3.7 A NEGATIVE FEEDBACK BIOCHEMICAL SYSTEM: THE GOODWIN EQUATIONS FOR $N=3$

Derivation of the equations and translation to nondimensional form

In Section 3.2 we introduced a three-variable model of a biochemical process where x_1 is an mRNA species, x_2 is the enzyme coded by the mRNA and x_3 is the product of the enzyme catalyzed reaction. In the previous analysis we treated x_1 as an input variable. Now we consider the case originally considered by Goodwin (1965) where the input consists of a constant nucleotide pool necessary for mRNA synthesis and where x_3 directly represses the synthesis of x_1. In this simple model it is assumed that ρ molecules of x_3 combine with the gene coding for the mRNA to form a nonfunctional operator–inhibitor complex. The rate of x_1 synthesis is assumed to be directly proportional to the fraction of time that the operator is not repressed. To calculate this fraction as a function of x_3, suppose that there is a large population of identical gene copies. Let G_T be the total number of genes in the population, G_A, the number of active genes and G_R the number of repressed genes. The fraction of time that a single gene is unrepressed is equal to the fraction of unrepressed genes in a large population, G_A/G_T. This ratio is found by using an argument analogous to those of classical enzyme kinetics. It is supposed that the repressor–operator reaction can be approximated as the direct combination of ρ molecules of repressor. This is clearly not the case chemically since the possibility of higher-order molecular collisions is vanishingly small. Strictly speaking one should consider the sequential addition of repressor molecules. However, the reaction of (1) below is taken as a first approximation. The approximation is surprisingly successful for a wide range of concentrations under certain circumstances; in particular, when binding the first repressor is the rarest step.

$$G_A + \rho x_3 \underset{h_2}{\overset{h_1}{\rightleftharpoons}} G_R;$$

$$dG_A/dt = -h_1 G_A x_3^{\rho} + h_2 G_R. \tag{1}$$

This reaction is assumed to be close to equilibrium so

$$G_T = G_A + G_R = G_A(1+\alpha x_3^\rho), \quad \text{where } \alpha \equiv h_1/h_2.$$

$$G_A/G_T = 1/(1+\alpha x_3^\rho).$$

The rate of x_1 synthesis is taken as being directly proportional to this function. Let k_0 be the constant of proportionality. It is also assumed that the rate of mRNA destruction is directly proportional to its concentration. As in Section 3.2 the synthesis and destruction of x_2 and x_3 are assumed to be first order. (Note that (2) is slightly more general than (3.2.2) since it admits the possibility $b_2 \neq g_2$.)

$$\begin{aligned} dx_1/dt &= [k_0/(1+\alpha x_3^\rho)] - b_1 x_1, \\ dx_2/dt &= g_1 x_1 - b_2 x_2, \\ dx_3/dt &= g_2 x_2 - b_3 x_3. \end{aligned} \tag{2}$$

The approximation associated with assuming a $(\rho+1)$-order reaction has already been discussed, but there is another approximation implicit in modeling gene control processes by continuous ordinary differential equations, namely that the law of mass action (large numbers) applies. However, in typical systems there may be very few copies of repressor molecules (Gilbert & Müller-Hill, 1966; Lin & Riggs, 1975). Berg & Blomberg (1977) have compared the binding laws derived for a system containing a discrete number of molecules with the classical mass action equation and found that the difference between them increases as the number of molecules in the system decreases. However, it is fair to conclude that the order of error introduced by this assumption is probably small compared to the others involved in the model, notably reducing mRNA synthesis and enzyme synthesis to a single equation. It is possible to produce (2) by an argument involving feedback inhibition in a metabolic system where the problem of limited numbers of molecules is less severe. In any case the initial purpose of the present exercise is to learn something in general about the behavior of continuous negative feedback systems.

Equation (2) contains seven parameters (k_0, α, b_1, b_2, b_3, g_1 and g_2). Substantial simplification is achieved by transforming the problem to non-dimensional coordinates. This is done in Example 3.7.1.

Example 3.7.1. Find scaling factors for concentrations and time to simplify (2) as much as possible; namely find scaling factors for t, x_1, x_2 and x_3 and hence new variables τ, z_1, z_2 and z_3 and new parameters k_1, k_2 and k_3 so that (2) becomes

$$\frac{dz_1}{d\tau} = \frac{1}{1+z_3^\rho} - k_1 z_1, \tag{3}$$

$$dz_2/d\tau = z_1 - k_2 z_2,$$

$$dz_3/d\tau = z_2 - k_3 z_3.$$

Solution:
Clearly the first step is to eliminate α from the denominator of the nonlinearity. Define intermediate variables $y_j = \alpha^{1/\rho} x_j$. With these,

$$\frac{dy_1}{dt} = \frac{\alpha^{1/\rho} k_0}{1+y_3^\rho} - b_1 y_1,$$

$$\frac{dy_2}{dt} = g_1 y_1 - b_2 y_2,$$

$$\frac{dy_3}{dt} = g_2 y_2 - b_3 y_3.$$

Define scale factors w and h_j such that $\tau = wt$ and $z_j = h_j y_j$. The differential equation becomes

$$\frac{dz_1}{d\tau} = \left(\frac{\alpha^{1/\rho} k_0 h_1}{w}\right)\left(\frac{1}{1+z_3^\rho/h_3^\rho}\right) - \left(\frac{b_1}{w}\right) z_1,$$

$$\frac{dz_2}{d\tau} = \left(\frac{g_1 h_2}{w h_1}\right) z_1 - \left(\frac{b_2}{w}\right) z_2,$$

$$\frac{dz_3}{d\tau} = \left(\frac{g_2 h_3}{h_2 w}\right) z_2 - \left(\frac{b_3}{w}\right) z_3.$$

The desired simplification results if

$$\frac{\alpha^{1/\rho} k_0 h_1}{w} = 1, \qquad \frac{g_1 h_2}{w h_1} = 1, \qquad \frac{g_2 h_3}{h_2 w} = 1, \qquad h_3 = 1.$$

Taking the product of the first three conditions fixes w:

$$w = (g_1 g_2 \alpha^{1/\rho} k_0)^{\frac{1}{3}}.$$

Solving the first three conditions sequentially we find

$$h_1 = \frac{(g_1 g_2 \alpha^{1/\rho} k_0)^{\frac{1}{3}}}{\alpha^{1/\rho} k_0}, \qquad h_2 = \frac{(g_1 g_2 \alpha^{1/\rho} k_0)^{\frac{2}{3}}}{g_1 \alpha^{1/\rho} k_0}, \qquad h_3 = 1.$$

So, as is necessary, the fourth condition is automatically satisfied. The transformation equations can be generalized to arbitrary dimension (Rapp, 1976).

It is important to consider now the significance of the parameter ρ in (3). As ρ increases the synthesis versus inhibitor function becomes steeper (Figure 3.7.1). In the terminology of Section 3.3, control becomes

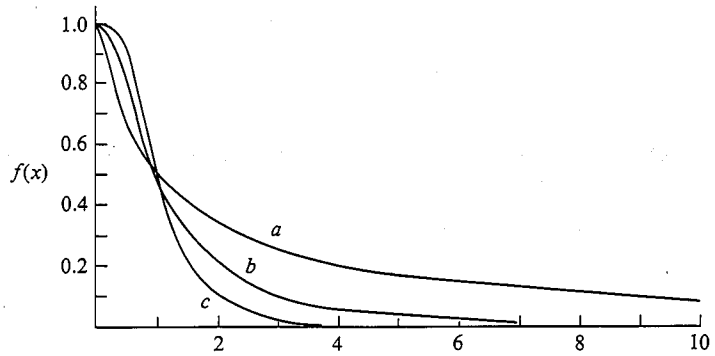

Figure 3.7.1. The effect of increasing ρ on the nonlinearity $f(x)=1/(1+x^{\rho})$. In Curve *a*, $\rho=1$; in Curve *b*, $\rho=2$ and in Curve *c*, $\rho=3$. As ρ increases the transition from high synthesis rate (large f) to low synthesis rate (small f) becomes sharper.

tighter as ρ increases. Accordingly we would expect that increasing ρ might destabilize the system. We begin the analysis of (3) by establishing two simple global properties of the equation.

Global properties

We now show that all biologically significant behavior of (3) is contained in a finite region of positive concentration space. In addition it is shown that this region contains a single steady state.

Example 3.7.2 (*Griffith*, 1968). Suppose that initially z_1, z_2 and z_3 are positive or zero. Since these variables are concentration functions this must be the case. Show that after a sufficiently large time the following inequalities are satisfied.

$$0 \leqslant z_1(\tau) \leqslant \max\,(z_1(0), (1/k_1)),$$

$$0 \leqslant z_2(\tau) \leqslant \max\,(z_2(0), (1/k_1k_2)),$$

$$0 \leqslant z_3(\tau) \leqslant \max\,(z_3(0), (1/k_1k_2k_3)).$$

Solution:
For $j=1$, 2 and 3, dz_j/dt is positive when $z_j=0$ so if $z_j(0)$ is non-negative then $z_j(t)$ must be positive for all time $\tau>0$. This establishes the lower bound. Consider the z_1 governing equation

$$\frac{dz_1}{d\tau}=\frac{1}{1+z_3^{\rho}}-k_1z_1.$$

Since $z_3 \geqslant 0$, we know that $1+z_3^{\rho} \geqslant 1$, so that

$$\frac{dz_1}{d\tau}=\frac{1}{1+z_3^{\rho}}-k_1z_1 \leqslant 1-k_1z_1.$$

Thus $\dot{z}_1$ is negative for all $z_1>(1/k_1)$. Accordingly if $z_1(0)$ is less than $1/k_1$ we know that it will never exceed $1/k_1$. If $z_1(0)$ is greater than $1/k_1$, the derivative will be negative and so z_1 will decrease until after some finite time it is less than $1/k_1$. Hence

$$0 \leqslant z_1(\tau) \leqslant \max\,[z_1(0), (1/k_1)].$$

The bound on z_2 is established by a similar argument. After a sufficient time $z_1<1/k_1$. Consequently

$$dz_2/d\tau = z_1-k_2z_2 \leqslant (1/k_1)-k_2z_2.$$

For all sufficiently large time and for all $z_2>1/k_1k_2$, we now know that $\dot{z}_2$ is negative, i.e.

$$0 \leqslant z_2(\tau) \leqslant \max\,[z_2(0), (1/k_1k_2)].$$

The bound on z_3 is established by an analogous argument. Let B denote the set in z_1–z_2–z_3 space formed by the rectangular box with vertices $(0, 0, 0)$ and $(1/k_1, 1/k_1k_2, 1/k_1k_2k_3)$ with sides parallel and perpendicular to the coordinate axes. If an initial point is in set B, the solution of the differential equation remains in B. Sets with this property are called **invariant**.

In Example 3.7.3 the next property of the system is established: it has only one physical steady state (where here we use the word 'physical' to mean non-negative, as is required of concentration functions).

Example 3.7.3. Show that there is only one steady state in positive concentration space and that it is in set B.

Solution:
Let the superscript '0' denote steady state values. They are defined by the steady state equation $dz_j/dt=0$.

$$0=\frac{1}{1+(z_3^0)^{\rho}}-k_1z_1^0, \qquad 0=z_1^0-k_2z_2^0, \qquad 0=z_2^0-k_3z_3^0.$$

Using the second and third equation to eliminate z_1^0 and z_2^0 and the definition $c_0=k_1k_2k_3$ we find that

$$c_0z_3^0=1/[1+(z_3^0)^{\rho}] \equiv f(z_3^0). \tag{4}$$

As shown in Figure 3.7.2 the left-hand side of this equation increases from zero to infinity while the right-hand side

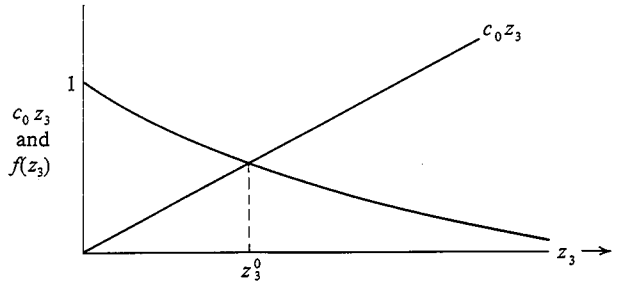

Figure 3.7.2. Solution of the steady equation $f(z_3) = c_0 z_3$ for z_3^0. The uniqueness of the solution for positive z_3 follows from the monotonicity of the functions (they are increasing or decreasing for all positive z_3).

decreases from one to zero so there must be an intersection (and hence a solution to the steady state equations). Because both $c_0 z_3^0$ and $f(z_3^0)$ are strictly monotone (i.e. always increasing or always decreasing) there is only one intersection. Thus there is only one positive z_3^0 and hence only one positive z_1^0 and z_2^0 satisfying the steady state equation.

To show that this steady state is in invariant set B, we make the following manipulations of the steady state equation.

$$c_0 z_3^0 = 1/[1+(z_3^0)^\rho],$$

$$c_0 z_3^0 + c_0 (z_3^0)^{\rho+1} = 1,$$

$$f_a(z_3^0) \equiv c_0 (z_3^0)^{\rho+1} = 1 - c_0 z_3^0 \equiv f_b(z_3^0).$$

The functions f_a and f_b are plotted in Figure 3.7.3. Their unique intersection occurs at the positive solution of the steady state

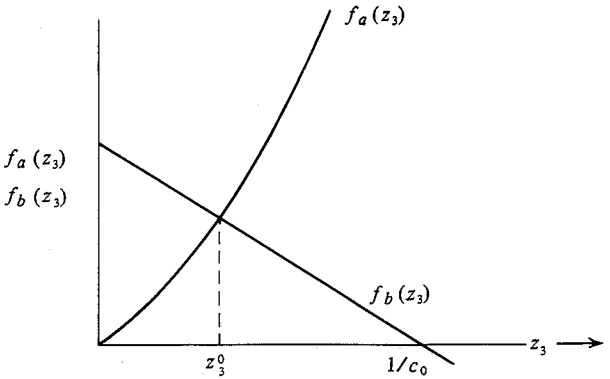

Figure 3.7.3. Proof that $0 < z_3^0 < (1/c_0)$. In this diagram $f_a(z_3) = c_0 (z_3)^{\rho+1}$ and $f_b(z_3) = 1 - c_0 z_3$.

equation. Since f_b is zero at $1/c_0$ we have

$$z_3^0 < 1/c_0 = 1/k_1 k_2 k_3.$$

Similarly

$$z_2^0 = k_3 z_3^0 < 1/k_1 k_2, \qquad z_1^0 = k_2 z_2^0 < 1/k_1,$$

so (z_1^0, z_2^0, z_3^0) is in set B.

Both of these results are fairly easy to prove, but are more valuable than they first appear since they can be used in conjunction with some recent mathematical results to identify conditions which ensure the existence of oscillations. Examples 3.7.2 and 3.7.3 have been generalized to a nonlinear n-dimensional multiple loop system that contains (3) as a special case (Mees & Rapp, 1978).

Local stability analysis

The analysis of the stability properties of steady states is the essential first step in the analysis of a nonlinear system. A steady state is said to be locally stable if all sufficiently close initial points possess solutions that tend to the steady state as $t \to \infty$. The local stability analysis begins by translating the original nonlinear differential equation (here (3)) to an approximate linear differential equation that describes the behavior of small variations around the steady state. Let z_j' be the variation from z_j^0, i.e. $z_j = z_j^0 + z_j'$. The equation for z_1' is found by substituting these new variables into the equation for z_1:

$$dz_1/d\tau = f(z_3) - k_1 z_1,$$

$$\frac{d}{d\tau}(z_1^0 + z_1') = \frac{d}{d\tau} z_1' = f(z_3^0 + z_3') - k_1(z_1^0 + z_1').$$

Here we have used the fact that z_1^0 is a constant and hence has a zero derivative. Using Taylor's theorem (A.1.30) we have the following expansion of $f(z_3)$:

$$f(z_3) = f(z_3^0 + z_3')$$

$$= f(z_3^0) + f'(z_3^0) z_3' + \frac{f''(z_3^0)(z_3')^2}{2!} + \ldots.$$

Recall that we are considering only small displacements from the steady state so z_3' is small and thus

$$z_3' \gg (z_3')^2 \gg (z_3')^3,$$

so we have a linear approximation

$$f(z_3^0+z_3')=f(z_3^0)+f'(z_3^0)z_3'.$$

Substituting this into the differential equation gives

$$dz_1'/d\tau=f(z_3^0)+f'(z_3^0)z_3'-k_1(z_1^0+z_1').$$

The steady state condition is

$$f(z_3^0)-k_1z_1^0=0,$$

so the differential equation becomes

$$dz_1'/d\tau=f'(z_3^0)z_3'-k_1z_1'.$$

The translation process for z_2 and z_3 is simpler since these equations are already linear:

$$dz_2/d\tau=z_1-k_2z_2,$$

$$\frac{d}{d\tau}(z_2^0+z_2')=z_1^0+z_1'-k_2(z_2^0+z_2').$$

Since $z_1^0-k_2z_2^0=0$ this becomes

$$dz_2'/d\tau=z_1'-k_2z_2'.$$

Similarly for z_3 we find

$$dz_3'/d\tau=z_2'-k_3z_3'.$$

The set of equations collected in (5) below is referred to as the linear variational equations taken about the steady state:

$$\begin{aligned} dz_1'/d\tau&=f'(z_3^0)z_3'-k_1z_1',\\ dz_2'/d\tau&=z_1'-k_2z_2',\\ dz_3'/d\tau&=z_2'-k_3z_3'. \end{aligned} \tag{5}$$

If this system of differential equations is stable, i.e. $z_j'(\tau)\to 0$ as $\tau\to\infty$ for $j=1$, 2 and 3, then the steady state is stable. The stability of the linear variational equations will be determined by the Nyquist criterion in a manner identical to the analyses in Sections 3.3 and 3.4. Equation (5) could be investigated directly, without transforms, but the transform method can be used for more general situations such as those with time delays. It is assumed that initially the system is at the steady state, so $z_j'(0)=0$. Taking Laplace transforms we find

$$\begin{aligned} s\hat{z}_1'&=f'(z_3^0)\hat{z}_3'-k_1\hat{z}_1',\\ s\hat{z}_2'&=\hat{z}_1'-k_2\hat{z}_2',\\ s\hat{z}_3'&=\hat{z}_2'-k_3\hat{z}_3'. \end{aligned}$$

The second and third equation can be used to eliminate $\hat{z}_1'$ and $\hat{z}_2'$:

$$\begin{aligned} (s+k_1)\hat{z}_1'&=f'(z_3^0)\hat{z}_3',\\ (s+k_2)\hat{z}_2'&=\hat{z}_1',\\ (s+k_3)\hat{z}_3'&=\hat{z}_2',\\ (s+k_1)(s+k_2)(s+k_3)\hat{z}_3'&=f'(z_3^0)\hat{z}_3',\\ \hat{z}_3'&=f'(z_3^0)[1/(s+k_1)(s+k_2)(s+k_3)]\hat{z}_3'. \end{aligned}$$

We define $G(s)$ as

$$G(s)=1/(s+k_1)(s+k_2)(s+k_3), \tag{6}$$

so

$$\hat{z}_3'=f'(z_3^0)G(s)\hat{z}_3'.$$

The control loop corresponding to this differential equation is shown in Figure 3.7.4.

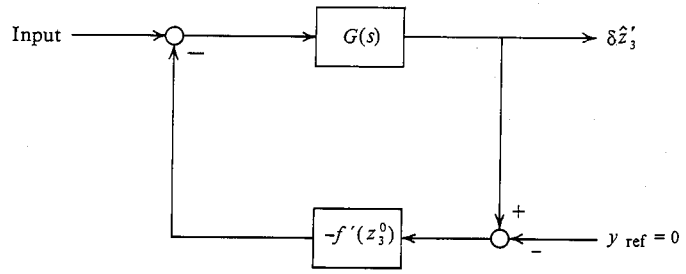

Figure 3.7.4. Local stability analysis by application of the Nyquist criterion follows from a restatement of the stability equations as a feedback system.

The stability of the variational equations, and hence of the steady state, is now found by application of the Nyquist criterion.

Example 3.7.4. Let $G(s)$ be given by (6). The Nyquist locus has the same form as that in Figure 3.3.7; $G(0)$ is a positive real number and the locus spirals into the origin in a clockwise direction intersecting the negative real axis once. Let ω_1 be the value of ω at this intersection. Show that the control system of Figure 3.7.4 is unstable if and only if

$$W_1(k_1, k_2, k_3)=\rho(1-c_0z_3^0)|G(i\omega_1)/G(0)|>1,$$

where $c_0=k_1k_2k_3$.

Solution:
The analysis follows the program of Example 3.3.6. Here the gain constant analogous to k is $-f'(z_3^0)$ (recall that since f is

monotone decreasing; i.e. decreasing for all positive z_3, $f'(z_3^0)$ is negative and thus $-f'(z_3^0)$ is positive). The control loop is unstable if the point $1/f'(z_3^0)$ (analogous to $-1/k$) is encircled by $G(i\omega)$. Since $1/f'(z_3^0)$ is a real number we conclude that this can happen only if

$$G(i\omega_1) < 1/f'(z_3^0) < G(0).$$

Since $1/f'(z_3^0)$ is negative and $G(0)$ is positive, the inequality on the right is satisfied automatically so the instability condition is

$$G(i\omega_1) < 1/f'(z_3^0).$$

Again using the known sign properties of these numbers this condition can be restated:

$$-|G(i\omega_1)| < -1/|f'(z_3^0)|,$$

$$|G(i\omega_1)| > 1/|f'(z_3^0)|,$$

$$|f'(z_3^0)\|G(i\omega_1)| > 1.$$

The function $|f'(z_3^0)\|G(i\omega_1)|$ is a function of the parameters k_1, k_2 and k_3. Defining W_1 as shown, we can conclude that $W_1 > 1$ is a necessary and sufficient condition for instability:

$$W_1(k_1, k_2, k_3) \equiv |f'(z_3^0)\|G(i\omega_1)| > 1.$$

It now only remains to transform W_1 into the desired final form. By definition

$$G(0) = 1/k_1k_2k_3 = 1/c_0,$$

so

$$W_1(k_1, k_2, k_3) = \frac{|f'(z_3^0)|}{c_0} |G(i\omega_1)/G(0)|.$$

It is necessary to show that $|f'(z_3^0)|/c_0 = \rho(1 - c_0z_3^0)$. But

$$f(z_3) = \frac{1}{(1+z_3^\rho)}, \qquad f'(z_3) = \frac{-\rho z_3^{\rho-1}}{(1+z_3^\rho)^2}.$$

At the steady state

$$c_0z_3^0 = 1/(1+(z_3^0)^\rho),$$

so

$$f'(z_3^0) = -\rho c_0^2 (z_3^0)^{\rho+1}.$$

Again using the steady state equation we find

$$c_0z_3^0 = 1/(1+(z_3^0)^\rho),$$

$$c_0z_3^0(1+(z_3^0)^\rho) = c_0z_3^0 + c_0(z_3^0)^{\rho+1} = 1,$$

$$c_0(z_3^0)^{\rho+1} = 1 - c_0z_3^0,$$

$$f'(z_3^0) = -\rho c_0(1 - c_0z_3^0),$$

and thus

$$\frac{|f'(z_3^0)|}{c_0} = \rho|1 - c_0z_3^0|.$$

In Example 3.7.3 it was shown that $z_3^0 < 1/c_0$ so

$$|1 - c_0z_3^0| = 1 - c_0z_3^0,$$

and the desired result is obtained:

$$W_1(k_1, k_2, k_3) = \rho(1 - c_0z_3^0)|G(i\omega_1)/G(0)|.$$

It should be noted that this result is immediately valid for a system of arbitrary dimension n where

$$G(s) = \prod_{j=1}^{n} \frac{1}{s + k_j}.$$

The behavior of $W_1(k_1, k_2, k_3)$ should now be examined. Even for $n = 3$ this is a comparatively complicated function. However, most of the more important information can be obtained by considering the special case of its maximum value. We begin by evaluating the maximum of $|G(i\omega_1)/G(0)|$. It should be realized that because $c_0 = k_1k_2k_3$, the factors $\rho(1 - c_0z_3^0)$ and $|G(i\omega_1)/G(0)|$ are coupled so that *ab initio* one should not determine the maximum of the two factors separately. However, it can be shown that the maximum value of $|G(i\omega_1)/G(0)|$ is obtained when $k_1 = k_2 = k_3$ (Rapp, 1975). The common value of the k's (and hence of c_0) is immaterial so the two factors can be maximized separately.

$$\max |G(i\omega_1)/G(0)| = [\cos(\pi/3)]^3,$$

so

$$\max W_1(k_1, k_2, k_3) = \max[\rho(1 - c_0z_3^0)] \cos(\pi/3)^3.$$

It can be shown (Rapp, 1975) that

$$\max \rho(1 - c_0z_3^0) = \lim_{c_0 \to 0} \rho(1 - c_0z_3^0) = \rho.$$

The maximum of W_1 has been found

$$\max W_1(k_1, k_2, k_3) = \rho \cos(\pi/3)^3 = \rho/8.$$

For $\rho = 1, \ldots 7$, max $W_1 < 1$; so for any positive set of k's the steady state is locally stable. For $\rho = 8$, max $W_1 = 1$, exactly, and since the maximum is only reached in the limit $c_0 \to 0$, we conclude that also for $\rho = 8$ the steady state is stable for any set of k's. However, for $\rho > 9$ it is possible to find finite positive k's that produce a system with $W_1 > 1$, i.e. that produce a system that has an unstable steady state. It is interesting to note that both W_1 and max W_1 are directly proportional to ρ. Thus as predicted, increasing ρ tends to destabilize the steady state. As ρ increases the synthesis versus inhibitor function becomes steeper and there is a more sharply defined on–off control (i.e. tightness of control is increased approaching the on–off controller in the Chancellor of the Exchequer oscillator). The implications of local instability for global behavior are considered next.

Oscillations: biological examples of tightness of control and local instability

It can be demonstrated that if the steady state of (3) is unstable (i.e. $W_1 > 1$) then the system possesses a periodic solution (Hastings, Tyson & Webster, 1975). The proof given by Hastings, Tyson & Webster proves this proposition for a much more general n-dimensional system. A simpler proof of the three-dimensional case of (3.7.3) has been published separately (Tyson 1975). However, even the proof of the simple case is beyond the scope of this book. An intuitive justification of the result can be argued along the lines previously presented for the case of oscillations in positive feedback loops. As shown in Example 3.7.2 the solution will always remain in a finite region. This is true even if the steady state is unstable. So again an oscillation results as a compromise between a bounded response and an unstable steady state.

However, granting that the mathematical result is intuitively acceptable, it does not necessarily follow that it is obvious that negative feedback systems can form the basis of biological oscillations. Indeed since local instability can result only if $\rho \geqslant 9$, systems analogous to (3) might seem unlikely candidates as biochemical oscillators. However, several considerations suggest that this is not necessarily the case.

First, it should be pointed out that the number of intermediate reactions in a biochemical system would often be larger than the $n = 3$ case of (3). A more satisfactory model would be one with a larger number of reactants:

$$\frac{dz_1}{d\tau} = \frac{1}{1+z_n^\rho} - k_1 z_1,$$

$$dz_j/d\tau = z_{j-1} - k_j z_j; \qquad j = 2, \ldots n.$$

The local instability condition is again

$$W_1(k_1, \ldots k_n) \equiv \rho(1 - c_0 z_n^0)|G(i\omega_1)/G(0)| > 1,$$

where

$$G(s) = \prod_{j=1}^{n} 1/(s + k_j).$$

The maximum of W_1 has been established for this more general system (Rapp, 1975):

$$\max W_1 = \rho \cos(\pi/n)^n.$$

As n increases, $\cos(\pi/n)^n$ increases, and the minimum value of ρ admitting the possibility of local instability decreases rapidly (Table 3.7.1).

Table 3.7.1. *Instability and tightness of control*

For a specified value of n, $\cos(\pi/n)^n$ is given. The minimum integer value of ρ such that max $W_1 > 1$ is found from the equation max $W_1 = \rho \cos(\pi/n)^n$.

n	$\cos(\pi/n)^n$	min ρ
3	0.125	9
4	0.250	5
5	0.347	3
6	0.422	3
7	0.482	3
8	0.531	2
9	0.571	2
10	0.605	2

Time delays between reaction steps have the same destabilizing effect as increasing the number of chemical intermediates. As the sum of all separate delays approaches infinity $|G(i\omega_1)/G(0)| \to 1$ so an equation with arbitrary dimension n, and any value of ρ except $\rho = 1$ can be unstable if the time delays are sufficiently large. (In fact it is possible for these systems to oscillate even if $n = 1$ and $\rho = 2$ (Rapp, 1974). This example is a mathematical curiosity, and does not correspond to any chemical analog but the example does make the point particularly vividly.)

Thus it is seen that the requirement for a large value of ρ is an artifact resulting from considering the system for the special case of $n = 3$ with no time delays. However, it should be pointed out that ρ can be fairly large. The DNA of bacteriophage λ has two operators recognized by the same repressor. Each operator binds six repressor molecules sequentially

(Maniatis & Ptashne, 1973). Here $\rho = 6$ but because the binding is sequential a more accurate nonlinearity would be the function

$$(z_n^6 + \alpha_5 z_n^5 + \ldots \alpha_1 z_n + \alpha_0)^{-1}.$$

Also the possibility of oscillations in negative feedback metabolic systems becomes more plausible when it is noted that the measured activity versus inhibitor functions of allosteric enzymes can be very steep. Specific examples are found in the enzymes that regulate cyclic AMP (cAMP) concentration. The inhibition of adenylate cyclase (the enzyme that synthesizes cAMP from ATP) by calcium (Marcus & Aurbach, 1971) and EGTA (Bradham, Holt & Sims, 1970; Johnson & Sutherland, 1973) and the activation of this enzyme by magnesium (Drummond & Duncan, 1970; Hepp, Edel & Wieland, 1970) and by epinephrine (Birnbaumer & Rodbell, 1969) all follow functions that are so steep that they approach the form of a mechanical on–off switch. This is also true of the activation of the cAMP catabolic enzyme phosphodiesterase by calcium (Kakiuchi, Yamazaki, Teshima & Uenishi, 1973; Teo & Wang, 1973). This suggests that if these components were incorporated into negative feedback loops oscillations could result. It has been argued that this is indeed the case (Rapp & Berridge, 1977) and that oscillations in calcium–cAMP control loops form the basis of several high frequency biological rhythms including potential oscillations in pancreatic β-cells, smooth muscle and the cardiac pacemaker. The feedback loop proposed for the cardiac pacemaker is shown in Figure 3.7.5. It is proposed that, as in the case of brain tissue, calcium, in conjunction with a calcium-dependent receptor protein, activates adenylate cyclase (Brostrom, Huang, Breckenridge & Wolff, 1975) and produces an increase in cAMP levels. In cardiac muscle cAMP stimulates the sequestration of calcium in the sarcoplasmic reticulum (Tada, Kirchberger & Katz, 1975). The existence of an oscillation in intracellular calcium is well established and calcium is the contraction trigger in all types of muscle (Morad & Goldman, 1973). An oscillation in cAMP concentration has been measured directly (Brooker, 1973, 1975; Wollenberger *et al.*, 1973). Because cAMP and calcium directly affect the membrane, oscillations in internal calcium and cAMP would produce

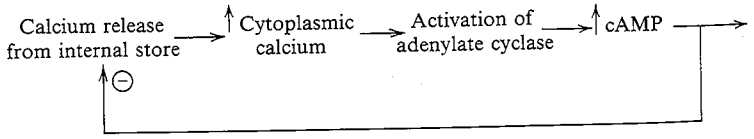

Figure 3.7.5. The interrelation of calcium and cAMP in cardiac muscle. Calcium increases the concentration of cAMP which in turn stimulates the sequestration of cytosol calcium.

observed oscillations in membrane potential. An alternative and more generally accepted model of the basis of pacemaker activity is based on the nonlinear properties of the ion-conducting channels of the cardiac sarcolemma (Jack, Noble & Tsien, 1975).

It has been suggested that other biological rhythms may be the result of oscillations in negative feedback loops. These include oscillations in cyclic enzyme synthesis (Masters & Donachie, 1966; Knorre, 1968, 1973; Donachie & Masters, 1969) and circadian rhythms (Benson & Jacklet, 1977).