

The Qualitative Dynamics of a Class of Biochemical Control Circuits

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Summary

The dynamical behavior of a class of biochemical control circuits that regulate enzyme or protein synthesis by end-product feedback is analyzed. Both inducible and repressible systems are studied and it is proven that in the former unique steady states are globally asymptotically stable. This precludes periodic solutions in these systems. A similar result holds for repressible systems under certain constraints on kinetic parameters and binding constants. However, when the reaction sequence is sufficiently long, or when a large enough number of effector molecules bind to each repressor molecule, repressible systems can show zero-amplitude ("soft") bifurcations: these are predicted by Hopf's bifurcation theorem.

I. Introduction

Enzyme and protein synthesis is a major component of cellular activity throughout much of the division cycle in proliferating cells. While the total mass of a growing cell increases continuously throughout interphase, the majority of enzymes are synthesized periodically at specific points in the cell cycle [Mitchison, 1971]. A major question is whether these periodic syntheses are the result of autonomous periodicities in the molecular pathways that produce the enzyme, or whether they result from entrainment of the pathway by some other intracellular pacemaker. Since biosynthetic pathways can be controlled at more than one level using different modes of control, there is undoubtedly no unique answer.

In some pathways a key enzyme, usually the first in a sequence, is under allosteric control by a product synthesized further down the sequence (Atkinson, 1965). Synthetic pathways can also be controlled at the genetic level by controlling the rate of synthesis of one or more unstable enzymes in the pathway. In this mode of control, which is generally interpreted according to the Jacob-Monod model, an "effector" molecule from the cytoplasm binds to the repressor of the structural gene and either inhibits or facilitates production of mRNA for the enzyme. The former is called repression and the latter induction of enzyme synthesis. In many pathways several modes are used, in particular, allosteric control of a single enzyme is often coupled with repression of all enzymes in the pathway [Atkinson, 1965]. Such multi-mode control leads to very complex dynamical equations for the

temporal behavior of the key components in the pathway. Consequently, it is imperative to first understand simpler systems in which only a single mode of control is used. This is our objective here.

A model for the feedback control of enzymatic activity was proposed by Yates and Pardee (1956) and studied by Morales and McKay (1967), by Walter (1969, 1971) and by Hunding (1974). Goodwin (1965) proposed and analyzed a model for control of enzyme synthesis at the genetic level; this model has been studied further by Griffith (1968), Goodwin (1969) and Tyson (1975). Dynamically the two models are indistinguishable when the Goodwin model is applied to enzyme repression; they differ of course when Goodwin's model is used to describe enzyme induction, as in Griffith (1968).

Considerable confusion has arisen from some of the preceding literature concerning the existence or non-existence of periodic solutions for these simple feedback control models. Goodwin (1965) reported stable periodic solutions on the basis of analog computations but some of Griffith's results cast doubt on the existence of these oscillations. Similarly, Morales and McKay (1967) reported stable periodic solutions based on analog computations for the Yates-Pardee model, but Walter (1971) reported that digital simulation of the same equations produced only highly damped oscillations. The uncertainties concerning oscillations stem from the difficulty of a complete analytical study of the equations and the consequent reliance on numerical simulations. In this paper we analyze these equations in detail, with a view towards establishing the existence or non-existence of periodic solutions by a combination of analytic and numerical techniques.

In the following section we derive the basic differential equations for both inducible and repressible systems and obtain some qualitative properties of their solutions. In that section it is shown that a two-step control circuit can be analyzed completely and that oscillations are never possible, a conclusion already reported by Griffith (1968).

Section 3 is devoted to the analysis of inducible systems. Conditions for uniqueness of the steady state are given and it is proven that uniqueness implies global asymptotic stability. Consequently, inducible systems with a single steady state can never show sustained autonomous oscillations. If such circuits oscillate it must be due to either coupling with another circuit, to another mode of control somewhere in the circuit, or to periodic changes in the concentration of some substrate.

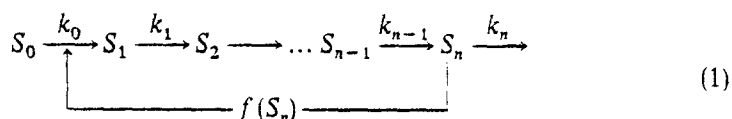
Repressible or negative feedback systems are studied in Section 4. It is found that there is a very simple relation between the minimum length of the control circuit and the number of effector molecules bound by the repressor which guarantees that the steady state is asymptotically stable¹. Stable periodic solutions are unlikely in these cases. When the steady state is unstable, the Hopf theorem gives local existence of a periodic solution in a neighborhood of the bifurcation curve. Tyson (1975) and Hastings, Tyson and Webster (1975) have proven that a

¹ A reviewer brought Hunding's paper, in which this same relation appears, to our attention.

periodic solution exists whenever the steady state is unstable and it is probably the same solution given by the Hopf theorem. This appears to be the case for the five step circuit we analyze in that section. For that particular system the region of parameter space in which the steady state is proved to be globally asymptotically stable is only slightly smaller than the region of asymptotic stability.

II. Kinetic Equations for the Control Circuits

The control circuits studied here consist of an n -step sequence of enzyme-catalyzed reactions that convert a substrate S_0 to an endproduct S_n , with feedback of S_n to the first step in the sequence:



Although each reaction is enzyme-catalyzed, it is assumed that the concentrations of intermediate species are sufficiently small and that the corresponding enzyme concentrations are constant so that the 2-nd through $(n-1)$ -st reactions can be considered first order and irreversible. Only the enzyme for the first step in the sequence is unstable, and its concentration is governed by the equilibrium between the rates of synthesis and degradation. Furthermore, it is assumed that S_0 varies significantly only on a time scale that is much longer than the time scale of the control action and hence S_0 is regarded as constant.

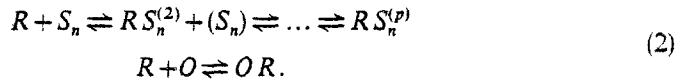
The simple schematic at (1) obscures the fact that the effector molecule S_n may act at one of several steps between gene transcription and assembly of the enzyme at the ribosomes. Different biochemical pathways are controlled at different steps (Mitchison, 1971) or at more than one step (Atkinson, 1965) but for concreteness, only control at the level of gene transcription is considered here. All other steps in the production of enzyme for the first reaction are unaffected by S_n and equilibrate to stationary states more rapidly than the controlled step. In turn, the controlled step equilibrates more rapidly than steps 1 through n in the control circuit².

There are at least two distinct types of control that are used at the genetic level; control by induction and control by repression (Yagil and Yagil, 1971). In the former an increase in S_n stimulates the production of enzyme and this in turn further increases the throughput through the pathway; thus the system functions as a positive feedback control system. In a repressible system an increase in the level of S_n decreases the rate of enzyme synthesis and the system behaves like a negative feedback control system. Negative feedback control systems are

² When the time scales of the steps in enzyme production are not sufficiently separated from those in the sequence 1, ..., n , one can simply reinterpret S_1 as mRNA and one of the succeeding species as the enzyme. This is the interpretation used by Goodwin (1965). The governing equations and the dynamical behavior of the circuit remain the same.

notoriously susceptible to oscillations and the same proves to be true in repressible systems.

Consider first an inducible system (in Yagil's terminology these are negatively inducible (Yagil, 1975)). In accordance with the Jacob-Monod model of control, the effector S_n reacts with the repressor R of an operon and thereby provides an alternate kinetic pathway for R :



Here $RS_n^{(p)}$ is a repressor-effector complex involving p effector molecules, O is the free operon concentration, and OR the concentration of operon-repressor complex³. When $p > 1$ the concentration of intermediate complexes is considered negligible and the total repressor concentration taken as $R_t = R + RS_n^{(p)}$. Evidently the total operon concentration O_t is $O_t = O + OR$, and with the assumption that the rates of enzyme production and degradation are proportional to the fraction of free operons and enzyme concentration, respectively, the quasi-steady enzyme concentration is also proportional to the fraction of free operons. Consequently

$$f(S_n) \propto \frac{O}{O_t} = \frac{1 + K_1 S_n^p}{(K_2 R_t + 1) + K_1 S_n^p}, \quad (3)$$

and the rate of the first reaction in the sequence at (1) is

$$k_o S_o f(S_n) = \frac{k_o S_o (1 + K_1 S_n^p)}{K + K_1 S_n^p}. \quad (4)$$

Here K_1 and K_2 are the equilibrium constants for the first and second steps of (2) and $K \equiv K_2 R_t + 1$. The qualitative features of this rate law for $p > 1$ are shown in Fig. 1 a.

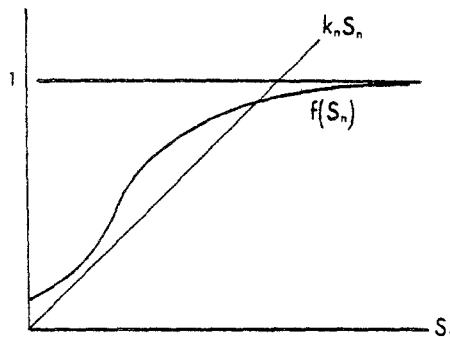


Fig. 1 a

³ In view of the small number of operons and repressor molecules, a stochastic model would be more appropriate here but of course would be more difficult to treat. The fraction of operons being transcribed in our deterministic model [cf. Equation 3] would be interpreted as the probability that any given operon was free to be transcribed.

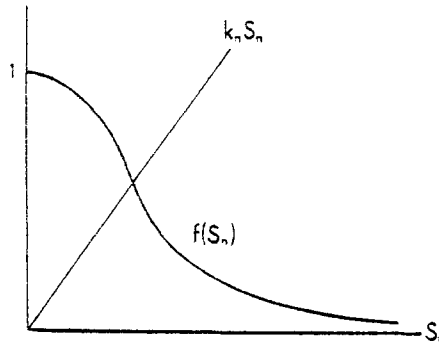


Fig. 1 b

Fig. 1. Qualitative features of the feedback term (a) Inducible systems, (b) Repressible systems

When gene transcription is repressed by high concentrations of the effector, the kinetic mechanism is



This leads to a rate expression of the form

$$k_o S_o f(S_n) = \frac{k_o S_o (1 + K_1 S_n^p)}{1 + (K_1 + K_1 K_2 R_t) S_n^p} \quad (6)$$

Since $K_1 S_n^p = R S_n^{(p)}/R$, whenever the fraction of repressor bound to operon is small $R S_n^{(p)}/R \ll 1$ and the rate law is approximately given by

$$k_o S_o f(S_n) \sim \frac{k_o S_o}{1 + K_1 K_2 R_t S_n^p} \quad (7)$$

This is the form of rate law used by Goodwin (1965) and others cited in the preceding section. Its basic features are shown in Fig. 1(b).

In either the inducible or repressible case, the differential equations governing the dynamic behavior of the control circuit are

$$\begin{aligned} \frac{dS_1}{dt} &= -k_1 S_1 + k_o S_o f(S_n), \\ \frac{dS_2}{dt} &= k_1 S_1 - k_2 S_2, \\ &\vdots \\ \frac{dS_n}{dt} &= k_{n-1} S_{n-1} - k_n S_n. \end{aligned} \quad (8)$$

and the nonlinear term is given either by (4) or (7). These equations can be simplified by introducing the dimensionless variables

$$\tau \equiv k_o t, \quad \bar{k}_j \equiv k_j/k_o, \quad \bar{S}_j \equiv S_j/S_o, \quad \bar{K}_1 = K_1 S_o^p,$$

$$\bar{f}(\bar{S}_n) = \begin{cases} \frac{1}{1 + \bar{K}_1 \bar{S}_n^p}, & \text{repressible case,} \\ \frac{1 + \bar{K}_1 S_n^p}{\bar{K} + \bar{K}_1 S_n^p}, & \text{inducible case,} \end{cases} \quad (9)$$

and the vectors and matrix

$$S = \begin{pmatrix} \bar{S}_1 \\ \vdots \\ \bar{S}_n \end{pmatrix}, \quad F(S) = \begin{pmatrix} \bar{f}(\bar{S}_n) \\ 0 \\ \vdots \\ 0 \end{pmatrix},$$

$$L = \begin{bmatrix} -\bar{k}_1 & 0 & \dots & 0 \\ \bar{k}_1 & -\bar{k}_2 & & 0 \\ 0 & & & \vdots \\ \vdots & & & \\ 0 & \dots & \bar{k}_{n-1} & -\bar{k}_n \end{bmatrix}, \quad (10)$$

for then (8) reads

$$\frac{dS}{d\tau} = LS + F(S). \quad (11)$$

Hereafter the overbars will be dropped.

The first step in the analysis of this equation is to establish some general properties of its solution(s). Because $F(S)$ is continuously differentiable for nonnegative S_n , the solution of (11) exists and is unique on some finite time interval $[0, T)$. Along any of the hyperplanes $S_i = 0$, $i = 1, \dots, n$, $dS_i/dt \geq 0$ and consequently any trajectory that begins in the nonnegative orthant remains there for all time. By computing the inner product of the vector field of (11) with the inward normals to the planes $S_i = \phi/k_i$, $\phi > 1$, one can show that the rectangular solid with vertices at $(0, 0, \dots, 0)$ and $\phi(1/k_1, \dots, 1/k_n)$ and with sides parallel to the coordinate axes is invariant under the flow of (11). Therefore the solution of (11) enters a compact subset of the nonnegative orthant and exists for all time.

In view of this fact, a qualitative picture of the dynamical behavior can be obtained by first determining the number of steady state solutions (critical points) of (11) and their stability properties and then deciding whether or not periodic solutions exist.

Steady states are nonnegative solutions S^* of the vector equation

$$S^* = -L^{-1}F(S^*) \quad (12)$$

or the equivalent scalar equations

$$k_1 S_1^* = k_2 S_2^* = \dots = k_n S_n^* = f(S_n^*). \quad (13)$$

From Fig. 1 it is clear that the steady state is always unique in repressible systems whereas there may be one, two or three steady states in an inducible

system. Near a critical point, the behavior of the solution of (11) is governed by the linear system in $\zeta \equiv S - S^*$,

$$\frac{d\zeta}{d\tau} = \left[L + \frac{\partial F}{\partial S} \Big|_{S=S^*} \right] \zeta \equiv \bar{L}\zeta, \quad (14)$$

whose solution is

$$\zeta = e^{\bar{L}t} \zeta(0). \quad (15)$$

By the spectral representation theorem (Kato, 1966)

$$e^{\bar{L}t} = \sum_{j=1} e^{\lambda_j t} \{P_j + \text{nilpotents}\} \quad (16)$$

where P_j is the projection onto the linear manifold spanned by the eigenvector(s) belonging to the eigenvalue λ_j . These eigenvalues are solutions of the characteristic equation

$$\det [\bar{L} - \lambda I] = 0, \quad (17)$$

which, when expanded, reads

$$\lambda^n + \left(\sum_{i=1}^n k_i \right) \lambda^{n-1} + \left(\sum_{i>j}^n k_i k_j \right) \lambda^{n-2} + \dots + \prod_{i=1}^{n-1} k_i (k_n - f'(S_n^*)) = 0. \quad (18)$$

The real parts of the λ 's determine stability and if some λ 's are complex, the solution is oscillatory but not in general periodic.

The following two sections deal with the questions of stability of critical points and the existence of periodic solutions to (11) when $n \geq 3$. These questions are easily answered for $n=2$ by use of the Poincare-Bendixson theorem and Bendixon's criterion. By virtue of the latter, there exists no periodic solutions confined to the first quadrant, because the divergence of the vector field is negative throughout that quadrant. The former theorem then implies that the only ω limit points are critical points. Because the steady state is unique in any repressible system, it is necessarily asymptotically stable and globally asymptotically stable⁴. The same is true in two-step inducible systems for which there is only one critical point. When three steady states exist in an inducible system, one can only say that the set of steady states is globally attracting in the sense that any trajectory beginning in the first quadrant approaches one of the steady states as $t \rightarrow +\infty$.

III. Inducible Systems

A. Multiplicity and Stability of Steady States

In an inducible system, a steady state is a nonnegative solution of the equations

$$k_1 S_1^* = k_2 S_2^* = \dots = k_n S_n^*, \quad (19)$$

⁴ Throughout, "global" always refers to the nonnegative orthant of concentration space. A mathematically precise definition of global asymptotic stability is given in the following section.

$$k_n S_n^* = \frac{1 + K_1 S_n^{*p}}{K + K_1 S_n^{*p}}, \quad (20)$$

and as previously noted, these have either one or three solutions. The $n+3$ parameters $(k_1, \dots, k_n, K, K_1, p)$ determine the steady state concentrations but of these, only $k_n, K, K_1,$ and p enter into S_n^* and the remaining $n-1$ fix S_1^*, \dots, S_{n-1}^* . On the boundary in parameter space that divides regions of one steady state from those of three steady states, the derivatives of the left and right sides of (20) must also be equal. Therefore this boundary is found by eliminating S_n^* from (20) and

$$k_n = \frac{p k_n (k_n K S_n^* - 1)}{1 + K_1 S_n^{*p}}. \quad (21)$$

Since these equations are independent of k_1, \dots, k_{n-1} , the boundary is a cylinder in n -dimensional space whose generators are orthogonal to the planes $k_n = \text{constant}$.

It is convenient to define

$$\mu = k_n S_n^*, \quad v = K_1 S_n^{*p} \quad (22)$$

for then (20), (21) can be written

$$\mu = \frac{1+v}{K+v}, \quad 1+v = p(K\mu - 1). \quad (23)$$

Elimination of μ leads to

$$v^2 + v[K(1-p) + (1+p)] + K = 0. \quad (24)$$

From this equation it follows that arbitrary values of p and K will generally not give nonnegative values of k_n, K_1 and S_n^* along the boundary. For these to be nonnegative it is necessary that

$$p^2 - 2p \left(\frac{K+1}{K-1} \right) + 1 \geq 0. \quad (25)$$

This inequality is satisfied for sufficiently large K and the minimum K that suffices for various integral p is given in the following table.

Table 1

p	1	2	3	4	5	6
K_{\min}	∞	9	4	2.78	2.25	1.96

If, for example, we assume a nuclear diameter of 2.5 microns and 10 repressor molecules/nucleus, then the measured value of 10^{13} moles⁻¹ for K_2 of the *lac* operon [Bourgeois and Monod, 1970] gives

$$K = K_2 R_i + 1 > \sim 10^5. \quad (26)$$

Therefore, whenever repressor is tightly bound to the operon one can expect to find values of k_n and K_1 that lead to three steady states, provided $p > 1$. For $p = 1$, no value of K will suffice.

When p and K are such that three solutions can exist, one finds from (22) and (24) that the boundary of the three solution region of the $K_1 - k_n$ plane is given by

$$(k_n)_{\pm} = \frac{1 + v_{\pm}}{v_{\pm}^{1/p} (K + v_{\pm})} K_1^{1/p}. \tag{27}$$

The region thus defined is shown in Fig. 2. Suppose that p , K and K_1 are held fixed. At small k_n only the upper steady state, in which the gene is fully induced, exists. In this steady state S_n is large, the enzyme concentration is close to the

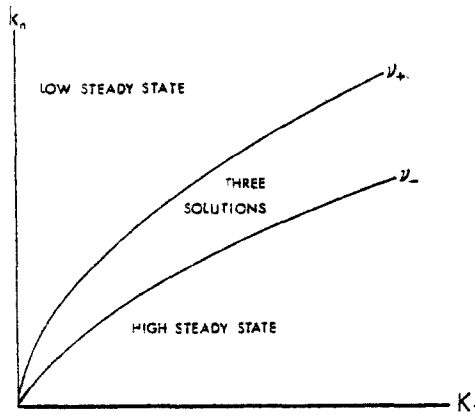


Fig. 2. Regions of one and three steady states in the $K_1 - k_n$ plane. Curves labelled v_{\pm} correspond to the two roots of Equation 24

maximum possible, and the dimensionless total throughput $k_n S_n$ is close to one. When K_1 is also small, k_n can only be increased slightly before two other steady states appear. With further increase of k_n the intermediate and upper steady states disappear and only the lower steady state in which S_n is small exists. If K_1 is large and k_n small, k_n can be increased substantially before two other steady states appear. Hence the larger K_1 is, the more the total throughput $k_n S_n$ is buffered against change in the enzyme concentration of the last step.

Asymptotic stability or instability of any steady state is governed by the roots of (18), written here as

$$P(\lambda) = P_o(\lambda) - f'(S_n^*) \prod_{i=1}^{n-1} k_i = 0 \tag{28}$$

where $P_o(\lambda) \equiv \det(\lambda I - L)$. Fig. 1 (a) shows that when three steady states exist, $f'(S_n^*) > k_n$ at the intermediate state and so $P(0) < 0$. Since every coefficient in $P_o(\lambda)$ is nonnegative, $P'(\lambda) > 0$ for all $\lambda > 0$ and there exists one and only one real positive solution to (28). Although this equation may also have complex conjugate roots with a positive real part, it is of little interest to determine when this occurs because the intermediate steady state is always unstable.

For any steady state other than the intermediate one when three exist, $f'(S_n^*) < k_n$ and one expects that these steady states are stable. This is shown in the following theorem.

Theorem 1: *When the steady state of an inducible system is unique, it is asymptotically stable. When three steady states exist, the upper and lower states are asymptotically stable and the intermediate state is unstable.*

Proof: A sufficient condition that a steady state be asymptotically stable is that the matrix \bar{L} of the linearized system have only eigenvalues with negative real parts. At any but the intermediate steady state, $f'(S_n^*) < k_n$ and $P(0) > 0$. Since $P'(\lambda) > 0$ for $\lambda > 0$, \bar{L} never has any real nonnegative eigenvalues. By Geršgorin's theorem (Minc and Marcus, 1964), the eigenvalues lie in the closed region of the complex λ plane consisting of the union of the disks

$$G_j \equiv \begin{cases} \{\lambda : |\lambda + k_j| \leq |k_j|\}, & j = 1, \dots, n-1 \\ \{\lambda : |\lambda + f'(S_n^*)| \leq |k_n|\} & j = n. \end{cases} \quad (29)$$

The first $n-1$ disks lie in the left-half plane and are tangent to the imaginary axis. By virtue of the preceding remarks, the n -th disk is wholly contained in the left-half plane. Therefore no eigenvalues of \bar{L} lie in the closed right-half plane when S^* is the upper or lower of three steady states or the single steady state.

B. Global Stability Results

With the preceding result on the local behavior of solutions, one can predict what happens when a system at a steady state is slightly perturbed. To complete the qualitative description of the solutions, information on the existence or non-existence of periodic or oscillating solutions is needed. When the steady state in an inducible system is unique, such solutions are precluded by the following theorem. Before stating it we need the following

Definition: *A steady state S^* of the autonomous system (11) is globally asymptotically stable if it is stable and $\lim_{t \rightarrow \infty} S(S_0, t) = S^*$ for all initial points S_0 in the positive orthant of concentration space.*

Theorem 2: *When the steady state in an inducible system is unique, it is globally asymptotically stable.*

To prove this, we first need a number of other results.

The standard technique for proving that a steady state is globally asymptotically stable is to show that there exists a scalar-valued function $V(S)$ (a Lyapunov function) that satisfies the following conditions (Brauer and Nohel, 1969)

- (i) $V(S) > 0$ for all $S_i > 0$,
 - (ii) V is non-increasing along the trajectories of (11),
 - (iii) $S = S^*$ is the only invariant subset of the set $\{S : \langle \nabla V, LS + F(S) \rangle = 0\}$,
 - (iv) $V(S) \rightarrow \infty$ as $\|S - S^*\| \rightarrow \infty$, $S_i > 0$.
- (30)

(Here $\langle \cdot, \cdot \rangle$ and $\|\cdot\|$ are the Euclidean inner product and norm on R_n .) Fortunately, the existence of $V(S)$ can be demonstrated once and for all for a large class of $F(S)$.

Problems similar to (11), in which there is only one nonlinear term and the nonlinearity depends only on a linear combination of the dependent variables, have been studied in control theory where they go under the name of Lure's problem (Lee and Markus, 1967). More precisely, Lure's problem concerns the system

$$\frac{dx}{dt} = Lx + b \cdot U(y) \quad (31)$$

where x , b and c are n -vectors, $y \equiv \langle c, x \rangle$, and U is a scalar valued function for which $U(0) = 0$ and $yU(y) \geq 0$. For such equations an appropriate choice for the Lyapunov function consists of a quadratic form plus an integral of the nonlinearity

$$V(x) = \langle x, Hx \rangle + \gamma \int_0^y U(y) dy. \quad (32)$$

Here H is a symmetric positive definite matrix and γ is a real scalar (Aizerman and Gantmacher, 1964). If γ and H can be chosen in such a way that the conditions on V are satisfied, the zero solution of (31) is globally asymptotically stable. It turns out that this can be done for a whole class of nonlinear functions $U(y)$. Before stating the general result we need the concept of absolute stability (Aizerman and Gantmacher, 1964).

Definition: Suppose that U is single-valued, that $U(0) = 0$, and that

$$0 \leq y U(y) \leq \Omega y^2. \quad (33)$$

For fixed $\Omega > 0$, the system (31) is absolutely stable in the sector $[0, \Omega]$ provided the origin is globally asymptotically stable for any $U(y)$ that satisfies (33).

Given that $U(y)$ satisfies (33), conditions on the linear part of (31) that are sufficient to guarantee absolute stability can be derived directly from (32). However, it proves more convenient to work in terms of the frequency response of the linear portion; the following theorem in essence expresses these necessary conditions in terms of the frequency response.

Theorem 3 [Popov's Theorem]: Suppose that L has only eigenvalues with negative real parts and that the numerator and denominator of

$$G(\lambda) \equiv \langle c, (\lambda - L)^{-1} b \rangle \quad (34)$$

have no roots in common. Then a sufficient condition that (31) be absolutely stable in the sector $[0, \Omega]$ is that there exist a real γ such that the complex function

$$T(\lambda) \equiv \frac{1}{\Omega} - (1 + \gamma \lambda) G(\lambda) \quad (35)$$

satisfies

$$\operatorname{Re} T(i\omega) > 0$$

for all real $\omega \geq 0$.

A proof of this theorem and generalizations of it can be found in (Aizerman and Gantmacher, 1964).

To use Popov's theorem to prove Theorem 2, we cast (11) into the form of (31). Define

$$\begin{aligned} x_i &= S_i - S_i^*, \\ U(x_n) &= -k_1 S_1^* + f(x_n + S_n^*), \\ b^T &= (1, 0, \dots, 0) \text{ and } c^T = (0, 0, \dots, 1). \end{aligned} \tag{36}$$

Now (11) takes the standard form

$$\frac{dx}{d\tau} = Lx + bU(y), \quad y = \langle c, x \rangle = x_n. \tag{37}$$

The first step is to verify that $G(\lambda)$ has no common roots in its numerator and denominator. Because L is lower triangular, its resolvent is also and has the form

$$(\lambda - L)^{-1} = \begin{bmatrix} 1/(k_1 + \lambda) & 0 & \dots & 0 \\ l_1 & 1/(k_2 + \lambda) & 0 & \dots & 0 \\ \vdots & m_1 & & & \\ \vdots & \vdots & & & \\ l_{n-1} & m_{n-2} & & & 1/(k_n + \lambda) \end{bmatrix}. \tag{38}$$

In view of the structure of b and c , only the $(n, 1)$ element of $(\lambda - L)^{-1}$ has to be computed and one finds that

$$G(\lambda) = \langle c, (\lambda - L)^{-1} b \rangle = l_{n-1} = \frac{1}{k_n} \prod_{i=1}^n \frac{k_i}{k_i + \lambda} \tag{39}$$

and

$$G(i\omega) = \frac{1}{k_n} \prod_{v=1}^n \frac{k_v}{\sqrt{k_v + \omega^2}} \cdot \exp\left(-i \sum_{j=1}^n \Theta_j\right) \tag{40}$$

where $\Theta_j \equiv \tan^{-1}(\omega/k_j)$.

According to the definition (35) of $T(\lambda)$,

$$T(i\omega) = \frac{1}{\Omega} - (1 + i\omega\gamma) G(i\omega) \tag{41}$$

and

$$\text{Re } T(i\omega) = \frac{1}{\Omega} - \text{Re } G(i\omega) + \omega\gamma \text{Im } G(i\omega).$$

Popov's theorem asserts that if there exists a real γ such that $\text{Re } T(i\omega) > 0$ for $\omega \geq 0$, then the origin in (37) is globally asymptotically stable. Geometrically this means that there must exist a γ such that the locus traced by the point $(\text{Re } G, \omega \text{Im } G)$ as ω varies lies to the left of the line

$$\omega \text{Im } G = \frac{\text{Re } G}{\gamma} - \frac{1}{\gamma\Omega}. \tag{42}$$

Clearly the modulus of G is monotone decreasing in ω and

$$\lim_{\omega \rightarrow \infty} G(i\omega) = 0. \tag{43}$$

Consequently the locus of $(\text{Re } G, \omega \text{Im } G)$ spirals inward toward zero and the maximum of $\text{Re } G$ for $\text{Im } G = 0$ occurs at $\omega = 0$. If Ω is such that $T(0) > 0$ and γ is chosen according to

$$\gamma^{-1} = \frac{\frac{d(\omega \text{Im } G)}{d\omega}}{\frac{d(\text{Re } G)}{d\omega}} \Big|_{\omega=0}, \tag{44}$$

(34) will be satisfied for all $\omega \geq 0$. Since

$$T(0) = \frac{1}{\Omega} - \text{Re } G(0) = \frac{1}{\Omega} - \frac{1}{k_n}, \tag{45}$$

$T(0) > 0$ provided $k_n > \Omega$. By definition Ω is the boundary of the sector within which U is completely contained and Fig. 1 (a) shows that $k_n > \Omega$ always holds when there is only one steady state. This proves that (11) is absolutely stable under this condition and hence that the steady state of (11) is globally asymptotically stable whenever it is unique.

This theorem shows that under the conditions given, the necessary condition for absolute stability is also sufficient. As a corollary, it follows that for $p = 1$ and any n , the unique steady state is globally asymptotically stable.

The preceding results give as complete a characterization of the dynamic behavior of an inducible system as is possible without specific restrictions on the kinetic and binding coefficients. In specific cases, it can happen that when three steady states exist the intermediate state has an $n - 1$ dimensional stable manifold that partitions the phase space in such a way that the set of critical points is globally attracting, in the sense used previously. In such cases, periodic solutions can be ruled out. It appears that this can only be decided on a case by case basis.

IV. Repressible Systems

A. Stability of the Steady State

The steady state in a repressible system is the unique solution of the system of equations

$$\begin{aligned} k_1 S_1^* &= k_2 S_2^* = \dots = k_n S_n^*, \\ k_n S_n^* &= \frac{1}{1 + K_1 S_n^{*p}} = f(S_n^*). \end{aligned} \tag{46}$$

Since $f'(S_n^*) < 0$, it follows from (18) or (28) that the characteristic equation for the linear system can never have a real positive root. Accordingly, the stability of a steady state can only change by virtue of a pair of complex conjugate eigenvalues crossing the imaginary axis. In the absence of the nonlinear feedback term $f(S_n)$, the linear system whose matrix is L has n real negative eigenvalues $\lambda_1 = -k_1, \dots, \lambda_n = -k_n$. With the addition of the feedback term, the resulting

matrix \bar{L} may have one or more pairs of complex conjugate eigenvalues. The characteristic equation for \bar{L} is

$$P(\lambda) = P_o(\lambda) - f'(S_n^*) \prod_{i=1}^{n-1} k_i = 0, \quad (47)$$

and consequently, as $f'(S_n^*)$ decreases, successive pairs of complex roots appear. At the point at which a real pair first becomes complex, the real part is strictly negative, but as $f'(S_n^*)$ decreases further the real part may become positive. However, at most one pair of complex roots has zero real part for fixed $f'(S_n^*)$ and any n , as the following proposition shows.

Proposition 1: Define $\rho = f'(S_n^*)$ and write $\lambda = \xi + i\omega$. For fixed k_1, \dots, k_n $P(\lambda) = 0$ has at most one pair of purely imaginary roots $\pm i\omega_o$ at any fixed ρ_o . Moreover, when $\lambda = \pm i\omega_o$, then $(d\xi/d\rho)_{\rho=\rho_o} \neq 0$.

Proof: $P_o(\lambda) = \prod_{j=1}^n (k_j + \lambda)$,

$$P_o(i\omega) = \prod_{j=1}^n \sqrt{k_j^2 + \omega^2} \left\{ \cos \sum_j \theta_j + i \sin \sum_j \theta_j \right\}, \quad (48)$$

where $\theta_j \equiv \tan^{-1}(\omega/k_j)$. The characteristic equation $P(\lambda) = 0$ has a pair of purely imaginary roots $\pm i\omega_o$ if and only if

$$\operatorname{Re} P_o(i\omega_o) = \rho \prod_{i=1}^{n-1} k_i, \quad \operatorname{Im} P_o(i\omega_o) = 0. \quad (49)$$

Since $P_o(0) > 0$ and the modulus of P_o increases with ω , there exists at most one ω_o such that $P(i\omega_o) = 0$. Furthermore,

$$P'(\lambda) = \frac{\partial(\operatorname{Im} P)}{\partial \omega} - i \frac{\partial(\operatorname{Re} P)}{\partial \omega} = \frac{\partial(\operatorname{Im} P_o)}{\partial \omega} - i \frac{\partial(\operatorname{Re} P_o)}{\partial \omega}, \quad (50)$$

and because the modulus of P_o increases, both partial derivatives cannot vanish simultaneously. Therefore the pair $\pm i\omega_o$ is simple. Finally,

$$\frac{d\lambda}{d\rho} = -\frac{\partial P/\partial \rho}{\partial P/\partial \lambda} = -\frac{\prod_{i=1}^{n-1} k_i}{|\partial P_o/\partial \lambda|^2} \left[\frac{\partial(\operatorname{Im} P_o)}{\partial \omega} + i \frac{\partial(\operatorname{Re} P_o)}{\partial \omega} \right], \quad (51)$$

and if $\partial(\operatorname{Im} P_o)/\partial \omega = 0$ when $\operatorname{Im} P_o(i\omega_o) = 0$, it would necessarily follow that $\operatorname{Re} P_o(i\omega_o) = 0$ as well. However, $P_o(\lambda) = 0$ has only real solutions. This proves the proposition.

The necessary and sufficient conditions for (47) to have a pair of pure imaginary roots are given by the well-known Routh-Hurwitz stability criterion. However, in view of the special structure of (11) it is advantageous to take a more geometric approach in deriving conditions for pure imaginary roots. As previously, define new variables

$$\begin{aligned}
 x_i &= S_i - S_i^*, \\
 U(x_n) &= k_1 S_1^* - f(S_n^* + x_n), \\
 b^T &= (-1, 0, \dots, 0) \quad \text{and} \quad c^T = (0, \dots, 0, 1),
 \end{aligned} \tag{52}$$

whereupon (11) reads

$$\frac{dx}{d\tau} = Lx + bU(y), \quad y = \langle c, x \rangle = x_n. \tag{53}$$

Clearly $x=0$ corresponds to the steady state, and if we linearize U about this steady state we get

$$\frac{dx}{d\tau} = (L + U'(0) b c^T) x. \tag{54}$$

A sufficient condition that $x=0$ be asymptotically stable is that the matrix

$$\bar{L} = L + U'(0) b c^T \tag{55}$$

has only eigenvalues with negative real parts. For this, it is necessary and sufficient that

$$P(\lambda) = \det[\lambda - (L + U'(0) b c^T)] \neq 0 \tag{56}$$

in the closed half-plane $\text{Re } \lambda \geq 0$. Because L is itself a stability matrix,

$$\det(\lambda - L) \neq 0 \quad \text{for} \quad \text{Re } \lambda \geq 0, \tag{57}$$

and

$$P(\lambda) = \det(\lambda - L) \det[I - U'(0)(\lambda - L)^{-1} b c^T]. \tag{58}$$

The matrix $(\lambda - L)^{-1} b c^T$ is of rank one or less; therefore

$$\begin{aligned}
 \det[I - U'(0)(\lambda - L)^{-1} b c^T] &= 1 - U'(0) \text{trace}\{(\lambda - L)^{-1} b c^T\} \\
 &= 1 - U'(0) \langle c, (\lambda - L)^{-1} b \rangle \\
 &= 1 - U'(0) G(\lambda).
 \end{aligned} \tag{59}$$

The condition (56) for asymptotic stability now takes the form

$$1 - U'(0) G(\lambda) \neq 0 \tag{60}$$

for $\text{Re } \lambda \geq 0$. Clearly $G(\lambda)$ has no poles in the right-half-plane and the principle of the argument shows that (60) is equivalent to

$$1 - U'(0) \text{Re } G(i\omega) > 0 \tag{61}$$

whenever $\text{Im } G(i\omega) = 0$ for $\omega \geq 0$. Evidently this result is just a special case of Popov's theorem; that it is also equivalent to the Routh-Hurwitz criterion follows from (56).

Equation (61) requires that

$$U'(0) < \frac{1}{\text{Re } G(i\omega)} \tag{62}$$

for stability and thereby gives the critical condition for marginal oscillatory stability directly. If we define

$$\Omega_c^{-1} = \max_{\substack{\omega \in [0, \infty) \\ \text{Im } G = 0}} \text{Re } G(i\omega), \quad (63)$$

then marginal oscillatory stability occurs when

$$U'(0) = \Omega_c. \quad (64)$$

At $\omega=0$, $G(0) = -1/k_p$ and because $|G|$ decreases as ω increases, the maximum of $\text{Re } G$ occurs at the first positive ω at which $\text{Im } G=0$. At this ω , $\sum_j \theta_j = \pi$ and

$$\text{Re } G = \frac{1}{k_n} \prod_{i=1}^n \frac{k_i}{\sqrt{k_i^2 + \omega_0^2}} = \Omega_c^{-1}, \quad (65)$$

where ω_0 is the solution of

$$\tan^{-1}(\omega/k_1) + \dots + \tan^{-1}(\omega/k_n) = \pi. \quad (66)$$

Ω_c depends only on the parameters k_1, \dots, k_n of the linear system while $U'(0)$ depends only on k_p, K_1 , and p . Therefore one can fix k_1, \dots, k_n and find the stability boundary by varying K_1 and p . Even this becomes unwieldy unless n is small so we shall first restrict the number of parameters we allow to vary.

By direct computation, one finds from (65) and (66) that

$$\frac{\partial \ln \Omega_c}{\partial k_j} = k_n \omega_0^2 \sum_{q=1}^n \frac{k_j^3 - k_q^3}{k_q^2 k_j^3 (k_q^2 + \omega_0^2)} \cdot \left[\sum_{q=1}^n \frac{k_q}{k_q^2 + \omega_0^2} \right]^{-1}, \quad j=1, \dots, n-1. \quad (67)$$

Certainly $\partial \ln \Omega_c / \partial k_j = 0$ $j=1, \dots, n-1$, if $k_1 = k_2 = \dots = k_n$. If k_p is the largest and k_q the smallest of k_1, \dots, k_{n-1} , one finds that

$$\frac{\partial \Omega_c}{\partial k_p} > 0, \quad \frac{\partial \Omega_c}{\partial k_q} < 0. \quad (68)$$

If k_p and k_q are fixed, Ω_c is largest when the remaining k 's are equally spaced between k_q and k_p . Conversely, if the average k is fixed, the variational problem

$$\min \Omega_c(k_1, \dots, k_n), \quad k_j > 0, \quad \frac{k_1 + \dots + k_{n-1}}{n-1} = k, \quad (69)$$

has the solution $k_1 = k_2 = \dots = k_{n-1} = k$. Since a minimum Ω_c for fixed k corresponds to the most easily destabilized system, we shall hereafter consider only this case and with only a slight loss of generality we set $k=1$. Only p, K_1 , and k_n will be treated as variable parameters and $k_n > 0, K_1 \geq 0, p \geq 0$.

With these restrictions on k_1, \dots, k_{n-1} , (65) and (66) reduce to

$$\Omega_c = (1 + \omega_0^2)^{(n-1)/2} \sqrt{k_n^2 + \omega_0^2}, \quad (70)$$

$$(n-1) \tan^{-1} \omega + \tan^{-1}(\omega/k_n) = \pi.$$

From these one can show that

$$\frac{\partial \Omega_c}{\partial k_n} > 0, \quad \frac{\partial^2 \Omega_c}{\partial k_n^2} > 0$$

and

$$\lim_{k_n \rightarrow 0} \Omega_c = \sec^{n-1} \left(\frac{\pi}{2(n-1)} \right) \tan \left(\frac{\pi}{2(n-1)} \right) > 0. \tag{71}$$

From (46) and (52) it follows that

$$U'(0) = -f'(S_n^*) = p k_n [1 - k_n S_n^*],$$

$$\lim_{k_n \rightarrow 0} -f'(S_n^*) = 0, \quad \lim_{k_n \rightarrow \infty} -f'(S_n^*) = 0,$$

and that

$$\max_{k_n \in (0, \infty)} [-f'(S_n^*)] = \frac{(p+1)^2}{4p} \left(\frac{p-1}{p+1} \right)^{\frac{p-1}{p}} K_1^{1/p}. \tag{72}$$

Finally, at fixed k_p , k_n , S_n^* decreases as K_1 increases and consequently

$$\frac{\partial}{\partial K_1} (-f'(S_n^*)) > 0.$$

With these facts, one can qualitatively sketch both $U'(0)$ and Ω_c as functions of k_n and K_1 . The result is shown in Fig. 3 and the conclusions that follow from the figure are summarized in Proposition 2.

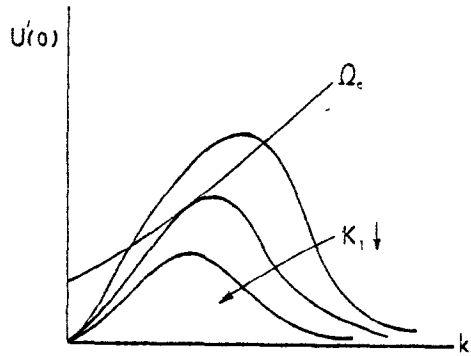


Fig. 3. Slope of $U(y)$ at the steady state and the critical Ω vs. k_n

Proposition 2: *When $k_1 = k_2 = \dots = k_{n-1}$ in a repressible system, the steady state is asymptotically stable for all $K_1 > 0$ if k_n is sufficiently small or sufficiently large, and for all $k_n > 0$ if K_1 is sufficiently small. At any fixed K_1 there is at most one interval $(k_n^L, k_n^U) \subset [0, \infty)$ such that the steady state is unstable for $k_n \in (k_n^L, k_n^U)$.*

The stability boundary in the k_n, K_1 plane is the locus given by the equations

$$\Omega_c(k_n) = U'(0), \quad k_n S_n^* = f(S_n^*). \tag{73}$$

In view of (46), these can be written

$$\Omega_c(k_n) = p k_n (1 - k_n S_n^*), \quad k_n K_1 S_n^{*p+1} + k_n S_n^* - 1 = 0. \tag{74}$$

These equations do not necessarily have a real solution for every pair (p, n) , for p must satisfy

$$p = \frac{\Omega_c(k_n)}{k_n(1 - k_n S_n^*)} > \frac{\Omega_c(k_n)}{k_n}, \quad (75)$$

by virtue of the fact that $1 - k_n S_n^* < 1$ for all $k_n > 0$. If the right-most term of (75) is minimized with respect to k_n , one finds that $k_n = 1$ and therefore

$$\inf_{(k_n, K_1) > (0, 0)} [p] = \Omega_c(1) = \sec^n \left(\frac{\pi}{n} \right). \quad (76)$$

This remarkably simple result shows that for any fixed n , there is a minimum number of effector molecules that must bind with repressor in order that the n -step circuit can have an unstable steady state. The p 's that correspond to various n are given in the following Table⁵.

Table 2

n	2	3	4	5	6	7	8
$\inf p$	∞	8	4	2.88	2.48	2.06	1.94

Viewed another way, the results in the table show that if four effector molecules bind to repressor, there must be at least five steps in the circuit to produce an unstable steady state. Similarly, if only two effector molecules bind to repressor at least *eight* steps are required. This proves that the steady state in the four-step problem studied by Morales and McKay (1967) is stable. A specific example for $p=4$ will be considered shortly, but first we derive some results on global asymptotic stability.

B. Global Stability Results

In the preceding section it was possible to prove that a unique steady state is always globally asymptotically stable because the nonlinearity is confined to the sector $[0, k_n]$ and this is sufficient for absolute stability. This is no longer the case in repressible systems; when the steady state is asymptotically stable in some subset of the $k_n - K_1$ plane Popov's theorem implies global stability only in a smaller subset. The following propositions give the precise results.

Proposition 3: *Suppose that for fixed p, n and K_1^* the steady state is asymptotically stable for all $k_n > 0$. Then there exists a $\bar{K}_1 \in (0, K_1^*]$ such that the steady state is globally asymptotically stable for all $K_1 \leq \bar{K}_1$.*

⁵ The results given are independent of the prior choice $k_1 = k_2 = \dots = k_{n-1}$. Indeed, if $\Omega_c / \prod_{i=1}^n k_i$ is minimized directly, one finds by symmetry that $k_1 = k_2 = \dots = k_n = k$. By scaling time one can choose $k = 1$.

Proof: From Proposition 2 it follows that the steady state is asymptotically stable for all $K_1 \leq K_1^*$. Furthermore, it is clear from Fig. 2 that if

$$\max_{k_n \in (0, \infty)} [-f'(S_n^*)] \leq \Omega_c(0) \tag{77}$$

for fixed K_1 , then the nonlinearity is confined to $[0, \Omega_c(0)]$ and (53) is absolutely stable. From (72) one has

$$\max_{k_n \in (0, \infty)} [-f'(S_n^*)] = \frac{(p+1)^2}{4p} \left(\frac{p-1}{p+1}\right)^{\frac{p-1}{p}} K_1^{1/p} \tag{78}$$

and from (71)

$$\Omega_c(0) = \sec^{n-1} \left(\frac{\pi}{2(n-1)} \right) \tan \left(\frac{\pi}{2(n-1)} \right). \tag{79}$$

Therefore, \tilde{K}_1 given by

$$\tilde{K}_1 = \left[\frac{4p}{(p+1)^2} \left(\frac{p+1}{p-1}\right)^{\frac{p-1}{p}} \sec^{n-1} \left(\frac{\pi}{2(n-1)} \right) \tan \left(\frac{\pi}{2(n-1)} \right) \right]^p \tag{80}$$

will suffice. Evidently $\tilde{K}_1 \leq K_1^*$.

If the inequality at (77) is satisfied the steady state is certainly asymptotically stable for all $K_1 \leq \tilde{K}_1$ and $k_n > 0$. Consequently, to apply the result one only has to compute \tilde{K}_1 from (80). We have done this for $p=4$ and several values of n ; the results are given in Table 3.

Table 3

n	2	3	4	5	6
\tilde{K}_1	∞	30.5	1.17	0.137	0.055
$(K_1^*)_{\max}$	∞	∞	∞	94.7	32.1

For comparison, the largest K_1^* for which the steady state is asymptotically stable for all k_n is also shown. The values of $(K_1^*)_{\max}$ for $n=5$ and $n=6$ were computed from (73). It is clear from Fig. 2 that (77) is a very stringent condition and one can expect that global asymptotic stability holds over a wider range of K_1 . The following proposition widens the range somewhat. However, the important conclusion from Proposition 3 is that the steady state is globally asymptotically stable for all k_n at sufficiently small K_1 .

Proposition 4: *Suppose that for fixed p, n and K_1^* the steady state is asymptotically stable for all $k_n > 0$. Then there exists a k_n^* such that the steady state is globally asymptotically stable for $k_n > k_n^*$.*

Proof: Let \hat{k}_n be the k_n at which $-f'(S_n^*)$ attains its maximum. Because the steady state is asymptotically stable, $\Omega_c(\hat{k}_n) > [-f'(S_n^*)]_{\max}$ and since Ω_c is increasing in k_n , $\Omega_c(k_n) > \Omega_c(\hat{k}_n) \forall k_n > \hat{k}_n$. The nonlinearity is certainly contained in the sector $[0, [-f'(S_n^*)]_{\max}]$ and by the preceding, this sector is contained in

the sector $[0, \Omega_c]$ for $k_n > \hat{k}_n$. Therefore the steady state is globally asymptotically stable for $k_n > \hat{k}_n$. Consequently any $k_n^* \geq \hat{k}_n$ suffices. The minimum k_n^* for any fixed K_1 is the solution of

$$\Omega_c(k_n^*) = \max[-f'(S_n^*)] = \frac{(p+1)^2}{4p} \left(\frac{p-1}{p+1}\right)^{\frac{p-1}{p}} K_1^{1/p}. \quad (81)$$

It is always the case that $(k_n^*)_{\min} \leq \hat{k}_n$.

Even if the steady state is not asymptotically stable for all k_n , it always is for sufficiently large k_n ; the following shows that the same is true of global stability.

Proposition 5: *Suppose that for p, n and K_1 fixed, the steady state is linearly unstable for $k_n \in (k_n^+, k_n^-)$. Then there exists a $\bar{k}_n > k_n^+$ such that the steady state is globally asymptotically stable for $k_n \geq \bar{k}_n$.*

The proof of this is similar to that of the preceding proposition.

The region of the k_n, K_1 plane defined by the conclusions of the three propositions is shown in Fig. 4 for a case in which the steady state is linearly unstable for some k_n and K_1 . More precise results on the region of global asymptotic stability can be derived for specific choices of p and n . One example will be studied shortly.

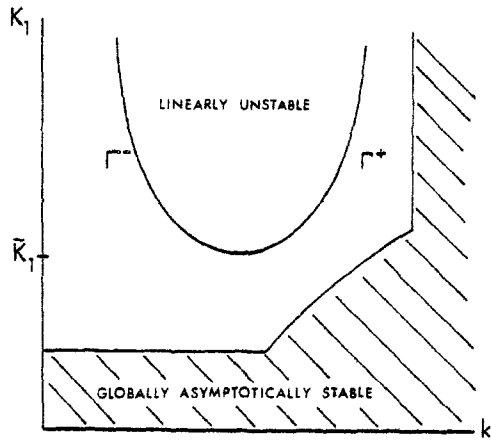


Fig. 4. Regions of linear instability and global asymptotic stability in the $k_n - K_1$ plane

C. Bifurcation of Periodic Solutions

To complete the qualitative analysis of the general repressible system, we have to determine the behavior of solutions when k_n and K_1 fall into the linearly unstable region. Suppose that K_1 is fixed at some value greater than \bar{K}_1 (Fig. 4). As k_n crosses the left boundary Γ^- of the unstable region, the real part of a

pair of complex conjugate eigenvalues changes from negative to positive. By an argument identical to that used in Proposition 1, one can show that $d\zeta/dk_n \neq 0$ along Γ^- . Consequently, the Hopf bifurcation theorem [Ruelle and Takens, 1971] implies that a unique periodic solution bifurcates at zero amplitude as Γ^- is crossed. The periodic solution falls under one of three possibilities: (i) it exists only for $k_n < k_n^L$, (ii) it exists only for $k_n = k_n^L$ or, (iii) it exists only for $k_n > k_n^L$. At $k_n = k_n^L$ the period is that of the linear system, namely, $2\pi/\omega_0$. A similar situation obtains along Γ^+ : a unique solution emerges or disappears as k_n crosses k_n^U and this solution falls under one of the above possibilities.

The Hopf theorem assures existence of the periodic solution, but more must be done to determine the direction of bifurcation and the stability of the bifurcating solution. Disregarding the case where the solution exists only at $k_n = k_n^L$ or at $k_n = k_n^U$, there are two possibilities along either Γ^- or Γ^+ . Along Γ^- , either an unstable periodic solution bifurcates to the left ($k_n < k_n^L$) or an orbitally stable periodic solution bifurcates to the right ($k_n > k_n^L$). Similarly, along Γ^+ either a stable solution bifurcates to the left ($k_n < k_n^U$) or an unstable solution bifurcates to the right ($k_n > k_n^U$). Which of these two possibilities obtain along Γ^\pm can be decided by a lengthy calculation, for which a general procedure is available

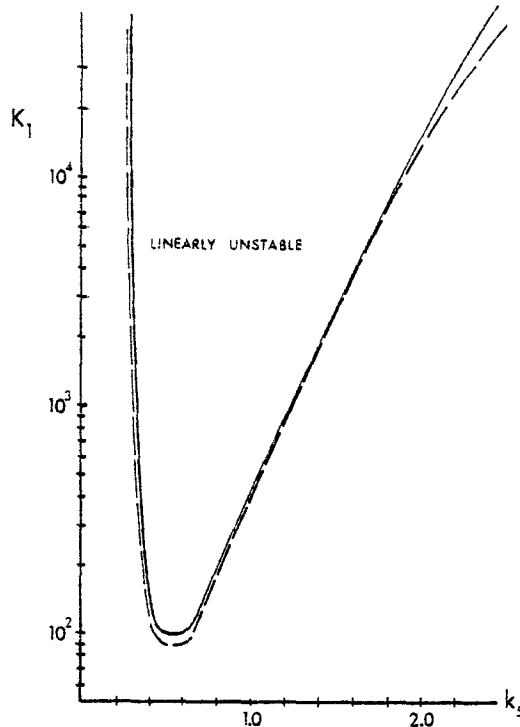


Fig. 5. Regions of linear instability and global asymptotic stability in the $k_5 - K_1$ plane. Solid curve: linear stability boundary. Broken curve: global stability boundary

[Hsü and Kazarinoff, 1975]. However, we shall not pursue this for the general system, for the effort required is currently unwarranted. Some computational results given in the sequel suggest what may happen in general.

D. Stability and Periodic Solutions for $p=4, n=5$

Consider a repressible system which has five steps in the control sequence and for which four molecules of S_5 bind to each repressor molecule. The boundary of the linearly unstable region of the k_5, K_1 plane is given by

$$\Omega_c(k_5) = 4k_5(1 - k_5 S_5^*), \quad k_5 K_1 S_5^{*5} + k_5 S_5^* - 1 = 0. \quad (82)$$

These equations have been solved numerically and the boundary is shown as a solid line in Fig. 5. Above this curve the steady state is linearly unstable and elsewhere it is asymptotically stable.

The region of the plane in which Popov's theorem implies that the steady state is globally asymptotically stable is found as follows. The nonlinearity always lies in the sector $[0, \chi]$ where χ is the maximum chord slope:

$$\chi \equiv \max_{x_5} \left[\frac{f(S_5^*) - f(x_5 + S_5^*)}{x_5} \right]. \quad (83)$$

If $\chi < \Omega_c$ for fixed k_5 and K_1 , the steady state is globally asymptotically stable. The boundary of the desired region can therefore be obtained by solving

$$\Omega_c x_5 = f(S_5^*) - f(x_5 + S_5^*), \quad \Omega_c = -f'(x_5 + S_5^*) \quad (84)$$

in conjunction with the equations

$$\Omega_c = (1 + \omega_0^2)^{\frac{n-1}{2}} \sqrt{k_5^2 + \omega_0^2}, \quad (85)$$

$$k_5 S_5^* = \frac{1}{1 + K_1 S_5^{*4}},$$

$$4 \tan^{-1} \omega_0 + \tan^{-1} (\omega_0/k_5) = \pi.$$

The last equation can be solved explicitly to give

$$\omega_0^2 = 2k_5 + 3 - 2\sqrt{k_5^2 + 2k_5 + 2} \quad (86)$$

and so Ω_c can be found explicitly for any k_5 . The remaining three equations contain the four variables x_5, S_5^*, K_1 and k_5 and one can expect that they generally define a curve in the k_5, K_1 plane. One finds that

$$x_5 = \frac{8k_5 S_5^* - 5 \pm \sqrt{25 - 16 S_5^* (K_1 + k_5)}}{8K_1}, \quad (87)$$

$$K_1 = \frac{1 - k_5 S_5^*}{k_5 S_5^{*5}},$$

and

$$k_5(1 - k_5 S_5^* + K_1 x_5) S_5^{*5} + (k_5 S_5^* - 1)(k_5 S_5^* - K_1 x_5)(x_5 + S_5^*)^4 = 0.$$

This system of equations defines the boundary of the region of global asymptotic stability. These were solved numerically and the results are shown as the broken curve in Fig. 5. Except at large k_n and K_1 , the broken curve follows closely the solid curve that defines the limit of linear stability. Consequently, but for the small region between these curves, an asymptotically stable system is also globally asymptotically stable. It seems doubtful that this conclusion would change significantly for larger n although the two curves may not follow quite as closely.

The Hopf theorem predicts that periodic solutions bifurcate upon crossing Γ^\pm and the preceding results show that if bifurcation is to the left along Γ^- , the unstable solution can at most exist in a very small interval $[k_n, k_n^L]$. A similar statement holds for unstable solutions that may bifurcate to the right at Γ^+ . Although we cannot rule out the possibility of unstable bifurcating solutions, they seem unlikely in view of the following computations.

Equations (53) were integrated numerically at numerous points across the linearly unstable region. Fig. 6(a) shows the amplitude and Fig. 6(b) the period of the

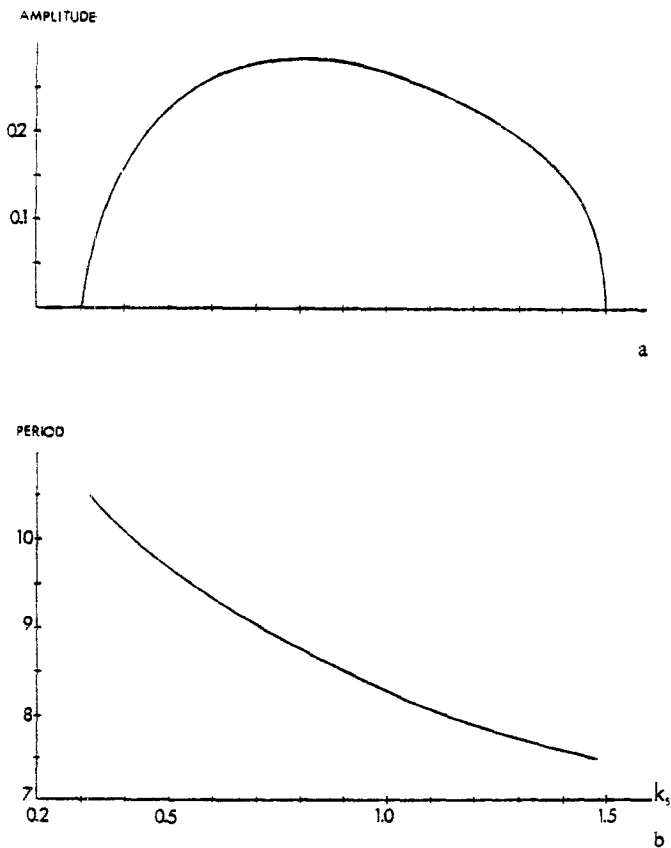


Fig. 6. (a) Amplitude vs. k_s and (b) Period vs. k_s of the periodic solution for the five-step circuit with $p=4$, $K_1=2883$

periodic solution as a function of k_5 for fixed K_1 . The quantity shown is the root mean square amplitude $(\sum (\Delta S_i)^2)^{1/2}$. As closely as can be determined numerically, the stable solution bifurcates to the right at k_5^L . As k_5 increases the amplitude first increases, goes through a maximum, and then decreases. At the right-hand boundary of the linearly unstable region the periodic solution bifurcates to the left. The period decreases monotonically as k_5 increases from k_5^L to k_5^U .

In Fig. 7 we show the amplitude as a function of K_1 for fixed k_5 . The solutions have the interesting property that the period is essentially insensitive to changes in K_1 ; all points shown in the figure have a period within $\pm 5\%$ of $T=8.75$. Furthermore, the amplitude rises sharply at first but levels off quickly and thereafter is essentially constant. Therefore when the affinity of repressor for effector is sufficiently large, both the period and amplitude are essentially constant.

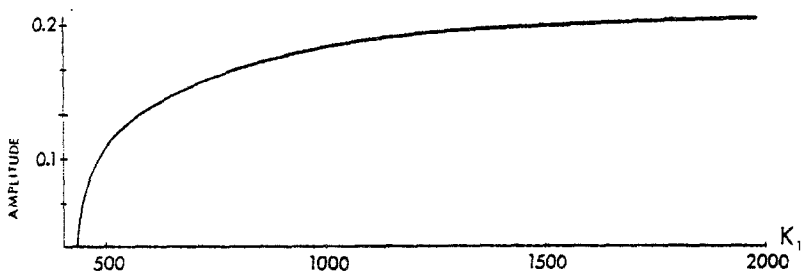


Fig. 7. Amplitude vs. K_1 for the same system as in Fig. 6 but with $k_5 = 1.0$

Discussion

The major question that motivated this investigation was whether simple "straight-through" molecular circuits for the feedback control of gene transcription are capable of sustained autonomous oscillations. If the control circuit functions by positive feedback, and if there is only one steady state, one can categorically rule out sustained oscillations: the steady state is always globally asymptotically stable for any choice of kinetic constants in the first $n-1$ steps. Consequently, such circuits are very strongly buffered against perturbations in the enzyme or substrate concentrations: for any concentration fluctuations the system returns to the steady state. When three steady states exist, one can make no general statement about the absence of oscillations; a case-by-case analysis is needed. What is interesting when there are three steady states is the possibility of switching between the two stable states by, for example, changing the enzyme concentration for the final step in the sequence. If the concentration of this last enzyme is under control of another circuit, its concentration can be raised or lowered and the net production of some key protein for differentiation can be switched on or off.

Such switching between steady states is impossible in circuits that use negative feedback, but under appropriate conditions these circuits can oscillate. One

interesting result is that a very simple relation exists between the number of effector molecules bound to repressor and the minimum length of the circuit needed to produce an unstable steady state. If p is fixed, and n is below the required minimum, the steady state is asymptotically stable for *any* choice of kinetic and binding constants. If p is above this minimum, it is still the case that the steady state is asymptotically stable if k_n is small enough or large enough. Thus either long-lived (small k_n) or short-lived (large k_n) end products lead to a stable steady state. Asymptotic stability does not preclude large amplitude oscillations *a priori*, but we could show that when $k_1 = \dots = k_{n-1}$ and either K_1 is small or k_n is sufficiently large, the steady state is also globally asymptotically stable.

The computational results given in the preceding section show that Popov's theorem can give a very good estimate of the region of global asymptotic stability. While results were only given for the case $k_1 = k_2 = \dots = k_{n-1}$, the technique is certainly applicable in general. One only has to solve a system comparable to (84) and (85) over the range of the variable k_j 's. Since all k_j 's for the intermediate steps enter both the characteristic equation and Equations (85) and (86) symmetrically, only a restricted region of parameter space needs to be explored.

All the preceding analysis is based on the assumption that all enzymes and substrates are uniformly distributed in space. In reality cellular enzymes are often immobilized on various organelles and any intermediate product in the control circuit must diffuse to the succeeding enzyme. If intermediates are small and the distances short (as they are within a single cell), diffusion will be sufficiently rapid to maintain spatial uniformity. However, for larger molecules the time delay due to transport may be significant; this could be another source of oscillations. A simplified model wherein enzymes are spatially localized has been studied by Glass and Kauffman (1972), who found that in a two-enzyme system spatial separation of enzymes could indeed produce oscillations where none would occur in a uniform system. A very careful analysis by Aronson (1975), of a somewhat similar system of two immobilized enzymes leads to a similar conclusion: when the product of enzyme *A* inhibits enzyme *B* but *B*'s product activates the enzyme for *A*, time-periodic solutions exist for appropriate values of the kinetic parameters. The results are not quite directly applicable to the case at hand, because once the possibility of spatial non-uniformity is allowed, transport mechanisms other than diffusion must also be considered. The most important one is probably transport across the nuclear membrane.

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