

The Dynamics of Feedback Control Circuits in Biochemical Pathways

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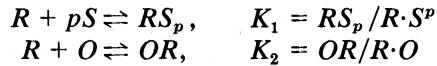
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I. Introduction

A. REGULATION OF ENZYME SYNTHESIS

Bacteria are able to use any of a variety of substances as their sole source of carbon because they can synthesize the enzymes needed to catabolize these substances. Usually these enzymes are produced only in the presence of their substrate or one of its analogues, and for this reason they are said to be inducible. In contrast, the enzymes in the biosynthetic pathways leading to essential metabolites such as amino and nucleic acids are synthesized in the cell when there is *no* external source of the metabolite. When the metabolite is available in the external growth medium, synthesis of these anabolic enzymes is repressed.

The operon model of Jacob and Monod (1961) provides the most commonly used framework for the analysis of enzyme induction and repression. In this model, each structural gene that codes for an enzyme or protein is linked with an operator gene that serves to regulate initiation of transcription. When a repressor molecule is bound to the operator gene, transcription is blocked. The binding of a repressor molecule to the operator is in turn modulated by a so-called effector molecule. In the case of inducible enzymes, the repressor is bound to the operator in the absence of effector, and transcription is blocked. When the substrate for such an enzyme is present, an effector molecule (usually the substrate, an analogue of it, or a product of it) can bind with repressor and thereby preclude binding of the latter to the operator. This permits transcription of the structural gene. The function of the effector is to provide an alternate kinetic pathway for repressor; this process can be modeled as a pair of competing reactions (Yagil and Yagil, 1971):



Here R = repressor, O = operator, and S = effector. Here and hereafter we shall use the same symbol for a chemical species and its concentration.

We assume that these reactions occur quickly and are therefore always in equilibrium. Furthermore, we assume that the binding of

effector to repressor is all-or-none; that is, we neglect the concentrations of the intermediate complexes RS , RS_2 , \dots , RS_{p-1} . Finally, we assume that the number of operator regions is small compared with the total number of repressor molecules. Under these assumptions the total repressor concentration is

$$R_t = R + RS_p = R(1 + K_1 S^p)$$

and the total operator concentration is

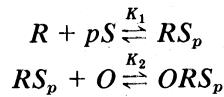
$$O_t = O + OR = O(1 + K_2 R)$$

The fraction of operator regions free of repressor is then

$$f(S) = \frac{O}{O_t} = \frac{1 + K_1 S^p}{K + K_1 S^p} \quad (1)$$

where $K = 1 + K_2 R_t > 1$. Notice that $f(S)$ is a monotone increasing function of S (Fig. 1a).

For repressible enzymes, the effector molecule permits or enhances the binding of repressor to the operator. This process can be described by the reactions



In this case

$$f(S) = \frac{1 + K_1 S^p}{1 + K_1 K S^p} \quad (2)$$

which is monotone decreasing (Fig. 1b).

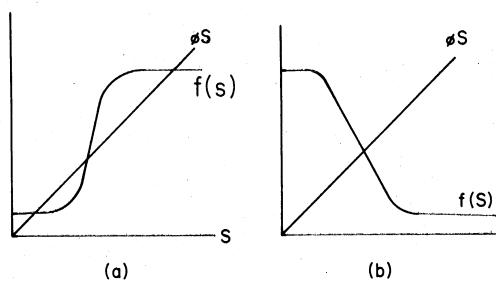


FIG. 1. The fraction $f(S)$ of operator regions that is free of repressor as a function of effector concentration S for (a) an inducible enzyme and (b) a repressible enzyme.

If one assumes that the rate of mRNA synthesis is directly proportional to the fraction of operator regions that are not blocked, the parameters p , K_1 , and $K_2 R_t$ can be determined from rate measurements. Yagil and Yagil (1971) have reviewed data on a number of regulatory gene systems; some of their results are collected in Table I.

B. REGULATION OF ENZYME ACTIVITY

Repression of enzyme synthesis by metabolite provides gross control over the pathway leading to this metabolite. When the metabolite is plentiful, the whole pathway shuts down on a time scale comparable to the half-life of the most labile enzyme in the sequence. Much finer and faster control is achieved by modulation of enzyme activity. For example, Umbarger (1956) found that, if isoleucine is added to the growth medium of bacterial cells, the biosynthesis of isoleucine is immediately and fully quenched. The first enzyme in the sequence unique to the synthesis of isoleucine, threonine dehydrogenase, is very sensitive to inhibition by the end product (see Fig. 2a). Often such feedback loops are coupled together (Fig. 2b). Indeed, the control of interconnected anabolic and catabolic pathways can be very complicated and may differ widely from one organism to another. Stadtman (1970) has provided an excellent review of a variety of mechanisms of enzyme regulation. Here we limit ourselves to the simplest feedback loops, with the expectation that experience gained in analyzing simple systems can be used to advantage in the analysis of more complicated control

TABLE I
QUANTITATIVE CHARACTERIZATION OF SOME GENE CONTROL SYSTEMS^a

Enzyme	Effector	p	K_1	$K_2 R_t$
Inducible				
β -Galactosidase	Isopropylthio-galactoside	1.91	$2.5 \times 10^{10} M^{-2}$	2.5×10^3
Histidine-NH ₃ -lyase	Imidazole propionate	2.04	$1.7 \times 10^{10} M^{-2}$	26
Urocanase	Histidine	2.3	$4.3 \times 10^{12} M^{-2}$	10^2
Mannitol dehydrogenase	Ribitol	3.13	—	—
Repressible				
IMP dehydrogenase	Guanine	0.91	—	—
XMP aminase	Guanine	0.68	—	—
Alkaline phosphatase	PO ₄ ³⁻	0.93	$2 \times 10^3 M^{-1}$	5×10^3

^a From Yagil and Yagil (1971).

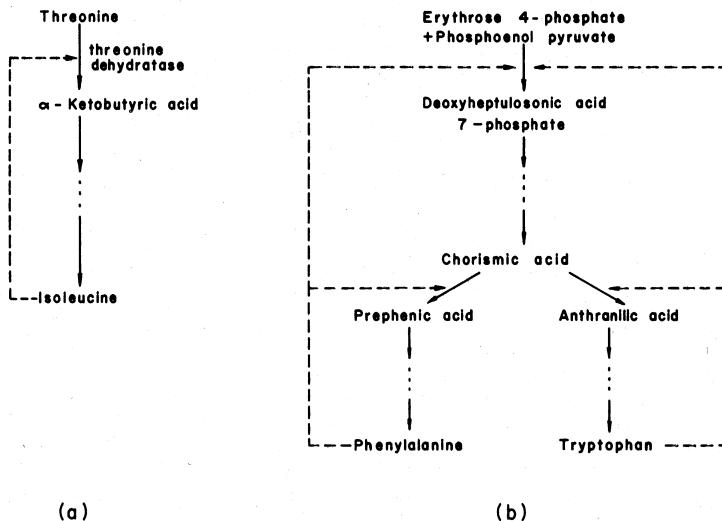


FIG. 2. Feedback inhibition in amino acid biosynthesis. (a) Single negative feedback loop. (b) Coupled negative feedback loops.

systems. Table II contains a short list of biosynthetic pathways controlled by feedback inhibition.

Feedback activation in metabolic pathways is not so common. One example is ADP activation of phosphofructokinase in the glycolytic pathway (Mansour, 1963; Atkinson, 1965), but this falls outside the scope of models we discuss because ADP is common to so many pathways. An example more amenable to analysis occurs in the dark reactions of photosynthesis (Buchanan and Schürmann, 1972): ribulose 1,5-diphosphate carboxylase, which catalyzes the initial CO_2 incorporation reaction, is activated by fructose 6-phosphate, one of the intermediates on the way to glucose (Fig. 3).

Enzymes whose activity is subject to modulation contain binding sites for substrates, activators, and inhibitors. Several models have been suggested for the operation of such "allosteric" enzymes (Monod *et al.*, 1965; Koshland, 1970). For a fixed concentration of activator or inhibitor, the rate of a reaction involving such an enzyme is usually related to the substrate concentration S by a Hill function:

$$V(S) = \frac{V_{\max} S^{n_H}}{K_{0.5}^{n_H} + S^{n_H}} \quad (3)$$

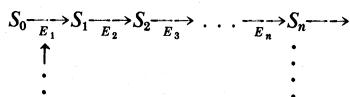
All three parameters (V_{\max} , $K_{0.5}$, and n_H) will depend on the concentra-

TABLE II
FEEDBACK INHIBITION IN BIOSYNTHETIC PATHWAYS^{a,b}

S_n	E_1	n	Mode
Valine	Acetolactate synthase	4	K
Leucine	α -Isopropylmalate synthase	4	
Isoleucine (+)	Threonine dehydratase	5	K
Arginine	Amino acid acetyltransferase	8	
Histidine	ATP phosphoribosyl transferase	9	V
Proline	Glutamate kinase and dehydrogenase	3	
Threonine	Aspartate kinase	5	K
Cytidine triphosphate (+)	Aspartate transcarbamoylase	8	K
Lysine (+)	Aspartate kinase	9	V
Thymidine triphosphate	Deoxycytidilate deaminase	4	K

^a Information compiled from Lehninger (1975) and Monod *et al.* (1965).

^b We tabulate S_n = end product, E_1 = regulatory enzyme, n = length of feedback loop:



Under Mode we indicate, when known, whether the inhibitor decreases V_{\max} (= maximum reaction rate) or increases $K_{0.5}$ (= substrate concentration at half-maximum velocity). The (+) next to S_n entries indicates, when known, that the inhibitory effect is cooperative—that is, that p in Eq. (4) can be larger than unity.

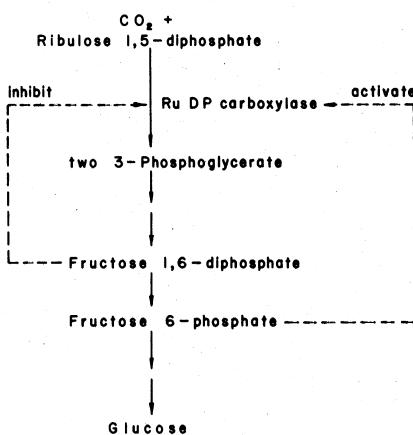


FIG. 3. Feedback activation and inhibition in the dark reactions of photosynthesis.

tion A of activator and on the concentration I of inhibitor. Indeed, these variations can be used to determine certain molecular properties of the enzyme such as number of subunits, binding constants, and conformational equilibria (see, for example, Johannes and Hess, 1973). As illustrated in Table II, most regulatory enzymes involved in feedback control of biosynthesis are "K systems"; that is, V_{\max} is relatively independent of I , but $K_{0.5}$ increases dramatically. At fixed substrate concentration, $S = S_0$, we write

$$V(S_0, I) = \frac{V(S_0, 0)}{1 + (I/I_0)^p} \quad (4)$$

where $V(S_0, 0)$ is just the reaction velocity at $S = S_0$ and $I = 0$, and $V(S_0, I_0) = V(S_0, 0)/2$. The parameters I_0 and p can be determined from the slope and intercept of the double logarithmic graph:

$$\log \left[\frac{V(S_0, 0)}{V(S_0, I)} - 1 \right] = p \log I - p \log I_0 \quad (5)$$

Values of p and I_0 for two of the enzymes from Table II are reported in Table III.

II. Kinetic Equations

In the limiting cases to be elaborated shortly, the dynamic behavior of a sequence of reactions under allosteric control can be deduced from results derived for systems controlled at the enzyme synthesis level. Therefore, kinetic equations will be derived for only the latter case. A schematic of the steps in the single feedback control loop is shown in Fig. 4.

Messenger RNA that codes for the unstable enzyme is produced by transcription of the structural gene SG, possibly followed by intranu-

TABLE III
OBSERVED VALUES OF THE SEMIEMPIRICAL RATE PARAMETERS p AND I_0 OF EQ. (4)

Inhibitor	Enzyme	Substrate	(Conc.)	p	I_0
Isoleucine	Threonine dehydratase ^a	Threonine	$(2.5 \times 10^{-3} M)$ $(10^{-2} M)$	1.5 1.9	$7.6 \times 10^{-4} M$ $2.5 \times 10^{-3} M$
dTTP	dCMP deaminase ^b	dCMP	$(2 \times 10^{-3} M)$ $(5 \times 10^{-3} M)$	3.4 2.7	$2.6 \times 10^{-2} M$ $5.1 \times 10^{-2} M$

^a Computed from Fig. 2 of Changeaux (1961).

^b Computed from Fig. 2B of Scarano *et al.* (1963).

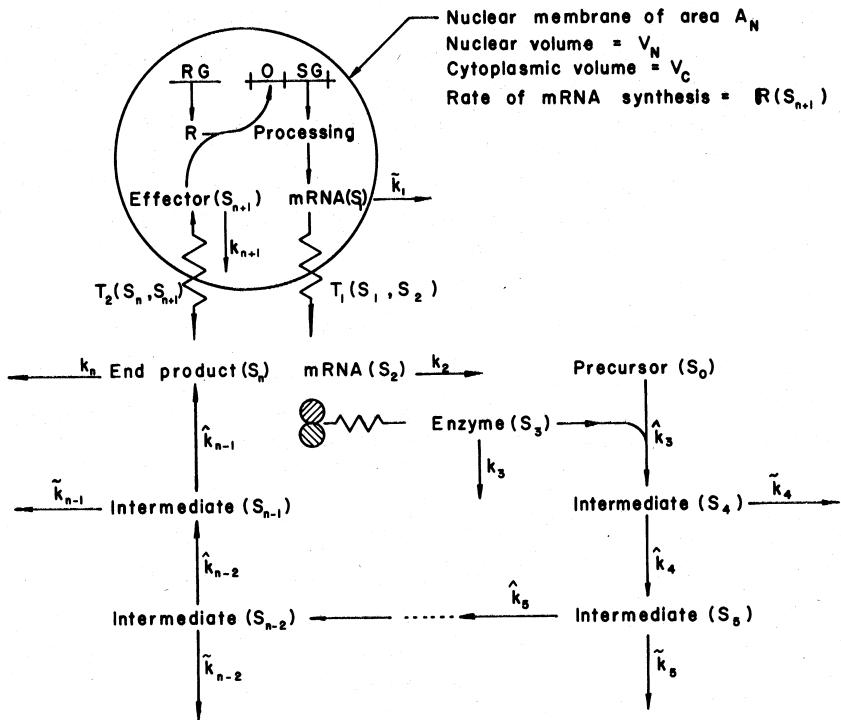


FIG. 4. Scheme for control of repressible enzyme; nuclear mRNA (S_1), cytoplasmic mRNA (S_2), and enzyme (S_3) are assumed to be labile. The precursor is assumed to be present at constant concentration. Each of the enzyme-catalyzed transformations of intermediates is assumed to be well below saturation.

clear processing such as cleavage. Some mRNA may be degraded enzymatically within the nucleus, and the remainder is transported into the cytoplasm, either by passive diffusion, by facilitated transport, or by active transport. In the cytoplasm, mRNA is both translated into the unstable enzyme at ribosomes and enzymatically degraded. The kinetics of mRNA degradation are assumed to be first order, both in the nucleus and in the cytoplasm.

The enzyme produced at the ribosome catalyzes the first step in a sequence of reactions that converts a substrate S_0 into an end product that acts as the effector of transcription control. Although each reaction in the sequence is enzyme-catalyzed, the concentrations of intermediate species are presumed to be small, and the enzyme concentrations are held fixed; as a result, the intermediate steps are treated as first-order

and irreversible. As is indicated in the figure, we allow for the possibility that each intermediate is also converted to something other than its successor in the control loop. The end product of the sequence either diffuses or is transported into the nucleus where it combines with the repressor R .

Regardless of whether the enzyme is repressible or inducible,* the kinetic equations that govern the transient behavior of the control circuit can be written

$$\begin{aligned} \frac{dS_1}{dt} &= \mathcal{R}(S_{n+1}) - \frac{A_N}{V_N} T_1(S_1, S_2) - \hat{k}_1 S_1 \\ \frac{dS_2}{dt} &= \frac{A_N}{V_C} T_1(S_1, S_2) - k_2 S_2 \\ \frac{dS_3}{dt} &= \hat{k}_2 S_2 - k_3 S_3 \\ \frac{dS_4}{dt} &= \hat{k}_3 S_3 - k_4 S_4 \\ &\vdots \\ \frac{dS_j}{dt} &= \hat{k}_{j-1} S_{j-1} - k_j S_j, \quad j = 5, \dots, n-1 \\ &\vdots \\ \frac{dS_n}{dt} &= \hat{k}_{n-1} S_{n-1} - k_n S_n - \frac{A_N}{V_C} T_n(S_n, S_{n+1}) \\ \frac{dS_{n+1}}{dt} &= \frac{A_N}{V_N} T_n(S_n, S_{n+1}) - k_{n+1} S_{n+1} \end{aligned} \tag{6}$$

where $k_j \equiv \hat{k}_j + \hat{k}_j$ for $4 \leq j \leq n-1$.

As written, the reaction rates all depend on the current concentration of the appropriate species. In view of the time delay involved in DNA transcription and mRNA translation, it would be reasonable to replace the rate of mRNA production by $\mathcal{R}[S_{n+1}(t - \tau_1)]$ and the rate of enzyme production by $\hat{k}_2 S_2(t - \tau_2)$, where τ_1 and τ_2 measure the delay. One effect of time delay is illustrated in Section IV,B.

Little is known about the mechanism for transfer of mRNA into the cytoplasm, and so for simplicity we shall assume that $T_1(S_1, S_2) = P_1 S_1$,

* We consider enzymes induced by end product rather than substrate. For instance, β -galactoside permease, which transports lactose into bacterial cells, is induced by its end product, intracellular lactose. (That the normal effector is an isomer of lactose does not affect our argument.) Of course, Eq. (1) does not apply to the *lac* operon because β -galactosidase, which catalyzes the hydrolysis of end product ($S_4 = S_n =$ intracellular lactose), belongs to the same operon as the permease. The kinetics of the parallel induction of the hydrolase would have to be included as well.

which is appropriate as an approximation to facilitated or active transport. Transport of the end product into the nucleus is assumed to occur by passive diffusion only, and consequently $T_n(S_n, S_{n+1}) = P_n(S_n - S_{n+1})$. If P_n is large (in appropriate dimensionless variables) and the rate of degradation of S_n small, then $S_n \sim S_{n+1}$, and as a first approximation we set them equal and ignore S_{n+1} . A more careful analysis of reaction and interphase transport shows that other limiting cases may occur when transport is rapid, but more precise knowledge concerning the relative rates of the processes is needed to decide when these cases are applicable (Othmer, 1975).

With these simplifications the equations reduce to

$$\begin{aligned} dS_1/dt &= \mathcal{R}(S_n) - k_1 S_1 \\ dS_2/dt &= \hat{k}_1 S_1 - k_2 S_2 \\ dS_3/dt &= \hat{k}_2 S_2 - k_3 S_3, \quad dS_4/dt = \hat{k}_3 S_3 - k_4 S_4 \\ dS_j/dt &= \hat{k}_{j-1} S_{j-1} - k_j S_j, \quad j = 5, \dots, n \end{aligned} \quad (7)$$

We write $\mathcal{R}(S_n)$ as

$$\mathcal{R}(S_n) = k_0 \bar{C} f(S_n) \quad (8)$$

where \bar{C} is the effective concentration of intranuclear ribonucleotide triphosphates,* and k_0 is the rate of incorporation of monomers into the growing RNA chains (a property of RNA synthetase). For an inducible system, $f(S_n)$ is given by Eq. (1). For a repressible system we shall use a slight modification of the function $f(S_n)$ given by Eq. (2). Whenever the fraction of repressor activated by effector is small (that is, whenever $R S_n^{(p)} \ll R$, or equivalently $R_t \approx R$), then to a good approximation $f(S_n) \approx (1 + K_1 K_2 R_t S_n^p)^{-1}$. We adopt this form hereafter:

$$f(S_n) = \begin{cases} \frac{1 + K_1 S_n^p}{K + K_1 S_n^p} & \text{inducible case} \\ \frac{1}{1 + K_1 K_2 R_t S_n^p} & \text{repressible case} \end{cases} \quad (9)$$

The kinetic equations at (7) can be simplified by introducing dimensionless variables. This can be done in various ways, and different choices for the dimensionless groups will emphasize different aspects of

* We introduce this concentration parameter so that all rate constants symbolized by a lower-case k have dimensions sec⁻¹.

the dynamics. Here we define

$$\begin{aligned} x_i &\equiv a_i S_i, \quad \tau \equiv bt, \quad \kappa_j \equiv k_j / b \\ a_n &\equiv \begin{cases} K_1^{1/p} & \text{inducible case} \\ (K_1 K_2 R_t)^{1/p} & \text{repressible case} \end{cases} \\ a_{j-1} &\equiv \frac{\hat{k}_{j-1}}{b} a_j \\ b &\equiv (k_0 \hat{k}_1 \dots \hat{k}_{n-1} a_n \bar{C})^{1/n} \end{aligned} \quad (10)$$

The kinetic equations now read

$$\begin{aligned} dx_1/d\tau &= f(x_n) - \kappa_1 x_1 \\ dx_j/d\tau &= x_{j-1} - \kappa_j x_j, \quad 2 \leq j \leq n \end{aligned} \quad (11)$$

where

$$f(x_n) \equiv \begin{cases} \frac{1 + x_n^p}{K + x_n^p} & \text{inducible case} \\ \frac{1}{1 + x_n^p} & \text{repressible case} \end{cases} \quad (12)$$

Our formulation of Eq. (7) is sufficiently general to encompass most of the analyses of feedback systems reported heretofore, and our choice of dimensionless variables is easily translated into those used by others.

One can easily show (Griffith, 1968a,b) that any solution of Eq. (11) that begins with all $x_i \geq 0$ remains bounded and is therefore well-defined for all $\tau > 0$. Indeed, the rectangular solid with vertices at $(0, 0, \dots, 0)$ and $(A/\kappa_1, A/\kappa_1 \kappa_2, \dots, A/\kappa_1 \dots \kappa_n)$, for any $A > 1$, is invariant under the flow of (11) for all $\tau > 0$.

For later purposes it is convenient to rewrite Eq. (11) in one of several equivalent vector forms, namely:

$$\mathbf{x}' = \mathbf{L}\mathbf{x} \pm \mathbf{b}f(x_n) \equiv \mathbf{f}(\mathbf{x}) \quad (13)$$

and

$$\xi' = \mathbf{L}\xi + \mathbf{b}\mathcal{F}(\mathbf{c}^T\xi) \quad (14a)$$

$$= \mathcal{L}\xi + \mathbf{b}g(\mathbf{c}^T\xi) \quad (14b)$$

Here $\mathbf{x}^T = (x_1, \dots, x_n)$, $\xi = \mathbf{x} - \mathbf{x}^*$, $\mathbf{b}^T = (\pm 1, 0, \dots, 0)$, $\mathbf{c}^T =$

$(0, \dots, 1)$,

$$\begin{aligned} L &= \begin{bmatrix} -\kappa_1 & 0 & 0 & \cdots & 0 \\ 1 & -\kappa_2 & 0 & \cdots & 0 \\ 0 & & & & \vdots \\ \vdots & & & & \vdots \\ 0 & \cdots & & 1 & -\kappa_n \end{bmatrix} \\ \mathcal{L} &= L \pm f'(x_n^*) \mathbf{b} \mathbf{c}^T = \begin{bmatrix} -\kappa_1 & 0 & \cdots & f'(x_n^*) \\ 1 & -\kappa_2 & 0 & \cdots & 0 \\ \vdots & & & & \vdots \\ 0 & \cdots & & 1 & -\kappa_n \end{bmatrix} \quad (15) \end{aligned}$$

$$\mathcal{F}(\mathbf{c}^T \xi) = \mathcal{F}(\xi_n) = \pm [f(x_n^* + \xi_n) - \kappa_1 x_1^*]$$

and

$$g(\mathbf{c}^T \xi) = g(\xi_n) = \mathcal{F}(\xi_n) \mp f'(x_n^*) \xi_n$$

The upper sign pertains to the inducible case, and the lower sign to the repressible case.

The constant vector \mathbf{x}^* is a steady-state solution of Eq. (11), and its components satisfy the equations

$$x_1^* = \kappa_2 x_2^* = \kappa_2 \kappa_3 x_3^* = \cdots = \kappa_2 \dots \kappa_n x_n^* \quad (16)$$

and

$$f(x_n^*) = \phi x_n^* \quad (17)$$

The scalar ϕ is given by

$$\phi = \prod_{i=1}^n \kappa_i = \prod_{i=1}^{n-1} \left(\frac{k_i}{\hat{k}_i} \right) \cdot \frac{k_n}{k_0 \bar{C} K_1^{1/p}} \quad (18)$$

for the case of induction. For repressible systems, simply replace $K_1^{1/p}$ by $(K_1 K_2 R_t)^{1/p}$. Notice that ϕ depends on all the rate constants k_i , \hat{k}_i , $1 \leq i \leq n-1$, provided that $k_i \neq \hat{k}_i$, and on the ratio $k_n/K_1^{1/p}$, where k_n measures the lability of end product and K_1 measures the tightness of the binding of end product to repressor. As can be seen in Fig. 1, Eq. (17) can have one, two, or three roots for an inducible system but only one root for a repressible system.

Asymptotic stability or instability of a steady state is governed by the eigenvalues of \mathcal{L} , the matrix of the linearized version of Eq. (14b). These eigenvalues are solutions of the characteristic equation

$$0 = P(\lambda) = \det(\lambda I - \mathcal{L}) = \prod_{j=1}^n (\lambda + \kappa_j) - f'(x_n^*) \quad (19)$$

In the special case $\kappa_1 = \kappa_2 = \dots = \kappa_n \equiv \kappa$, the roots of $P(\lambda)$ are simply

$$\lambda_j = -\kappa + \begin{cases} [f'(x_n^*)]^{1/n} \rho_{+}^j & \text{inducible case} \\ [-f'(x_n^*)]^{1/n} \rho_{-}^j & \text{repressible case} \end{cases} \quad (20)$$

for $j = 1, \dots, n$, where $(\rho_{+}^j)^n = 1$ and $(\rho_{-}^j)^n = -1$. In the general case there is no simple expression for the eigenvalues.

The following two sections are devoted to the study of the existence and stability of steady-state solutions of Eq. (11) for arbitrary n . The special cases $n = 2, 3$ have been widely studied; see, for example, Goodwin (1965), Griffith (1968 a and b), Lavenda (1972), and Tyson (1975). The special case leading to Eq. (20) was studied by Hunding (1974), and numerous numerical results for $n > 3$ have been reported (C. Walter, 1970). General analytical results for $n > 3$ are given in Othmer (1976a), where a different nondimensionalization of the equations is used.

III. Inducible Systems

A. MULTIPLICITY AND STABILITY OF STEADY STATES

In an inducible system there may be up to three solutions x_n^* of Eq. (17), written here as

$$h(x_n) = \phi x_n - f(x_n) = 0$$

These solutions depend only on the parameters p , ϕ , and K . On the locus in (p, ϕ, K) -space that divides regions of one solution from regions of three solutions, both $h(x_n) = 0$ and $h'(x_n) = 0$. Written out, these simultaneous equations are

$$\phi x_n = \frac{1 + x_n^p}{K + x_n^p} \quad (21)$$

and

$$\phi = \frac{p(K - 1)x_n^{p-1}}{(K + x_n^p)^2} \quad (22)$$

These can be combined to give

$$x_n^{2p} - [K(p - 1) - (p + 1)]x_n^p + K = 0 \quad (23)$$

In order that this equation have a positive real solution x_n for $K > 1$ and $p \geq 1$, it is necessary and sufficient that

$$K > K_{\min} = (p + 1/p - 1)^2 \quad (24)$$

TABLE IV
THE MINIMUM K NEEDED FOR MULTIPLE STEADY STATES AT
INTEGRAL p VALUES

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

The minimum K that suffices is shown as a function of p in Table IV. Evidently the steady state is always unique when $p = 1$.

If $K > K_{\min}$, then there exist two positive real roots $x_n^{\pm} = x_n(K, p)$ of Eq. (23). When these are used in Eq. (21), the following relation is obtained for the surface $\phi = \phi_{\pm}(K, p)$ in parameter space that divides regions of one steady state from those of three:

$$\phi = \phi_{\pm}(K, p) = \frac{1 + [x_n^{\pm}(K, p)]^p}{K + [x_n^{\pm}(K, p)]^p} [x_n^{\pm}(K, p)]^{-1} \quad (25)$$

From Table I we observe that $p = 2$ is the most common value for inducible enzymes and that $K = 1 + K_2 R_t > K_{\min}(2) = 9$. Indeed, $K \gg K_{\min}$, and this can be exploited to simplify Eq. (25) considerably. For $p = 2$ and K large, we have

$$[x_n^{\pm}(K, 2)]^2 \approx \begin{cases} K + \mathcal{O}(1) \\ 1 + \mathcal{O}(K^{-1}) \end{cases}$$

and so

$$\phi_{\pm}(K, 2) \approx \begin{cases} 1/2 \sqrt{K} \\ 2/K \end{cases} \quad (26)$$

The loci defined by these relations are shown in Fig. 5. We shall return to this figure in the discussion of hysteresis effects in Section V,A.

The next step in the analysis is to consider asymptotic stability of the steady states. This is governed by the roots of the characteristic equation (19). When three steady states exist, $f'(x_n^*) > \phi$ at the intermediate one (cf. Fig. 1a), and therefore $P(0) < 0$. Since $P'(\lambda) > 0$ for $\lambda \geq 0$, it follows that $P(\lambda)$ has exactly one positive real root. Consequently the intermediate steady state is always unstable.

At any other steady state (the unique one or the upper and lower of three), $f'(x_n^*) < \phi$, and $P(\lambda)$ has no nonnegative real roots. Furthermore:

Theorem 1. *When the steady state of an inducible system is unique, it*

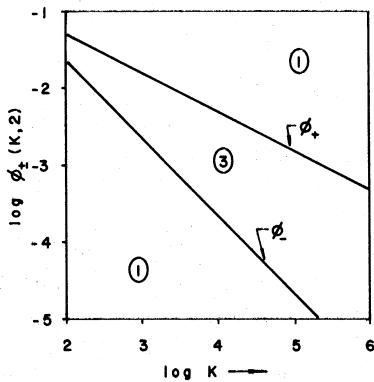


FIG. 5. An approximation to the region of multiple steady states in positive feedback loops for large K . Between the curves $\phi_+(K, 2)$ and $\phi_-(K, 2)$, given by Eq. (26), there are three positive steady states, and elsewhere the steady state is unique.

is asymptotically stable. When three steady states exist, the upper and lower are asymptotically stable and the intermediate one is unstable.

PROOF. We must show that, if $f'(x_n^*) < \phi$, then $\operatorname{Re} \lambda_k < 0$, $1 \leq k \leq n$, where the λ_k 's are the eigenvalues of the matrix \mathcal{L} . To this end we introduce the matrix

$$\mathbf{B} = \mathcal{L} + \kappa_{\max} \mathbf{I}$$

where κ_{\max} is the largest of the decay constants $\kappa_1, \dots, \kappa_n$. Denote the eigenvalues of \mathbf{B} by r_k , $1 \leq k \leq n$. \mathbf{B} is a nonnegative matrix; that is, $B_{ij} \geq 0$ for all i, j , and a weak version of the Perron-Frobenius theorem (Gantmacher, 1960; Minc and Marcus, 1964) implies that it always has a nonnegative eigenvalue r_1 such that

$$|r_j| \leq r_1, \quad 2 \leq j \leq n$$

Since \mathbf{B} obviously has some nonzero eigenvalues, $r_1 > 0$. The eigenvalues of \mathcal{L} are simply $\lambda_k = r_k - \kappa_{\max}$, $1 \leq k \leq n$, so the theorem is established if we can show that $r_1 - \kappa_{\max} < 0$. But if that were not so, then \mathcal{L} would have an eigenvalue $\lambda_1 = r_1 - \kappa_{\max} \geq 0$, which we have already shown to be impossible.

This theorem does not preclude the possibility that the domain of attraction of an asymptotically stable steady state is vanishingly small. The following shows that this cannot happen under certain conditions.

Theorem 2. Suppose that the differential equation for an inducible system is written in the form (14b), and suppose that K , p , and ϕ are

such that

$$0 < f'(x_n^*) < \phi - \epsilon$$

for $\epsilon > 0$. Then if $\|\xi(0)\| < \epsilon_1/\delta N^2$, where ϵ_1 , δ , and N are positive constants, $\|\xi(\tau)\| \rightarrow 0$ exponentially as $\tau \rightarrow \infty$.

PROOF. Write the solution of Eq. (14b) as

$$\xi(\tau) = e^{\mathcal{L}\tau}\xi(0) + \int_0^\tau e^{\mathcal{L}(\tau-\tau_1)}\mathbf{b}g(\xi_{\tau_1}) d\tau_1$$

Then, in the Euclidean norm $\|\cdot\|$,

$$\|\xi(\tau)\| \leq \|e^{\mathcal{L}\tau}\| \cdot \|\xi(0)\| + \int_0^\tau \|e^{\mathcal{L}(\tau-\tau_1)}\| \cdot \|\mathbf{b}\| \cdot |g(\xi_{\tau_1})| d\tau_1 \quad (27)$$

Because $f'(x_n^*)$ is bounded away from ϕ , there is an $\epsilon_1 > 0$ such that the largest real eigenvalue of \mathcal{L} is strictly less than $-\epsilon_1$, and all other eigenvalues of \mathcal{L} have real part less than $-\epsilon_1$. It follows that there exists an $N > 0$ such that

$$\|e^{\mathcal{L}\tau}\| < Ne^{-\epsilon_1\tau}$$

for $\tau > 0$. Furthermore, $g(0) = g'(0) = 0$, and there exists a $\delta > 0$ such that, for sufficiently small ξ_n ,

$$|g(\xi_n)| \leq \delta \xi_n^2 \leq \delta \|\xi\|^2$$

We define $\eta(\tau) = e^{\epsilon_1\tau}\|\xi(\tau)\|$ and then write Eq. (27) as

$$\eta(\tau) \leq N\eta(0) + \delta N \int_0^\tau e^{-\epsilon_1\tau_1} \eta^2(\tau_1) d\tau_1$$

The solution of this inequality is dominated by the solution of the equality

$$\hat{\eta}(\tau) = N\hat{\eta}(0) + \delta N \int_0^\tau e^{-\epsilon_1\tau_1} \hat{\eta}^2(\tau_1) d\tau_1$$

which is (W. Walter, 1970)

$$\hat{\eta}(\tau) = \frac{\epsilon_1 N \hat{\eta}(0)}{\epsilon_1 - \delta N^2 \hat{\eta}(0)(1 - e^{-\epsilon_1\tau})}$$

It follows that

$$\|\xi(\tau)\| \leq \frac{\epsilon_1 N e^{-\epsilon_1\tau} \|\xi(0)\|}{\epsilon_1 - \delta N^2 \|\xi(0)\| (1 - e^{-\epsilon_1\tau})}$$

and so if $\|\xi(0)\| < \epsilon_1/\delta N^2$, then $\|\xi(\tau)\| \rightarrow 0$ exponentially.

The theorem shows that an asymptotically stable steady state is stable with respect to finite amplitude disturbances if $f'(x_n^*)$ is bounded away from ϕ . This precludes small-amplitude periodic solutions that lie near a stable steady state, but not large-amplitude periodic solutions. The following analysis shows that these, too, can be ruled out in certain cases.

B. GLOBAL STABILITY RESULTS

When the steady state is unique, it can happen that all trajectories tend to it as $t \rightarrow \infty$. The following definition introduces some terminology for this case.

Definition. A steady state x^* of the autonomous system $x' = f(x)$ is globally asymptotically stable if it is asymptotically stable and

$$\lim_{\tau \rightarrow \infty} x(x_0, \tau) = x^*$$

for all initial points x_0 in the positive orthant of concentration space. This leads to the following result.

Theorem 3 (Othmer, 1976a). *When the steady state in an inducible system is unique, it is globally asymptotically stable.*

The proof of this requires some preliminary results.

Feedback control problems of the form of Eq. (14a) have been widely studied, and a variety of techniques are available for their analysis (Narendra and Taylor, 1973). A proof of global stability usually proceeds by constructing a Lyapunov function that is defined throughout the state space and has the appropriate sign-definiteness. A useful choice of the Lyapunov function for Eq. (14a) consists of a quadratic form plus an integral of the nonlinearity

$$V(\xi) = \xi^T H \xi + \gamma \int_0^{\xi_n} \mathcal{F}(\xi_n) d\xi_n \quad (28)$$

where H is positive definite and γ is a real scalar (Aizerman and Gantmacher, 1964). If H and γ can be chosen to fulfill the conditions on a Lyapunov function, the origin will be globally stable for Eq. (14a). It turns out that this can be done for a whole class of nonlinear functions $\mathcal{F}(\xi_n)$. Before stating the general result we need the concept of absolute stability (Aizerman and Gantmacher, 1964).

Definition. Suppose that \mathcal{F} is single-valued, that $\mathcal{F}(0) = 0$, and that

$$0 \leq \xi_n \mathcal{F}(\xi_n) \leq \Omega \xi_n^2 \quad (29)$$

For fixed $\Omega > 0$, the system (14a) is *absolutely stable* in the sector $[0, \Omega]$ provided the origin is globally asymptotically stable for any $\mathcal{F}(\xi_n)$ that satisfies Eq. (29).

Given that $\mathcal{F}(\xi_n)$ satisfies Eq. (29), conditions on the linear part of (14a) that are sufficient to guarantee absolute stability can be derived directly from Eq. (28). However, in general it is easier to work with the frequency response of the linear portion. The following theorem gives sufficient conditions for absolute stability in terms of the frequency response.

Theorem 4 [Popov's Theorem]. Suppose that L has only eigenvalues with negative real parts and that the numerator and denominator of

$$G(\lambda) \equiv c^T(\lambda I - L)^{-1}b$$

have no roots in common. Then a sufficient condition that $\xi' = L\xi + b\mathcal{F}(c^T\xi)$ be absolutely stable in the sector $[0, \Omega]$ is that there exist a real γ such that the complex function

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

satisfies

$$\operatorname{Re} T(i\omega) > 0 \quad (30)$$

for all real $\omega \geq 0$.

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

The proof of Theorem 3 is now easy. One first must verify the conditions on $G(\lambda)$. A short computation gives

$$G(\lambda) = [(\lambda I - L)^{-1}]_{n,1} = \prod_{j=1}^n (\kappa_j + \lambda)^{-1} \quad (31)$$

which obviously satisfies the stated conditions. Now

$$G(i\omega) = \prod_{j=1}^n (\kappa_j^2 + \omega^2)^{-1/2} \cdot \exp \left(-i \sum_{j=1}^n \Theta_j \right) \quad (32)$$

where

$$\Theta_j \equiv \tan^{-1}(\omega/\kappa_j) \quad (33)$$

Furthermore,

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

Since $|G|$ is monotone decreasing, the maximum of $\operatorname{Re} G(i\omega)$ occurs at $\omega = 0$, and so, if Ω is such that $T(0) > 0$, Eq. (30) will be satisfied with the

choice $\gamma = 0$. Since

$$T(0) = \frac{1}{\Omega} - \operatorname{Re} G(0) = \frac{1}{\Omega} - \frac{1}{\phi}$$

$T(0) > 0$, provided that $\Omega < \phi$. By definition, Ω is the boundary of the sector in which $\mathcal{F}(\xi_n)$ lies, and it is always true that $\Omega < \phi$ when there is only one steady state.

This result gives a complete picture of the dynamics of an inducible system when the steady state is unique. The analogous result for the case of three steady states would be:

Conjecture. The two stable steady states are globally attracting in the sense that all trajectories, except those starting on a particular $(n - 1)$ dimensional manifold, tend to one of the stable steady states as $t \rightarrow \infty$.

The exceptional manifold separates the positive orthant of R^n into domains of attraction of the two stable steady states. Within this manifold periodic solutions may appear. For instance, for $n \geq 5$ the linearization around the intermediate steady state can have a pair of complex conjugate eigenvalues with a positive real part. This is most readily seen in the special case $\kappa_1 = \kappa_2 = \dots = \kappa_n = \kappa$, for then the eigenvalues are simply

$$\lambda_j = -\kappa + \rho_+^j [f'(x_n^*)]^{1/n}, \quad (\rho_+^j)^n = 1$$

For $n \geq 5$ and sufficiently large ρ , at least one pair of λ 's has a positive real part. The Hopf theorem (see Section IV,C) implies that a periodic solution bifurcates when a pair of λ 's crosses the imaginary axis. Such periodic solutions are necessarily unstable when their amplitude is small, because the steady state always has a one-dimensional unstable manifold corresponding to a real eigenvalue. Whether there can be other large-amplitude stable periodic solutions is still an open question.

IV. Repressible Systems

A. LOCAL STABILITY RESULTS

In this section we consider repressible systems, and now $f'(x_n) < 0$ for $x_n \in [0, \infty)$. In this case Eq. (17) has a unique solution (cf. Fig. 1b). The stability of the steady state is governed by the location in the complex plane of the roots of the characteristic equation (19):

$$P(\lambda) = \det(\lambda I - \mathcal{L}) = \prod_{j=1}^n (\lambda + \kappa_j) - f'(x_n^*) \quad (34)$$

When written in terms of L , this reads

$$\begin{aligned} P(\lambda) &= \det [\lambda I - L + f'(x_n^*) b c^T] \\ &= \det (\lambda I - L) \det [I + f'(x_n^*)(\lambda I - L)^{-1} b c^T] \end{aligned}$$

[Recall that $b^T \equiv (-1, 0, \dots, 0)$ for repressible systems.] The steady state will be asymptotically stable provided that

$$P(\lambda) \neq 0 \quad \text{for } \operatorname{Re} \lambda \geq 0 \quad (35)$$

Because $\kappa_j > 0$, $j = 1, \dots, n$, L is a stability matrix, and the criterion (35) for stability becomes

$$\begin{aligned} 0 &\neq \det [I + f'(x_n^*)(\lambda I - L)^{-1} b c^T] \\ &= 1 + f'(x_n^*)G(\lambda) \quad \text{for } \operatorname{Re} \lambda \geq 0 \end{aligned} \quad (36)$$

where

$$G(\lambda) \equiv c^T(\lambda I - L)^{-1}b = - \prod_{j=1}^n (\lambda + \kappa_j)^{-1}$$

It is easy to see that $1 + f'(x_n^*)G(0) > 0$ and that $|\operatorname{Re} G|$ is monotone decreasing in $\operatorname{Re} \lambda$ along any locus $\operatorname{Im} G = 0$ in the complex λ plane. Therefore the stability criterion reduces to

$$1 + f'(x_n^*) \operatorname{Re} G(i\omega) > 0$$

whenever $\operatorname{Im} G(i\omega) = 0$ for $\omega \geq 0$. Consequently, the locus of marginal stability in parameter space is given by

$$-f'(x_n^*) = \Omega_c \quad (37)$$

where

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

This maximum occurs at the smallest ω for which $\arg G(i\omega) = 0$; that is, at $\omega = \omega_0$, where

$$\sum_{j=1}^n \tan^{-1} \left(\frac{\omega_0}{\kappa_j} \right) = \sum_{j=1}^n \Theta_j = \pi \quad (38)$$

Thus

$$\Omega_c = \prod_{j=1}^n (\kappa_j^2 + \omega_0^2)^{1/2} = \phi \prod_{j=1}^n (\sec \Theta_j) \quad (39)$$

and this depends on all κ_j 's separately. By contrast, $f'(x_n^*)$ depends on n, p , and the product $\phi = \kappa_1 \dots \kappa_n$. This suggests that we fix n, p , and

ϕ and minimize Ω_c as the parameters $\kappa_1, \dots, \kappa_n$ vary, subject to

$$\begin{aligned}\phi &= \prod_{j=1}^n \kappa_j = \text{constant} \\ \sum_{j=1}^n \Theta_j &= \pi\end{aligned}$$

Clearly all κ_j 's enter symmetrically in Eq. (39), and therefore the minimum occurs when $\kappa_1 = \kappa_2 = \dots = \kappa_n = \phi^{1/n}$. In this case $\Theta_j = \pi/n$, and

$$\Omega_{c,\min} = \phi \sec^n(\pi/n)$$

It is easy to see that

$$-f'(x_n^*) = p\phi(1 - \phi x_n^*)$$

and therefore the steady state is asymptotically stable provided that

$$p\phi(1 - \phi x_n^*) < \phi \sec^n(\pi/n) \quad (40)$$

This proves the following result.

Theorem 5. The steady state in a repressible system is asymptotically stable for all parameter values $\{\kappa_1, \dots, \kappa_n\}$ that satisfy $\prod_{j=1}^n \kappa_j = \phi$, if

$$\phi x_n^*(p, \phi) > (p - p_0)/p \quad (41)$$

where

$$p_0 = p_0(n) \equiv \sec^n(\pi/n) \quad (42)$$

If $p < p_0(n)$, then Eq. (41) is satisfied for all ϕ , and the steady state is asymptotically stable for all $\kappa_1, \dots, \kappa_n$. On the other hand, if $p > p_0(n)$, then Eq. (41) is violated at sufficiently small ϕ because

$$\lim_{\phi \rightarrow 0} \phi x_n^* = \lim_{\phi \rightarrow 0} \frac{1}{1 + x_n^{*p}} = \lim_{x_n^{*p} \rightarrow \infty} \frac{1}{1 + x_n^{*p}} = 0$$

Since Ω_c is smallest when $\kappa_1 = \dots = \kappa_n \equiv \kappa$, the critical value of $\kappa = \phi^{1/n}$ is

$$\kappa_0(n, p) = \left(\frac{p - p_0}{p x_n^*} \right)^{1/n}$$

For any parameters $\{\kappa_1, \dots, \kappa_n\}$ such that $\prod \kappa_j > \kappa_0^n$, the steady state is asymptotically stable, whereas for $\prod \kappa_j < \kappa_0^n$ there exists some set $\{\kappa_1, \dots, \kappa_n\}$ close to $\{\kappa, \dots, \kappa\}$, $\kappa \equiv (\prod \kappa_j)^{1/n}$, for which the steady

TABLE V
CRITICAL VALUES OF THE FEEDBACK PARAMETER p AND
DECAY CONSTANT κ AS FUNCTIONS OF n , THE NUMBER OF
ELEMENTS IN THE CONTROL LOOP^a

n	$p_0(n)$	$\kappa_0(2, n)$	$p_+(n)$	$p_1(n)$
3	8.0	—	5.37	—
4	4.0	—	2.76	—
6	2.37	—	1.86	—
8	1.88	0.671	1.61	2174.0
10	1.65	0.827	1.48	203.0
16	1.36	0.928	1.31	19.2
24	1.23	0.960	1.21	6.69
32	1.17	0.973	1.16	4.09

^a For $p > p_0(n)$ there exists an $\kappa_0(p, n)$ such that the steady-state solution is unstable for all $0 < \kappa < \kappa_0(p, n)$. See Eqs. (42) and (43). For $p > p_+(n)$ we have $\mu_2 < 0$, and small-amplitude periodic solutions exist for $-1 \leq \mu_2 = \kappa - \kappa_0 < 0$. See Eq. (59). For $p > p_1(n)$ there exist values of κ small enough such that a second family of small-amplitude periodic solutions bifurcates from the steady state.

state is unstable.* At the critical κ value,

$$\phi x_n^* = \frac{1}{1 + x_n^{*p}} = \frac{p - p_0}{p}$$

and it follows that

$$\phi_0(p, n) = [\kappa_0(p, n)]^n = \frac{(p - p_0)^{(p+1)/p}}{pp_0^{1/p}} \quad (43)$$

Values of $p_0(n)$ and $\kappa_0(p, n)$ for some integer values of n are provided in Table V. Some results on the region of instability in the $K_1 - k_n$ plane in the special case $k_1 = k_2 = \dots = k_{n-1}$ are given in Othmer (1976a).

* The minimum value of p for which the steady state can exhibit instability is larger than p_0 if the parameter set $\{\kappa_1, \dots, \kappa_n\}$ is not close to some set $\{\kappa, \dots, \kappa\}$. For instance, for $n = 3$ one can show that at the point of marginal stability

$$p\phi x_3^* = p - p_{\min}$$

where

$$p_{\min} = \frac{\Omega_c}{\phi} = 8 + \frac{(\kappa_1 - \kappa_2)^2}{\kappa_1 \kappa_2} + \frac{(\kappa_2 - \kappa_3)^2}{\kappa_2 \kappa_3} + \frac{(\kappa_3 - \kappa_1)^2}{\kappa_3 \kappa_1} \quad (44)$$

Obviously, $\Omega_{c,\min} = 8\phi$ at $\kappa_1 = \kappa_2 = \kappa_3$. Furthermore, p_{\min} increases dramatically as the decay constants become unequal; for example, for $\kappa_1 = \kappa_2 = 10\kappa_3$, $p_{\min} \approx 24$.

In conclusion, we remark that Theorem 2 applies equally well to repressible systems with minor modifications in the hypotheses to bound the eigenvalues of \mathcal{L} away from the imaginary axis.

B. GLOBAL STABILITY RESULTS

Asymptotic stability is governed by the slope $f'(x_n^*)$ of the nonlinearity in the feedback loop and the critical gain Ω_c of the linear system. According to Popov's theorem (Theorem 4), the equilibrium is globally asymptotically stable provided that we can find a real γ such that

$$\operatorname{Re} T(i\omega) = \frac{1}{\chi} - \operatorname{Re} G(i\omega) + \omega \gamma \operatorname{Im} G(i\omega) > 0 \quad (45)$$

for $\omega \geq 0$. The constant χ defines the upper boundary of the sector in which \mathcal{F} lies:

$$0 \leq \xi_n \mathcal{F}(\xi_n) \leq \chi \xi_n^2$$

It is geometrically obvious that χ is the maximum chord slope:

$$\chi \equiv \max_{\xi_n \in [-x_n^*, \infty)} \left[\frac{f(x_n^*) - f(x_n^* + \xi_n)}{\xi_n} \right] \quad (46)$$

and so if $\chi < \Omega_c$, Eq. (45) will be satisfied if we choose

$$\gamma^{-1} = \left. \frac{(d/d\omega)[\omega \operatorname{Im} G(i\omega)]}{(d/d\omega)[\operatorname{Re} G(i\omega)]} \right|_{\omega=\omega_0}$$

(Fig. 6). Here ω_0 is the first nonzero solution of $\operatorname{Im} G(i\omega) = 0$; that is, ω_0

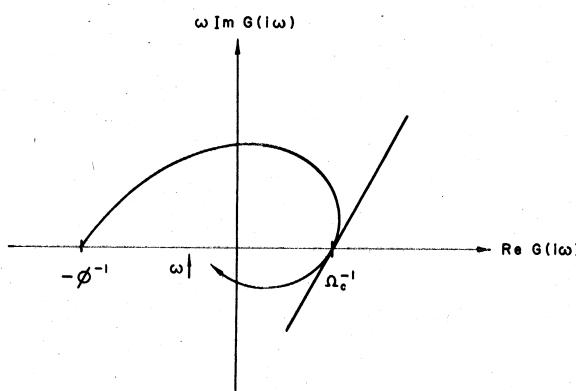


FIG. 6. The locus of the point $[\operatorname{Re} G(i\omega), \omega \operatorname{Im} G(i\omega)]$ for a negative feedback system as ω varies between 0 and ∞ . The straight line is the Popov line with slope γ^{-1} .

is the solution of Eq. (38). Consequently, to find conditions for global stability we have to find conditions under which $\chi < \Omega_c$.

The simplest case is $p = 1$, for which

$$\chi = 1/(1 + x_n^*) < 1 \quad (47)$$

Theorem 6. For $p = 1$ and fixed $n > 1$, the steady state in a repressible system is globally asymptotically stable for all κ_j , $j = 1, \dots, n$, that satisfy

$$\prod_{j=1}^n \kappa_j > \phi_{\min} = \frac{\cos^{2n}(\pi/n)}{1 + \cos^n(\pi/n)} \quad (48)$$

PROOF. We know that, for any $\phi > 0$,

$$\Omega_{c,\min} = \phi \sec^n(\pi/n)$$

and therefore, from Eq. (47), global asymptotic stability follows whenever

$$1/(1 + x_n^*) < \phi \sec^n(\pi/n)$$

For $p = 1$,

$$\phi x_n^* = 1/(1 + x_n^*)$$

so

$$1 + x_n^* = \frac{1 + (1 + 4\phi^{-1})^{1/2}}{2}$$

and (48) follows directly.

When $\phi < \phi_{\min}$, Eq. (48) is violated and Popov's theorem no longer guarantees global stability. However, a more refined analysis (Allwright, 1977) establishes global stability of the steady state for $p = 1$ and for all $\phi > 0$.

Now suppose that $p > 1$. We write the condition for global stability as*

* Equations (44) and (49) imply that for $n = 3$ the steady state is globally asymptotically stable if

$$\frac{\chi(p, \phi)}{\phi} < 8 + \frac{(\kappa_1 - \kappa_2)^2}{\kappa_1 \kappa_2} + \frac{(\kappa_2 - \kappa_3)^2}{\kappa_2 \kappa_3} + \frac{(\kappa_3 - \kappa_1)^2}{\kappa_3 \kappa_1}$$

This inequality was first derived by Ingwerson (1961) and by Bergen and Williams (1962).

$$\frac{\chi(p, \phi)}{\phi} < \frac{\Omega_c(\kappa_1, \dots, \kappa_n)}{\phi} \quad (49)$$

Since

$$\lim_{\phi \rightarrow 0} \frac{\chi(p, \phi)}{\phi} = \lim_{\phi \rightarrow 0} \frac{1}{\phi x_n^*} = \infty$$

and

$$\Omega_c/\phi > \sec^n(\pi/n) \quad \text{for all } \phi$$

inequality (49) is violated for sufficiently small values of ϕ . To obtain an upper bound for ϕ_{\min} we observe that, at fixed p , $\chi(p, \phi) \leq \chi_{\max}(p)$, where $\chi_{\max}(p)$ is the slope of $f(x)$ at its inflection point:

$$\chi_{\max}(p) = \max_{\phi > 0} [-f'(x_n^*)] = \frac{p^2 - 1}{4p} \left(\frac{p + 1}{p - 1} \right)^{1/p}$$

Insisting that $\chi_{\max}(p) < \Omega_{c,\min}(\phi)$, we obtain:

Theorem 7. For fixed n and $p > 1$ the steady state of a repressible system is globally asymptotically stable for all parameter sets $\{\kappa_1, \dots, \kappa_n\}$ that satisfy

$$\prod_{j=1}^n \kappa_j = \phi > \cos^n(\pi/n) \cdot \chi_{\max}(p) \quad (50)$$

It is not difficult to calculate ϕ_{\min} numerically. In Fig. 7 we plot the left-hand side of Eq. (49) as a function of ϕ for $p = 2, 3, 4, 5$. The minimum value of the right-hand side, $\sec^n(\pi/n)$, is also indicated. The intersection points define $\phi_{\min}(n, p)$; that is,

$$\chi(p, \phi_{\min})/\phi_{\min} = \sec^n(\pi/n) \quad (51)$$

In Table VI we compare ϕ_{\min} with ϕ_0 . Notice that $\phi_0 = \phi_{\min}$ for $p = 3, n = \infty$.* In this case only can we say that the steady state is globally asymptotically stable for all $\kappa_i \geq 0$ if and only if it is (locally) asymptotically stable for all $\kappa_i \geq 0$.

Regions of global asymptotic stability in the $k_n - K_1$ plane are available in propositions 3, 4, and 5 of Othmer (1976a). The use of Popov's theorem for the analysis of negative feedback systems was apparently first suggested by Viniegra-Gonzalez (1973). By a different method an der Heiden (1976b) has obtained similar global stability results.

* This follows from (a) $\chi(3, \hat{\phi}) = \hat{\phi} = \phi_0(3, \infty) = 2^{4/3}/3$ and (b) $\chi(p, \phi)/\phi$ is a monotone decreasing function of ϕ for $\phi \geq \hat{\phi}$.

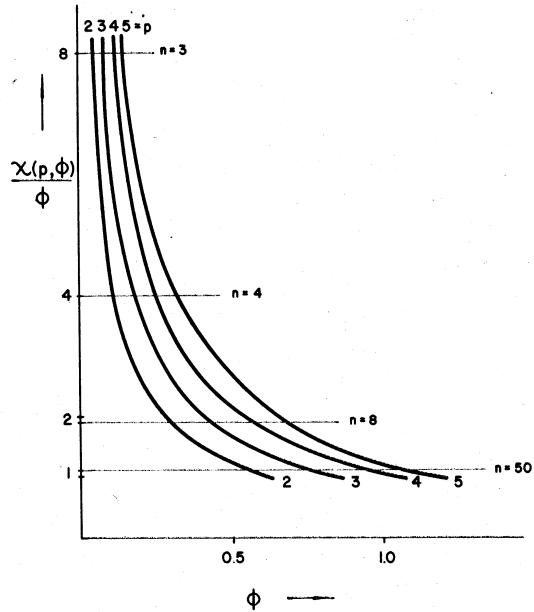


FIG. 7. Popov's theorem implies that the steady state is globally stable if $\chi(p, \phi)/\phi < \sec^n(\pi/n)$.

C. SMALL-AMPLITUDE PERIODIC SOLUTIONS

In Section IV,A it was shown that the steady state of a repressible system can be made unstable if (a) $p > p_0(n)$, (b) $\kappa \equiv (\prod \kappa_j)^{1/n} < \kappa_0(p, n)$, and (c) the parameter set $\{\kappa_1, \dots, \kappa_n\}$ is sufficiently close, in an appropriate norm, to $\{\kappa, \dots, \kappa\}$. Under these conditions the following result holds.

Theorem 8. Fix n and $p > p_0(n)$. There exist parameter values $\{\kappa_1, \dots, \kappa_n\}$ sufficiently close to $\{\kappa_0(p, n), \dots, \kappa_0(p, n)\}$, for which system (11) with $f(x_n) = (1 + x_n^p)^{-1}$ admits periodic solutions. These periodic solutions all belong to a unique, one-parameter family of periodic solutions, and the amplitudes of these periodic solutions are, in an appropriate sense, small.

This theorem follows directly from

Theorem 9 (Hopf's Theorem)

(i) *Conditions.* Let $\mathbf{x}' = \mathbf{f}(\mathbf{x}, \mu)$ be a real analytic autonomous system of differential equations with $\mathbf{x}, \mathbf{f}(\mathbf{x}, \mu) \in \mathbb{R}^n$, and $\mu \in \mathbb{R}$. Suppose that $\mathbf{f}(0, \mu) = \mathbf{0}$ for all $\mu \in [-c, c]$ for some $c > 0$. Let \mathcal{L}_μ be the linearization of $\mathbf{f}(\mathbf{x}, \mu)$ around $(0, \mu)$. Suppose that \mathcal{L}_0 has exactly two

TABLE VI
GLOBAL STABILITY RESULTS FOR REPRESSIBLE
SYSTEMS^a

$n =$	3	4	5	8	16	∞
$p = 1$	0.00	0.00	0.00	0.00	0.00	0.00
	0.02	0.05	0.09	0.19	0.31	0.50
	0.00	0.00	0.00	0.01	0.21	0.50
	2	0.04	0.11	0.17	0.29	0.44
		0.00	0.00	0.01	0.31	0.58
	3	0.07	0.18	0.27	0.42	0.62
		0.00	0.00	0.01	0.31	0.58
		0.11	0.25	0.35	0.57	0.78
	4	0.00	0.00	0.22	0.54	0.99
		0.11	0.25	0.35	0.57	0.79
		0.00	0.15	0.40	0.69	0.88
	5	0.14	0.36	0.46	0.69 ⁺	0.92
		1.00	1.00	1.00	1.00	1.00
∞	1.13	1.25	1.35	1.53	1.74	2.00

^a For given n and p we tabulate ϕ_0 and ϕ_{\min} as defined by Eqs. (43) and (51). Notice that $\phi_0 < \phi_{\min}$, except for $p = 3$, $n = \infty$, when $\phi_0 = \phi_{\min}$. For $\phi < \phi_0$ the steady state is unstable if $\kappa_1 = \dots = \kappa_n$, or nearly so. For $\phi > \phi_{\min}$ Popov's theorem assures global stability of the steady state. For $\phi_0 < \phi < \phi_{\min}$ the steady state is locally stable, but we make no claim about global stability.

purely imaginary eigenvalues, $\lambda(0) = -\bar{\lambda}(0) = i\omega_0$ whose continuous extensions $\lambda(\mu)$, $\bar{\lambda}(\mu)$ satisfy the transversality condition

$$\operatorname{Re} \left(\frac{d\lambda(\mu)}{d\mu} \right)_{\mu=0} \neq 0$$

(ii) *Existence.* Under these conditions there exists an $\epsilon_0 > 0$ and a functional relation $\mu = \mu(\epsilon)$ such that for each $\epsilon \in (-\epsilon_0, \epsilon_0)$ there exists a periodic solution $\tilde{x}(t, \epsilon)$ with period $T(\epsilon)$ of $\dot{x}' = f(x, \mu)$. At $\epsilon = 0$ we have $\mu(0) = 0$, $\tilde{x}(t, 0) = 0$, and $T(0) = 2\pi/\omega_0$, and $\tilde{x}(t, \epsilon) \neq 0$ for all sufficiently small $\epsilon \neq 0$.* Moreover $\mu(\epsilon)$, $\tilde{x}(t, \epsilon)$, and $T(\epsilon)$ are analytic at $\epsilon = 0$. These periodic solutions exist only for $\mu > 0$, or only for $\mu < 0$, or only for $\mu = 0$.

(iii) *Uniqueness.* Furthermore, for each $L > T(0)$ there exist $a > 0$, $b > 0$ such that if $|\mu| < b$ then, except for the bifurcating periodic

* The frequency ω_0 is the solution of Eq. (38) if all other eigenvalues have negative real parts.

solutions $\tilde{x}(t, \epsilon)$ with $\epsilon > 0$, there is no nonconstant periodic solution with period less than L that lies entirely in $\{x: \|x\| < a\}$.

PROOF. See Hopf (1942) or Ruelle and Takens (1971).

PROOF OF THEOREM 8. That the conditions of Hopf's theorem hold for repressible systems is the content of Proposition 1 in Othmer (1976a).

We may paraphrase Hopf's theorem and conclusions that can be drawn from it as follows:

(i) At $\mu = 0$ the constant solution $x = 0$ of the system $\dot{x} = f(x, \mu)$ loses stability by a single pair of complex conjugate eigenvalues $\lambda, \bar{\lambda}$ crossing the imaginary axis.

(ii) Whenever this happens we can be sure that there exists a one-parameter family of small-amplitude periodic solutions for μ sufficiently close to zero: only for $\mu = 0$ (which is unusual), or only for $\mu > 0$ (called supercritical bifurcation), or only for $\mu < 0$ (called subcritical bifurcation). If all the other eigenvalues of \mathcal{L}_0 have negative real parts, then bifurcating periodic solutions that exist only for $\operatorname{Re} \lambda > 0$ are orbitally asymptotically stable, and bifurcating periodic solutions that exist only for $\operatorname{Re} \lambda < 0$ are unstable.

(iii) Within a small neighborhood of the origin these bifurcating periodic solutions are unique.

In the remainder of this subsection we shall restrict our attention to the special case $\kappa_1 = \kappa_2 = \dots = \kappa_n \equiv \kappa = \phi^{1/n}$. Just as in the analogous case for an inducible system, the eigenvalues can be found explicitly:

$$\lambda_j = -\kappa + p^j [-f'(x_n^*)]^{1/n}, \quad 1 \leq j \leq n \quad (52)$$

where $-f'(x_n^*) = p\phi(1 - \phi x_n^*)$. Let n and p be fixed, let κ vary, and let $\mu = \kappa - \kappa_0$, where at $\kappa = \kappa_0(p, n)$ a pair of complex conjugate eigenvalues crosses the imaginary axis. Then a one-parameter family of periodic solutions exist only for $\kappa < \kappa_0(p, n)$, or only for $\kappa > \kappa_0(p, n)$, or only for $\kappa = \kappa_0(p, n)$. Momentarily we shall determine which of these alternatives applies.

For n sufficiently large, more pairs of complex conjugate eigenvalues may cross the imaginary axis and generate more families of periodic solutions. For instance, we see from Eq. (52) that for $n \geq 7$ a second bifurcation occurs at $\kappa = \kappa_1(p, n)$ where $\kappa_1(p, n)$ is defined by the relation

$$\kappa^n x_n^* = (p - p_1)/p, \quad p_1 = p_1(n) = \sec^n(3\pi/n)$$

What happens at these higher bifurcations is of considerable mathematical interest but of little importance in the biological context because $p_1(n)$ is exceedingly large for reasonable values of n (see Table V).

Having established the existence of a Hopf bifurcation for our system

$$\dot{\xi}' = \mathcal{L}_\mu \xi + g(\xi, \mu) \quad (53)$$

we now proceed to calculate the direction of bifurcation—that is, whether the periodic solutions exist for $\mu = 0$, or for $\mu < 0$, or for $\mu > 0$.* Consider the expansion of $\mu(\epsilon)$ about $\mu(0) = 0$:

$$\mu(\epsilon) = \mu_1 \epsilon + \mu_2 \epsilon^2 + \dots \quad (54)$$

Hopf proved that $\mu_1 = \mu_3 = \dots = 0$ (hence follows the last statement in part *ii* of the theorem). If $\mu_2 \neq 0$, then for ϵ sufficiently small the direction of bifurcation ($\mu < 0$ or $\mu > 0$) is determined by the sign of μ_2 . Poore (1976) has shown that

$$8 \operatorname{Re} [\lambda'(0)] \mu_2 = \operatorname{Re} \{-u_l f_{ijk}^l v_i v_j \bar{v}_k + 2u_l f_{jk}^l v_j (\mathcal{L}_0^{-1})_{kr} f_{pq}^r v_p \bar{v}_q + u_l f_{jk}^l \bar{v}_j [(\mathcal{L}_0 - 2i\omega_0 I)^{-1}]_{kr} f_{pq}^r v_p v_q\} \quad (55)$$

where:

- (a) $\lambda'(0) = [d\lambda(\mu)/d\mu]_{\mu=0}$.
- (b) \mathbf{u} and \mathbf{v} are left and right eigenvectors of \mathcal{L}_0 corresponding to the purely imaginary eigenvalue $i\omega_0$, normalized so that $\mathbf{u} \cdot \mathbf{v} = 1$. $\bar{\mathbf{v}}$ is the complex conjugate of \mathbf{v} .
- (c) f^l is the l th component of $\mathbf{f}(x)$, and

$$f_{ijk}^l = \frac{\partial^3 f^l}{\partial x_i \partial x_j \partial x_k}$$

$$f_{jk}^l = \frac{\partial^2 f^l}{\partial x_j \partial x_k}$$

(d) I is the $n \times n$ identity matrix.

(e) Repeated indices imply sums.

MacDonald (1977) first noticed that Eq. (55) is particularly simple when applied to Eq. (11) because only two of the partial derivatives are nonzero—namely, f_{nn}^1 and f_{nnn}^1 .† Equation (55) reduces to three terms:

* The perceptive reader might think that we have already eliminated the possibility of small-amplitude periodic solutions for $\mu > 0$, since the generalization of Theorem 2 to cover repressible systems implies that, when the steady state is asymptotically stable ($\mu = \kappa - \kappa_0 > 0$), then it is attracting in a domain $\|\xi\| < \epsilon_1/\delta N^2$. However, as $\kappa \rightarrow \kappa_0^+$, $\epsilon_1 \rightarrow 0$ as $\kappa - \kappa_0$. Thus the domain of attraction shrinks linearly with μ . On the other hand, the amplitude of bifurcating periodic solutions generally approach zero as $\mu^{1/2}$, and thus they would lie outside the domain of attraction for μ sufficiently small. Therefore, one cannot rule out the existence of unstable small-amplitude periodic solutions on the basis of Theorem 2.

† One of the authors (J.T.) has determined the sign of μ_2 using a formula derived by Hsü and Kazarinoff (1976). Though the same results are obtained, the calculations are considerably more complicated, so here we follow MacDonald's work.

$$8 \operatorname{Re} [\lambda'(0)] \mu_2 = \operatorname{Re} (u_1 v_n^2 \bar{v}_n \{-f_{nnn}^1 + 2(f_{nn}^1)^2 (\mathcal{L}_0^{-1})_{n1} + (f_{nn}^1)^2 [(\mathcal{L}_0 - 2i\omega_0)^{-1}]_{n1}\}) \quad (56)$$

In the special case $\kappa_1 = \dots = \kappa_n \equiv \kappa$, the necessary factors are easily evaluated:

$$\begin{aligned} f_{nnn}^1 &= -\kappa^{3n} \frac{p_0 p^2}{(p - p_0)^2} [p^2 - (6p_0 + 3)p + 2(3p_0^2 + 3p_0 + 1)] \\ f_{nn}^1 &= \kappa^{2n} \frac{p_0 p}{p - p_0} (p - 2p_0 - 1) \\ u_1 = v_n &= n^{-1/2} \kappa^{-(n-1)/2} (1 + i \tan z)^{-(n-1)/2} \\ (\mathcal{L}_0^{-1})_{n1} &= -\kappa^{-n} (1 + p_0)^{-1} \\ [(\mathcal{L}_0 - 2i\omega_0)^{-1}]_{n1} &= -\kappa^{-n} [p_0 + (1 + 2i \tan z)^n]^{-1} \end{aligned}$$

where $\tan z = \omega_0 / \kappa$, and we use the abbreviation $z = \pi/n$. Equation (56) becomes

$$\begin{aligned} &\{-8 \operatorname{Re} [\lambda'(0)] n^2 \kappa^{-(n+2)} p_0 p^{-2} (p - p_0)^2 \cos z\} \mu_2 \\ &= \operatorname{Re} \left\{ (1 + i \tan z) \left[p^2 - (6p_0 + 3)p + 2(3p_0^2 + 3p_0 + 1) \right. \right. \\ &\quad \left. \left. - \left(\frac{2p_0}{1 + p_0} + \frac{p_0}{p_0 + (1 + 2i \tan z)^n} \right) (p - 2p_0 - 1)^2 \right] \right\} \quad (57) \end{aligned}$$

Since the quantity in braces on the left-hand side of Eq. (57) is positive definite for all positive $p \neq p_0$, the sign of μ_2 is determined by the sign of

$$\begin{aligned} Q &= p^2 - (6p_0 + 3)p + 2(3p_0^2 + 3p_0 + 1) \\ &\quad - \left(\frac{2p_0}{1 + p_0} + R \right) (p - 2p_0 - 1)^2 \end{aligned} \quad (58)$$

where

$$R = \frac{1 + (1 + 3 \sin^2 z)^{n/2} (\cos n\theta + \tan z \sin n\theta)}{1 + (1 + 3 \sin^2 z)^n + 2(1 + 3 \sin^2 z)^{n/2} \cos n\theta}$$

and

$$\theta = \tan^{-1} (2 \tan z)$$

Rearranging Eq. (58) we find that

$$Q = ap^2 + bp + c \quad (59)$$

where

$$\begin{aligned} a &= -\frac{p_0 - 1}{p_0 + 1} - R \\ b &= (2p_0 + 1) \left(\frac{p_0 - 3}{p_0 + 1} + 2R \right) \\ c &= 2(1 + 3p_0 + 2p_0^2 - p_0^3)(1 + p_0)^{-1} - (2p_0 + 1)^2 R \end{aligned}$$

For all integers $n \geq 3$ we find that $a < 0$, $b < 0$, $c > 0$, and thus $Q < 0$ for all $p > p_+(n)$, where $p_+(n)$ is the unique positive root of $Q(p) = 0$. The functions $p_+(n)$ and $p_0(n)$ are compared in Table V and Fig. 8. We see that $\mu_2 < 0$ whenever $p > p_0(n)$, at least for $n \leq 32$. This implies that the bifurcating periodic solutions appear for $\kappa < \kappa_0(p, n)$ —that is, when the steady state is unstable. The periodic solutions are orbitally asymptotically stable.

That $\mu_2 < 0$ for n sufficiently large and for all $p > p_0(n)$ can be established by expanding p_0 and R in powers of $z = \pi/n$. We find that

$$p_0 = 1 + \frac{1}{2}\pi z + \frac{1}{8}\pi^2 z^2 + \frac{1}{48}\pi^3 z^3 + \frac{1}{12}\pi z^3 + \mathcal{O}(z^4) \quad (60)$$

$$R = \frac{1}{2} - \frac{3}{8}\pi z + \frac{9}{128}\pi^3 z^3 + \frac{3}{16}\pi z^3 + \mathcal{O}(z^4) \quad (61)$$

Substituting these expansions into Eq. (58) and determining the positive root, we find that

$$p_0 - p_+ = \frac{1}{4}\pi^3 z^3 + \pi z^3 + \mathcal{O}(z^4) \quad (62)$$

That is, $p_+(n) < p_0(n)$ for n sufficiently large, and thus $\mu_2 < 0$ for all $p > p_0(n)$.

If we remove the restriction $\kappa_1 = \kappa_2 = \dots = \kappa_n$, analysis becomes difficult, since we cannot even find the eigenvalues $\lambda_1, \dots, \lambda_n$ in general. However, for the simplest case $n = 3$ the analysis can be carried through to the point of Eq. (59), where a , b , and c are complicated functions of κ_1 , κ_2 , and κ_3 . At this point one must rely

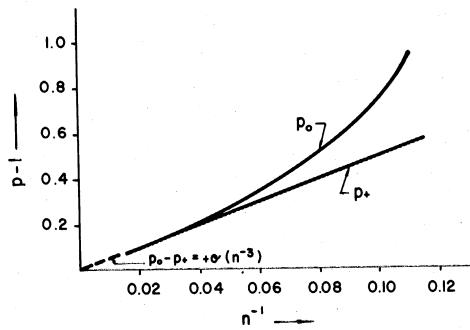


FIG. 8. Critical values of the feedback parameter p as a function of n , the number of elements in the loop. For $p > p_0$ the steady state becomes unstable for sufficiently small values of κ ; see Eq. (43). For $p > p_+$ the quadratic function in Eq. (59) is negative, and the bifurcating periodic solutions are orbitally asymptotically stable. Since $p_0 > p_+$ for $n \leq 32$ at least, we find no instances of periodic solutions when the steady state is stable.

again on the digital computer: choosing values of κ_1 , κ_2 , and κ_3 and calculating the sign of μ_2 . For all the combinations tried, the bifurcating periodic solutions appear only when the steady state is unstable. Moreover, the periodic solutions are least stable (that is, the characteristic exponent is least negative) when $\kappa_1 = \kappa_2 = \kappa_3$.

We summarize with:

Observation 1. In every case for which the direction of bifurcation has been determined, the small-amplitude periodic solutions exist only when the steady state is unstable. At the first bifurcation point, $\kappa = \kappa_0(p, n)$, the periodic solutions are orbitally asymptotically stable.

D. PERIODIC SOLUTIONS IN THE LARGE

In the previous subsection we found that, as κ decreases below $\kappa_0(p, n)$, the steady state loses stability, and small-amplitude periodic solutions exist for $|\kappa - \kappa_0(p, n)|$ sufficiently small. The following theorem establishes the existence of periodic solutions for all $\kappa \in [0, \kappa_0(p, n)]$ —indeed, for all parameter sets $\{\kappa_1, \dots, \kappa_n\}$ for which the steady state is unstable.

Theorem 10 (Hastings *et al.*, 1977)

(i) *Conditions.* Let $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ be a real autonomous system of differential equations with $\mathbf{x}, \mathbf{f}(\mathbf{x}) \in \mathbb{R}^n$ and $\mathbf{f} \in C^n$ on the set $\bar{P} = \{\mathbf{x}_i \geq 0\}$. Suppose that $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ has a unique steady state \mathbf{x}^* in $P = \text{interior of } \bar{P}$. Suppose that the Jacobian matrix, $\mathcal{L}(\mathbf{x}) = \partial \mathbf{f} / \partial \mathbf{x}$, has the form

$$\mathcal{L}(\mathbf{x}) = \begin{bmatrix} -\kappa_1 & 0 & 0 & \cdots & -d_1 \\ d_2 & -\kappa_2 & 0 & \cdots & 0 \\ 0 & d_3 & -\kappa_3 & \cdots & 0 \\ \vdots & \vdots & \vdots & & \vdots \\ 0 & 0 & 0 & \cdots & d_n & -\kappa_n \end{bmatrix} \quad (63)$$

and suppose that $\mathcal{L}(\mathbf{x}^*)$ has no repeated eigenvalues. Suppose that the following inequalities hold in P :

$$\frac{\partial f_i}{\partial x_j} < 0 \quad \text{and} \quad \frac{\partial f_j}{\partial x_{j-1}} > 0, \quad \text{both for } 2 \leq j \leq n \quad (64)$$

$$\frac{\partial f_1}{\partial x_n} < 0 \quad (65)$$

Suppose further that

$$f_1(0, 0) \geq 0, \quad 1 \leq i \leq n \quad (66)$$

$$f_1(x_n, 0) \geq 0 \quad \text{for all } x_n \geq 0 \quad (67)$$

$$f_1(x_n, x_1) < 0 \quad \text{if } x_n > x_n^* \text{ and } x_1 > x_1^* \quad (68a)$$

$$f_1(x_n, x_1) > 0 \quad \text{if } x_n < x_n^* \text{ and } x_1 < x_1^* \quad (68b)$$

$$\frac{\partial f_1}{\partial x_1} \text{ is bounded above in } \bar{P} \quad (68c)$$

(ii) *Existence of "oscillatory" solutions.* Under these conditions, if $\mathcal{L}(\mathbf{x}^*)$ has any eigenvalues with positive real part, then there exists a positively invariant set $V = \bigcup_{1 \leq i \leq n} V_i$, where the V_i 's are arranged, in an appropriate sense, in a circuit around the steady state. Any solution of $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ that starts in V remains bounded for all $\tau > 0$ and progresses through the V_i 's successively and interminably without approaching \mathbf{x}^* as $\tau \rightarrow \infty$.

(iii) *Existence of periodic solution(s).* Moreover, at least one of these oscillatory solutions is periodic; that is, it closes on itself after one circuit around \mathbf{x}^* .

By design the hypotheses on $\mathbf{f}(\mathbf{x})$ are sufficiently general to include a variety of negative feedback models. The form of \mathcal{L} , Eq. (63), is typical of a single feedback loop. Hypotheses (64) and (65) specify a negative feedback loop without being too specific about the form of $f_j(x_{j-1}, x_j)$. In particular, $f_j(x_{j-1}, x_j)$ need not be linear; for example, the theorem covers rate laws of the form

$$f_j(x_{j-1}, x_j) = \frac{V_{j-1}x_{j-1}}{K_{j-1} + x_{j-1}} - \frac{V_jx_j}{K_j + x_j} \quad (69)$$

(Rate laws of this form appear in metabolic pathways when the substrate concentration, x_j , is comparable to the Michaelis constant, K_j , of the enzyme. See, for example, Morales and McKay, 1967.) The rather complicated assumptions on $f_1(x_n, x_1)$ are introduced to cover functions of the form

$$f_1(x_n, x_1) \sim \frac{x_1}{1 + x_n^p}$$

which appear in models of the control of stem cell proliferation (Nazarenko and Sel'kov, 1978).

The proof of Theorem 10 is long and complicated, but the underlying ideas are simple. The first step is to construct a region \bar{V} in n -space that is positively invariant under the flow of the differential equation; solutions that begin in this region either tend to \mathbf{x}^* as $\tau \rightarrow \infty$ or oscillate. Then one shows that the former alternative does not occur by showing that solutions may tend to \mathbf{x}^* as $\tau \rightarrow \infty$ only by leaving \bar{V} . (The proof to this point is outlined in more detail in Appendix A.) To prove the existence of at least one closed orbit, one constructs an appropriate closed subset of \bar{V} which is homeomorphic to an $(n - 1)$ cell, is mapped continuously into itself by the flow $\mathbf{x}' = \mathbf{f}(\mathbf{x})$, and does not contain the steady state. Part (iii) then follows from Brouwer's fixed-point theorem.

For $n = 3$ the entire proof is elementary because the geometric aspects are transparent (Tyson, 1975). Other discussions of this method of proof are given in Pliss (1966) and Noldus (1969). The latter treats feedback systems of the kind considered in earlier sections of this paper.

Theorem 10 leaves three important questions unanswered. Is the periodic solution unique? Is the periodic solution orbitally asymptotically stable? Are there any attracting, nonperiodic, recurrent solutions? (For $n \geq 3$ there may exist solutions within P which do not tend to a periodic solution but which, loosely speaking, periodically return close to where they have been.) At the present time rigorous analysis of these global questions is out of grasp. In the next subsection we use an approximation scheme to characterize the periodic solutions further.

In Appendix B we outline a method for calculating periodic solutions in the large for the special case $p \rightarrow \infty$ —that is, for a step function nonlinearity.

E. FOURIER APPROXIMATION OF PERIODIC SOLUTIONS

In 1968 Viniegra-Gonzalez (1973) suggested the appropriateness of describing function analysis of periodic solutions of negative feedback cellular control systems, but only recently has a thorough pursuit of this method for determining existence, uniqueness, and stability of periodic solutions been undertaken by Rapp (1975a,b, 1976).

Describing function analysis, or “harmonic balancing,” is an approximation scheme based on Fourier analysis of the expected periodic functions. To see how the technique works (Rapp, 1976) we first write Eq. (11) as a single n th-order differential equation in $x_n(\tau)$:

$$(D + \kappa_1)(D + \kappa_2) \dots (D + \kappa_n)x_n(\tau) = f[x_n(\tau)] \quad (70)$$

where $D = d/d\tau$, and

$$f(x_n) = 1/(1 + x_n^p) \quad (71)$$

We suppose that Eq. (70) has a periodic solution $\tilde{x}_n(\tau)$ of period $T = 2\pi/\omega$ and expand $\tilde{x}_n(\tau)$ as a Fourier series:

$$\tilde{x}_n(\tau) = \operatorname{Re} \sum_{k=0}^{\infty} z_k e^{ik\omega\tau} \quad (72)$$

Since $f(\cdot)$ is single-valued, $f[\tilde{x}_n(\tau)]$ is also periodic of period $T = 2\pi/\omega$ and can be expanded:

$$f(\tilde{x}_n) = \operatorname{Re} \sum_{k=0}^{\infty} a_k e^{ik\omega\tau} \quad (73)$$

The coefficients a_k and z_k are related by

$$\begin{aligned} a_0 &= \frac{1}{2\pi} \int_0^{2\pi} f(\operatorname{Re} \sum z_k e^{ik\theta}) d\theta \\ a_j &= \frac{1}{\pi} \int_0^{2\pi} f(\operatorname{Re} \sum z_k e^{ik\theta}) e^{-ij\theta} d\theta, \quad j \geq 1 \end{aligned}$$

Substituting Eqs. (72) and (73) in Eq. (70), we obtain

$$\operatorname{Re} \sum_{k=0}^{\infty} z_k (ik\omega + \kappa_1) \cdots (ik\omega + \kappa_n) e^{ik\omega t} = \operatorname{Re} \sum_{k=0}^{\infty} a_k e^{ik\omega t}$$

Equating terms of like frequency $k\omega$ ($k \geq 0$), we generate an infinite set of "balance" equations:

$$\begin{aligned} z_0 \phi &= a_0, \quad \phi = \kappa_1 \dots \kappa_n \\ z_1 (i\omega + \kappa_1) \cdots (i\omega + \kappa_n) &= a_1 \\ z_2 (2i\omega + \kappa_1) \cdots (2i\omega + \kappa_n) &= a_2 \\ &\vdots \end{aligned} \tag{74}$$

The obvious approximation is to truncate this series, most conveniently at level $k = 1$:

$$z_0 = \frac{1}{2\pi\phi} \int_0^{2\pi} f(z_0 + z_1 \cos \theta) d\theta \tag{75a}$$

$$(i\omega + \kappa_1) \cdots (i\omega + \kappa_n) = \frac{1}{\pi z_1} \int_0^{2\pi} f(z_0 + z_1 \cos \theta) (\cos \theta - i \sin \theta) d\theta \tag{75b}$$

In principle Eq. (75a) can be solved for $z_0 = z_0(z_1)$, and then Eq. (75b) takes the form

$$G(i\omega) = -1/F_1(z_1) \tag{76}$$

where $G(i\omega)$ is the Nyquist function used in Section IV,A, and

$$F_1(z_1) = \frac{1}{\pi z_1} \int_0^{2\pi} f[z_0(z_1) + z_1 \cos \theta] (\cos \theta - i \sin \theta) d\theta \tag{77}$$

is known as the describing function. Truncation at $k = 1$ constitutes dual input describing function (DIDF) analysis. The practicality of the method for this case lies in the fact that the amplitude z_1 and the frequency ω of the periodic solution are separated in Eq. (76). It is convenient to solve Eq. (76) graphically; on plotting $G(i\omega)$ and $-1/F_1(z_1)$ in the complex plane, every intersection corresponds to a periodic solution. Furthermore, the stability of the periodic solution can be

predicted from the behavior of $1/F_1(z_1)$ close to the point of intersection (Rapp, 1975a). Suffice it to say that, if $1/F_1(z_1)$ is a decreasing function of z_1 at the point of intersection, then the periodic solution is orbitally asymptotically stable. If $1/F_1(z_1)$ is increasing, then the periodic solution is an anti-limit cycle (that is, stable as $t \rightarrow -\infty$).

Before continuing, a few words are in order to justify truncating the infinite set of Eqs. (74) at $k = 1$, or rather to justify keeping only the fundamental term in Eq. (72): $\hat{x}_n(\tau) = z_0 + z_1 \cos \omega\tau$. We must convince ourselves that $z_k \ll z_1$ for $k \geq 2$, so write Eq. (74) as

$$z_k = -G(ik\omega)a_k$$

First of all, the coefficients a_k are expected to decrease in magnitude with increasing k . Indeed, $f(x_n)$ of the form specified in Eq. (71) looks much like a step function for $p \geq 2$, and for a square wave $|a_k| \sim 1/k$. Furthermore, $|G(ik\omega)|$ decreases rapidly for increasing k (as we have seen), which means that the linear part of Eq. (70) tends to filter out higher harmonics of $f(\hat{x}_n)$. We illustrate these effects in Appendix B.

We have already seen that the complex-valued function $G(i\omega)$ "spirals" into the origin as $\omega \rightarrow \infty$; that is, $|G(i\omega)|$ and $\arg G(i\omega)$ decrease monotonically as ω increases.

More difficult is to determine the properties of $F_1(z_1)$. First of all, we establish that F_1 is a real-valued function of z_1 (Rapp, 1975a). Defining $\eta = z_0 + z_1 \cos \theta$, we transform the imaginary part of $F_1(z_1)$ to

$$\text{Im } F_1(z_1) = \frac{1}{\pi z_1^2} \int_{z_0+z_1}^{z_0+z_1} f(\eta) d\eta$$

which is identically zero, since $f(\cdot)$ is a single-valued function. Second, $F_1(z_1)$ is a negative function of z_1 . To show this (Rapp, 1976) write Eq. (77) as

$$F_1(z_1) = \frac{2}{\pi z_1} \int_0^{\pi/2} [f(z_0 + z_1 \cos \theta) - f(z_0 - z_1 \cos \theta)] \cos \theta d\theta$$

Since $z_0 \geq z_1 \geq 0$ and $\cos \theta \geq 0$ on $[0, \pi/2]$ and $f(\cdot)$ is monotonically decreasing positive function, the integrand $[f(z_0 + z_1 \cos \theta) - f(z_0 - z_1 \cos \theta)] \cos \theta \leq 0$, and thus $F_1(z_1)$ is negative.

Notice that we insist that $z_0 \geq z_1 \geq 0$ in order that $\hat{x}_n(\tau) \geq 0$ for all τ . Examining Eq. (75a) we see that at fixed z_1 the left-hand side is an increasing function of z_0 , whereas the right-hand side is a decreasing

function of z_0 . Thus a unique root such that $z_0 \geq z_1 \geq 0$ exists if and only if

$$z_1 \leq \frac{1}{2\pi\phi} \int_0^{2\pi} f(z_1 + z_1 \cos \theta) d\theta$$

but this can be true only for $0 \leq z_1 \leq A_m$, where the maximum amplitude A_m satisfies

$$A_m = \frac{1}{2\pi\phi} \int_0^{2\pi} f(A_m + A_m \cos \theta) d\theta$$

As z_1 varies over $[0, A_m]$, $F_1(z_1)$ varies along the negative real axis: $F_{1,\min} \leq F_1(z_1) \leq F_{1,\max} < 0$. To satisfy Eq. (76) we must have

$$\frac{-1}{F_{1,\min}} \leq G(i\omega_0) \leq \frac{-1}{F_{1,\max}} \quad (78)$$

where ω_0 is the frequency (there may be more than one) at which $G(i\omega)$ crosses the positive real axis; that is, $\text{Re } G(i\omega_0) > 0$ and $\text{Im } G(i\omega_0) = 0$.

In his papers Rapp examines further the properties of $F_1(z_1)$ for $f(x_n) = (1 + x_n^p)^{-1}$ with $p = 1, 2, 3, 4$. For $p = 1$ he finds that $1/F_1(z_1)$ is an increasing function of z_1 , which implies that for $p = 1$ there can be no stable periodic solutions—granted the accuracy of the approximation. For $p = 1$ Allwright (1977) has shown that the steady state is globally asymptotically stable. Thus DIDF analysis predicts the existence of unstable periodic solutions when this is clearly impossible. This unexpected failure of the method has been discussed by Rapp and Mees (1977).

For $p = 2$ Rapp finds that $1/F_1(z_1)$ is monotone decreasing; that is,

$$1/F_{1,\min} = 1/F_1(0)$$

The same is true for $p = 3$, if $\phi < 2.618$, and for $p = 4$, if $\phi < 1.880$. In this case we make:

Observation 2 (Rapp, 1976). *If the global minimum of $F_1(z_1)$ is obtained as $z_1 \rightarrow 0$, then the dual input describing function method predicts the existence of a periodic solution $\tilde{x}_n(\tau) = z_0 + z_1 \cos \omega_0 \tau$ if and only if the steady state is unstable with respect to infinitesimal perturbations.*

PROOF. Numerical work indicates that $-1/F_{1,\max} > G(i\omega_0)$, so DIDF analysis predicts a periodic solution if and only if

$$G(i\omega_0) \geq -1/F_{1,\min} = -1/F_1(0)$$

assuming the validity of the condition. We have already seen that the steady state (x_n^*) is unstable if and only if $G(i\omega_0) \geq -1/f'(x_n^*)$, so we must establish that $F_1(0) = f'(x_n^*)$. Expand $F_1(z_1)$ in a Taylor series:

$$\begin{aligned} F_1(z_1) &= \frac{1}{\pi z_1} \int_0^{2\pi} \left[f(z_0) + z_1(\cos \theta) f'(z_0) \right. \\ &\quad \left. + \frac{1}{2} z_1^2 (\cos^2 \theta) f''(z_0) + \dots \right] \cos \theta d\theta \\ &= 0 + f'(z_0) + \frac{z_1^2}{\pi} \int_0^{2\pi} \left[\frac{1}{3!} (\cos^4 \theta) f'''(z_0) + \dots \right] d\theta \end{aligned}$$

Taking the limit as $z_1 \rightarrow 0$, we find that $F_1(0) = f'(z_0)$, where $z_0 = z_0(0)$ is determined by Eq. (75a):

$$\phi z_0 = \frac{1}{2\pi} \int_0^{2\pi} \frac{d\theta}{1 + z_0^{-p}} = \frac{1}{1 + z_0^{-p}}$$

which is the same equation satisfied by x_n^* .

Combining this with the global stability results in Table VI, we make:

Observation 3. Granted the accuracy of dual input describing function analysis, for $p = 2, 3, 4$ there exists a periodic solution if and only if the steady state is unstable. The periodic solution is stable.

In Appendix B we apply DIDF analysis to Eq. (70) with $f(x_n)$ a step (down) function.

V. Discussion

A. HYSTERESIS EFFECTS

Inducible enzymes undoubtedly have adaptive value in that they provide an organism with the capability of utilizing a variety of food-stuffs without having to continually maintain a high concentration of the enzymes needed to catabolize them. That inducible systems exhibit multiple steady states in certain regions of parameter space has the further adaptive value of buffering the system against fluctuations in the concentration of substrate.

To investigate this phenomenon quantitatively we must determine the dependence of S_3^* , the steady-state enzyme concentration, on S_0 , the parametrically varied substrate concentration. From the scaling equations (10) we find that

$$S_3^* = \frac{k_4 k_5 \dots k_n}{k_3 k_4 \dots k_{n-1}} \frac{x_n^*}{K_1^{1/p}} \quad (79)$$

According to Eq. (18),

$$\phi = \frac{k_1 k_2 \dots k_{n-1} k_n}{k_0 \bar{C} \hat{k}_1 \dots \hat{k}_{n-1} K_1^{1/p}}$$

and the substrate concentration enters ϕ via the relation $\hat{k}_3 = c_3 S_0$ (see Fig. 4). The second-order rate constant c_3 is the product of an equilibrium constant for the enzyme-substrate binding, $S_0 + S_3 \rightleftharpoons (S_0 S_3)$, and a first-order rate constant for the breakdown of the enzyme-substrate complex, $(S_0 S_3) \rightarrow S_4 + S_3$. Thus ϕ is inversely proportional to S_0 , and it is convenient to introduce

$$\sigma_0 \equiv \phi^{-1} = \frac{k_0 \bar{C} \hat{k}_1 \hat{k}_2 c_3 \hat{k}_4 \dots \hat{k}_{n-1} K_1^{1/p}}{k_1 k_2 \dots k_n} S_0 \quad (80)$$

In the common case $p = 2$, $K \gg 1$ (cf. Table I), the steady states satisfy

$$x_n^3 - \sigma_0 x_n^2 + K x_n - \sigma_0 = 0 \quad (81)$$

For $\sigma_0 \ll K$ this polynomial has one and only one positive real root:

$$x_n^{\text{off}} = \sigma_0 / K + \mathcal{O}(K^{-3})$$

For $\sigma_0 \gg K$ the only real root is

$$x_n^{\text{on}} = \sigma_0 + \mathcal{O}(\sigma_0^{-1})$$

Finally, if

$$2\sqrt{K} < \sigma_0 < K/2 \quad (82)$$

then Eq. (81) admits three positive real roots. Using Eqs. (79) and (80) we find that

$$S_3^* = \frac{k_0 \bar{C} \hat{k}_1 \hat{k}_2}{k_1 k_2 k_3} \cdot \begin{cases} K^{-1}, & \text{if } \sigma_0 \ll 2\sqrt{K} \\ 1, & \text{if } \sigma_0 \gg K/2 \end{cases}$$

These conclusions are illustrated in Fig. 9.

To see whether the range of σ_0 given by Eq. (82) is significant under normal physiological conditions, we have to estimate the parameters that appear in Eq. (79). We can make the following estimates:

- i. Volume of cell $\sim 10^{-15} \text{ l} = 10^{-9} \mu\text{l}$. This is appropriate for bacteria of linear dimension $\sim 1 \mu\text{m}$. Notice that a concentration of $1 \mu\text{M}$ is equivalent to 10^3 molecules per cell.
- ii. Number of copies of mRNA $\sim 10^2$ (Watson, 1970, p. 439).
- iii. Number of enzyme molecules when fully induced $\sim 10^3$ (Watson, 1970, p. 438).

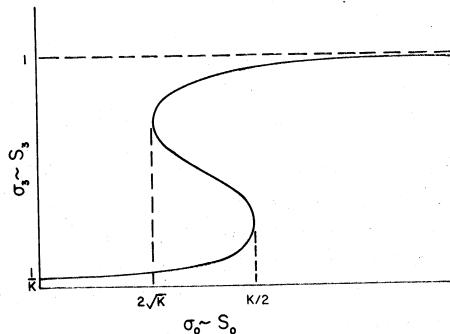


FIG. 9. Steady-state enzyme concentration S_3^* as a function of substrate concentration S_0 for an inducible enzyme.

- iv. $k_0 \bar{C} \sim 1$ molecule sec $^{-1}$ $\sim 10^{-3} \mu M$ sec $^{-1}$. That is, several minutes are required to synthesize 100 copies of mRNA (Watson, 1970, p. 453).
- v. $\hat{k}_1 \sim k_2$. Since bacterial cells lack a nuclear membrane, we do not distinguish intranuclear from cytoplasmic mRNA.
- vi. $\hat{k}_2 \sim 10^{-2}$ sec $^{-1}$. Assuming 100 molecules of mRNA per cell, this corresponds to synthesis of 1 protein molecule per second, or 1000 protein molecules in ~ 10 minutes (Watson, 1970, p. 439).
- vii. $c_3 \sim 1 \mu M^{-1}$ sec $^{-1}$. Since $c_3 \sim V_{max}/K_M [E_t]$, this is equivalent to $V_{max} \sim 10^{-3} \mu M$ sec $^{-1}$, $K_M \sim 10^3 \mu M$, and $[E_t] \sim 1 \mu M$.
- viii. $K_1^{1/p} \sim 10^{-1} \mu M^{-1}$. This is the value of K_1 for the *lac* inducer IPTG (see Table I).
- ix. $k_1 \sim 10^{-2}$ sec $^{-1}$, $k_3 \sim 10^{-3}$ sec $^{-1}$, $k_n \sim 10^{-1}$ sec $^{-1}$. That is, mRNA has a half-life of ~ 1 minute, enzyme is ten times as stable, and inducer is ten times as labile.

With these assumptions, $\sigma_0 \sim S_0/1 \mu M$, and

$$S_3^* = \begin{cases} K^{-1} \mu M, & \text{fully repressed} \\ 1 \mu M, & \text{fully induced} \end{cases}$$

For $K = 2500$ (see Table I), $S_3^* < 1$ molecule/cell when the operon is fully repressed. The region of multiple steady states,

10^5 molecules/cell $\sim 10^2 \mu M < S_0 < 10^3 \mu M \sim 10^6$ molecules/cell buffers the system against dramatic fluctuations in enzyme concentration between the two extremes, 0 and $1 \mu M$. Notice that the system is buffered against an order of magnitude fluctuation in S_0 only if $K >$

2500. Might this be why K is observed to be large in many systems? (See Yagil and Yagil, 1971, Table I, where $\alpha_b = K^{-1}$.)

Were positive feedback to play a role in the control of growth (as yet there seems to be no evidence for this), then multiple steady-state behavior could model multiple modes of growth, such as passive maintenance/active proliferation. For example, under certain conditions the model of growth control diagrammed in Fig. 10 has three modes of behavior: (i) the tissue can deteriorate (stem cell population $\rightarrow 0$); (ii) the tissue can regulate (stem cell population finite and stable with respect to small perturbations); (iii) the tissue can grow uncontrollably (stem cell population $\rightarrow \infty$). Sufficiently large perturbations can shift a regulated system out of state (ii) into states (i) or (iii). A similar model has been studied by Sel'kov (1970) in relation to the malignant transformation.

B. APPLICATIONS OF NEGATIVE FEEDBACK

It is widely appreciated that negative feedback control loops readily give rise to sustained oscillations, and they have been invoked in numerous models of periodic phenomena. For instance, it is observed in synchronous cultures of bacteria that the synthesis of many enzymes occurs only periodically, in specific portions of the cell cycle, and that the order of enzyme synthesis corresponds closely to the order of replication of the structural genes (Masters and Pardee, 1965). This would seem to suggest that the timing of enzyme synthesis is controlled by the sequential replication of the genes. However, Masters and Donachie (1966) have shown that periodic enzyme synthesis continues in the absence of DNA synthesis, and they suggested that the periodicity is

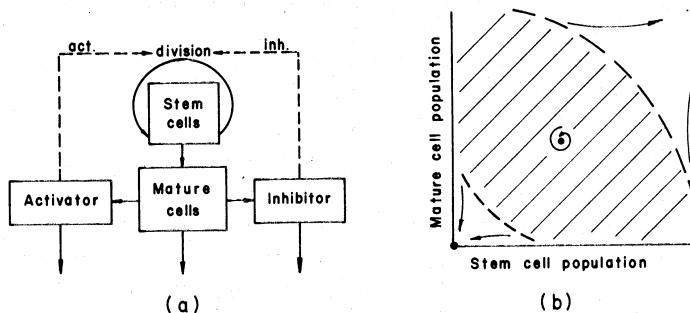


FIG. 10. Positive and negative feedback in control of growth. (a) "Wiring" diagram. (b) Schematic illustration of modes of behavior. In the shaded region growth is self-regulating, close to the origin the tissue degenerates, and far from the origin the tissue grows without bound.

a result of end-product repression of mRNA synthesis. That the period of each separate oscillator should correspond exactly to the cell cycle time and that the relative phases of the oscillators should correspond exactly to the order of replication of the structural genes has been attributed to nonlinear entrainment (Goodwin, 1966).

Recently Rapp and Berridge (1976) have presented evidence that oscillations in internal Ca^{2+} and cyclic AMP may be due to a negative feedback instability in their common control loops. They suggest that many high-frequency biological rhythms, particularly those associated with membrane potential changes, may be driven by these second messenger oscillations.

Danziger and Elmergreen (1954) have suggested that periodic catatonic schizophrenia may be caused by an underlying periodicity in the endocrine system. Their model is a three-component, piecewise linear, negative feedback oscillator. Cronin (1973) studied these equations in some detail and showed that solutions remain bounded for all $t > 0$. That there exists at least one periodic solution whenever the steady state is unstable is easily demonstrated, as in Tyson (1975). Uniqueness and global stability should yield to the methods developed by Hastings (1977) for piecewise linear systems. MacDonald (1976b) has applied the describing function method to these equations.

Endogenous oscillatory activity is observed in isolated neurons (Chen *et al.*, 1971; Gainer, 1972). an der Heiden (1976a) recently suggested that perhaps this activity is the result of negative feedback, since a neuronal model which incorporates cell fatigue reduces to a single, three-component negative feedback loop under certain simplifying assumptions.

Endogenous rhythms of period close to 24 hours are well known. Mergenhagen and Schweiger (1975) have shown that inhibition of enzyme synthesis at the translation level blocks the circadian clock in *Acetabularia*. Tyson *et al.* (1976) take this as evidence for a suspicion that end-product repression of ribosomal activity may be involved in circadian rhythms. The large period could be accommodated easily by a sufficiently long chain of intermediates.

Time-keeping is a major necessity during morphogenesis (Robertson and Cohen, 1972). Goodwin and Cohen (1969) have presented a hypothetical negative feedback model for a developmental clock. When certain rules for spatial coupling are also assumed, phase differences in the oscillators can serve as a developmental map as well.

Negative feedback is thought to play an important role in the control of growth. In its simplest form the idea is that stem cells grow and divide to produce new stem cells and a certain percentage of nondividing, differentiating cells. At one or more stages of development feedback

signals are generated and transmitted to the stem cell population (see Fig. 11). In this case, however, the susceptibility to oscillation is more of a nuisance than a benefit. Weiss and Kavanau (1957) analyzed numerically a number of negative feedback models of chick growth and were careful to choose their parameters in the nonoscillatory regime. King-Smith and Morley (1970) eliminated oscillatory behavior from their negative feedback model of granulopoiesis by introducing a second feedback loop. In certain pathological conditions oscillations of the granulocyte population may appear (Wheldon, 1975).

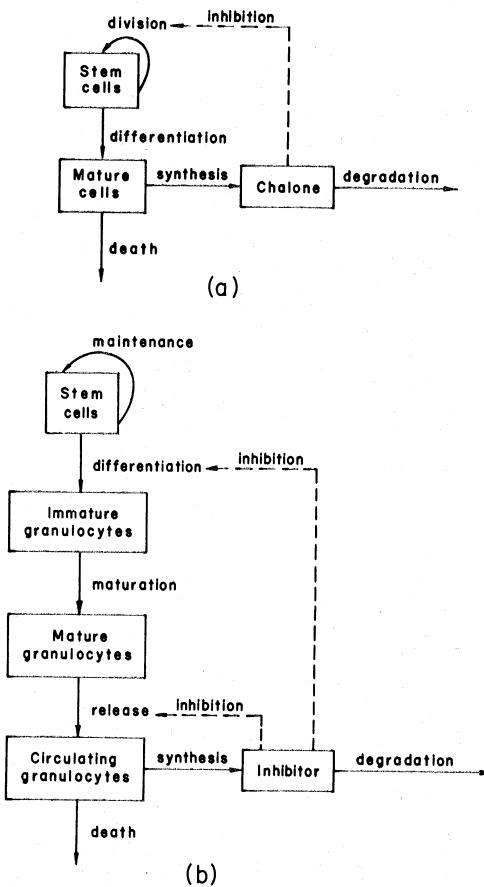


FIG. 11. Two models of control of cell proliferation. (a) Simple model of feedback inhibition of growth (Weiss and Kavanau, 1957). (b) Model of granulopoiesis (King-Smith and Morley, 1970).

We have dealt with only the simplest "straight-through" feedback loops, and for these there is both numerical (C. Walter, 1970) and analytical evidence (Section IV) to support the contention that, when the degree of cooperativity p and the chain length n are related by $p < \sec^n(\pi/n)$, there can be no sustained oscillations. Since it is biologically unlikely that $p > 4$, oscillations are possible only for sequences of five or more elements (mRNA, enzyme, at least two intermediates, end product). Since $p \approx 2$ is more reasonable, the pathway must be longer. However, this ignores time delays, coupling between control loops, and spatial localization of one or more enzymes. Any of these may lead to oscillations at smaller values of p and/or n .

To illustrate this point in more detail, let us consider the effect of a continuous time delay in the transcription of DNA to RNA. Suppose that we replace $f(x_n)$ in Eq. (11) by $f(y_{m+1})$, where

$$y_{m+1} = \int_{-\infty}^{\tau} x_n(s) G_m(\tau - s) ds$$

and

$$G_m(w) = (w^m/m!) \exp(-\kappa w), \quad m = \text{integer}$$

That is, the rate of transcription depends on the previous history of end-product concentration, most heavily on the end-product concentration at $\tau - m\kappa^{-1}$. Now define (Vogel, as cited in MacDonald, 1976a)

$$y_k = \int_{-\infty}^{\tau} x_n(s) G_{k-1}(\tau - s) ds, \quad 1 \leq k \leq m + 1$$

Equation (11) becomes

$$\begin{aligned} dx_1/d\tau &= f(y_{m+1}) - \kappa_1 x_1 \\ dx_j/d\tau &= x_{j-1} - \kappa_j x_j, \quad 2 \leq j \leq n \\ dy_1/d\tau &= x_n - \kappa y_1 \\ dy_l/d\tau &= y_{l-1} - \kappa y_l, \quad 2 \leq l \leq m + 1 \end{aligned}$$

which is just a simple feedback loop of length $n + m + 1$. Since at fixed κ the effective time lag increases with m , we see that the effect of time delay of this sort is to increase the length of the feedback loop and thus make oscillations possible at smaller values of p . Including time delays for processing heterogeneous nuclear RNA and for translation of cytoplasmic messenger RNA could generate quite large effective chain lengths.

C. SPATIALLY NONUNIFORM SYSTEMS

The analysis in the preceding sections applies only when spatial nonuniformities in concentration decay rapidly on the characteristic time scale for reaction. This is undoubtedly true at the single-cell level when the enzymes and intermediates are all soluble and free to diffuse about. However, when larger distances are involved or when some enzymes are immobilized, transport may play a significant role in the dynamics of the control circuit. In such cases, instabilities of the uniform state may arise from the interaction of reaction and transport, and these instabilities may lead to nonuniform spatial or spatiotemporal concentration patterns (Turing, 1952; Othmer and Scriven, 1971).

To analyze the role of transport in the dynamics of a control circuit, we consider the simplest model of an assemblage of cells—namely, a single-phase continuum model wherein all cellular structure is ignored. Suppose that all species involved in a feedback loop are free to diffuse throughout a region V of three-dimensional space and that there is no flux across the boundary of V . The dynamics of the nonuniform system are governed by the partial differential equation

$$\begin{aligned} \frac{\partial \mathbf{x}}{\partial \tau} &= \mathcal{D} \nabla^2 \mathbf{x} + \mathbf{Lx} \pm \mathbf{bf}(x_n) && \text{in } V \\ \mathbf{n} \cdot \nabla \mathbf{x} &= 0 && \text{on } \partial V \\ \mathbf{x}(\mathbf{r}, 0) &= \mathbf{x}_0(\mathbf{r}) \end{aligned} \quad (83)$$

Here \mathcal{D} is an $n \times n$ matrix of dimensionless diffusion constants $\mathcal{D}_{ij} \equiv \mathcal{D}_{ij}^* / bL^2$, where \mathcal{D}_{ij}^* is the dimensional constant and L is a characteristic length. For simplicity we assume that \mathcal{D} is diagonal.

In view of the no-flux boundary condition, Eq. (83) always admits spatially uniform solutions, either steady states \mathbf{x}^* or uniform periodic solutions. One expects that, if diffusion is rapid enough, the system will ultimately evolve to a uniform state. The following result provides an estimate of how large the smallest diffusivity must be.

Theorem 11 (Othmer, 1976b). *Let*

$$K = \max_{\mathbf{x} \geq 0} \|\mathcal{L}(\mathbf{x})\| \quad (84)$$

and let μ_1 be the smallest non-zero eigenvalue of the scalar problem:

$$\begin{aligned} \nabla^2 u + \mu u &= 0 && \text{in } V \\ \mathbf{n} \cdot \nabla u &= 0 && \text{on } \partial V \end{aligned}$$

If

$$\min_i (\mathcal{D}_i) \mu_1 > K \quad (85)$$

then all spatial nonuniformities decay exponentially in time.

The proof will be omitted.

The dimensional version K^* of the parameter K provides a measure of the sensitivity of the reaction rate to concentration changes, and its reciprocal is one measure of the kinetic relaxation time. Similarly, $[(\min_i \mathcal{D}_i^*) \mu_1^*]^{-1}$ is the longest diffusional relaxation time. If, for instance, $K^* \sim 10^{-1} \text{ sec}$, then in a one-dimensional system of length L , Eq. (85) will be satisfied if

$$(\min_i \mathcal{D}_i^*)/L^2 > \sim 0.04 \text{ sec}^{-1}$$

When $L = 10 \mu\text{m}$ (a typical cell diameter), the condition is met for $\mathcal{D}_i^* > \sim 4 \times 10^{-8} \text{ cm}^2/\text{sec}$, which is well within the range of diffusivities measured *in vitro*. Therefore, unless K is much larger than the given estimate, spatial nonuniformity within a cell can be ignored when enzymes are soluble.

When the distances are greater, one can no longer be assured that the system will always evolve to a uniform state. The next best thing is to determine whether or not uniform states are stable to small nonuniform disturbances. A partial answer is given by:

Theorem 12. Suppose that a uniform steady state \mathbf{x}^* of an inducible or a repressible system is asymptotically stable as a solution of Eq. (13). Then it is asymptotically stable as a solution of Eq. (83).

PROOF. To prove this we must simply show that all small-amplitude disturbances, uniform and nonuniform, decay in time. The linearized version of Eq. (83), which is analogous to the linearized version of Eq. (14b), is

$$\partial \xi / \partial \tau = \mathcal{D} \nabla^2 \xi + \mathcal{L} \xi \quad (86)$$

with appropriate initial conditions and zero flux boundary conditions. Solutions of Eq. (86) can be written (Othmer and Scriven, 1969):

$$\xi(\mathbf{r}, \tau) = \sum_p e^{(\mathcal{L} - \mu_p \mathcal{D})\tau} \mathbf{y}_p(0) \mathbf{u}_p(\mathbf{r}) \quad (87)$$

where $\mathbf{y}_p(0)$ is the amplitude vector for the p th eigenfunction of the Laplacian. One eigenvalue, μ_0 , is zero, and the remaining μ_p 's are strictly positive. Asymptotic stability is governed by the set of eigenvalues of $\mathcal{L} - \mu_p \mathcal{D}$, $p = 0, 1, 2, \dots$, and these eigenvalues are the solutions of the characteristic equation

$$\det(\lambda I - \mathcal{L} + \mu_p \mathcal{D}) = 0 \quad (88)$$

By comparison with Eq. (19), it follows that Eq. (88) can be written

$$\prod_{j=1}^n (\lambda + \kappa_j + \mu_p \mathcal{D}_j) - f'(x_n^*) = 0 \quad (89)$$

A comparison with the proof of Theorems 1 and 5 shows that, if Eq. (89) has no roots with a positive real part for $\mu_p \equiv 0$, then it has no roots with a positive real part for any $\mu_p > 0$. Therefore, all small-amplitude disturbances decay exponentially, and the uniform steady state is asymptotically stable.

This theorem precludes diffusive instabilities, and consequently there is no possibility for the formation of nonuniform steady states by bifurcation from a uniform state that has become unstable to nonuniform disturbances. In an inducible enzyme system that has three steady states, there is, in addition to the two stable states, always an unstable intermediate state. Simple examples can be constructed which show that bifurcations can occur from this unstable state. However, these bifurcating solutions are necessarily unstable and therefore are of little interest. The instability of these solutions can be shown in special cases by making use of results due to Pismen and Kharkats (1968) and Luss and Ervin (1972), and in general by direct construction of the bifurcating solutions. Edelstein (1972) has numerically computed the nonuniform solutions that bifurcate from an unstable state in a two-species system with an immobilized inducible enzyme, but his results all represent unstable solutions.

It would be desirable to prove that, when the system of ordinary differential equations for a repressible enzyme has an asymptotically stable periodic solution, then it is also asymptotically stable as a solution of Eq. (83). One can prove this in the special case for which the diffusivities are all "nearly" equal, and in the general case one can show that, if the steady state is stable to nonuniform disturbances, then all small-amplitude uniform periodic solutions are asymptotically stable (Othmer, 1976b). In light of these results and the conclusion of Theorem 12, we conjecture that the spatially uniform periodic solutions in a repressible system are asymptotically stable. This will probably be difficult to establish, particularly in view of the fact that it is not even established yet that the large-amplitude solutions are stable as solutions of the ordinary differential equation (see Section IV,D). Nonetheless, it is unlikely that the simple single-loop feedback circuits studied in earlier sections ever lead to any interesting nonuniform spatial patterns when coupled with diffusive transport. They are therefore uninteresting as

kinetic models for the control of spatial pattern formation in developing systems. The next step is to consider more complicated coupled circuits, the simplest of which involves two enzymes, each of whose products may activate or inhibit the other enzyme.

Such two enzyme systems have been investigated by several authors. Glass and Kauffman (1972) studied a linear four-compartment system in which the enzymes are located only in the end compartments. They supposed that the product of the enzyme at one end decays by a first-order reaction and diffuses through the compartments. At the other end it inhibits the second enzyme whose product in turn activates the first enzyme. It was found numerically that for appropriate diffusivities and decay rates the system could exhibit sustained oscillations. This case as well as those of mutual activation and mutual inhibition in continuous or compartmentalized systems have also been studied by Shymko and Glass (1974), Glass and Perez (1975), Thamnes (1974), Aronson (1975), and Aronson and Thamnes (1976).

The latter authors use the following system of equations to describe a one-dimensional system that has an enzyme localized at each boundary:

$$\begin{aligned}\frac{\partial u}{\partial t} &= \frac{\partial^2 u}{\partial \zeta^2} - q^2 u \\ \frac{\partial v}{\partial t} &= \frac{\partial^2 v}{\partial \zeta^2} - q^2 v \\ \frac{\partial u}{\partial \zeta}(0, t) &= -pq f[v(0, t)] \\ \frac{\partial v}{\partial \zeta}(1, t) &= pq\{1 - f[u(1, t)]\} \\ \frac{\partial u}{\partial \zeta}(1, t) &= \frac{\partial v}{\partial \zeta}(0, t) = 0\end{aligned}\tag{90}$$

Here $f(x) \equiv x^2/(1 + x^2)$, and u and v represent dimensionless enzyme concentrations. They find that when the diffusivity and the decay rate are large the unique steady state is globally asymptotically stable. However, there is an intermediate range of parameters in which the steady state is unstable and stable periodic solutions bifurcate along the locus of marginal stability. The results are particularly relevant in the context of gene control because one can interpret one "enzyme" as the operator for a structural gene, whereas the other could be an enzyme localized in the cytoplasm. Their conclusions point up the fact that, when transport is important, very simple schemes in nonuniform systems can mimic the behavior of far more complicated networks in homogeneous systems.

In all the foregoing studies, the spatial localization of the enzymes is critical for the existence of periodic solutions. One can show that, were the enzymes free to diffuse about, the uniform steady state would always be stable. The time lag due to diffusion of products between the enzymes is necessary for the oscillations. This points up the need for further analysis of models that attempt to account for intracellular structure. One such model is given in Othmer (1975).

Appendix A: Existence of Oscillatory Solutions for Negative Feedback Loops

In this appendix we sketch the proof (Hastings *et al.*, 1977) of part (ii) of Theorem 10 under slightly more restrictive conditions than those in part (i). We shall assume that

$$f_1(x_n, 0) > 0 \quad \text{for all } x_n \geq 0 \quad (67')$$

$$\partial f_1 / \partial x_1 < 0 \quad \text{in } P \quad (68')$$

$$\lim_{x \rightarrow \infty} f_i(c, x) < 0 \quad \text{for all } c \geq 0, \quad 1 \leq i \leq n \quad (A1)$$

Conditions (63)–(66) remain the same, conditions (67'), (68') replace (67), (68), and condition (A1) is added.

Condition (63) implies that $\mathbf{x}' = \mathbf{f}(\mathbf{x})$ can be written in component form as

$$\begin{aligned} x_1' &= f_1(x_n, x_1) \\ x_j' &= f_j(x_{j-1}, x_j), \quad 2 \leq j \leq n \end{aligned} \quad (A2)$$

Conditions (64)–(66) and (67'), (68'), (A1) together imply that system (A2) has a unique constant solution $\mathbf{x}^* = (x_1^*, \dots, x_n^*)$ in P . Condition (A1) implies the existence of a point $\mathbf{x} = \{A_1, \dots, A_n\} \in P$ satisfying

$$\begin{aligned} f_1(0, A_1) &< 0 \\ f_j(A_{j-1}, A_j) &< 0, \quad 2 \leq j \leq n \end{aligned}$$

These inequalities, along with (66) and (67'), imply that the set $\bar{S} = \{\mathbf{x} \in R^n: 0 \leq x_i \leq A_i, 1 \leq i \leq n\}$ is a positively invariant manifold; that is, if $\mathbf{x}(0) \in \bar{S}$, then $\mathbf{x}(\tau) \in S$ for all $\tau > 0$. Of necessity, $\mathbf{x}^* \in \bar{S}$.

The planes $x_i = x_i^*$, $1 \leq i \leq n$, divide \bar{S} into 2^n subsets $\bar{S}_0, \dots, \bar{S}_{2^n-1}$. It is convenient to use a binary notation for these subsets. Let $a_1(j) \dots a_n(j)$ be the binary expansion of the integer $j \in [0, 2^n - 1]$. For example, if $n = 5$ and $j = 13$, then the binary expansion is 01101. Now a point $\mathbf{x} \in \bar{S}$ is in \bar{S}_j if

$$(-1)^{a_i(j)}(x_i - x_i^*) \leq 0, \quad 1 \leq i \leq n$$

In the example $n = 5, j = 13$, we define \bar{S}_{13} as the set

$$\begin{aligned} 0 &\leq x_1 \leq x_1^* \\ x_2^* &\leq x_2 \leq A_2 \\ x_3^* &\leq x_3 \leq A_3 \\ 0 &\leq x_4 \leq x_4^* \\ x_5^* &\leq x_5 \leq A_2 \end{aligned}$$

and $S_{13} = \text{interior of } \bar{S}_{13}$.

Using this notation we state (without proof) an important lemma which tells how trajectories of system (A2) move from box to box.

Lemma 1. Suppose that j has the binary expansion $a_1(j) \dots a_n(j)$ and that $x(0) \in S_j$. Then either $x(\tau) \in S_j$ for all $\tau > 0$ and $x(\tau) \rightarrow x^*$ as $\tau \rightarrow \infty$, or else $x(\tau)$ intersects the boundary of S_j and immediately enters one of the subsets S_k , $k \neq j$, for which:

- (i) $a_1(j) = a_1(k)$ if $a_1(j) \neq a_n(j)$
- (ii) $a_l(j) = a_l(k)$ if $a_l(j) = a_{l-1}(j)$, $2 \leq l \leq n$

In other words, k is obtained from j by changing at least one of the $a_i(j)$ according to the rule: $a_1(j)$ can change if $a_1(j) = a_n(j)$, while for $2 \leq l \leq n$, $a_l(j)$ can change if $a_l(j) \neq a_{l-1}(j)$. For example, if $n = 5$ and $j = 13$, then either $x(\tau)$ stays in S_{01101} for all $\tau > 0$, or $x(t)$ enters one of the sets $S_{00101}, S_{01111}, S_{01100}, S_{00111}, S_{00100}, S_{01110}, S_{00110}$.

Consider the set

$$\bar{V} = \bar{S}_{000\dots00} \cup \bar{S}_{100\dots00} \cup \bar{S}_{110\dots00} \cup \bar{S}_{111\dots00} \cup \dots \cup \bar{S}_{111\dots11} \cup \bar{S}_{011\dots11} \cup \bar{S}_{001\dots11} \cup \dots \cup \bar{S}_{000\dots01}$$

where \bar{V} is the union of $2n$ subsets \bar{S}_j . If $x(0) \in \bar{V}$, then the lemma implies that either

- (a) $x(\tau)$ eventually remains in some $S_j \subset V$ and hence tends to x^* , or
- (b) $x(\tau)$ repeatedly circulates through the $S_j \subset V$ in the order indicated in the definition of \bar{V} .

Our next step is to show that alternative (a) is impossible. This is where the assumptions about the Jacobian matrix \mathcal{L} are used. The union of all trajectories $x(\tau)$ that approach x^* as $\tau \rightarrow \infty$ is called the stable manifold \mathcal{S} . Close to x^* the stable manifold is tangent to the linear space L spanned by the real and imaginary parts of the eigenvectors of \mathcal{L} associated with eigenvalues with negative real parts. It is the position of L with respect to \bar{V} that we now investigate.

From conditions (63)–(65), (68') we find that the characteristic equa-

tion for \mathcal{L} is

$$P(\lambda) = \prod_{j=1}^n (\lambda + \kappa_j) + \Phi = 0 \quad (\text{A3})$$

where $\kappa_j > 0$ ($1 \leq j \leq n$) and $\Phi = d_1 \dots d_n > 0$. We recall that any roots of $P(\lambda)$ with positive real parts must occur in complex conjugate pairs λ and $\bar{\lambda}$.

As before, we view the eigenvalues of \mathcal{L} , λ_k for $1 \leq k \leq n$, as functions of the parameter set $\kappa = (\kappa_1, \dots, \kappa_n)$ with Φ constant. It is always possible to arrange the eigenvalues such that

$$\begin{aligned} \operatorname{Im} \lambda_k(\kappa) &\geq 0 & \text{if } 1 \leq k < \frac{n}{2} + 1 \\ \operatorname{Im} \lambda_k(\kappa) &\leq 0 & \text{if } \frac{n}{2} + 1 \leq k \leq n \end{aligned}$$

and

$$\begin{aligned} \operatorname{Re} \lambda_{k_1}(\kappa) &\geq \operatorname{Re} \lambda_{k_2}(\kappa) & \text{if } 1 \leq k_1 < k_2 < \frac{n}{2} + 1 \\ \operatorname{Re} \lambda_{k_3}(\kappa) &\leq \operatorname{Re} \lambda_{k_4}(\kappa) & \text{if } \frac{n}{2} + 1 \leq k_3 < k_4 \leq n \end{aligned}$$

If \mathcal{L} has any eigenvalues with positive real parts, they must include $\lambda_1(\kappa)$ and $\lambda_n(\kappa)$. Define

$$\mathcal{L} = \{2, 3, \dots, n - 1\}$$

If the steady state is unstable, then the stable manifold \mathcal{S} is tangent to a linear space L contained in the linear space spanned by $\operatorname{Re} w_l$ and $\operatorname{Im} w_l$, where w_l is the eigenvector of \mathcal{L} corresponding to the eigenvalue λ_l with $l \in \mathcal{L}$.

Following Hastings *et al.*, we prove:

Lemma 2. Suppose that all the eigenvalues of \mathcal{L} are simple. Suppose that $l \in \mathcal{L}$. If λ_l and w_l are real, then w_l points into $\bar{P} - \bar{V}$. If λ_l and w_l are complex, then $\operatorname{Re} w_l$ and $\operatorname{Im} w_l$ point into $\bar{P} - \bar{V}$.

PROOF. Consider $u_l = \operatorname{Re} w_l$, w_l real or complex. We must determine as closely as possible the signs of the components u_l^i of u_l . These will be determined by the arguments ψ_l^i of the components w_l^i of w_l . From the equation $(\mathcal{L} - \lambda_l I)w_l = 0$ and the form of \mathcal{L} , it is apparent that

$$(\lambda_l + \kappa_{j+1})w_l^{j+1} = d_j w_l^j, \quad 1 \leq j \leq n - 1$$

Hence, not restricting ψ_l^j to $[0, 2\pi]$, we can assume that

$$\psi_l^j = \psi_l^{j+1} + \theta_l^{j+1} \quad (\text{A4})$$

where θ_l^{j+1} is the argument of $\lambda_l + \kappa_{j+1}$. It can be assumed further that $\operatorname{Im} \lambda_l \geq 0$, from which $0 \leq \theta_l^j \leq \pi$. We can also take $u_l^1 = w_l^1 = -\Phi$ and $\psi_l^1 = \pi$.

From (A3) we have

$$-\Phi = \prod_{j=1}^n (\lambda_l + \kappa_j) = \left(\prod_{j=1}^n |\lambda_l + \kappa_j| \right) e^{i\pi\theta_l(\kappa)}$$

where

$$\theta_l(\kappa) = \frac{1}{\pi} \sum_{j=1}^n \theta_l^j(\kappa)$$

Obviously, $\theta_l(\kappa)$ is continuous and integer-valued, and thus $\theta_l(\kappa) = \text{constant}$. Evaluating $\theta_l(\kappa)$ at the convenient point $\kappa = (1, 1, \dots, 1)$, we find

$$\sum_{j=1}^n \theta_l^j(\kappa) = (2l + 1)\pi \quad (\text{A5})$$

Let $a = a_1 \dots a_n$ be any binary number such that

$$\begin{aligned} a_i &= 0 && \text{if } u_l^i < 0 \\ a_i &= 1 && \text{if } u_l^i > 0 \\ (a_i &= 0 \text{ or } 1 && \text{if } u_l^i = 0) \end{aligned}$$

Then by assumption $a_1 = 0$. We must show that $\bar{S}_a \not\subset \bar{V}$. If $\bar{S}_a \subset \bar{V}$, then either $a_i = 0$ for all $i \in [1, n]$, or there exists an $m < n$ such that

$$\begin{aligned} a_i &= 0 && \text{if } 1 \leq i \leq m \\ a_i &= 1 && \text{if } m < i \leq n \end{aligned}$$

If $a_i = 0$, $1 \leq i \leq n$, then each ψ_l^i , $1 \leq i \leq n$, lies in some interval $(\frac{\pi}{2} + 2N\pi, \frac{3\pi}{2} + 2N\pi)$. Then by (A4), either

$$\sum_{j=2}^n \theta_l^j \leq \pi \quad (\text{A6})$$

or $\theta_l^j = \pi$ for some j , $2 \leq j \leq n$. But this implies that λ_l is real and each $\theta_l^j = 0$ or π . Since $\psi_l^1, \dots, \psi_l^n$ are necessarily odd multiples of π , $\theta_l^2 = \dots = \theta_l^n = 0$, and (A6) is still valid. However, (A6) contradicts (A5), since $l \geq 2$ and $0 \leq \theta_l^1 \leq \pi$. Thus u_l does not point into $\bar{S}_{0\dots 0}$.

If $a_i = 0$ for $1 \leq i \leq m$ and $a_i = 1$ for $m < i \leq n$, then

$$\sum_{j=2}^m \theta_l^j \leq \pi \quad (\text{A7})$$

and

$$\theta_l^1 + \sum_{j=m+2}^n \theta_l^j \leq \pi \quad (\text{A8})$$

Since $0 \leq \theta_l^{m+1} \leq \pi$, (A7) and (A8) give $\sum_{j=1}^n \theta_l^j \leq 3\pi$ with equality only if λ_l is real. But if λ_l is real, then $\sum_{j=2}^m \theta_l^j = 0$, and $\sum_{j=1}^n \theta_l^j \leq 2\pi$. In any case, (A5) is violated, and $\bar{S}_a \not\subset \bar{V}$.

For $v_l = \text{Im } w_l$ the proof goes through with little change.

On any solution $x(\tau)$ tending to x^* as $\tau \rightarrow \infty$, we can locate a sequence of points $x(\tau_j)$, $\tau_j \rightarrow \infty$, which lie on a curve tangent to some u_k or v_k , $2 \leq k \leq n/2$. Thus if a solution $x(\tau)$ which starts in \bar{V} were to approach x^* as $\tau \rightarrow \infty$, then Lemma 2 implies that $x(\tau)$ would have to leave \bar{V} . This is impossible, because \bar{V} is a positively invariant set. Having eliminated possibility (a), we can conclude that any solution of Eq. (A2) that starts in \bar{V} must circulate forever through the $2n$ boxes making up \bar{V} without approaching x^* as $\tau \rightarrow \infty$ (provided that x^* is unstable). That is to say, whenever the steady state of a negative feedback loop is unstable, there exist nondamped, oscillatory solutions.

Appendix B: Periodic Solutions of Negative Feedback Loops with a Step Function Nonlinearity

The equation

$$\begin{aligned} dx_1/d\tau &= f(x_n) - \kappa_1 x_1 \\ dx_j/d\tau &= x_{j-1} - \kappa_j x_j, \quad 2 \leq j \leq n \end{aligned} \quad (\text{B1})$$

with

$$f(x_n) = \begin{cases} 1, & \text{if } 0 < x_n < 1 \\ 0, & \text{if } 1 < x_n < \infty \end{cases} \quad (\text{B2})$$

can be solved exactly because in each of the two halves of phase space ($x_n < 1$ and $x_n > 1$) Eq. (B1) is linear and the solution can be expressed

in terms of the eigenvalues and eigenvectors of the matrix:

$$L = \begin{bmatrix} -\kappa_1 & 0 & 0 & \cdots & 0 & 0 \\ 1 & -\kappa_2 & 0 & \cdots & 0 & 0 \\ 0 & 1 & -\kappa_3 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 1 & -\kappa_n \end{bmatrix}. \quad (B3)$$

To illustrate the technique we treat the special case $n = 3$ and $\kappa_1 = \kappa_2 = \kappa_3 = \kappa$. For $x_3 > 1$ the general solution of the homogeneous linear ordinary differential equation is

$$\begin{pmatrix} x_1(\tau) \\ x_2(\tau) \\ x_3(\tau) \end{pmatrix}_h = e^{-\kappa\tau} \left\{ a_3 \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} + a_2 \left[\begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \tau + \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix} \right] + a_1 \left[\begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \frac{\tau^2}{2} + \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix} \tau + \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} \right] \right\} \quad (B4)$$

which satisfies the initial condition

$$\begin{pmatrix} x_1(0) \\ x_2(0) \\ x_3(0) \end{pmatrix} = \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix} \quad (B5)$$

For $x_3 < 1$ the solution of the nonhomogeneous equation is simply

$$\begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix} = \begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix}_h + \begin{pmatrix} \kappa^{-1} \\ \kappa^{-2} \\ \kappa^{-3} \end{pmatrix} \quad (B6)$$

To find a periodic solution we start with the initial condition

$$\begin{pmatrix} x_1(0) \\ x_2(0) \\ x_3(0) \end{pmatrix} = \begin{pmatrix} a_1 \\ a_2 \\ 1 \end{pmatrix}, \quad a_1 > \kappa^2, \quad a_2 > \kappa \quad (B7)$$

The conditions on a_1 and a_2 assure that $x_3 > 1$ for $\tau = 0^+$. Thus Eq. (B4) holds for $0 < \tau < \tau_1$:

$$\begin{aligned} x_1(\tau) &= a_1 e^{-\kappa\tau} \\ x_2(\tau) &= (a_2 + a_1\tau)e^{-\kappa\tau} \\ x_3(\tau) &= (1 + a_2\tau + \frac{1}{2}a_1\tau^2)e^{-\kappa\tau} \end{aligned} \quad (B8)$$

where τ_1 is the time of first return of x_3 to $x_3 = 1$. At $\tau = \tau_1$

$$e^{\kappa\tau_1} = 1 + a_2\tau_1 + \frac{1}{2}a_1\tau_1^2 \quad (B9)$$

which determines $\tau_1 = \tau_1(a_1, a_2)$. Furthermore, at $\tau = \tau_1$,

$$\begin{aligned} x_1(\tau_1) &= a_1 e^{-\kappa \tau_1} \equiv b_1(a_1, a_2) < \kappa \\ x_2(\tau_1) &= (a_2 + a_1 \tau_1) e^{-\kappa \tau_1} \equiv b_2(a_1, a_2) < \kappa^2 \end{aligned} \quad (\text{B10})$$

The conditions on b_1 and b_2 assure that $x_3 < 1$ for $\tau = \tau_1^+$. Thus Eq. (B6) holds for $\tau_1 < \tau < \tau_2$:

$$\begin{aligned} \kappa x_1(\tau) &= 1 - (1 - b_1 \kappa) e^{-\kappa \tau} \\ \kappa^2 x_2(\tau) &= 1 - [(1 - b_2 \kappa^2) + \kappa(1 - b_1 \kappa)\tau] e^{-\kappa \tau} \\ \kappa^3 x_3(\tau) &= 1 - [(1 - \kappa^3) + \kappa(1 - b_2 \kappa^2)\tau + \frac{1}{2}\kappa^2(1 - b_1 \kappa)\tau^2] e^{-\kappa \tau} \end{aligned} \quad (\text{B11})$$

where τ_2 is the time of next return of x_3 to $x_3 = 1$. At $\tau = \tau_2$,

$$(1 - \kappa^3) e^{\kappa \tau_2} = (1 - \kappa^3) + \kappa(1 - b_2 \kappa^2)\tau_2 + \frac{1}{2}\kappa^2(1 - b_1 \kappa)\tau_2^2 \quad (\text{B12})$$

and

$$\begin{aligned} x_1(\tau_2) &= \kappa^{-1}[1 - (1 - b_1 \kappa)e^{-\kappa \tau_2}] \equiv c_1(a_1, a_2) > \kappa^2 \\ x_2(\tau_2) &= \kappa^{-2}\{1 - [(1 - b_2 \kappa^2) + \kappa(1 - b_1 \kappa)\tau]e^{-\kappa \tau_2}\} \equiv c_2(a_1, a_2) > \kappa \end{aligned} \quad (\text{B13})$$

A periodic solution satisfies the conditions

$$\begin{aligned} c_1(a_1, a_2) &= a_1 \\ c_2(a_1, a_2) &= a_2 \end{aligned} \quad (\text{B14})$$

Equation (B14) has the trivial solution $c_1(\kappa^2, \kappa) = \kappa^2$, $c_2(\kappa^2, \kappa) = \kappa$, which is just the steady state. It is a simple exercise (Tyson, 1975) to show that (B14) has at least one nontrivial solution as well. For $\kappa = 0.75$ the mapping $(a_1, a_2) \rightarrow (c_1, c_2)$ is illustrated in Fig. 12.

For a three-component system with a step function nonlinearity, Hastings (1977) has recently proved that, for all $\phi \equiv \kappa_1 \kappa_2 \kappa_3 < 1$, every solution except those on the one-dimensional stable manifold tend to some nonconstant periodic solution. Furthermore, for $\phi = \frac{1}{2}$ there is a unique, globally orbitally asymptotically stable periodic solution. The same result applies for p sufficiently large and ϕ sufficiently close to $\frac{1}{2}$.

Dual input describing function analysis. We seek an approximate solution of Eq. (B1) of the form $x_3(\tau) = z_0 + z_1 \cos \omega \tau$; see Section IV,E. For simplicity we require that $z_0(z_1) = 1$ for all $z_1 \in [0, 1]$. Equation (75a) implies that

$$1 = \frac{1}{2\pi\phi} \int_{\pi/2}^{3\pi/2} d\theta = \frac{1}{2\phi}, \quad \phi = \kappa_1 \dots \kappa_n \quad (\text{B15})$$

That $z_0 = 1$ simplifies considerably the describing function $F_1(z_1)$, Eq.

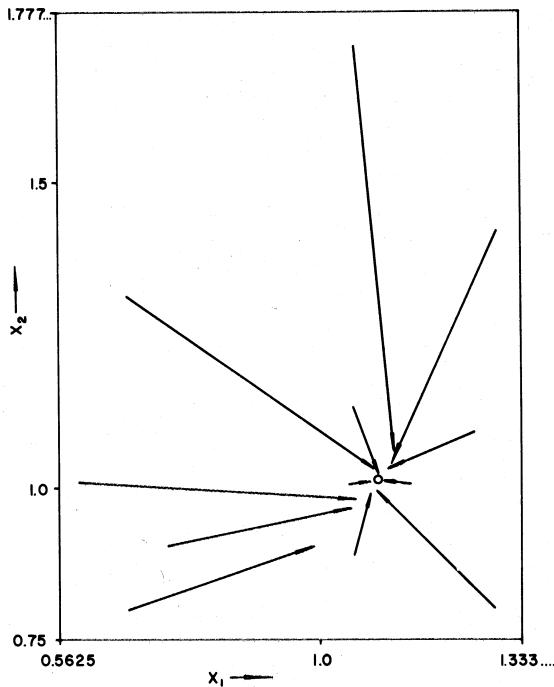


FIG. 12. Poincaré map for system (B.1) and (B.2) with $n = 3$ and $\kappa_1 = \kappa_2 = \kappa_3 = 0.75$. The tail of an arrow is (a_1, a_2) and the head is (c_1, c_2) . (Our thanks to M. Witten for carrying out these calculations.)

(77). Indeed

$$\begin{aligned} F_1(z_1) &= \frac{1}{\pi z_1} \int_{\pi/2}^{3\pi/2} \cos \theta \, d\theta \\ &= -\frac{2}{\pi z_1}, \quad z_1 \in [0, 1] \end{aligned} \tag{B16}$$

To simplify matters further we assume that $\kappa_1 = \dots = \kappa_n = \kappa$. By Eq. (B15) we have

$$\kappa^n = \frac{1}{2} \tag{B17}$$

and furthermore,

$$G(i\omega) = -(\kappa + i\omega)^{-n} = -2 \frac{(1 - i\bar{\omega})^n}{(1 + \bar{\omega}^2)^n} \tag{B18}$$

where $\bar{\omega} = \omega/\kappa$. From Eq. (33) we derive

$$\bar{\omega}_0 = \tan(\pi/n) \quad (B19)$$

and

$$G(i\omega_0) = 2(1 + \bar{\omega}_0^2)^{-n/2} \quad (B20)$$

For a periodic solution to exist, Eq. (78), we must have

$$2[1 + \tan^2(\pi/n)]^{-n/2} \leq \pi/2 \quad (B21)$$

This is satisfied for all $n \leq 20$. We choose $n = 3$. Equation (76) predicts a periodic solution of amplitude

$$z_1 = \frac{1}{2}\pi \approx 0.159 \quad (B22)$$

Thus

$$x_3(\tau) \approx 1 + 0.159 \cos \omega_0 \tau, \quad (B23)$$

where $\omega_0 = \sqrt{3}\kappa \approx 1.375$.

To check the validity of approximation (B23), we compare it with the exact solution derived earlier. For $\kappa^{-3} = 2$ —that is, $\kappa \approx 0.794$ —Eq. (B14) is satisfied for $a_1 \approx 1.08$, $a_2 \approx 1.02$. Equation (B9) has the root $\tau_1 \approx 2.30$, for which $b_1 \approx 0.174$, $b_2 \approx 0.565$, and Eq. (B12) has the root $\tau_2 \approx 2.31$. The period of the exact solution, $T = \tau_1 + \tau_2 \approx 4.61$, compares well with the period $T = 2\pi/\omega_0 \approx 4.57$ of Eq. (B23). For that half of the oscillation for which $x_3(\tau) \geq 1$, we have

$$\begin{aligned} x_1(\tau) &= 1.08e^{-\kappa\tau} \\ x_2(\tau) &= (1.02 + 1.08\tau)e^{-\kappa\tau} \\ x_3(\tau) &= (1 + 1.02\tau + 0.54\tau^2)e^{-\kappa\tau}, \quad 0 \leq \tau \leq 2.30 \end{aligned} \quad (B24)$$

with $\kappa \approx 0.794$. For the analogous section of the approximate solution

$$\begin{aligned} x_1(\tau) &= 0.630 - 0.201 \cos \omega_0 \tau - 0.347 \sin \omega_0 \tau \\ x_2(\tau) &= 0.794 + 0.126 \cos \omega_0 \tau - 0.219 \sin \omega_0 \tau \\ x_3(\tau) &= 1 + 0.159 \cos \omega_0 \tau, \quad -\pi/2\omega_0 \leq \tau \leq +\pi/2\omega_0 \end{aligned} \quad (B25)$$

with $\omega_0 \approx 1.37$. Equations (B24) and (B25) are plotted in Fig. 13. They differ by no more than 1% for $x_3(\tau)$, they agree fairly well on $x_2(\tau)$, but the approximation to $x_1(\tau)$ is much poorer.

Finally, to illustrate our remarks in Section IV,E on the neglect of higher harmonics in the DIDF method, we estimate $|z_k|$, for $k \geq 2$. For $f(x_n)$ given by Eq. (B2) and $x_n(\tau)$ given by Eq. (B23) we derive

$$f[x_n(\tau)] = \frac{1}{2} + \sum_{k \text{ odd}} (-1)^{(k+1)/2} \frac{2}{k\pi} \cos(k\omega_0 \tau) \quad (B26)$$

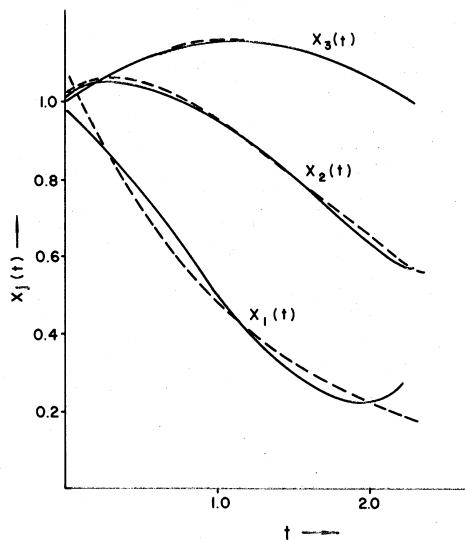


FIG. 13. Comparison of DIDF solution with exact solution of negative feedback loop with step function nonlinearity. The solid line (—) is the DIDF approximation, Eq. (B.25), and the dashed line (---) is the exact solution, Eq. (B.24). Results are given for only half of an oscillation.

Furthermore, from Eqs. (B23) and (B24),

$$G(ik\omega_0) = 2(1 + 3k^2)^{-3/2} \quad (\text{B27})$$

In Table VII we list $|a_k|$, $|G(ik\omega_0)|$, and $|z_k| = |G(ik\omega_0)| \cdot |a_k|$ as functions of k . Notice how quickly $|z_k|$ drops off. For k large, $|z_k| \sim 4\sqrt{3}/9\pi k^4$.

TABLE VII
JUSTIFICATION OF THE NEGLECT OF HIGHER
HARMONICS IN EQ. (72)^a

k	$ a_k $	$ G(ik\omega_0) $	$ z_k $
0	0.5	2.0	1.0
1	0.637	0.25	0.159
2	0	0.0427	0
3	0.212	0.0135	0.00287
4	0	0.00583	0
5	0.127	0.00302	0.000384
6	0	0.00176	0
7	0.091	0.00111	0.000101

^a Calculations based on Eqs. (B26) and (B27).

Notation (in order of appearance)

S	concentration of effector substance
R	concentration of free repressor molecules
O	concentration of free operator regions
p	number of binding sites for effector on a repressor molecule
K_1, K_2	equilibrium constants for binding of effector to repressor and of repressor to operator, respectively
R_t	total concentration of repressor
O_t	total concentration of operator regions (bound and free)
$f(S) = O/O_t$	fraction of total operator regions that are free of repressor and thus permit transcription of structural genes
K	$1 + K_2 R_t$
V, V_{\max}	reaction velocity
$K_{0.5}$	substrate concentration at half-maximum velocity
n_H	Hill exponent
I	concentration of inhibitor
S_1, S_2	concentration of mRNA in nucleus and cytoplasm, respectively
S_3	concentration of unstable enzyme
S_4, \dots, S_{n-1}	concentration of intermediates in biosynthetic pathway
S_n, S_{n+1}	concentration of end product in cytoplasm and nucleus, respectively
$k_i, \hat{k}_i, 1 \leq i \leq n+1$	first-order rate constants
\bar{C}	average intranuclear concentration of ribonucleotide triphosphates
k_0	first-order rate constant for incorporation of mononucleotides into growing mRNA chain
$\mathcal{R}(\cdot) = k_0 \bar{C} f(\cdot)$	rate of formation of mRNA
$T_1(S_1, S_2), T_n(S_n, S_{n+1})$	rate of transport of mRNA and of end product, respectively, between nucleus and cytoplasm
A_N	surface area of nucleus
V_N, V_C	volume of nucleus and cytoplasm, respectively
$x_i = a_i S_i, 1 \leq i \leq n$	dimensionless concentration variables
b	parameter defined at Eq. (10)
$\tau = bt$	dimensionless time variable
$\kappa_i = k_i/b, 1 \leq i \leq n$	dimensionless rate ("decay") constants
$f(x_n) = f(S_n)$	fraction of free operator regions
\mathbf{x}^*	dimensionless steady-state concentration
$\phi = \prod_{i=1}^n \kappa_i$	steady-state parameter
$\xi = \mathbf{x} - \mathbf{x}^*$	deviation from steady state
L	matrix of the linear part of the feedback loop
$\mathcal{L} = \mathbf{f}'_{\mathbf{x}}(\mathbf{x}^*)$	Jacobian of $\mathbf{f}(\mathbf{x})$ evaluated at $\mathbf{x} = \mathbf{x}^*$
T	transpose of a vector
$\mathbf{b}^T = (\pm 1, 0, \dots, 0)$	standard vectors
$\mathbf{c}^T = (0 \dots 0, 1)$	
$\mathcal{F}(\xi_n)$	nonlinear function defined by Eq. (15)
$g(\mathbf{c}^T \xi)$	strictly nonlinear part of $f(x_n)$
$\ \cdot\ $	the Euclidean norm

$P(\lambda) = \det (\lambda I - L)$	characteristic polynomial of \mathcal{L}
I	identity matrix
$\rho_{\pm j}$, $1 \leq j \leq n$	n th roots of ± 1
λ_k, w_k , $1 \leq k \leq n$	eigenvalues and eigenvectors, respectively, of \mathcal{L}
sector $[0, \Omega]$	subset of the plane which contains the graph of $\mathcal{F}(\xi_n)$
$G(\lambda)$	Nyquist function characterizing L
ω_0	smallest value of $\omega > 0$ such that $\text{Im } G(i\omega) = 0$
Ω_c	critical gain of L
$p_0 = p_0(n)$	critical value of the feedback parameter
$\kappa_0 = \kappa(p, n)$	critical value of the decay constant
$\phi_0 = \phi_0(p, n)$	critical value of the steady-state parameter
μ	bifurcation parameter
u, v	left and right eigenvectors of \mathcal{L}_0 corresponding to the purely imaginary eigenvalue $i\omega_0$
z	
$\tilde{x}_n(\tau)$	last component of periodic solution $\tilde{x}(\tau)$ of $x' = f(x)$
z_k , $1 \leq k < \infty$	coefficients in Fourier expansion of $\tilde{x}_n(\tau)$
a_k , $1 \leq k < \infty$	coefficients in Fourier expansion of $f[\tilde{x}_n(\tau)]$
$F_1(z_1) = a_1(z_1)/z_1$	describing function
\mathcal{D}	matrix of diffusion coefficients
∇^2	Laplacian operator
V	region of three-dimensional space

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