Multistability in Open Chemical Reaction Systems

By L. J. Aarons
DEPARTMENT OF PHARMACY, UNIVERSITY OF MANCHESTER, MANCHESTER M13 9PL
and B. F. Gray
SCHOOL OF CHEMISTRY, UNIVERSITY OF LEEDS, LEEDS LS2 9JT

1 Introduction

The stationary-state hypothesis (SSH) plays a central role in the evaluation of data obtained from chemical kinetics studied in a closed reaction vessel. It is often a good approximation and until the past ten years was regarded as widely valid except in explosive systems where the condition for its breakdown (as in simple branched-chain reactions) is identified with the explosion limit. Recently other spectacular cases have come to light where the SSH does not hold, *e.g.* in oscillatory chemical reactions, which are discussed elsewhere.[1] We shall be interested in cases where the equations representing the SSH have more than one solution, *i.e.*, depending on the initial conditions, the system can reach different final stationary states.

The above remarks which apply to closed systems apply quite rigorously to open reactors where the reactants flow in and the products flow out. In this case the SSH is exact and the steady state is genuinely steady. The examples discussed in this article deal strictly with open systems, but everything can be transposed to closed systems provided that the SSH is shown to be a valid approximation. To illustrate this we will consider a simple example.

Consider the reaction

$$\mathrm{A} \xrightarrow{k_1} \mathrm{X} \xrightarrow{k_2} \mathrm{B}$$

where X is perhaps a radical or an unstable intermediate. Neglecting reverse reactions the equations describing the system are

$$\mathrm{d}A/\mathrm{d}t = -k_1A \tag{1}$$

$$\mathrm{d}X/\mathrm{d}t = k_1A - k_2X \tag{2}$$

where A and X are the concentrations of A and X respectively. The SSH would postulate

$$\mathrm{d}X/\mathrm{d}t = 0,\ X_s = k_1A/k_2 \tag{3}$$

which will be a good approximation if $k_2 \gg k_1A$.[1] In a continuous stirred tank reactor (CSTR) the equations will be

[1] B. F. Gray, 'Kinetics of Oscillating Reactions', in 'Reaction Kinetics,' ed. P. G. Ashmore (Specialist Periodical Reports), The Chemical Society, London, 1975, Chapter 8.

$$dA/dt = -k_1A + k \tag{4}$$

$$dX/dt = +k_1A - k_2X - k_1'X \tag{5}$$

where k is a constant inward flux of A and $k_1'X$ represents removal of X by the flow. Under normal conditions the system will actually achieve the steady state

$$X_S = k_1A_S/(k_2 + k_1') \tag{6}$$

$$A_S = k/k_1 \tag{7}$$

In such a simple system, and in many other more complex systems, equations (3) and (6) for the steady-state intermediate concentration are linear and have only a single solution. This was implicitly assumed to be universally true for isothermal chemical systems until fairly recently, but the acknowledged existence of at least three steady states for a single first-order reaction when its heat effect is considered[2] might well have been expected to throw some doubt on this assumption (see Figure 1). This assumption is not correct and a number of

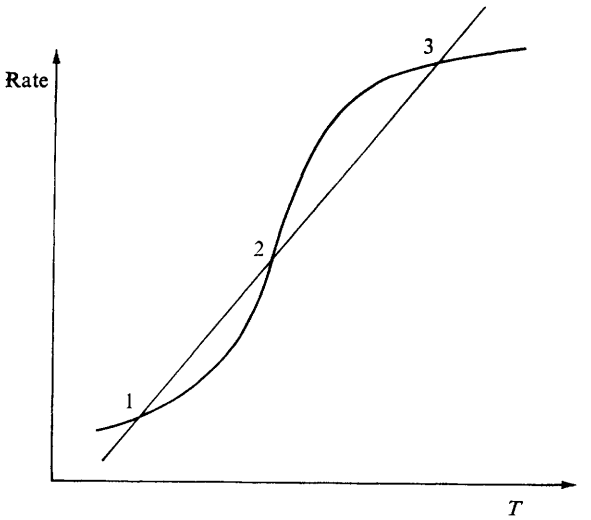

Figure 1 *Heat generation and loss curves for a simple exothermic reaction*

interesting cases are now known where the steady-state equation such as (6) [or (3) for a closed system] has more than one physically acceptable solution. Clearly, higher-order reactions must be involved as the equations must be non-linear, *i.e.* a quadratic or cubic, *etc.* In a closed system, where the equations of the SSH have more than one solution, this means that the overall reaction rate, calculated by substituting the steady-state values for the intermediates, can have more than one form.

[2] B. F. Gray, P. Gray, and N. A. Kirwan, *Combustion and Flame*, 1972, **18**, 439.

Of course in any chemical or physical system the behaviour cannot be understood solely in terms of the number of steady states since these are not physically significant unless shown to be *stable*. For example the simple pendulum has two steady states, but the one pointing vertically upwards is unstable in so far as infinitesimal perturbations will displace it downwards towards the lower stable state (if the pendulum is damped). Likewise it is necessary to show which of a set of multiple steady states in a chemical kinetic system is stable. This is done mathematically by looking into whether perturbations from the steady state in question grow or decay. Usually steady states alternate in stability: stable–unstable–stable, *etc.* The example in Figure 1 shows this sort of behaviour, *i.e.* the middle steady state is unstable, the upper and lower being stable. The middle one acts like a watershed and is known as a saddle point. The instability of steady state 2 can be seen qualitatively by considering a small upward perturbation of T. Since at this steady state the slope of the heat release curve is greater than that of the straight line (representing conductive losses), the former will increase more than the latter, thus reinforcing the perturbation and pushing the temperature still higher. A similar examination of steady states 1 and 3 shows a secondary effect tending to nullify the perturbation and thus giving stability.

Later, when we have analysed a few examples of purely kinetic multistability, we shall see how imposition of a slowly varying parameter on the system can cause rapid switches from one stable steady state to another, giving what superficially appears to be very odd behaviour such as discontinuities in reaction rate and spiky oscillations.

The importance of these rapid switches in complex biochemical networks cannot be overemphasized as they offer much greater possibilities of control than do ordinary smoothly responding chemical systems. For a control system to be effective it must satisfy two criteria:[3] there must be a sharp threshold in the concentration of the inducing substrate necessary to 'fire' the control and a rapid response of the control system. In practice it would be difficult to distinguish a control system which had true multistability from one which merely had rapid response, except by hysteresis effects. However, in terms of the two-effectiveness criteria a control system operating in a region of multiple steady states will always have a sharper threshold and faster response than the same system acting in a region of monostability. Jacob and Monod[4] have proposed several models for transcriptional control of gene expression, Chernavskii *et al.*[5] and Edelstein[6] have studied the multistability of these models. These genetic 'triggers' form the basis of the gradient theory of morphogenesis.[7] According to this theory gradients of 'morphogens' exist in embryonic tissue and differentiation occurs when one or more of the substrates exceeds (or falls below) a certain threshold, causing a switch in cellular biochemistry. Although the molecular

[3] M. A. Savageau, *Nature*, 1974, **252**, 546.
[4] F. Jacob and J. Monod, *J. Mol. Biol.*, 1961, **3**, 318.
[5] D. S. Chernavskii, L. N. Grigorov, and M. C. Poliakova, in 'Oscillating Processes in Biological and Chemical Systems', Nauka, Moscow, 1967.
[6] B. B. Edelstein, *J. Theor. Biol.*, 1972, **37**, 221.
[7] L. Wolpert, *J. Theor. Biol.*, 1969, **25**, 1.

nature of these switches has not been discovered as yet a system exhibiting multistability is a good candidate.

2 An Autocatalytic Example

Many of the kinetic schemes which are now known to show multistability contain an enzyme conversion step,

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

where enzyme combines with substrate to form an enzyme–substrate complex which then splits to enzyme plus product. This reaction sequence is basic in biochemical systems and when it is combined with an autocatalytically produced substrate,

$$\begin{gathered} A + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AS \\ E + S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P \end{gathered} \tag{8}$$

Edelstein[8] has shown that more than one steady state is possible under some circumstances. If we assume that A and P, the concentrations of A and P, are maintained constant by the flow processes, or in large excess and approximately constant in a closed system,[9] we have two kinetic equations for this system,

$$dS/dt = k_1 A.S - k_{-1}S^2 - k_2 E.S + k_{-2}(E_T - E) \tag{9}$$

$$dE/dt = -k_2 E.S - k_{-3}E.P + (k_3 + k_{-2})(E_T - E) \tag{10}$$

where E_T is the total enzyme concentration (complexed and free), assumed constant. This is realistic since cell membranes will allow influx and efflux of metabolites, but not enzymes. If we put the right-hand sides of equations (9) and (10) equal to zero and perform the algebra of eliminating E_s we obtain a cubic for S_s:

$$k_{-1}k_2 S_s^3 + [k_{-1}(k_{-3}P + k_{-2} + k_3) - k_1 k_2 A]S_s^2 + [k_2 k_3 E_T - k_1 A(k_{-3}P + k_{-2} + k_3)]S_s - k_{-2}k_{-3}PE_T = 0 \tag{11}$$

For a physically acceptable range of values of the coefficients of this cubic there are three positive roots, the middle one being unstable. The variation of the roots of this equation as a function of one of the independent parameters (A for example) is shown in Figure 2, and it can be seen that there is a critical situation where two of the roots merge (and become first imaginary and then complex): this situation is known mathematically as a bifurcation. At parameter values corresponding to the steady state 1′, the system will suddenly jump into the new steady state 3′. The calculation of the rate at which this transition occurs is fairly difficult[9] and need not concern us here; we can simply assume that the jump is very rapid once the unstable point 1′ is achieved.

[8] B. B. Edelstein, *J. Theor. Biol.*, 1970, **29**, 57.
[9] B. F. Gray and L. J. Aarons, *Symp. Faraday Soc.*, 1974, No. 9, p. 129.

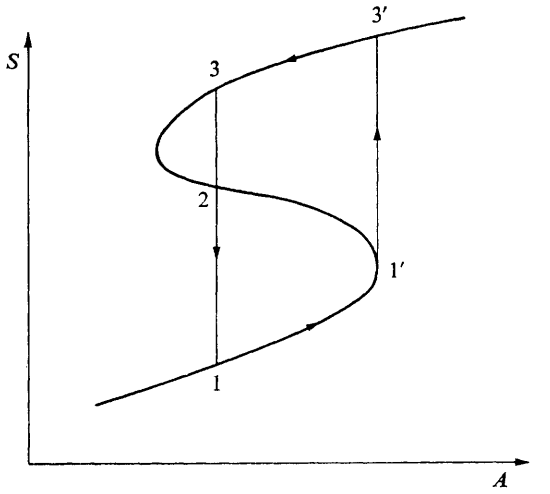

Figure 2 *Steady-state curve showing bistability*

At this point we introduce the concept of chemical hysteresis. If the system is initially on the lower branch of the steady-state curve and we gradually increase the parameter until at 1′ the system jumps to 3′ and then moves to the right along the upper branch. Similarly if the system is initially on the upper branch and the parameter is gradually reduced the system will jump to the lower branch at the other critical point and move to the left along this curve. Thus there are steady states which cannot be achieved by smoothly varying the parameter, unlike systems which have only one steady state where all points are accessible by simple sweeping through a parameter determining them. This phenomenon is known as hysteresis and whenever it is observed it indicates the existence of multistability.

The mechanism summarized by equation (8) works as a highly efficient control when the product of the reaction is suddenly required by an organism in an emergency, but not normally desirable in large quantities (such as adrenalin). There are many other types of kinetic scheme now known involving only a few variables which show multistability, and some of these are discussed below.

3 Product Inhibition

This type of reaction is often suggested as a source of chemical oscillations[10] in biological systems, and is also a possible candidate for multistability. Consider the following scheme

$$\begin{gathered} \mathrm{E} + \mathrm{S} \overset{k_1}{\rightarrow} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \overset{k}{\rightarrow} \text{Exterior} \\ 2\mathrm{P} + \mathrm{E} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{EP}_2 \end{gathered} \tag{12}$$

[10] J. Higgins, *Ind. and Eng. Chem.*, 1967, **59**, 19.

where E, S, and P represent enzyme, substrate, and product respectively. EP_2 is an enzyme–product complex, whose existence does not favour the progress of the reaction, effectively removing enzyme from availability. Again we assume realistically that the reactor cell is impermeable to enzyme, which is therefore conserved (over the time-scale of interest), so that

$$E_T = E + ES + EP_2 \tag{13}$$

The kinetic equations are

$$\begin{aligned} dS/dt &= -k_1ES + k_2E.S + K(S_0 - S) \\ dP/dt &= k_2E.S - kP \end{aligned} \tag{14}$$

where k is the efflux rate of P from the reactor and $K(S_0 - S)$ represents a diffusive influx term from a reservoir held at a constant concentration S_0. In the steady state,

$$\begin{aligned} ES_s &= k_1S_0E_s/k_2 \\ (EP_2)_s &= k_3E_sP^2/k_{-3} \end{aligned} \tag{15}$$

and with the conservation condition (13) we get

$$E_s = E_T/(1 + k_1S/k_2 + k_3P^2/k_{-3}) \tag{16}$$

so that the rate equations (14) become

$$\begin{aligned} \frac{dP}{dt} &= \frac{k_1E_TS}{1 + (k_1S/k_2) + (k_3P^2/k_{-3})} - kP \\ \frac{dS}{dt} &= \frac{-k_1E_TS}{1 + (k_1S/k_2) + (k_3P^2/k_{-3})} + K(S_0 - S) \end{aligned} \tag{17}$$

Equating these two equations to zero gives, after some manipulation,

$$K(S_0 - S_s) = k_1E_TS_s/[1 + (k_1S_s/k_2) + (k_3K^2/k_3k^2)(S_0 - S_s)^2] \tag{18}$$

The number of roots for S_s of this equation is easily investigated graphically by plotting the right- and left-hand sides on the same graph, roots of the equation being represented by interesections. These functions are plotted in Figure 3 for the case where $k_1/k_2 > 2k_3K^2S_0/k_{-3}k^2$. It is seen that it is possible for this equation to have either one or three steady states. The number actually occurring depends on the independent parameters S_0 and K and the rate constants. Some of these are intrinsically fixed for a given system (the rate constants at a given temperature), but the physical parameters K, S_0, and k can easily be varied. S_0 is the concentration of substrate in which the cell is immersed, K depends on the surface area of the interface and the diffusion coefficient in the membrane (easily altered rapidly by a nerve impulse), and k also depends on similar factors. Again we have a biochemically plausible simple reaction network showing the possibility of multistability in a basically isothermal system. One general result

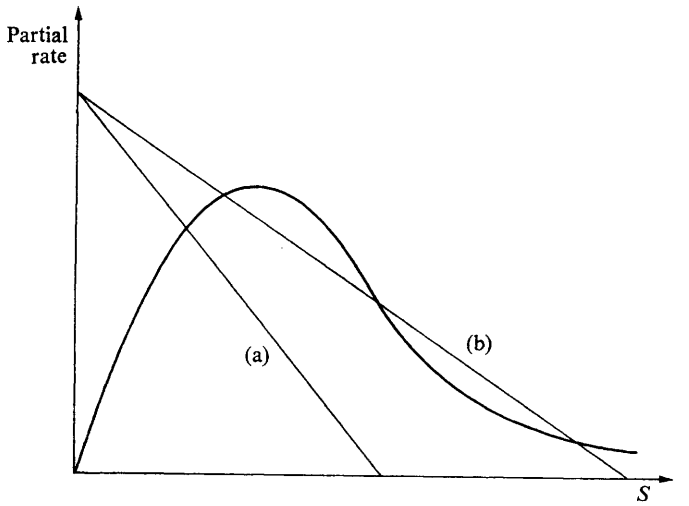

Figure 3 *Steady-state analysis of equation* (18). (a) *One steady state;* (b) *three steady states*

emerges from this example and that is that if more than one product molecule combines with the enzymes one is likely to achieve multistability.

A related and interesting case arises when two enzyme-catalysed reactions cross-inhibit each other, *i.e.*

$$\begin{aligned} E_1 + S_1 &\overset{k_1}{\rightarrow} E_1S_1 \overset{k_2}{\rightarrow} E_1 + P_1 \\ E_2 + S_2 &\overset{k_1}{\rightarrow} E_2S_2 \overset{k_2}{\rightarrow} E_2 + P_2 \\ 2P_2 + E_1 &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_1P_2P_2 \\ 2P_2 + E_2 &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_2P_1P_1 \end{aligned} \tag{19}$$

To simplify the algebra we have assumed that the two pathways are symmetrical with respect to rate constants. This provides a possible mechanism for switching rapidly between two metabolic pathways, one of which may have become unfavourable to the organism, *e.g.* the depletion of a convenient foodstuff in the diet or an increase in the proportion of the other component. In keeping with this we will only consider the variation of P_1 and P_2 explicitly and treat S_1 and S_2 as slowly varying parameters. The rate equations become, after some algebraic manipulation,

$$\begin{aligned} \frac{dP_1}{dt} &= \frac{k_1E_TS_1}{1 + (k_1S_1/k_2) + (k_3P_2^2/k_{-3})} - kP_1 \\ \frac{dP_2}{dt} &= \frac{k_1E_TS_2}{1 + (k_1S_2/k_2) + (k_3P_1^2/k_{-3})} - kP_2 \end{aligned} \tag{20}$$

The steady-state equations are

$$\frac{k_1 E_T S_1}{1 + (k_1 S_1/k_2) + (k_3 P_2^2/k_{-3})} = kP_1$$

$$\frac{k_1 E_T S_2}{1 + (k_1 S_2/k_2) + (k_3 P_1^2/k_{-3})} = kP_2$$

whence

$$S_1 = \frac{k^3[1 + (k_1 S_2/k_2) + (k_3 P_1^2/k_{-3})]^2 P_1 + k^3 k_3 (k_1 E_T S_2)^2 P_1/k_{-3}}{k^2[1 + (k_1 S_2/k_2) + (k_3 P_1^2/k_{-3})]^2 [k_1 E_T - (k\, k_1 P_1/k_2)]} \tag{21}$$

This function is plotted in Figure 4 for two different sets of parameters. Provided that $k_3/k_{-3} \gg k_1/k_2$ there is a region of multistability. Alternatively the steady state shows a monotonic dependence on S_1.

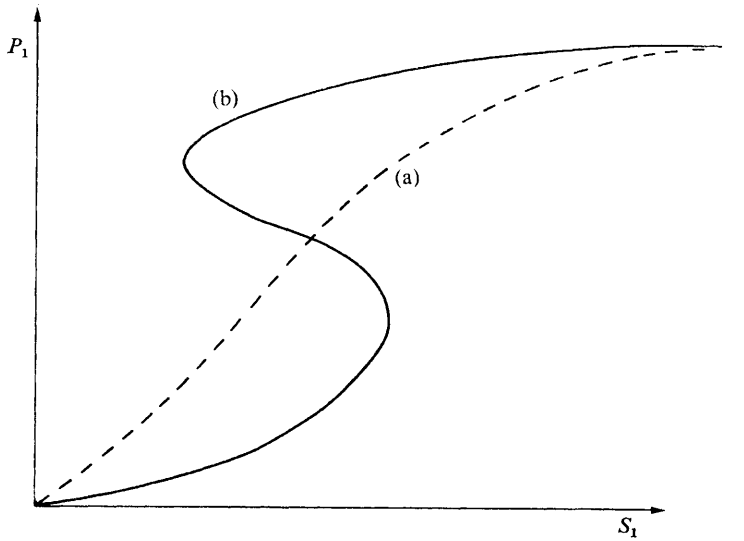

Figure 4 *Steady-state analysis of equation* (21). *Parametric values:* $k_1 = k_2 = S_2 = k = 1$, $E_T = 2$; (a) $k_3/k_{-3} = 2$, (b) $k_3/k_{-3} = 20$

If the system is originally on the lower branch [curve (b)] the effect of increasing S_1 beyond the jump point is to cause a transition to the upper branch which corresponds to a decrease in P_2. This model thus describes how an organism could switch rapidly from utilizing one substrate to another if this substrate becomes more easily available. The existence of the switch is determined by either large inhibition (k_3/k_{-3}) or a large Michaelis constant (k_2/k_1) or both.

4 Substrate Inhibition

Degn[11] has studied the oxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) by horseradish peroxidase in an open system where oxygen was continuously bubbled into solution. Oxygen inhibits the oxidation by combining with the enzyme (reversibly) to form an inactive complex. He followed both the oxygen concentration (in solution) polarographically and the enzyme–substrate complex spectrophotometrically. When the external oxygen concentration was increased slowly from zero to a constant level the oxygen concentration in the solution increased to a very low level where it remained constant. If, however, the external supply was pulsed and then returned to the original constant level, the internal concentration of oxygen rose rapidly to a new, much larger level where it remained constant. This strongly suggested the existence of two stable stationary states reached by different initial conditions. Degn proposed the following method to account for this:

$$S_0 \rightleftharpoons S + E \underset{}{\overset{k_1}{\rightharpoonup}} ES \overset{k_2}{\rightharpoonup} E + P \rightarrow \text{Exterior} \qquad (22)$$

$$S + ES \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} ES_2$$

The rate equation for S is

$$\frac{dS}{dt} = \frac{-k_1 E_T S}{1 + (k_1 S/k_2) + (k_1 k_3 S^2/k_2 k_{-3})} + K(S_0 - S) \qquad (23)$$

This equation is formally similar to equation (18) and hence regions of multistability exist. Switching from one steady state to the other is mediated by varying S_0 which here is the external oxygen concentration. The effect of this switching mechanism is to increase the internal concentration of the substrate rapidly when the external concentration is increased while the amount of product is rapidly reduced. Thus the cell can be made rapidly more permeable to substrate, but more likely this switch will initiate some other event. For example, if the substrate is converted into more than one product competitively the effect of the switch is to change from the production of one product to the other.[12] This then represents feedback to the environment and shows how an organism could adapt to changing external conditions. To illustrate this further consider the simple substrate reaction

$$S \rightarrow P^1 \rightarrow \text{Exterior}$$

together with equation (22). The form of the steady-stage equation is unchanged but now a transition from one steady state to the other causes a switch in production from P to P^1 or *vice versa.*

5 Induction

In many biochemical systems the enzyme is in an inactive or repressed state,

[11] H. Degn, *Nature*, 1968, **217**, 1047.
[12] J. Higgins, *Mem. Acad. Roy. Med. Belgique*, 1967, **6**, 235.

being reversibly bound to a protein molecule which is called a repressor (R). This repression can be lifted or induced if the repressor is made to combine with something else (called the inducer), thus liberating the free enzyme. Quite often the inducer is the substrate or product corresponding to the enzyme. Karfunkel and Seelig[13] have considered a cellular model for both substrate and product induction where the substrate and product, but not the enzyme or repressor are allowed to diffuse in and out of the cell. Schematically the reactions are

$$\rightarrow \mathrm{S} + \mathrm{E} \overset{k_1}{\rightarrow} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \overset{k}{\rightarrow} \qquad (24)$$

$$\mathrm{E} + \mathrm{R} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{ER}$$

It is assumed that the inducer (I) reacts with the repressor as follows,

$$2\mathrm{I} + \mathrm{R} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{RI}_2 \qquad (25)$$

where I is either S or P, and that the enzyme and repressor are conserved:

$$E_\mathrm{T} = E + ER + ES \qquad (26)$$

$$R_\mathrm{T} = R + ER + RI_2$$

The rate equations involving S and P are

$$\mathrm{d}S/\mathrm{d}t = -k_1 E.S + K(S_0 - S) \qquad (27)$$

$$\mathrm{d}P/\mathrm{d}t = k_1 E.S - kP$$

After applying the SSH and some unwieldy algebra the steady-state equations can be derived as usual. A graphical analysis of these equations is shown in Figure 5. It is seen that in the case of substrate induction there is only one possible steady state [defined as the intersection of the curves $K(S_0 - S)$ and $k_1 ES$], but for product induction there is a possibility of three steady states, (the curve representing $k_1 ES$ will be quantitatively different in the two cases owing to the different SSH's used, but the form will be the same[13] and so for simplicity only one curve is shown in the figure). As yet no experimental confirmation of these results is forthcoming, but unlike many hypothetical mathematical models this one is based on sound biochemistry.[14]

6 Coenzymes

Certain enzymes are found on analysis to be pure proteins, whereas others are found to consist of a non-protein part in addition to the protein. In such cases the protein part is known as the apoenzyme and the non-protein part as the coenzyme. Only when the two are combined in the haloenzyme is it active. NADH (discussed in Section 4) is actually the coenzyme of horseradish peroxidase. Proteins contain a number of ionizing groups such as CO_2H and in these cases the hydro-

[13] H. R. Karfunkel and F. F. Seelig, *J. Theor. Biol.*, 1972, **36**, 237.
[14] E. C. Webb, 'Enzymes and Metabolic Inhibitors', Academic Press, London, 1963, Vol. 1.

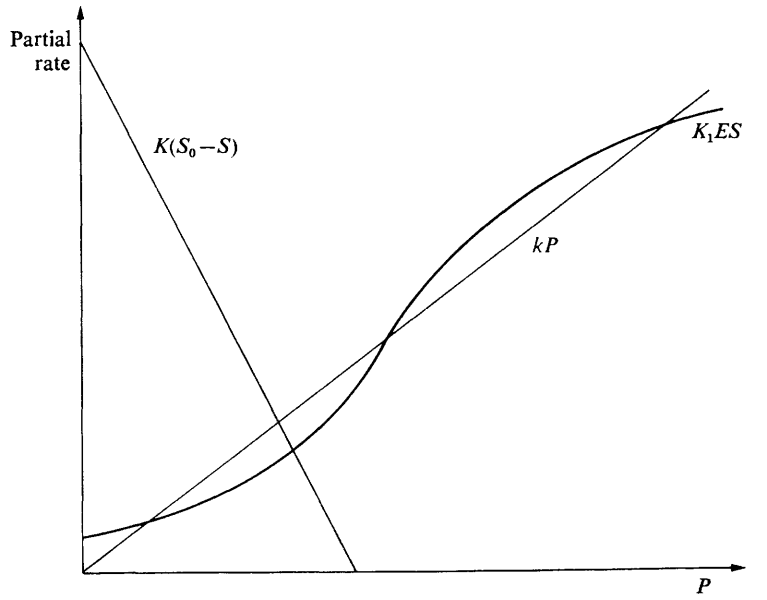

Figure 5 *Steady-state analysis of equation* (27)

gen ion may be thought of as the coenzyme and the ionized protein (CO_2^-) as the apoenzyme.[15,16] The apoenzyme has two binding sites, one for the coenzyme which it binds first and one for the substrate. If it binds a second molecule of coenzyme the enzyme is inactivated. Finally, the product of reaction always dissociates before the reacted form of the coenzyme. Denoting by C and C′ the coenzyme and its reacted form respectively the above mechanism can be summarized by the following scheme

$$\begin{aligned} &\rightarrow \mathrm{E} + \mathrm{C} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{EC} \\ &\mathrm{EC} + \mathrm{C} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{EC_2} \\ &\mathrm{EC} + \mathrm{S} \overset{k_3}{\rightarrow} \mathrm{ECS} \overset{k_4}{\rightarrow} \mathrm{EC'} + \mathrm{P} \rightarrow \\ &\mathrm{EC'} \overset{k_5}{\rightarrow} \mathrm{E} + \mathrm{C'} \end{aligned} \tag{28}$$

The corresponding rate equation is

$$\frac{dC}{dt} = \frac{-k_3E_TS}{1 + (k_3/k_4 + k_3/k_5)S + (k_{-1}/k_1C) + (k_2C/k_2)} + K(C_0 - C) \tag{29}$$

[15] L. Michaelis and H. Davidsohn, *Biochem. Z.*, 1911, **35**, 386.
[16] L. Michaelis and M. Rothstein, *Biochem. Z.*, 1920, **110**, 217.

The first term of equation (29) passes through a maximum as C is varied and hence the steady-state analysis proceeds similarly to that of substrate inhibition given in Section 4, the independent parameters being K, C_0, and S. The scheme is a particular example of a two-substrate reaction[17] (the coenzyme being the other substrate). It illustrates what seems to emerge as a necessary condition for multistability (and probably oscillation kinetics as well) in these biochemical systems. The enzyme must have two sites of attachment capable of binding substrate and repressor/inducer.

7 Sigmoid Kinetics

All of the schemes described above can be classed in the general category of sigmoid kinetics. Many simple enzyme-catalysed reactions show a hyperbolic relationship between rate and substrate concentration. However, others show a sigmoidal relationship between rate and concentration in which the slope of the curve initially increases and then decreases, instead of decreasing steadily as in the former case.[17] An equation that can be fitted to most cases of sigmoid kinetics is[18]

$$\text{Rate} = \frac{iS + jS^2}{k + lS + mS^2} \tag{30}$$

where i, j, k, and l are constants. This expression, initially zero, approaches j/m as S approaches infinity. Most of the rate laws discussed above are of this type. For example the general function depicted in Figure 6b is very similar to that

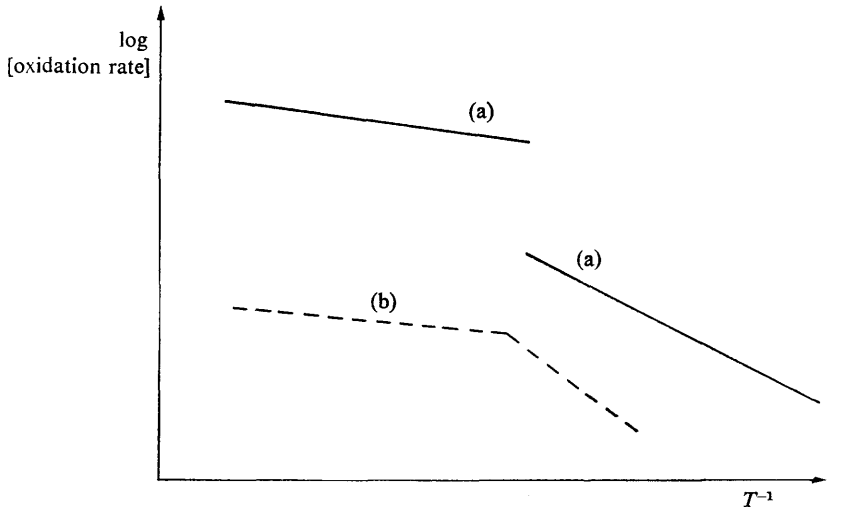

Figure 6 *Arrhenius plots for succinate oxidation by* (a) *rat liver mitochondria and* (b) *chilling sensitive cucumber fruit*

[17] K. J. Laidler and P. S. Bunting, 'The Chemical Kinetics of Enzyme Action', Clarendon Press, Oxford, 1973.
[18] W. Ferdinand, *Biochem. J.*, 1966, **98**, 278.

shown in Figure 5 for enzyme induction. Further, if $jl < im$ expression (30) passes through a maximum and rate laws similar to that of substrate inhibition (Section 4) are obtained. Thus another general condition for multistability in enzyme kinetics is that the rate laws show a sigmoid dependence.

A particular example of equation (30) is furnished by allosteric reactions. Allostery is a term coined by Monod, Wyman, and Changeaux and refers to the attachment of a substrate or modifier at a site other than the catalytic site, with resulting modification of the enzyme. Allosteric enzymes consist of a number of identical subunits which can exist in two or more conformations which have different binding abilities for the substrate. The equilibria between the various conformers and substrate are complex but in general the rate law for an allosteric reaction is given by the expression[17]

$$\text{Rate} \propto \frac{S(1 + S) + ScL(1 + cS)}{(1 + S)^2 + L(1 + cS)^2} \tag{31}$$

for a two-conformer model, where L is the equilibrium constant for interconconversion between the two conformers and c is the ratio of the binding abilities of the two conformers. Comparing (31) with (30) we see that $jl = (1 + c^2L)(2 + 2cL)$ and $im = (1 + cL)(1 + c^2L)$, and hence $jl \not< im$. However, if we now include product inhibition we get the general rate equations

$$\begin{aligned} \frac{dS}{dt} &= \frac{-(iS + jS^2)}{k + lS + mS^2 + nP^q} + K(S_0 - S) \\ \frac{dP}{dt} &= \frac{(iS + jS^2)}{k + lS + mS^2 + nP^q} - kP \end{aligned} \tag{32}$$

In the steady state $K(S_0 - S) = kP$, and so

$$0 = \frac{-(iS + jS^2)}{k + lS + mS^2 + n'(S_0 - S)^q} + K(S_0 - S) \tag{33}$$

If $q = 1$ the inhibition has the effect of reducing l and thus increasing the likelihood that the inequality $jl < im$ will be satisfied. If $q = 2$, l is reduced and m is increased so that this possibility is even further increased. Looking at equation (33) this will be most likely for strong inhibition (large n), rapid flux (large K), and slow consumption of P (small k).

8 Discontinuities in Arrhenius Plots

In this section we present some experimental results which suggest the existence of multiple steady states but which are as yet not fully understood. In a simple reaction, a plot of $R \log k$ against $1/T$ is a straight line of slope $-E$, where R is the universal gas constant, k is the reaction rate constant, and E is the activation energy for the reaction. Discontinuities in such plots are fairly common in bio-

logical systems[19,20] and have attracted considerable attention both recently and also in earlier studies.[21] In the 1920's, the temperature dependence of many biological processes was studied and shown to fit the Arrhenius equation.

Often, however, sharply defined discontinuities either in slope[22] or in both slope and rate, as shown in Figure 6, also appeared.[19,23,24] A certain amount of controversy has existed over the interpretation of these plots. One faction claims that the discontinuities do not exist,[25] whereas Crozier[21] and others claim an explanation on the basis of simultaneously occurring reactions taking over the rate-determining role in different temperature ranges. The latter theory is difficult to accept, particularly as it requires the reaction of lower activation energy to become dominant at higher temperatures; nor does it explain discontinuities in activation energy or in reaction rate. It has been suggested that only the existence of a phase change[19] would account for the phenomena, though no independent evidence for such a phase change is produced. An alternative proposal (although also not proven) is the existence of multiple steady states in which the temperature would be the independent factor determining the critical conditions. Such switching has been observed in thermokinetic systems (see next section), but has been neglected in biological systems because of the small temperature range involved. However, since the switch between steady states is so sensitive, there is no reason why this critical condition should not occur at physiological temperatures. Gray and Aarons[9] have considered the autocatalytic scheme discussed in Section 2 in a region of monostability and shown that multistability can be produced by thermokinetic feedback. In this case if k_{-1} has a strong temperature dependence such that the forward reaction is exothermic, then by considering the variation of temperature explicitly admitting heat losses hysteresis can be produced. This then provides a mechanism whereby a small change in temperature could cause a large switch in metabolic activity. Temperature regulation could operate using just such a mechanism, or at least small temperature variations may be more significant than they first appear.

9 Thermokinetic Multistability

As well as the simple type of 'thermal' multistability exhibited in flow reactors shown in Figure 1, a 'chemical' multistability can also arise due to sigmoid or other types of complex kinetics. The oxidation of propane[26] has been studied under flow reactor conditions as has that of acetaldehyde,[27] which also shows multistability, hysteresis, and oscillation. The latter system is reasonably well

[19] J. Kumato, J. K. Raison, and J. M. Lyons, *J. Theor. Biol.*, 1971, **31**, 47.
[20] M. H. Han, *J. Theor. Biol.*, 1972, **35**, 543.
[21] W. J. Crozier, *J. Gen. Physiol.*, 1924, **7**, 123.
[22] T. J. B. Stier, *J. Gen. Physiol.*, 1933, **16**, 815.
[23] J. M. Lyons and J. K. Raison, *Comp. Biochem. Physiol.*, 1970, **37**, 405.
[24] J. M. Lyons and J. K. Raison, *Plant Physiol. (Lancaster)*, 1970, **45**, 386.
[25] J. Belehradek, *Ann. Rev. Physiol.*, 1957, **19**, 59.
[26] B. F. Gray and P. G. Felton, *Combustion and Flame*, 1974, **23**, 295.
[27] B. F. Gray and P. G. Felton, and N. Shank, 2nd European Symposium on Combustion, Orleans, 1975.

understood kinetically and thermodynamically and a good representation of these strange observed facts has been given.[28]

In principle a system which shows 'chemical' multistability and which also has a significant heat release could show very odd behaviour, as its heat release rate could be multivalued at any given temperature depending on which 'kinetic' steady state it happened to be in. Thus in Figure 7 (to be compared with Figure 1)

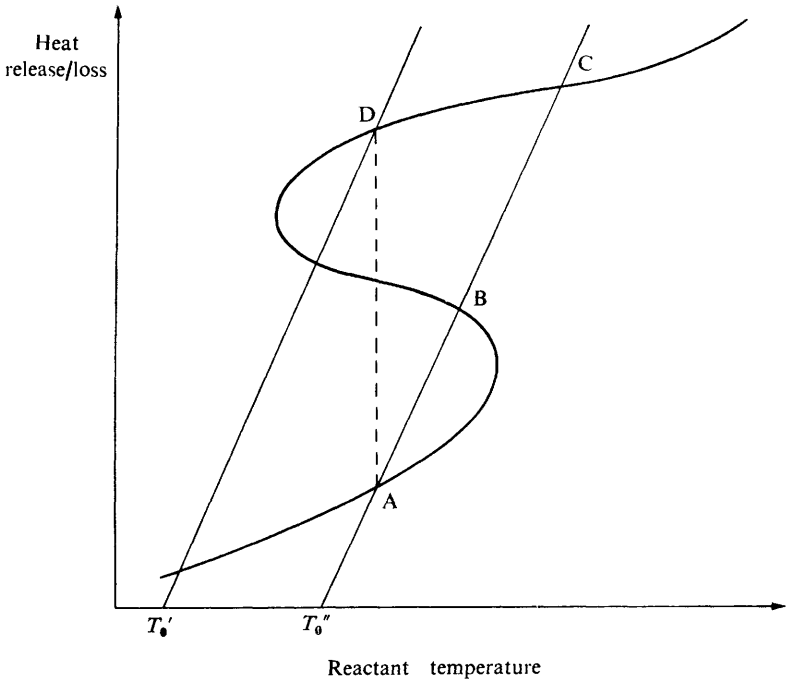

Figure 7 *Heat generation and loss curves for an exothermic system showing 'chemical' multistabllity*

the stable reaction mixtures represented by points A and D could be in thermal equilibrium with each other (*i.e.* in different reactors) but in completely different steady states and in contact with totally different ambient temperatures T_0' and T_0''. Such a system has not yet been realized experimentally.

All of the examples considered in this review have been concerned with spatially homogeneous systems. Chemical engineers who have been concerned with chemical reactions in tubular flow reactions have observed multiple steady states

[28] P. G. Felton, B. F. Gray, and N. Shank, *Combustion and Flame*, in press.

which are spatially inhomogeneous.[29,30] They have found that a simple first-order irreversible exothermic reaction, when its heat effect is taken into account, can show one or three steady-state profiles. Multistability is favoured by concentrated mixtures and long tubes.

10 Relaxation Oscillations

Finally we point out that if multistability exists it is possible for the system to perform oscillations in which the variables are continuously switched from one branch of the steady-state curve to the other.[9] This behaviour is referred to as a relaxation oscillator because of the rapid jumps followed by relatively slow motion.[31] In all the examples discussed above the steady states are functions of several independent parameters, either the concentration of some substance held in excess (the so-called 'pool' chemicals) or a physical parameter such as temperature. If the variation of these parameters is considered explicitly the SSH will still be valid, except at the critical points where transition to the other branch of the steady-state curve occurs. The motion along the steady-state curve will be governed by the differential equations for the 'pool' chemicals or temperature, but the jumps will be very rapid and of negligible time compared with the other motion. Several examples of this type of behaviour are to be found in the paper by Gray and Aarons.[9]

11 Conclusions

Chemists have been traditionally interested in closed chemical systems and have thus missed the wealth of information available from open systems. For a start one can deal with true steady states and the analysis is that of algebraic equations rather than differential equations. Consequently the idea of multistability has not occurred to many chemists. However, in open reacting biochemical systems—such as a living organism—the concept of switching from one steady state to another is very appealing, although in most cases the evidence is only circumstantial. Furthermore, in considering these systems most workers have concentrated on finding the conditions for limit cycle oscillations rather than for multistability. The requirement, in fact, for both of these phenomena is the same, *i.e.* some sort of feedback.

In the models considered above certain general trends can be found. Positive or negative feedback from either substrate or product can give rise to multistability. Switching between alternate pathways can be mediated by suitably linking them. A general feature of all the models is that the enzyme needs to have at least two sites—either for two molecules of repressor or for one molecule of repressor and one molecule of substrate—in order to achieve multistability. Finally, any scheme that exhibits sigmoid kinetics can show multiple steady states in an open chemical reactor under suitable conditions.

[29] L. R. Raymond and N. R. Amundson, *Canad. J. Chem. Eng.*, 1964, **42**, 173.
[30] D. Luss and N. R. Amundson, *Canad. J. Chem. Eng.*, 1967, **45**, 341.
[31] A. A. Andronov, A. A. Vitt, and S. E. Khaikin, 'Theory of Oscillators', Pergamon Press, Oxford, 1966.

Two final points can be made. Firstly, only enzyme–substrate reactions have been considered here, but nearly all the models can be cast in a genetic framework in which the feedback is to the gene and not the enzyme.[5,32] Secondly, if hysteresis exists there is the possibility that the system can perform relaxation oscillations in which the variables continuously switch from one branch of the steady-state curve to the other. This can be achieved by allowing the so-called 'pool' chemicals, normally held in excess, to vary. This reinforces the idea that in these systems multistability and limit cycle oscillations go hand in hand.

[32] L. J. Aarons and B. F. Gray, *J. Theor. Biol.*, 1975, **50**, 501.