Optimal Design of Feedback Control by Inhibition
Dynamic Considerations*

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Summary. The local stability of unbranched biosynthetic pathways is exam-
ined by mathematical analysis and computer simulation using a novel non-
linear formalism that appears to accurately describe biochemical systems.
Four factors affecting the stability are examined: strength of feedback
inhibition, equalization of the values among the corresponding kinetic
parameters for the reactions of the pathway, pathway length, and alter-
native patterns of feedback interaction. The strength of inhibition and
the pattern of feedback interactions are important determinants of
steady-state behavior. The simple pattern of end-product inhibition in
unbranched pathways may have evolved because it optimizes the steady-
state behavior and is temporally most responsive to change. Stability in
these simple systems is achieved by shortening pathway length either
physically or, in the case of necessarily long pathways, kinetically by
a wide divergence in the values of the corresponding kinetic parameters
for the reactions of the pathway. These conclusions are discussed in the
light of available experimental evidence.

INTRODUCTION

Of all biochemical control systems, the unbranched pathways
for the biosynthesis of amino acids and nucleotides in micro-
organisms are the most thoroughly studied at the molecular
level. For several of these pathways, e.g. tryptophan

(Creighton & Yanofsky, 1970) and histidine (Brenner & Ames, 1971), all of the molecular components have been studied and kinetically characterized to some degree. Despite our rather extensive knowledge at the molecular level, we are still relatively ignorant of the behavior of the intact system as it operates in the cell. We are only now beginning to understand the principles behind the design of these marvelous control systems.

In the previous study of the steady-state behavior of these types of systems it was found that the experimentally observed pattern of end-product inhibition tends to be optimal with respect to a variety of functional criteria (Savageau, 1974). This is consistent with the postulate of optimality as the basis for selection (Rosen, 1967). One would expect these control systems to have been selected also for local stability, since presumably one of the prime functions of the amino acid biosynthetic pathways is to provide a relatively constant supply of their end-products for protein synthesis.

The stability of linear models of chemical reaction networks was examined by several authors (e.g., see Hearon, 1953; Bak, 1963) before the general molecular architecture of control patterns in biosynthetic pathways had been discovered and appreciated. Consequently, the biological questions raised by more recent discoveries were not specifically addressed in these early analyses. Others have used mathematical models to investigate the conditions for stable oscillatory behavior in the circuits for enzyme synthesis as well as in biosynthetic pathways subject to feedback control by inhibition (Goodwin, 1963; Morales & McKay, 1967; Viniegra-Gonzalez & Martinez, 1969; Walter, 1970). For these models a particular approximation to the actual nonlinear systems is used, the so-called Goodwin equations. All of the nonlinearity is lumped into the description of the first or allosteric enzyme. The remainder of the system is assumed to be linear, composed of simple first-order reactions with identical kinetic parameters. Even with these simplifying assumptions, the resulting nonlinear model is difficult to analyze mathematically and much of the information has been gained by computer simulation. Recently, Hunding (1974) has reexamined the original analysis of Viniegra-Gonzalez & Martinez (1969) and shown that theirs is not a necessary condition for the existence of an unstable steady state or a stable limit-cycle. The same criticism applies to the independent derivation of this condition by Higgins et al. (1973). The more recent analysis by Viniegra-Gonzalez (1973), however, avoids these difficulties for the most part. Hunding (1974) and Hastings & Tyson

(1975) also have reexamined the computer simulation results of Walter and discounted some of his claims. Walter (1974) has gone on to simulate a modified Goodwin model in which the unregulated reactions are represented by specific "hyperbolic" or "sigmoidal" rate laws.

The power-law formalism used in this paper represents an alternative approach to the analysis of biochemical systems. Starting with the observation that biological systems are composed of networks of enzyme-catalyzed reactions and using the well-established postulates of enzyme catalysis, one can in principle write differential equations with rational function nonlinearities for an arbitrary system. It is practically impossible to deal with these equations in any general sense. Nevertheless, one can use a logical approach analogous to linearization and derive a nonlinear approximation to the original equations that involves multidimensional power-laws. This representation is guaranteed to accurately represent the original system over an appropriate range of the dynamic variables in the same manner that a linearization will, except that the appropriate range is greater than that for any linear approximation (Savageau, 1969).

The dynamic aspects of optimal control by feedback inhibition in biosynthetic pathways, the integration of these properties with the steady-state properties described in the previous paper (Savageau, 1974), and correlations with experimental data will be considered in this paper. For this purpose I shall assume that these systems normally operate in a stable steady state and focus on the conditions for local stability. Analytical techniques for examining local stability in terms of the parameters of the power-law formalism are presented in the Appendix. These techniques, along with computer simulations, are used to examine four factors affecting stability: (i) strength of feedback inhibition, (ii) degree of equality among the corresponding kinetic parameters of the unregulated reactions in the pathway, (iii) number of reactions in the pathway, and (iv) alternative patterns of feedback inhibition. Finally, the results are compared and found to agree with available experimental evidence, which is consistent with the assumption that local stability is an important factor in metabolic regulation and provides insight into how such stability is achieved in nature.

STRENGTH OF FEEDBACK INHIBITION AND STABILITY

The pathway represented in Fig.1 will be analyzed for local stability first when n=3. We shall derive an expression for the threshold of stability which is a function of the para-
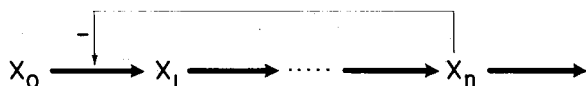
Fig.1. Model of an unbranched biosynthetic pathway. X_i's represent metabolite concentrations, the arrows from one X_i to another represent enzyme catalyzed reactions, and the arrow from X_n to the center of the arrow representing the first reaction denotes an allosteric modulation of the first reaction by the end-product X_n. The initial substrate X_0 is subject to independent experimental manipulation

meters of the system. The equations describing this system in the power-law formalism are

$$(1) \qquad \dot{X}_1 = \alpha_1 X_0^{g_{10}} X_3^{g_{13}} - \beta_1 X_1^{h_{11}}$$

$$\dot{X}_2 = \alpha_2 X_1^{g_{21}} - \beta_2 X_2^{h_{22}}$$

$$\dot{X}_3 = \alpha_3 X_2^{g_{32}} - \beta_3 X_3^{h_{33}}$$

The rate law for each reaction is represented by a product of power-laws, one for each of the reactants and modifiers associated with the reaction. The exponent g_{ij} represents the apparent kinetic order with respect to X_j, for the synthesis of X_i. α_i is the apparent rate constant for this reaction. When a rate law appears in a degradative term of the system's equations, the corresponding parameters are h_{ij} and β_i, respectively. Since the rate of degradation of X_i is the same as the rate of synthesis of X_{i+1} in Fig.1, we have the following equalities:

$$\alpha_2 = \beta_1 \quad ; \quad g_{21} = h_{11}$$

$$\alpha_3 = \beta_2 \quad ; \quad g_{32} = h_{22}$$

All the parameters in Eq.(1) are positive except for g_{13}; it represents the apparent kinetic order of the first reaction with respect to the end-product inhibitor and is therefore negative. X_0 is an independent concentration variable.

The linearized equations describing the behavior of this system for small variations about the normal operating point are readily obtained from Eq.(1) by using the techniques developed in the Appendix.

(2) $\dot{u}_1 = F_1 \left[-h_{11}u_1 + g_{13}u_3 \right]$

$\dot{u}_2 = F_2 \left[h_{11}u_1 - h_{22}u_2 \right]$

$\dot{u}_3 = F_3 \left[h_{22}u_2 - h_{33}u_3 \right]$

The characteristic equation for this system is

(3) $\lambda^3 + (F_1 h_{11} + F_2 h_{22} + F_3 h_{33}) \lambda^2 +$

$+ (F_1 F_2 h_{11} h_{22} + F_1 F_3 h_{11} h_{33} + F_2 F_3 h_{22} h_{33}) \lambda +$

$+ F_1 F_2 F_3 h_{11} h_{22} (h_{33} - g_{13}) = 0$

or

$\lambda^3 + \phi_1 \lambda^2 + \phi_2 \lambda + \phi_3 = 0$

From Routh's criterion for stability the critical condition among these coefficients is $\phi_1 \phi_2 > \phi_3$, which implies

(4) $-g_{13} < \left[2 + \dfrac{F_1 h_{11}}{F_2 h_{22}} + \dfrac{F_2 h_{22}}{F_1 h_{11}} + \dfrac{F_1 h_{11}}{F_3 h_{33}} + \dfrac{F_3 h_{33}}{F_1 h_{11}} + \right.$

$\left. + \dfrac{F_2 h_{22}}{F_3 h_{33}} + \dfrac{F_3 h_{33}}{F_2 h_{22}} \right] h_{33}$

This equation defines the conditions that must exist among the parameters in order for the system to be stable. If a change in the strength of inhibition g_{13} were accompanied by a compensatory change in the apparent rate constant α_1, then the steady-state rate of the first reaction would be unaffected by the change in g_{13}. In this way the stability of the same steady-state operating value can be examined in systems with different strengths of inhibition but otherwise identical. As the magnitude of g_{13} is increased in this fashion,
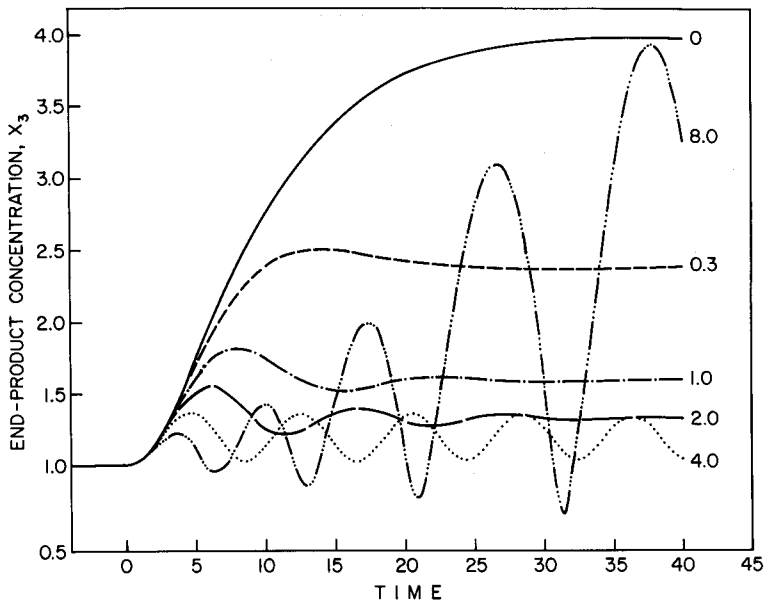
Fig.2. Strength of feedback inhibition and stability. The model in Fig.1 is simulated with n=3 and various strengths of inhibition $(-g_{13})$ as indicated. The threshold value of $-g_{13}$ for instability is about 4. The independent concentration variable X_O is perturbed at t=0. See text for further discussion.

the threshold for instability is approached and then exceeded.

This is graphically illustrated for the nonlinear system by a computer simulation in Fig.2. The method of simulation using the power-law formalism has been previously described (Savageau, 1970). Each curve in this figure represents the concentration of the end-product X_3 as a function of time after a perturbation in the level of the initial substrate X_O. The system is in a steady state with the concentrations normalized before the disturbance. At t=0 the initial substrate is increased and maintained at this elevated level during the subsequent time period. Each response represents the system having different strengths of end-product inhibition g_{13}. The magnitude of g_{13} in each case is shown in association with the appropriate temporal response. The case with $g_{13} = 0$ represents the pathway without inhibition.

As the strength of inhibition is increased, the overall gain is decreased (i.e., the new steady state differs less and less from the predisturbance steady state). However, in approaching the new steady state the system begins to show more and more overshoot and eventually exhibits oscillations. With a further increase in the strength of inhibition, the
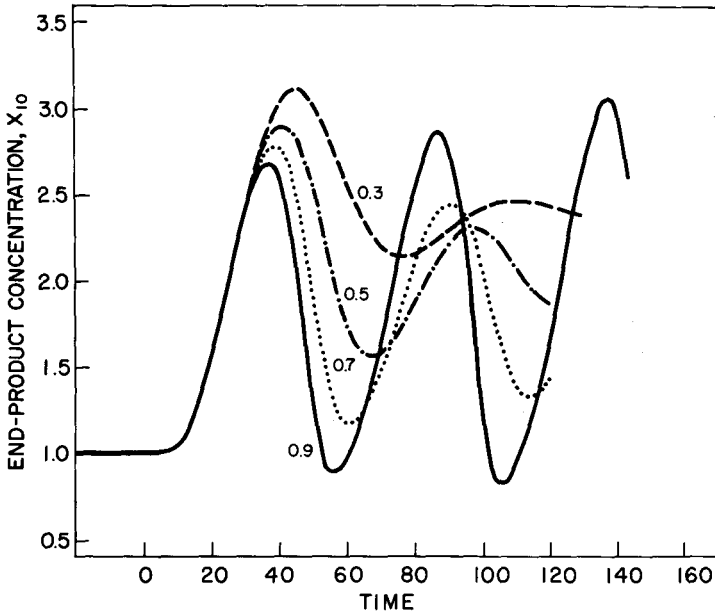
Fig.3. Strength of feedback inhibition and stability. The model in Fig.1 is simulated with n=10, and for various strengths of inhibition as in the previous figure. The threshold value of $-g_{1,10}$ for instability in this case is about 0.8

system becomes unstable and oscillates with increasing ampli-
tude. At this point, the system ceases to regulate the supply
of the end-product. Of course in any real system the concen-
trations would reach a limit for other physical reasons, but
the conclusion remains unchanged: the system is no longer
effectively regulated by feedback inhibition.

A necessary limitation on the strength of inhibition to
ensure stability is found for more general pathways than the
n=3 case we have been discussing. To illustrate just one such
case, I have shown in Fig.3 a nonlinear system identical to
the previous one except that n=10. The behavior is very simi-
lar to that already described in Fig.2. However, the threshold
value of $g_{1,10}$ which is necessary for instability is clearly
different from that for g_{13} in the previous case. We can con-
clude that, in general, there is a limit to the strength of
inhibition for a stable system and this limit depends in part
on the length of the pathway. The interrelationship between
strength of inhibition and pathway length will be more fully
explored in a later section.

Let us return for the moment to the case with n=3 and examine a second important feature of Eq.(4): namely, the effect of the other kinetic parameters in determining the threshold of instability. First, it will be helpful to emphasize the meaning of the kinetic parameters in Eq.(4). The F_i's are relatively complex expressions of the apparent kinetic orders and rate constants for the system (see Appendix). Nevertheless, they have a rather simple interpretation for the systems we are considering. F_i is the ratio of the steady-state rate for the pathway to the concentration of X_i in steady state. Consequently, if the K_M for the ith intermediate reaction is doubled, the substrate X_i for that reaction will increase until it is also doubled and the steady-state rate for the pathway is reestablished. The corresponding value for F_i is therefore halved by this doubling of the ith K_M while all other parameters remain unchanged. In other words,

$$(5) \qquad F_i = \frac{\text{steady-state rate}}{X_{i0}} \propto \frac{1}{K_{M_i}}$$

It is important to emphasize that for the pathway in steady state this common type of enzyme modification only affects the corresponding F_i parameter and not the apparent kinetic order h_{ii}. However, a change in the molecular activity of an enzyme can be reflected in changes of both F_i and h_{ii}.

From Eq.(4) it is clear that the system in Fig.1 can be made more stable by increasing the differences in value among the corresponding kinetic parameters for each reaction. Conversely, as the corresponding kinetic parameters become more nearly equal, the system becomes more unstable. The minimum value for any pair of terms in Eq.(4) such as $(F_1 h_{11})/(F_2 h_{22})$ and $(F_2 h_{22})/(F_1 h_{11})$ is 2; this can occur when the two steps have identical kinetic parameters. Therefore,

$$(6) \qquad -g_{13} < 8\,h_{33}$$

is a sufficient condition to ensure stability of the system even when the corresponding kinetic parameters for all the remaining reactions are identical. Independently, Viniegra-Gonzalez (1973) carried out a similar analysis of the Goodwin model.

This effect, and the strength of inhibition effect discussed in the previous section, are closely related by means
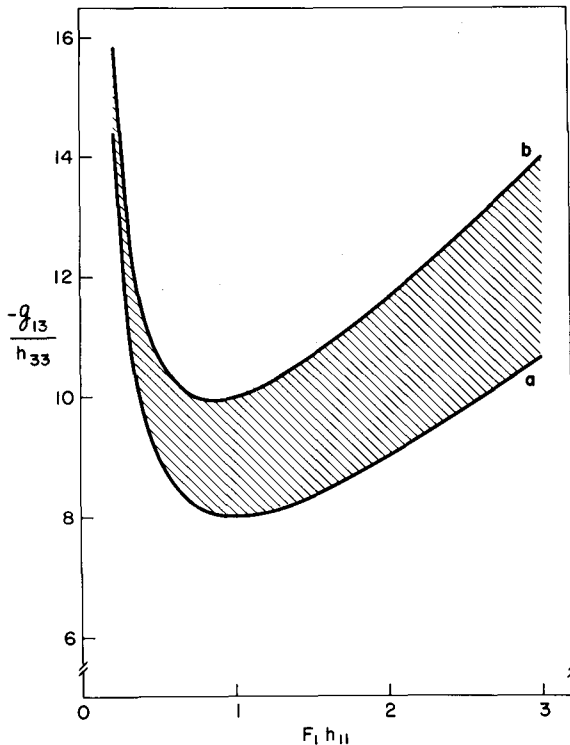
Fig.4. The threshold of stability as a function of the parameter values. Equality in Eq.(4) represents the threshold of stability and when $-g_{13}/h_{33}$ is plotted as a function of F_1h_{11}, the region of stability lies below the curve. (a) $F_2h_{22} = F_3h_{33} = 1$.

(b) $F_2h_{22} = 0.5$ and $F_3h_{33}=1.5$. The hyperbolic nature of these curves indicates that values of F_1h_{11} different from the other parameters allow a greater strength of inhibition for stability. The shaded area indicates the increase in the region of stability when F_2h_{22} and F_3h_{33} also differ

of Eq.(4). This equation shows that an unstable system can be stabilized by either decreasing the magnitude of g_{13} or increasing the differences in value among the corresponding kinetic parameters for the other reactions. The effect of parameter "mismatch" in promoting stability also can be shown by plotting the threshold of stability in the parameter space and showing that the region of stability increases when the parameters differ. This is done in Fig.4, where the shaded area represents the increased region for stability when the parameters F_2h_{22} and F_3h_{33} differ.

The stabilizing effect of unequal values among the corresponding kinetic parameters is not restricted to systems with n=3. The analytical treatment of cases with n > 3 is, however, much more difficult. Computer simulation of these systems provides valuable insight, as can be seen for the case with n=5 depicted in Fig.5. We begin with a matched system that is unstable (a); progressive stabilization is achieved as more and more of the corresponding kinetic parameters are made to differ, (b) and (c). Many other specific examples could be represented and the conclusion is the same: inequality among the corresponding kinetic parameters tends to promote the stability of the system.
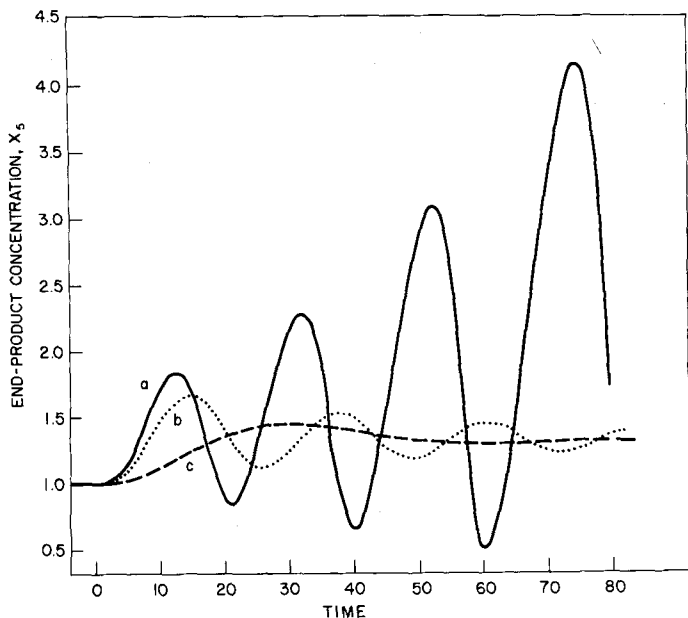
Fig.5. The stabilizing effect of unequal kinetic parameters. The model in Fig.1 is simulated, as in Fig.2, except with n=5 and various values of the K_M's for the last four reactions in the pathway. Relative values of the last four K_M's: (a) 1,1,1,1; (b) 4,1,1,0.25; (c) 16,4,0.25,0.0625

The generality of this conclusion can be seen from a physical argument, which also illuminates the basis for the effect. Consider the situation where the corresponding kinetic parameters of the reactions in a sequence are sufficiently different in value that some of the reactions occur with a much faster rate. From the kinetic point of view, the pathway may be considered to have fewer reactions than it actually does, and we shall see in the next section, this decrease in the effective length of the pathway leads to a more stable system.

PATHWAY LENGTH AND STABILITY

A general analytical expression representing the threshold for stability as a function of the path length n is difficult to obtain. However, as was indicated in the preceding section, a pathway with a suitable variation among the corresponding kinetic parameters of the individual reactions can be considered as a shorter length pathway for kinetic purposes. By eliminating the faster reactions from consideration the kinetic parameters for the remaining reactions in the pathway tend to be more nearly equal. Thus, for the purposes of this

section we need only consider pathways in which the corresponding kinetic parameters of each reaction are equal. Under these conditions, stability can be easily determined by direct examination of the roots of the characteristic equation.

The characteristic equation for the general system in Fig. 1 is obtained from the following determinant equation:

$$(7) \quad \begin{vmatrix} -(Fh + \lambda) & O & O & \cdots & Fg_{1n} \\ Fh & -(Fh + \lambda) & O & & O \\ O & Fh & -(Fh + \lambda) & & O \\ \cdot & & & \cdot & \cdot \\ \cdot & & & \cdot & \cdot \\ \cdot & & & \cdot & \cdot \\ O & O & O \cdots Fh & - & (Fh + \lambda) \end{vmatrix} = O$$

Since all the kinetic parameters of the reactions after the first are identical, this can be expanded to give

$$(8) \quad (Fh + \lambda)^n - \frac{g_{1n}(Fh)^n}{h} = O$$

where g_{1n} is the only negative parameter. The roots of Eq.(8) are obtained by solving directly for λ. The real parts of all the characteristic values must be negative for stability. Thus, $Re(\lambda_m) < O$ implies that

$$(9) \quad (-g_{1n}/h)^{1/n} \cos\left(\frac{2m-1}{n}\right)\pi - 1 < O$$

$$m = 1, 2 \ldots, n$$

For a given value of n, the largest value of $\cos\left(\frac{2m-1}{n}\right)\pi$ occurs when m=1, so that the condition for stability is the following:

$$(10) \quad (-g_{1n}/h)^{1/n} \cos(\pi/n) - 1 < O$$

This is always satisfied for n=1 and n=2. For $n \geq 3$ we can write Eq.(10) as

$$(11) \quad -g_{1n} < h \sec^n(\pi/n)$$

An analysis similar to this was carried out for the Goodwin model by Viniegra-Gonzalez & Martinez (1969) and Higgins et al. (1973), and was criticized by Hunding (1974). However,
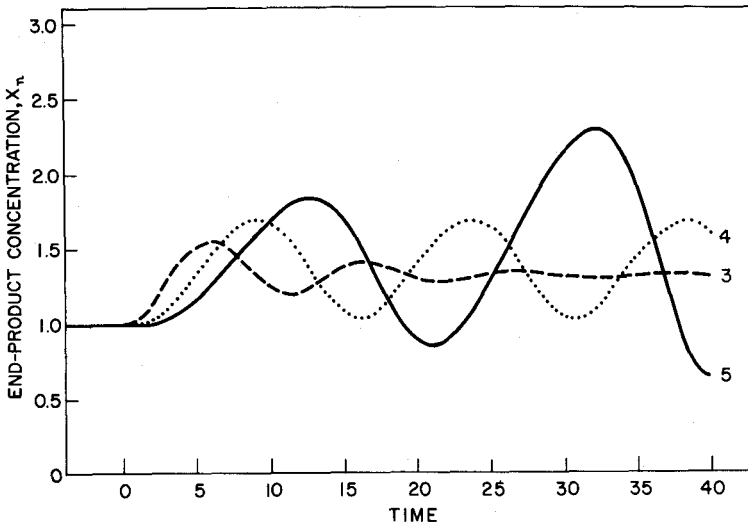
Fig.6. Pathway length and stability. The model in Fig.1 is simulated as in Fig.2, except with a fixed strength of inhibition ($-g_{1n}=2$) and various values of n as indicated. Pathways with $n > 4$ are unstable

his criticism does not apply to the analysis given above because comparisons were made under conditions in which the steady-state solution does not change as the strength of inhibition g_{1n} is changed. For a given strength of inhibition, provided it is greater in magnitude than h, the threshold of instability is approached as n increases. Eventually, for some sufficiently large n the threshold is surpassed and the system becomes unstable.

This can be graphically illustrated for the nonlinear systems by computer simulation as shown in Fig.6. For this simulation g_{1n} has been fixed and the responses of systems with increasing n have been plotted. For lower values of n the systems approach a new steady state with damped oscillations. A threshold is reached when n=4 and the system continuously oscillates. For larger values of n the systems begin to oscillate with increasing amplitude. Fig.7 shows a similar set of simulations for a lower fixed value of g_{1n}. The general behavior is the same except that the threshold value of n for instability is different.

From the above results we conclude that increasing the length of the pathway generally leads to instability of the system. This effect is closely related to the strength of inhibition effect, and this is seen from Eq.(11). Thus, for stability, longer pathways cannot be as strongly inhibited as shorter ones. The maximum strength of inhibition for stability as a function of the path length is shown in Table 1. The asymptotic value of $(-g_{1n}/h)$ is unity as n becomes large.
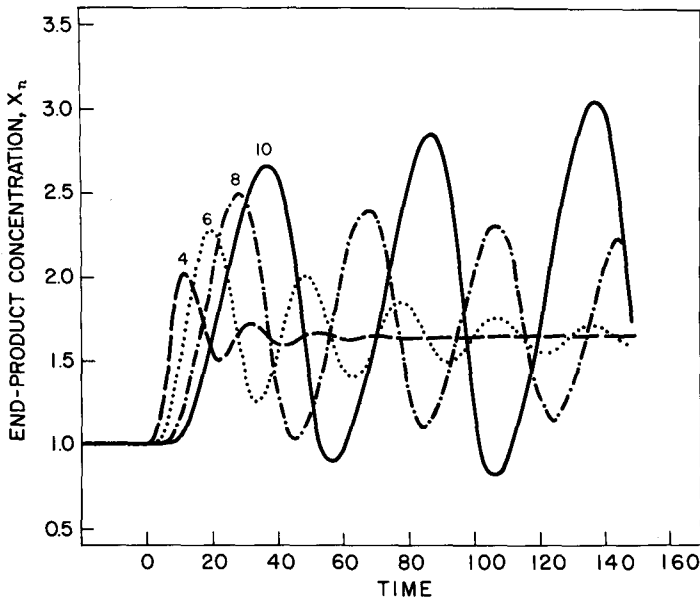
Fig.7. Pathway length and stability. The model in Fig.1 is simulated as in the previous figure, except for the strength of inhibition $-g_{1n}=$ 0.9. For this set of conditions, only pathways with $n > 9$ are unstable

Thus, as long as $|g_{1n}| < h$ the system will always be stable.

Let us now turn to an examination of alternative patterns of control to see what effects these have upon the stability of the system.

Table 1. Pathway length and the maximum strength of inhibition allowing for stability

Length of Pathway	Strength of Inhibition at the Threshold of Stability
n	$-g_{1n}/h$
1	*
2	*
3	8.0
4	4.0
5	2.9
6	2.4
7	2.1
8	1.9
9	1.8
10	1.7
.	.
.	.
.	.
∞	1.0

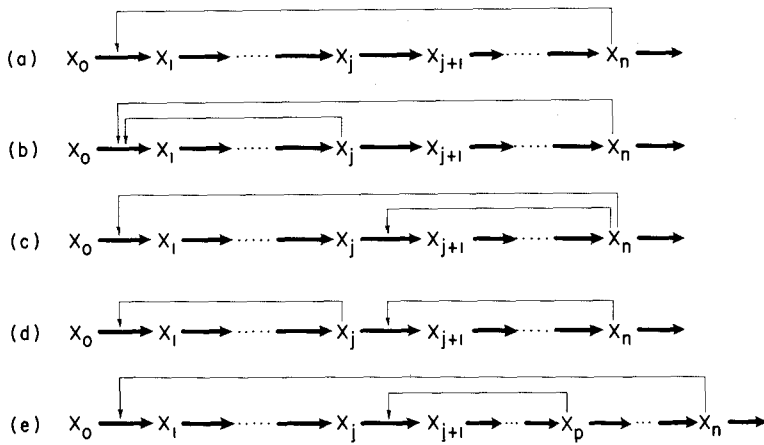*Stable for all allowable values of g_{1n}

Fig.8. Alternative patterns of control by feedback inhibition in un-
branched biosynthetic pathways

ALTERNATIVE PATTERNS OF CONTROL AND STABILITY

So far we have only considered pathways controlled by simple
end-product inhibition, because the bulk of experimental evi-
dence indicates that this is the predominant pattern of con-
trol in unbranched biosynthetic pathways (Monod et al., 1963).
Nevertheless, other patterns of control could conceivably be
employed for such pathways. Several such alternatives are
shown in Fig.8.

General Comparison for Systems with n=3

By allowing for all possible feedback interactions, the lin-
earized equations corresponding to Eq.(2) can be written as

$$(12) \qquad \dot{u}_1 = F_1 \left[(g_{11}-h_{11})u_1 + (g_{12}-h_{12})u_2 + (g_{13}-h_{13})u_3 \right]$$

$$\dot{u}_2 = F_2 \left[\qquad h_{11} u_1 + (h_{12}-h_{22})u_2 + (h_{13}-h_{23})u_3 \right]$$

$$\dot{u}_3 = F_3 \left[\qquad\qquad h_{22} u_2 + (h_{23}-h_{33})u_3 \right]$$

All the parameters in Eq.(12) are positive except those re-
presenting feedback inhibitions --g_{11}, g_{12}, g_{13}, h_{12}, h_{13},
h_{23}-- which are all negative. We shall require this general
system and the simple one in Fig.8a to be identical except for

the differences in their pattern of regulation. Under these conditions all the parameters in Eq.(12) are identical to the corresponding parameters in Eq.(2).

The characteristic equation for the general system is the following:

$$\lambda^3 + \left[(F_1h_{11}+F_2h_{22}+F_3h_{33})-(F_1g_{11}+F_2h_{12}+F_3h_{23})\right]\lambda^2 +$$

$$+ \left[(F_1F_2h_{11}h_{22}+F_1F_3h_{11}h_{33}+F_2F_3h_{22}h_{33})+(F_1F_2g_{11}h_{12}+\right.$$

$$+F_1F_3g_{11}h_{23}+F_2F_3h_{12}h_{23}-F_1F_2h_{11}g_{12}-F_2F_3h_{22}h_{13}-$$

$$\left.-F_1F_2g_{11}h_{22}-F_1F_3h_{11}h_{23}-F_1F_3g_{11}h_{33}-F_2F_3h_{12}h_{33}\right]\lambda +$$

$$+ \left[F_1F_2F_3h_{11}h_{22}(h_{33}-g_{13})+F_1F_2F_3(g_{11}h_{22}h_{13}+\right.$$

$$+h_{11}g_{12}h_{23}+g_{11}h_{12}h_{33}-h_{11}g_{12}h_{33}-g_{11}h_{12}h_{23}-$$

$$\left.-g_{11}h_{22}h_{33})\right] = 0$$

or

$$(13) \qquad \lambda^3 + \phi_1'\lambda^2 + \phi_2'\lambda + \phi_3' = 0$$

ϕ_1', ϕ_2', and ϕ_3' in Eq.(13) are all greater than the corresponding coefficients in Eq.(3). This is seen by inspection when the signs of the various apparent kinetic orders are taken into account. The critical condition for stability is $\phi_1'\phi_2' > \phi_3'$. For Eq.(13) this condition can be written as

$$(14) \qquad (\phi_1+A)(\phi_2+B) > (\phi_3+C)$$

where ϕ_1, ϕ_2, and ϕ_3 are defined in Eq.(3), and A, B, and C are sums of positive terms given in Eq.(13). Rearranging the terms in Eq.(14) yields

$$(15) \qquad (\phi_1\phi_2-\phi_3)+(AB+\phi_1B-C)+\phi_2A > 0$$

When the first term in parentheses is zero, the system in Fig. 8a will be on the boundary of instability. However, the remaining terms in Eq.(15) are all positive, since $(AB+\phi_1B-C)>0$, so that the system with the general pattern of feedback interaction is always further from the boundary of instability than the simple system in Fig.8a. Thus, the system with an arbitrary pattern of feedback inhibitions is always more stable than the system in Fig.8a.

The results in this section are not restricted to the case with n=3. A variety of control patterns has been compared with the simple pattern of end-product inhibition for equivalent systems with n > 3. These comparisions have been made using computer simulations, and in some cases, analytical techniques. In these comparisons, the system in Fig.8a has always been nearer than the other systems to the boundary of instability. This conclusion may be generally true for arbitrary patterns of control in systems with an arbitrary number of reactions, although we have no proof. It appears to be another extremum property possessed by the system in Fig.8a that, once proved, could be added to those already established for this system in steady state (Savageau, 1974).

DISCUSSION

In the preceding sections we have considered four different factors that affect the local stability of biosynthetic control systems. These factors were strength of end-product inhibition, equalization of the values for the corresponding kinetic parameters of the reactions in the pathway, pathway length, and alternative patterns of control. In a previous paper (Savageau, 1974), I showed that the pattern of control and the strength of end-product inhibition were important determinants of steady-state behavior in biosynthetic pathways. The results from this previous paper, together with those presented in the preceding sections, provide a rational explanation for the selection of kinetic parameters and regulatory patterns in unbranched biosynthetic pathways. This explanation will now be discussed in light of available experimental evidence.

Given the initial substrate and the end-product desired, their structural differences will determine roughly the number of elementary reactions required in the pathway. The free-energy difference and the energy required for the synthesis of the necessary enzymes would also be expected to influence the number and nature of the reactions in the pathway. By grouping biosynthetic pathways in families according to struc-

tural similarity of their end-products, and the extensive use of branching pathways, the cell has been able to minimize the length of most unbranched segments. For example, in the bacterium *Escherichia coli* 60% of the amino acids are synthesized by pathways shorter than 4 reactions, and during the evolution of higher forms it has been the longer pathways that have been dispensed with: 55% of the amino acids are not synthesized in mammals and those that are involve fewer than 4 reactions (Davis, 1961). In addition to the conservation of energy, these influences tend to promote the stability of metabolic control systems by shortening the pathway length, as we have already seen.

Nevertheless, there are several pathways of 5 to 10 reactions in microorganisms, and other means of stabilization are especially important for these pathways. Mismatch among the corresponding kinetic parameters for the reactions in the pathway will also promote stability. This mechanism allows the organism to effectively shorten the length of the pathway kinetically, even under conditions where it is impossible to physically reduce the number of reactions because of structural or thermodynamic reasons. This prediction could be verified by an examination of the kinetic parameters for the reactions in long biosynthetic sequences.

Although there are few pathways that have been experimentally examined in such detail, the available evidence does support the prediction of widely different kinetic parameters. A case in point is the histidine biosynthetic pathway of *Salmonella typhimurium*. This unbranched pathway consisting of 10 reactions has been under extensive biochemical and genetic analysis by Ames & Hartman for more than 20 years. In a recent review Brenner & Ames have reported the specific activities in crude extracts for the enzymes in this pathway. They range from a low of 0.3 to a high of 50 (μmoles of product formed/gm dry weight bacteria x minutes) with the other values distributed more or less equally between these extremes (Brenner & Ames, 1971). The K_M's for these same enzymes have been measured under a common set of conditions in crude extracts. The values range from a low of 1.1×10^{-5} M to a high of 60×10^{-5} M with intermediate values distributed between these (R.G.Martin & H.Whitfield, personal communication). The ratio of specific activity to K_M value for the enzymes of the pathway has an equally wide range of values. Under normal operating conditions, one can calculate the equivalent kinetic parameters in the power-law formulation (Savageau, 1971), and these will show a similar degree of variation for the reactions in the pathway.

The mismatch of the corresponding kinetic parameters in the histidine pathway cannot be due entirely to chance. If

the parameters were nearly equal, the system would surely be unstable from what we know of the strength of end-product inhibition and a path length of 10. Such a configuration would be under strong negative selective pressure.

Another mechanism for achieving stabilization is to reduce the strength of end-product inhibition. However, this solution does not appear to be of primary importance for most pathways. In 1956 Umbarger first reported end-product inhibition at the molecular level in the isoleucine pathway of *E. coli.* He observed that the apparent kinetic order for this inhibition was two. Since this initial observation, numerous regulatory enzymes have been examined and the apparent kinetic order generally tends to be between second- and fourth-order.

There is a structural basis for this kinetic fact. The study of quaternary structure has revealed that most enzymes are composed of subunits, not arbitrary numbers of subunits but mostly dimers and tetramers (Klotz et al. 1970). Furthermore, the symmetry of subunits and binding sites appears to be the same for most cases (Monod et al., 1965; Koshland & Neet, 1968). It is the multiplicity of binding sites that can lead to kinetic orders greater than unity, although under certain conditions other mechanisms can produce this result (Ferdinand, 1966).

These large apparent kinetic orders would normally make all pathways with greater than 4 reactions unstable, if it were not for the mismatch among the kinetic parameters for the reactions of the pathway. One reason why the cell has not utilized lower apparent kinetic orders to achieve stabilization may be due to constraints on the allowable structures for regulatory enzymes, although this seems to be an insufficient explanation. Another reason, having to do with the function of the intact control system, is that such a reduction would simultaneously detract from advantageous steady-state behavior. This was shown in a previous paper, where decreasing the strength of end-product inhibition (apparent kinetic order of the controlled reaction with respect to the end-product concentration) decreased the ability of the system to respond to a change in demand for the end-product (Savageau, 1974). The accumulation of intermediate metabolites was also shown to be aggravated by a reduction in the strength of inhibition. Thus, optimum steady-state behavior requires that this parameter be sufficiently high, and that stabilization be achieved in another way.

Alternative patterns of control by feedback inhibition could also be used to achieve stabilization as we have seen. However, these alternatives generally have less than optimal

steady-state behavior (Savageau, 1974). This conflict between two desirable goals appears to have been resolved in favor of optimal steady-state behavior, since the predominant pattern of control in unbranched pathways is simple end-product inhibition (Monod et al., 1963); however, this conflict may be more apparent than real. Surely a degree of stability is necessary for survival. But beyond this degree, overstabilization can be a disadvantage, since it may make the system's temporal response to change very sluggish. Thus one could argue that the system will be selected to be as near the boundary of instability as possible and yet have a sufficient margin of safety to ensure stability. In this way end-product inhibition will be the fast-acting, fine control it is normally assumed to be.

In summary, the simple pattern of control by end-product inhibition in unbranched biosynthetic pathways appears to have evolved because it optimizes the steady-state behavior and is temporally most responsive to change. Stability in such systems is achieved by shortening pathway length either physically or, in the case of long pathways, kinetically by a wide divergence in the corresponding kinetic parameters for the reactions of the pathway.

APPENDIX: ANALYTICAL EXAMINATION OF LOCAL STABILITY

Questions about the stability of nonlinear systems are generally quite difficult to answer. A notable exception is the case of local stability analysis. This type of stability is important in the design of biosynthetic control systems. In this appendix a systematic method of examining biochemical systems which can be accurately described by power-law nonlinearities (Savageau, 1969) is developed. A general two-variable case will be examined first; the general n-variable case follows as a natural extension.

The Two-Variable System

The general two-variable system is represented by the following equations

$$\text{(A1a)} \quad \frac{dX_1}{dt} = \alpha_1 X_1^{g_{11}} X_2^{g_{12}} - \beta_1 X_1^{h_{11}} X_2^{h_{12}}$$

(A1b) $\quad \dfrac{dX_2}{dt} = \alpha_2 X_1^{g_{21}} X_2^{g_{22}} - \beta_2 X_1^{h_{21}} X_2^{h_{22}}$

where the X's denote chemical concentrations, the α's and β's are apparent rate constants, and the g's and h's are apparent kinetic orders. The nonzero steady-state solution for this system can be obtained using the techniques previously described (Savageau, 1969). This solution may be written as follows:

(A2) $\quad X_{10} = \left[(\beta_1/\alpha_1)^{a_{22}} (\alpha_2/\beta_2)^{a_{12}} \right]^{1/(a_{11}a_{22}-a_{12}a_{21})}$

$\quad\quad X_{20} = \left[(\beta_2/\alpha_2)^{a_{11}} (\alpha_1/\beta_1)^{a_{21}} \right]^{1/(a_{11}a_{22}-a_{12}a_{21})}$

The additional "o" subscript denotes the steady-state value, and a_{ij} is defined as the difference $(g_{ij}-h_{ij})$.

If we consider only small variations about this steady-state operating point, we may substitute for each expression in Eq.(A1) its Taylor series and retain the first two terms. The resulting equations are

(A3a) $\quad \dfrac{d(X_{10}+x_1)}{dt} = \left[\alpha_1 X_{10}^{g_{11}} X_{20}^{g_{12}} + \alpha_1 g_{11} X_{10}^{g_{11}-1} X_{20}^{g_{12}} x_1 \right.$

$$\left. + \alpha_1 g_{12} X_{10}^{g_{11}} X_{20}^{g_{12}-1} x_2 \right]$$

$$- \left[\beta_1 X_{10}^{h_{11}} X_{20}^{h_{12}} + \beta_1 h_{11} X_{10}^{h_{11}-1} X_{20}^{h_{12}} x_1 \right.$$

$$\left. + \beta_1 h_{12} X_{10}^{h_{11}} X_{20}^{h_{12}-1} x_2 \right]$$

(A3b) $\quad \dfrac{d(X_{20}+x_2)}{dt} = \left[\alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}} + \alpha_2 g_{21} X_{10}^{g_{21}-1} X_{20}^{g_{22}} x_1 \right.$

$$\left. + \alpha_2 g_{22} X_{10}^{g_{21}} X_{20}^{g_{22}-1} x_2 \right]$$

$$-\left[\beta_2 X_{10}^{h_{21}} X_{20}^{h_{22}} + \beta_2 h_{21} X_{10}^{h_{21}-1} X_{20}^{h_{22}} x_1\right.$$

$$\left. + \beta_2 h_{22} X_{10}^{h_{21}} X_{20}^{h_{22}-1} x_2\right]$$

In Eq.(A3), x_1 and x_2 represent small variations about the normal operating values X_{10} and X_{20}. Since

$$\alpha_1 X_{10}^{g_{11}} X_{20}^{g_{12}} = \beta_1 X_{10}^{h_{11}} X_{20}^{h_{12}}$$

and

$$\alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}} = \beta_2 X_{10}^{h_{21}} X_{20}^{h_{22}},$$

and since the time derivatives of X_{10} and X_{20} are zero, we can simplify Eq.(A3) as follows:

(A4)
$$\frac{dx_1}{dt} = \alpha_1 X_{10}^{g_{11}-1} X_{20}^{g_{12}} (g_{11}-h_{11}) x_1$$

$$+ \alpha_1 X_{10}^{g_{11}} X_{20}^{g_{12}-1} (g_{12}-h_{12}) x_2$$

$$\frac{dx_2}{dt} = \alpha_2 X_{10}^{g_{21}-1} X_{20}^{g_{22}} (g_{21}-h_{21}) x_1$$

$$+ \alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}-1} (g_{22}-h_{22}) x_2$$

Divide the small variation x_i by the corresponding steady state value X_{i0} to obtain a new variable u_i which represents the percent variation in X_i. Then Eq.(A4) can be written

(A5)
$$\dot{u}_1 = \alpha_1 X_{10}^{g_{11}-1} X_{20}^{g_{12}} [a_{11}u_1 + a_{12}u_2]$$

$$\dot{u}_2 = \alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}-1} [a_{21}u_1 + a_{22}u_2]$$

where the dot ($\cdot$) denotes the time derivative. The factors in front of each equation are simply positive numbers, since X_{10} and X_{20} are constants given by Eq. (A2), and they are defined as follows:

(A6)
$$F_1 = \alpha_1 X_{10}^{g_{11}-1} X_{20}^{g_{12}}$$

$$F_2 = \alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}-1}$$

As a result, one can finally rewrite Eq. (A5) to give those shown below.

(A7)
$$\dot{u}_1 = F_1 \left[a_{11} u_1 + a_{12} u_2 \right]$$

$$\dot{u}_2 = F_2 \left[a_{21} u_1 + a_{22} u_2 \right]$$

The stability of such a linearized system is determined by an examination of the roots of the characteristic equation according to well-known methods. The characteristic equation in this case is

(A8)
$$\lambda^2 - (F_1 a_{11} + F_2 a_{22})\lambda + F_1 F_2 (a_{11} a_{22} - a_{12} a_{21}) = 0$$

Application of the Routh stability criterion (Timothy & Bona, 1968) yields the conditions for stability:

(A9)
$$F_1 a_{11} + F_2 a_{22} < 0$$

$$a_{11} a_{22} - a_{12} a_{21} > 0$$

The n-Variable System

The equations for the n-variable case corresponding to Eq. (A1) are

(A10)
$$X_i = \alpha_i \prod_{j=1}^{n} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n} X_j^{h_{ij}} \qquad i=1, 2, \ldots, n.$$

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By a direct extension of the procedures in the previous sub-section, we can write a set of linearized equations. These are

(A11) $\dot{u}_i = F_i \left[a_{i1}u_1 + a_{i2}u_2 + \ldots + a_{in}u_n \right]$

where

$$F_i = \alpha_i X_{10}^{g_{i1}} X_{20}^{g_{i2}} \ldots X_{iO}^{g_{ii}-1} \ldots X_{no}^{g_{in}}$$

for $i = 1, 2, \ldots, n.$

The stability of this system is determined by the nature of the roots of the characteristic equation, which is given by

(A12)
$$
\begin{vmatrix}
(F_1 a_{11}-\lambda) & F_1 a_{12} & F_1 a_{13} & \cdots & F_1 a_{1n} \\
F_2 a_{21} & (F_2 a_{22}-\lambda) & F_2 a_{23} & \cdots & F_2 a_{2n} \\
F_3 a_{31} & F_3 a_{32} & (F_3 a_{33}-\lambda) & \cdots & F_3 a_{3n} \\
\cdot & & \cdot & & \cdot \\
\cdot & & \cdot & & \cdot \\
\cdot & & \cdot & & \cdot \\
F_n a_{n1} & F_n a_{n2} & F_n a_{n3} & \cdots & (F_n a_{nn}-\lambda)
\end{vmatrix} = O
$$

It is analytically difficult to characterize the roots of this equation for an arbitrary case when n is larger than 3. Nevertheless, for any particular case it is straightforward to expand Eq.(A12) and ascertain the number of roots with positive real parts using the Routh criterion.

In the preceding sections these techniques are used for the analysis of the factors affecting the dynamic behavior of biosynthetic pathways.

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