OSCILLATIONS IN CONTROLLED BIOCHEMICAL SYSTEMS

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ABSTRACT Stability analysis of equations describing certain biochemical control mechanisms involving negative feedback suggests that limit cycle behavior might be possible if the control system involves a sufficient number of intermediate chemical steps. For the example considered in this paper, digital simulation of the nonlinear control system illustrates that limit cycle behavior actually arises for a sixth-order system. On the other hand, the corresponding fourth- and fifth-order systems are asymptotically stable.

INTRODUCTION

Under appropriate conditions the dynamic behavior of certain well-known biochemical control mechanisms can be described by equations 1 (for example applications, see Goodwin, 1965; Morales and McKay, 1967; Walter, 1968 *a*, *b*, 1969):

$$
\begin{aligned}
\dot{S}_1 &= F(S_{n+1}) - K_1 S_1 \\
&\vdots \\
\dot{S}_i &= K_{i-1} S_{i-1} - K_i S_i \qquad i = 2, \cdots, n+1.
\end{aligned}
\tag{1}
$$

In these equations $F(S_{n+1})$ is a nonlinear feedback function describing the effect of S_{n+1} on the rate of formation (or introduction) of S_1. The controlled system can be envisioned as a series of n consecutive first-order biochemical reactions, wherein S_1 is either stoichiometrically converted to S_{n+1} or in some other manner ultimately "causes" S_{n+1} to be formed.

Morales and McKay (1967) have used equations 1 to describe the kinetic behavior of metabolic end-product feedback of the Yates and Pardee (1956) type. Goodwin (1965), on the other hand, utilizes these equations to describe the dynamic behavior of the fundamental control circuit for macromolecular synthesis in certain cellular systems.

There are several important reasons for establishing the properties of the equations describing the dynamics of biochemical control systems. Perhaps, foremost is the fact that such equations are essential for the construction of a statistical mechanics of cellular processes. It seems clear from Goodwin's (1963) initial work that the macroscopic properties which emerge from this statistical mechanics will describe some very general and important features of cellular behavior.

Goodwin (1963, 1965) has pointed out that the fact that cells divide implies the existence of at least one autonomous oscillating "signal". This suggests that the dynamic equations describing biochemical control circuits should be capable of exhibiting limit cycle behavior. On this basis Goodwin (1963) suggested that sustained oscillations might arise "naturally" in controlled biochemical feedback systems. Subsequently Goodwin (1965)[1] and then Morales and McKay (1967) reported that they had found self-limiting, sustained oscillations in analog computer simulations of equations 1; the nonlinear negative feedback function used in these simulations appears in equation 2:

$$F(S_{n+1}) = \frac{K_0}{1 + \alpha[S_{n+1}]^{\rho}}. \tag{2}$$

Griffith (1968), on the other hand, has reported results which he interprets to cast serious doubt on the possibility that feedback of the type described by equation 2 from a (metabolic) product of a single gene can ever give rise in practice to undamped oscillations. In addition, I (Walter, 1968 *a*, *b*, 1969) have recently shown that sustained oscillations do not occur in at least some of the situations for which such behavior was reported. Both analog and digital computer simulations (i.e. electronic analog and numerical solutions) of several systems for which Morales and McKay (1967) reported sustained oscillations confirmed that only highly damped oscillations actually occur.

In this paper we examine the possibility that limit cycle behavior is possible for other situations described by equations 1 and 2. In particular, we investigate the effect of introducing additional phase lag (increasing n) into the examples considered previously.

DISCUSSION

To begin, we consider the linear transformation, $U_i = S_i - S_i^*$ $(i = 1, \cdots, n + 1)$ where S_i^* is the positive, real singular point of equation 1. Linearization of the transformed differential equations in the neighborhood of $U_i = 0$ $(i = 1, \cdots, n + 1)$ eads to the abridged equations:

$$\begin{aligned} \dot{U}_1 &= aU_{n+1} - K_1U_1 \\ &\vdots \\ \dot{U}_i &= K_{i-1}\,U_{i-1} - K_iU_i \qquad i = 2, \cdots, n + 1 \end{aligned} \tag{3}$$

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[1] Dr. Goodwin has kindly informed me that he now considers the limit cycle he observed in his analog computer simulation to be artifactual.

a, the linear feedback term is less than zero if the feedback is negative (as, for example, in equation 2), but greater than zero if the feedback is positive.

The characteristic equation for equation 3 is

$$\prod_{i=1}^{n+1} (K_i + h) - a \prod_{i=1}^{n} K_i = 0 \tag{4}$$

where the roots, h are the characteristic exponents. If we set $h = 0$, we obtain

$$K_{n+1} = a. \tag{5}$$

In what follows we shall refer to equation 5 as the "equation of zero roots". Similarity, if we set $h = i\omega$ ($i = \sqrt{-1}$), we obtain for various n:

$$n = 1 \qquad K_1 + K_2 = 0 \tag{6}$$

$$n = 2 \qquad (K_1 + K_2)(K_1 + K_3)(K_2 + K_3) + K_1K_2a = 0 \tag{7}$$

$$n = 3$$

$$\frac{\begin{gathered}(K_3 + K_4)(K_2^2 + K_2K_3 + K_2K_4 + K_3K_4)[K_1^2(K_1 + K_2 + K_3\, K_4) \\ + K_1K_2K_3 + K_1K_2K_4 + K_1K_3K_4 + K_2K_3K_4]\end{gathered}}{K_1K_2K_3(K_1 + K_2 + K_3 + K_4)^2} + a = 0 \tag{8}$$

$$n = 4 \qquad \sqrt{B^2 + 4C} - B + 2a = 0 \tag{9}$$

where

$$B = \frac{K_5}{a_0} (2a_0 + a_3^2a_4 - a_2a_3 - 2a_1a_4)$$

$$C = \frac{K_5^2}{a_0^2} (a_1a_2a_3a_4 - a_0a_3^2a_4 - a_1^2a_4^2 - a_1a_2^2 + a_0a_2a_3 + 2a_0a_1a_4 - a_0^2)$$

$$a_0 = \prod_{i=1}^{5} K_i, a_1 = \sum_{j=1}^{5} \frac{\prod_{i=1}^{5} K_i}{K_j}, a_2 = \frac{1}{2} \sum_{\substack{k,j=1 \\ k\neq j}}^{5} \frac{\prod_{i=1}^{5} K_i}{K_jK_k}, a_3 = \frac{1}{2} \sum_{\substack{i,j=1 \\ i\neq j}}^{5} K_iK_j, a_4 = \sum_{i=1}^{5} K_i.$$

In what follows we shall refer to equations 6–9 as the "neutrality equations". The rationale for the following use of the neutrality equations and the equation of zero roots is well-known in linear control theory and has been outlined by Sel'kov (1967) in connection with a similar analysis of a different control system.

RESULTS

With the aid of equations 5–9 we can partition the plane of the parameters, a and any one K_i, into areas for which at least one characteristic exponent changes sign

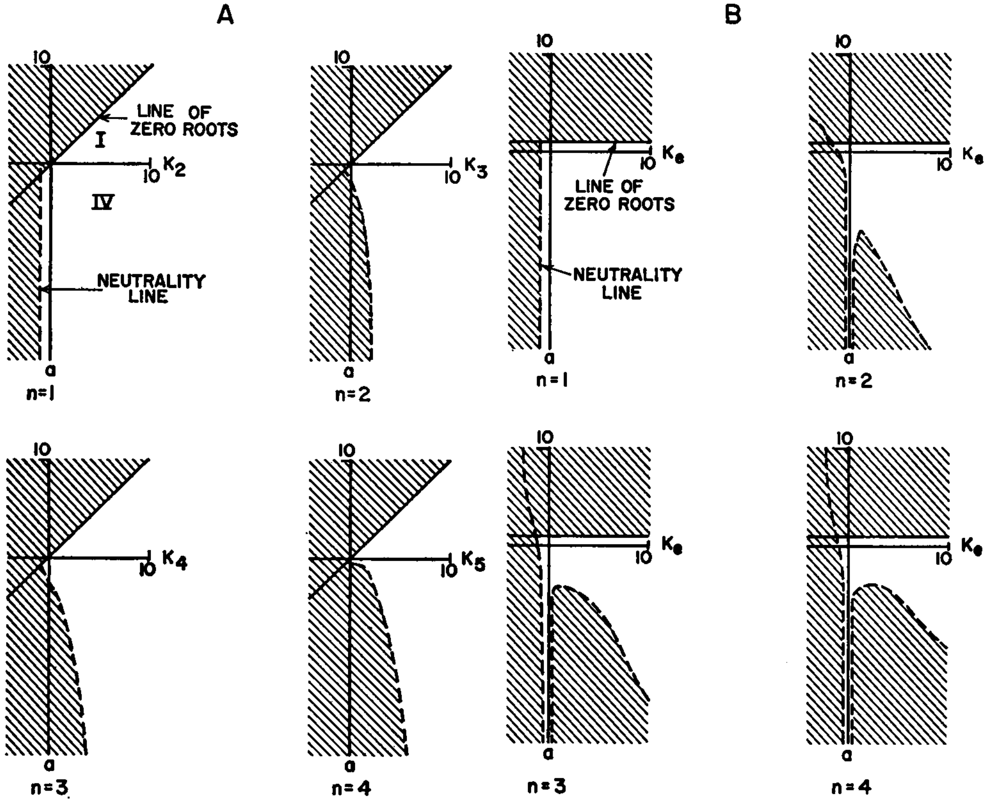

FIGURE 1 Partition of the plane of the linear feedback term, a, and any one of the rate constants into areas of instability (shaded portion) and asymptotic stability (unshaded portion). The solid line is the line of zero roots (equation 5) and the dashed line is the neutrality line (equations 6–9). In Fig. 1 a all the $K_i = 1$ $(i = 1, \cdots, n)$ and K_{n+1} is varied; in Fig. 1 b all the $K_i = 1$ $(i = 1, \cdots, n + 1)$ except $K_e (e \leq n)$ which is varied.

in its real part. In Fig. 1 are plotted the line of zero roots (solid line) and the neutrality line (dashed line) for $n = 1, 2, 3,$ and 4. In Fig. 1 a all the $K_i = 1$ $(i = 1, \cdots n)$ and K_{n+1} is varied; in Fig. 1 b all the $K_i = 1$ $(i = 1, \cdots, n + 1)$ except K_e $(e \leq n)$ which is varied. In the shaded areas of Fig. 1 the real part of at least one of the characteristic exponents is positive; the shaded areas therefore denote areas wherein the linear system must be unstable. For values of a and K_i corresponding to the unshaded area the singularity $U_i = 0$, is asymptotically stable in the small if the real parts of all the other characteristic exponents are also negative.

Since in the systems we are considering the K_i are positive rate constants we will limit our discussion to the first and fourth quadrants of Fig. 1. For $n = 1$ there is no shaded area for $a < 0$. Furthermore, it is easy to show by quadratic formula that when $n = 1$, the real parts of both roots of equation 4 are necessarily negative provided K_1 and K_2 are positive, real numbers and $a < 0$. Thus, linear negative feedback systems are always asymptotically stable when $n = 1$. There is, however, a large area of instability available to linear positive feedback systems ($a > 0$); the size of this area does not change for the various n.

For $n = 2$ there is a small area of instability in the fourth quadrant. This means that negative feedback systems can have unstable singularities when $n = 2$. When n gets progressively larger, this area of instability in the fourth quadrant also gets larger. This suggests that the potential for oscillatory behavior in negative feedback systems might become greater as n gets progressively larger. Since increasing n amounts to the introduction of additional phase lag, it is intuitively clear that sustained oscillations could be more likely for larger n.

A specific case for which Morales and McKay (1967) reported limited cycle behavior for equations 1 and 2 is: $n = 3$, $\rho = 4$, $\alpha = 0.08$, $K_0 = 5.1$, $K_1 = K_2 = K_3 = 1$, and $K_4 = 0.6$. For this case $a = -1.7277$; substitution into the characteristic equations yields

$$h^4 + 3.6h^3 + 4.8h^2 + 2.8h + 2.3277 = 0. \qquad (10)$$

The roots of this equation have negative real parts if

$$\begin{vmatrix} 3.6 & 2.8 & 0 \\ 1 & 4.8 & 2.2377 \\ 0 & 3.6 & 2.8 \end{vmatrix} > 0. \qquad (11)$$

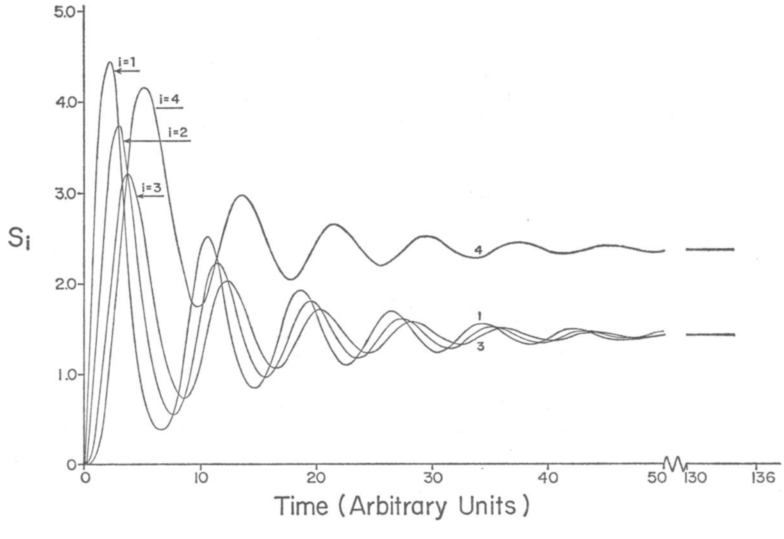

FIGURE 2 $S_i(t)$ obtained from a digital computer simulation (a numerical solution) of equations 1 and 2. $n = 3$, $\rho = 4$, $\alpha = 0.08$, $K_0 = 5.1$, $K_1 = K_2 = K_3 = 1$, and $K_4 = 0.6$.

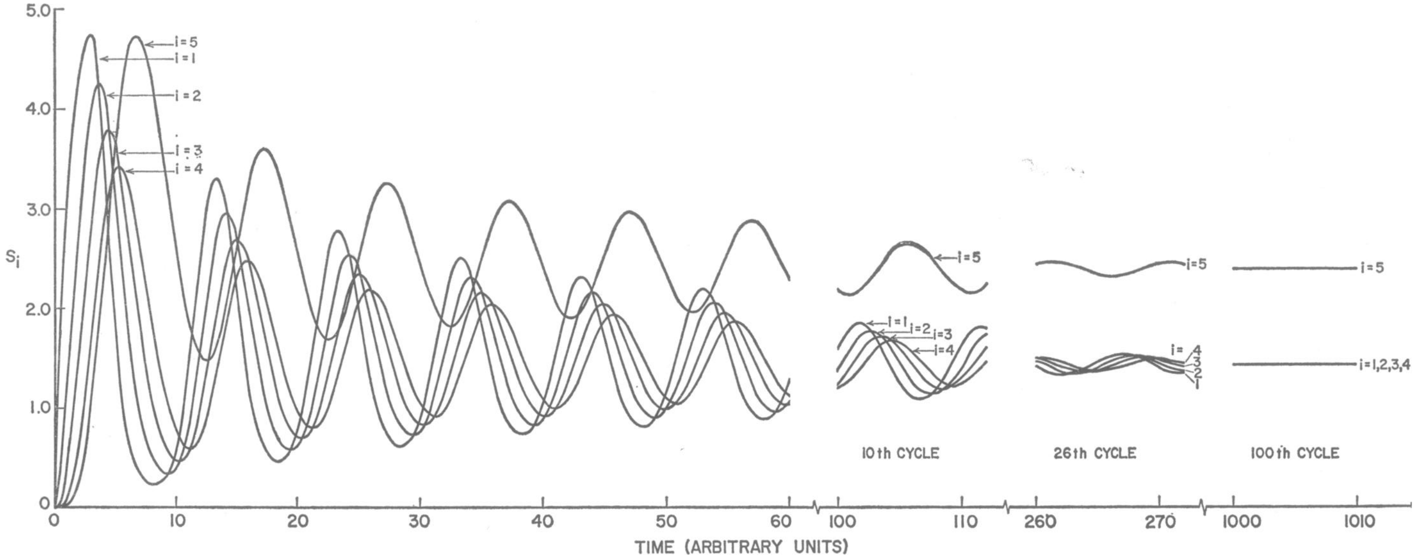

FIGURE 3 $S_i(t)$ obtained from a digital computer simulation of equations 1 and 2. $n = 4$, $\rho = 4$, $\alpha = 0.08$, $K_0 = 5.1$, $K_1 = K_2 = K_3 = K_4 = K_6 = 1$, and $K_5 = 0.6$.

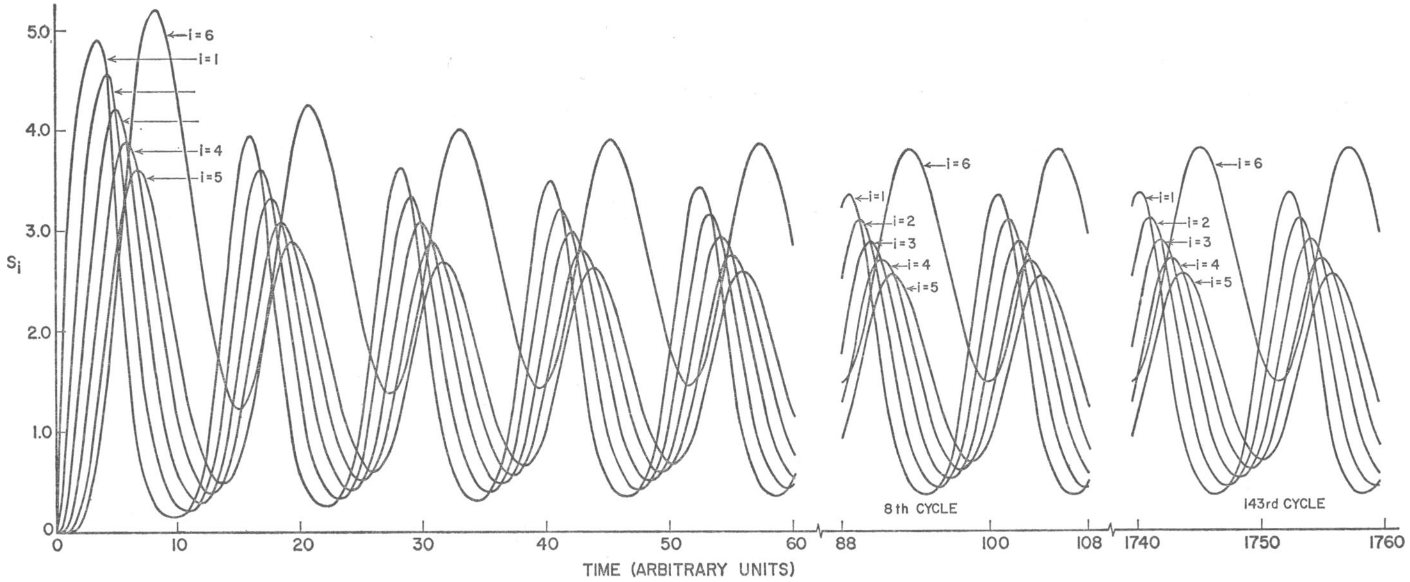

FIGURE 4 $S_i(t)$ obtained from a digital computer simulation of equa tions 1 and 2. $n = 5$, $\rho = 4$, $\alpha = 0.08$, $K_o = 5.1$, $K_1 = K_2 = K_3 = K_4 = K_5 = 1$, and $K_6 = 0.6$.

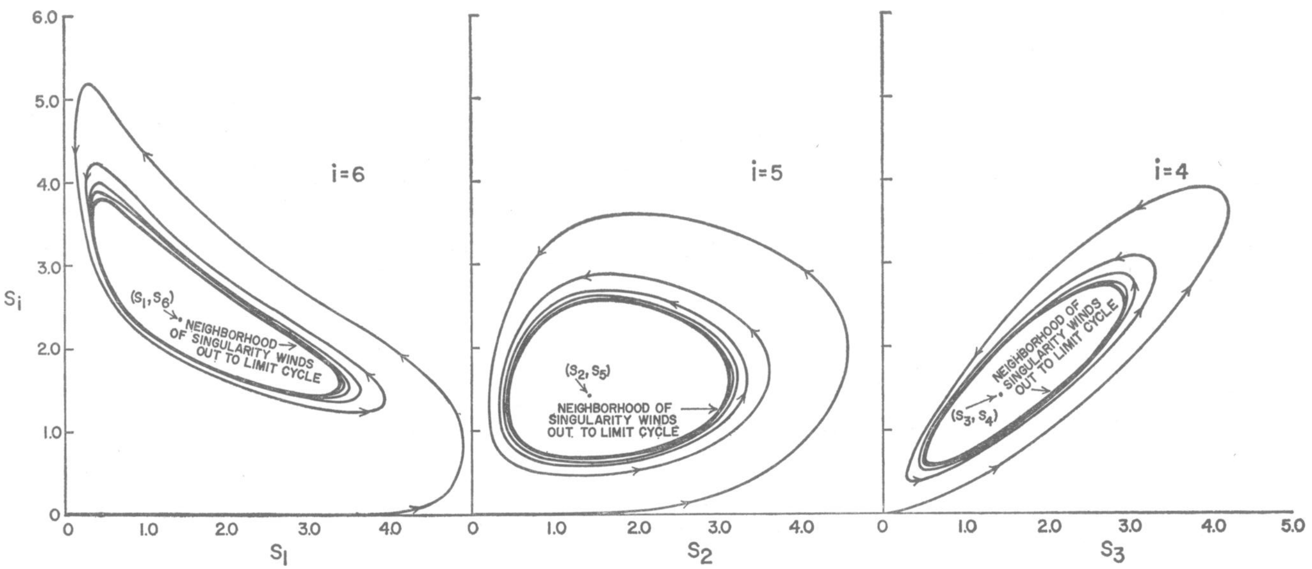

FIGURE 5 Phase-plane representation of the $S_i(t)$ obtained from a digital computer simulation of equations 1 and 2. $n = 5$, $\rho = 4$, $\alpha = 0.08$, $K_o = 5.1$, $K_1 = K_2 = K_3 = K_4 = K_5 = 1$, and $K_6 = 0.6$.

Expanding the determinant we obtain

$$48.384 - 39.011 > 0. \tag{12}$$

Thus, the characteristic exponents all have negative real parts. This means that for this case the solution of equations 1 is asymptotically stable in the small neighborhood of the singularity $S_i = S_i^*$. We cannot decide on this basis whether or not the nonlinear system is asymptotically stable in all the phase space accessible to the variables. We do know, however, that if we start the system in the neighborhood of the singularity $S_i = S_i^*$, sustained oscillations will not occur.

In Fig. 2 appears the actual time course of the variables $S_i(t)$, for the system under discussion here. Highly damped rather than sustained oscillations occur. At the first maximum the highest S_i is about 300 per cent of that S_i^*; at the seventh maximum, however, no S_i exceeds 101 per cent of the corresponding S_i^*; at a time sufficient to reach the fifteenth maximum, all the $S_i = S_i^*$.

Progressing to larger n, we next consider the system: $n = 4$, $\rho = 4$, $\alpha = 0.08$, $K_0 = 5.1$, $K_1 = K_2 = K_3 = K_4 = 1$, and $K_5 = 0.6$. In this case the roots of the characteristic equation,

$$h^5 + 4.6h^4 + 8.4h^3 + 7.6h^2 + 3.4h + 2.3277 = 0 \tag{13}$$

still have negative real parts. Thus, we remain in the sitution when equations 1 are asymptotically stable in the small neighborhood of the singularity $S_i = S_i^*$. In Fig. 3 appear $S_i(t)$ for this case. Although the damping is not as rapid as in Fig. 2, sustained oscillations do not occur in Fig. 3.

Progressing to even larger n, we next consider the system: $n = 5$, $\rho = 4$, $\alpha = 0.08$, $K_0 = 5.1$, $K_1 = K_2 = K_3 = K_4 = K_5 = 1$, and $K_6 = 0.6$. In this case the characteristic equation is

$$h^6 + 5.6h^5 + 13h^4 + 16h^3 + 11h^2 + 4h + 2.3277 = 0. \tag{14}$$

It is easy to prove that the characteristic exponents in equation 14 do not all have negative real parts. This means that if the number of steps in equations 1 and 2 is sufficiently large, the solution becomes unstable in the neighborhood of the singularity $S_i = S_i^*$. In Fig. 4 appear $S_i(t)$ for this case; as can be seen from this figure the oscillations are sustained. The limit cycle behavior is further illustrated in the phase-plane representations in Fig. 5. Note that if the system is initiated in the neighborhood of the singularity $S_i(0) = S_i^*$, the variables wind outward to the limit cycle; if the system is initiated in the phase space outside the limit cycle $S_i(0) = 0$, the variables wind inward toward the same closed trajectory.

CONCLUSIONS

The results reported in this paper establish for the first time that biochemical control circuits described by equations 1 and 2 can possess a limit cycle. I (Walter, 1968 *b*) have shown that such systems cannot experience sustained oscillations under any conditions when $n = 1$ and only under special conditions when $n = 2$ or 3. Griffith (1968) has independently shown that such systems cannot have a limit cycle when $n = 1$ and that they probably cannot experience sustained oscillations when $n = 2$ unless $\rho > 8$. However, the results presented here illustrate that the idea suggested by Goodwin (1965) and Morales and McKay (1967) (i.e. that sustained oscillations can arise naturally in "controlled" biochemical systems describable by equations 1 and 2) is fundamentally sound; furthermore, these results are inconsistent with Griffith's suggestion that feedback of the type described by equation 2 from the metabolic product of a single gene cannot give rise to undamped oscillations in practice. Clearly, it is unlikely (as Griffith indicates) that the stoichiometry of the feedback inhibition will be greater than eight in actual biochemical control circuits. However, if macromolecular control circuits involve a sufficient number of kinetically important steps, it is clearly possible for sustained oscillations to arise in metabolic repressor systems involving a single gene. Fig. 4, for example illustrates that such behavior is possible when $\rho = 4$ and $n = 5$.

It remains to be established how one should construct a statistical mechanics on a nonconservative dynamic system (e.g. equations 1) for which no first integral can be obtained. It seems clear, however, that the statistical mechanics of nonlinear, nonconservative cellular control systems must eventually be formulated in a manner that will differ from that used by the great Willard Gibbs in physics.

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