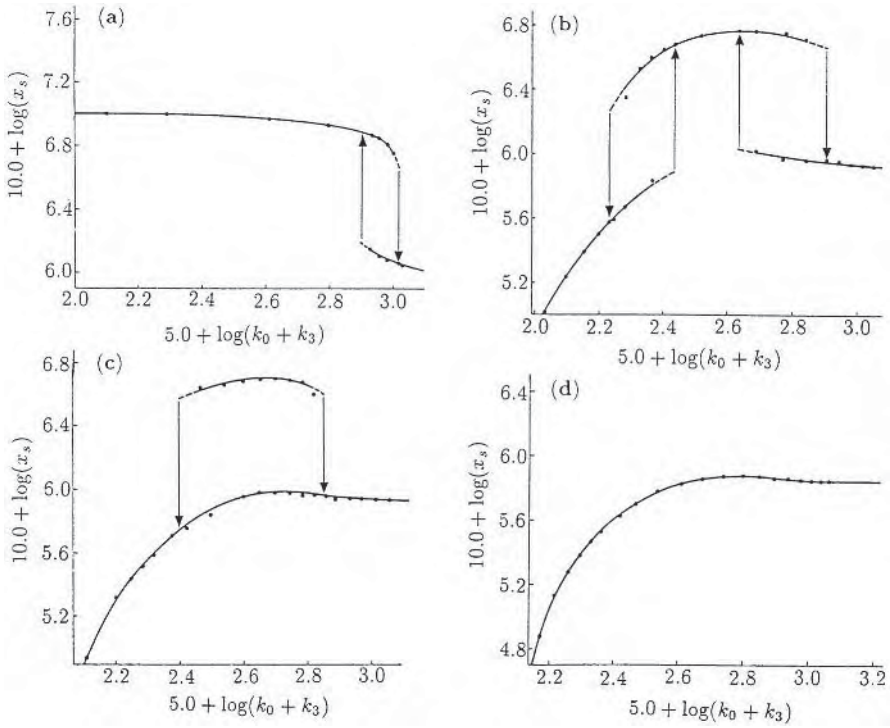

Figure 6.16. Experimentally determined steady state iodide concentrations for the iodate–arsenous acid reaction as a function of $k_0 + k_3$ for different values of k_3. Parameter values: $X_0 = 1.01 \times 10^{-3} M$, $Y_0 = 8.40 \times 10^{-5} M$ with the flow of $[H_3AsO_3]_0 = 4.99 \times 10^{-3} M$; (**a**) $k_3 = 0$, (**b**) $k_3 = 1.17 \times 10^{-3} s^{-1}$, (**c**) $k_3 = 9.71 \times 10^{-4} s^{-1}$, (**d**) $k_3 = 1.37 \times 10^{-3} s^{-1}$. Compare with Figures 6.15 (a), (b), (c) and (d) respectively. (Redrawn from Ganapathisubramanian and Showalter 1984)

$$0 = R + k_0 X_0 - (k_0 + k_3)X, \quad 0 = -R + k_0 Y_0 - (k_0 + k_3)Y,$$
$$R = (k_1^* + k_2^* X)XY.$$

These give the cubic polynomial for X_s

$$\begin{aligned} &k_2^*(k_0 + k_3)X_s^3 + [k_1^*(k_0 + k_3) - k_2^* k_0(X_0 + Y_0)]X_s^2 \\ &+ [(k_0 + k_3)^2 - k_1^* k_0(X_0 + Y_0)]X_s - k_0(k_0 + k_3)X_0 = 0. \end{aligned} \tag{6.135}$$

Values for k_1 and k_2 have been determined experimentally and X_0 and Y_0 and $[H^+]$ can be imposed, and so, from (6.134), k_1^* and k_2^* can be determined. Figure 6.15 shows the positive steady state iodide concentration X_s calculated numerically from the cubic equation (6.135) as a function of $k_0 + k_3$ for different values of k_3.

When the above iodate–arsenous acid reaction model is compared with the full reaction system, good quantitative results are obtained. Figure 6.15 shows that mushroom and isola multi-steady state behaviour is possible. The final step in demonstrating the existence of this type of behaviour is experimental confirmation. This has also been done by Ganapathisubramanian and Showalter (1984), whose results are reproduced in Figure 6.16. Note the comparison between these experimental results and those obtained with the model mechanism for this iodate–arsenous acid reaction. The results in Figure 6.16 clearly show the various hysteresis behaviours suggested by Figures 6.11 and 6.12.

Exercises

1 An allosteric enzyme E reacts with a substrate S to produce a product P according to the mechanism

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \overset{k_2}{\rightarrow} E + P,$$
$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \overset{k_4}{\rightarrow} C_1 + P,$$

where the k's are rate constants and C_1 and C_2 enzyme–substrate complexes. With lowercase letters denoting concentrations, and initial conditions $s(0) = s_0$, $e(0) = e_0$, $c_1(0) = c_2(0) = p(0) = 0$, write down the differential equation model based on the Law of Mass Action. If

$$\varepsilon = \frac{e_0}{s_0} \ll 1, \quad \tau = k_1 e_0 t, \quad u = \frac{s}{s_0}, \quad v_i = \frac{c_i}{e_0}$$

show that the nondimensional reaction mechanism reduces to

$$\frac{du}{d\tau} = f(u, v_1, v_2), \quad \varepsilon \frac{dv_i}{d\tau} = g_i(u, v_1, v_2), \quad i = 1, 2.$$

Determine f, g_1 and g_2 and hence show that for $\tau \gg \varepsilon$ the uptake of u is governed by

$$\frac{du}{d\tau} = -r(u) = -u\frac{A + Bu}{C + u + Du^2},$$

where A, B, C and D are positive parameters.

When $k_2 = 0$ sketch the uptake rate $r(u)$ as a function of u and compare it with the Michaelis–Menten uptake.

2 Two dimensionless activator–inhibitor mechanisms have reaction kinetics described by

$$\text{(i)} \quad \frac{du}{dt} = a - bu + \frac{u^2}{v}, \quad \frac{dv}{dt} = u^2 - v,$$
$$\text{(ii)} \quad \frac{du}{dt} = a - u + u^2 v, \quad \frac{dv}{dt} = b - u^2 v,$$

where a and b are positive constants. Which is the activator and which the inhibitor in each of (i) and (ii)? What phenomena are indicated by the nonlinear terms? Sketch the null clines. Is it possible to have positive multi-steady states with these kinetics? What can you say if substrate inhibition is included in (i); that is, u^2/v is replaced by $u^2/[v(1 + Ku^2)]$.

3 A gene product with concentration g is produced by a chemical S, is autocatalysed and degrades linearly according to the kinetics equation

$$\frac{dg}{dt} = s + k_1\frac{g^2}{1 + g^2} - k_2 g = f(g; s),$$

where k_1 and k_2 are positive constants and $s = [S]$ is a given concentration. First show that if $s = 0$ there are two positive steady states if $k_1 > 2k_2$, and determine their stability. Sketch the reaction rate dg/dt as a function of g for $s = 0$ (that is, $f(g; 0)$). By considering $f(g; s)$ for $s > 0$ show that a critical value s_c exists such that the steady state switches to a higher value for all $s > s_c$. Thus demonstrate that, if $g(0) = 0$ and s increases from $s = 0$ to a sufficiently large value and then decreases to zero again, a biochemical switch has been achieved from $g = 0$ to $g = g_2 > 0$, which you should find.

4 Consider the reaction system whereby two reactants X and Y degrade linearly and X activates Y and Y activates X according to

$$\frac{dx}{dt} = k_1\frac{y^2}{K + y^2} - k_2 x,$$
$$\frac{dx}{dt} = h_1\frac{x^2}{H + x^2} - h_2 y,$$

where $x = [X]$, $y = [Y]$, and k_1, k_2, h_1, h_2, K and H are positive constants. Nondimensionalise the system to reduce the relevant number of parameters. Show (i) graphically and (ii) analytically that there can be two or zero positive steady states. [Hint for (ii): use Descartes' Rule of Signs (see Appendix B).]

5 If the reaction kinetics $\mathbf{f}(\mathbf{u})$ in a general mechanism

$$\frac{d\mathbf{u}}{dt} = \mathbf{f}(\mathbf{u})$$

is a gradient system, that is,

$$\mathbf{f}(\mathbf{u}) = \nabla_{\mathbf{u}} F(\mathbf{u}),$$

which is guaranteed if curl $\mathbf{f}(\mathbf{u}) = 0$, show that the solution $\mathbf{u}$ cannot exhibit limit cycle behaviour. [Hint: Use an energy method; that is, first multiply the system by $d\mathbf{u}/dt$.]