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Stable Oscillations in Mathematical Models of Biological Control Systems

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Summary. Oscillations in a class of piecewise linear (PL) equations which have been proposed to model biological control systems are considered. The flows in phase space determined by the PL equations can be classified by a directed graph, called a state transition diagram, on an N-cube. Each vertex of the N-cube corresponds to an orthant in phase space and each edge corresponds to an open boundary between neighboring orthants. If the state transition diagram contains a certain configuration called a cyclic attractor, then we prove that for the associated PL equation, all trajectories in the regions of phase space corresponding to the cyclic attractor either (i) approach a unique stable limit cycle attractor, or (ii) approach the origin, in the limit $t \to \infty$. An algebraic criterion is given to distinguish the two cases. Equations which can be used to model feedback inhibition are introduced to illustrate the techniques.

Key words: Biological control systems — Limit cycles — Feedback inhibition.

1. Introduction

Biological oscillations have recently attracted widespread interest from both mathematicians and biologists. In the following we explicitly demonstrate stability and uniqueness properties of limit cycle oscillations in a class of differential equations which have been proposed to represent the interactions which occur in biological control systems (Glass, 1975a).

A fundamental observation underlying this work is that control elements in biological systems are often analogous to switches so that their activities depend on input variables in a non-linear switchlike fashion. In bacteria, regulation of metabolic pathways often occurs by modulation of catalytic activity of enzymes by metabolites which are not themselves substrates of the enzymes. In the phenomena of *end-product inhibition* or *feedback inhibition*, the last product of a synthetic sequence inhibits in a non-linear switchlike fashion the catalytic activity of an early

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allosteric enzyme in the synthetic sequence (Monod, Wyman and Changeux, 1965; Lehninger, 1970). Not only are the activities of enzymes subject to control, but in bacteria the synthesis of the enzymes is also often controlled by the presence or absence of critical metabolites in the growth medium. For example, in *E. coli*, lactose stimulates or induces the production of the enzyme β-galactosidase, and this induction shows an increasing sigmoidal dependence on lactose concentration (Bourgeois and Monod, 1970; Yagil and Yagil, 1971; Yagil, 1975). Repression of enzyme synthesis by end-products of synthetic sequences in which those enzymes participate is also well known (Lehningen, 1970; Yagil and Yagil, 1971; Yagil, 1975).

Many biological control systems are composed of two or more non-linear control elements, where the outputs from one element act as input stimuli elsewhere in the network. Several authors have described ways in which simple control elements can be used to synthesize networks which can regulate diverse phenomena such as biological oscillations and differentiation (Monod and Jacob, 1961; Sugita, 1963; Simon, 1965; Kauffman, 1969; Glass and Kauffman, 1973; Rössler, 1974; Othmer, 1976).

As a result of their particularly simple structure and their biological importance, feedback inhibition networks, represented schematically by

$$\xrightarrow{+} x_1 \xrightarrow{+} x_2 + \cdots \xrightarrow{+} x_N, \tag{1.1}$$

have attracted widespread interest. Since x_N inhibits the production of x_1, the concentration of x_N may be regulated at a constant level. However, in many circumstances, the resulting system is liable to oscillations (Goodwin, 1965; Griffith, 1968; Walter, 1971; Hunding, 1974; Tyson, 1975; Glass, 1975a, 1975b, 1977a; Othmer, 1976; Hastings, 1977; Hastings, Tyson and Webster, 1977).

In what follows, the non-linearities in biological control systems are represented in an extreme way by discontinuities in differential equations. This transforms the non-linear equations to piecewise linear (PL) equations. A PL equation will arise for example, if a steep sigmoidal control function is approximated by a step function. The study of the PL equations is motivated by an attempt to model biological systems within the framework of a tractable mathematical structure. We believe that these discontinuous systems can serve as mathematical models for real biological systems, and as approximations of continuous models (Glass, 1975a; Glass and Pasternack, 1978).

In Section 2 we derive a class of PL equations. In Section 3, we give a technique to classify the PL equations using directed graphs on N-cubes, N-dimensional hypercubes. The N-cube classification can be used to identify PL equations in $N \geqslant 2$ dimensions, which can display stable, limit cycle oscillations, as described by the theorem, Section 4. In Section 5, three special cases of the theorem are given. In Section 6, the results are discussed. The theorem stated in Section 4 is proved in the Appendix.

2. The PL Equations

Although we believe the PL equations are of broad general interest, we confine our discussion of the derivation of the PL equations to biochemical systems. The variables $y_1, y_2, \ldots, y_N$ which represent chemical concentrations are real non-negative variables. The rate of synthesis of y_i called g_i is assumed to depend on the concentrations of the other chemical constituents, whereas the rate of degradation of y_i is proportional to its own concentration. The network can be represented

$$\frac{dy_i}{dt} = g_i(y_1, y_2, \ldots, y_{i-1}, y_{i+1}, \ldots, y_N) - \gamma_i y_i \quad i = 1, 2, \ldots, N; N + 1 = 1, \tag{2.1}$$

where γ_i is the decay constant for the ith variable. A number of mathematical models of biochemical control systems can be written this way (Goodwin, 1965; Simon, 1965; Griffith, 1968; Walter, 1971; Hunding, 1974; Tyson, 1975; Glass, 1975a, 1977a; Othmer, 1976; Hastings, 1977; Hsü, 1977; Hastings, Tyson and Webster, 1977; Glass and Pasternack, 1978).

Of particular interest have been mathematical models of feedback inhibition. The systems of equations which have been proposed for feedback inhibition can be written in the form of (2.1) with the following restrictions on the g_i (Hastings, Tyson and Webster, 1977) (cf. (1.1)),

$$\frac{\partial g_1}{\partial y_N} < 0, \qquad \frac{\partial g_i}{\partial y_{j-1}} > 0 \quad \text{for } i = 2, \ldots, N, \qquad \frac{\partial g_i}{\partial y_j} = 0 \quad \text{otherwise.} \tag{2.2}$$

It has recently been proven for equations of the form (2.1), which satisfy (2.2) plus certain additional conditions, that a periodic solution exists (Hastings, Tyson and Webster, 1977). In addition, extensive numerical simulations and analysis of Hopf bifurcation phenomena in feedback inhibition schemes have been reported (Goodwin, 1965; Griffith, 1968; Walter, 1971; Hunding, 1974; Othmer, 1976; Glass, 1977a; Glass and Pasternack, 1978). Proof of stable limit cycle oscillations for a special case in three dimensions has been reported (Hastings, 1977).

In biochemical systems, synthetic rates are often empirically described by the Hill function (Monod, Wyman and Changeux, 1965; Yagil and Yagil, 1971; Yagil, 1975)

$$g(y) = \frac{\lambda y^m}{\theta^m + y^m}, \tag{2.3a}$$

$$g(y) = \frac{\lambda \theta^m}{\theta^m + y^m}, \tag{2.3b}$$

where λ, called the *production constant*, represents the maximal value of $g(y)$, m is a positive real number called the Hill coefficient, and θ is a positive real number. In the particular case where $m = 1$, (2.3a) represents Michaelis–Menten kinetics (Lehninger, 1970). Using the Hill functions (2.3) for the terms giving synthetic rates in (2.1), in combination with the criteria in (2.2), we derive as a mathematical model of feedback inhibition

$$\begin{aligned}\frac{dy_1}{dt} &= \frac{\lambda_1\theta_1^{m_1}}{\theta_1^{m_1}+y_N^{m_1}} - \gamma_1 y_1,\\ \frac{dy_i}{dt} &= \frac{\lambda_i y_{i-1}^{m_i}}{\theta_i^{m_i}+y_{i-1}^{m_i}} - \gamma_i y_i, \quad i = 2, 3, \ldots, N,\end{aligned} \tag{2.4}$$

where λ_i, θ_i, m_i, $i = 1, 2, \ldots, N$ are positive reals.

The PL equations which we consider, emerge as extreme limits of non-linear equations such as (2.4). A variable whose possible values are only 0 and 1 is called a *Boolean variable*, and a function whose possible values are only 0 and 1 is called a *Boolean function*. Suppose positive constants $\theta_1, \theta_2, \ldots, \theta_N$ are given. For each $i = 1, 2, \ldots, N$ associate to y_i a Boolean variable $\tilde{y}_i$ defined by

$$\begin{aligned}\tilde{y}_i &= 1 \quad \text{if } y_i > \theta_i\\ \tilde{y}_i &= 0 \quad \text{if } y_i < \theta_i.\end{aligned} \tag{2.5}$$

In order to model non-linear biochemical control networks, Glass (1975a) has proposed the PL equations

$$\frac{dy_i}{dt} = \lambda_i B_i(\tilde{y}_1, \tilde{y}_2, \ldots, \tilde{y}_{i-1}, \tilde{y}_{i+1}, \ldots, \tilde{y}_N) - \gamma_i y_i \quad i = 1, 2, \ldots, N;\ N+1 = 1, \tag{2.6}$$

where the B_i are Boolean functions, and λ_i, γ_i are once again positive real constants. For example, if we consider in (2.4) the case in which

$$\gamma_i = 1, \qquad \lambda_i = 1, \qquad \theta_i = 0.5, \qquad m_i = m \quad \text{for } i = 1, \ldots, N, \tag{2.7}$$

and consider the limit $m \to \infty$, Equation (2.4) can be written,

$$\begin{aligned}\frac{dy_1}{dt} &= 1 - H(\tilde{y}_N) - y_1,\\ \frac{dy_i}{dt} &= H(\tilde{y}_{i-1}) - y_i, \quad i = 2, 3, \ldots, N,\end{aligned} \tag{2.8}$$

where H is the Boolean function

$$H(1) = 1, \qquad H(0) = 0. \tag{2.9}$$

Stable limit cycle oscillations in (2.8) are demonstrated in Section 5.

Other control networks in addition to the feedback inhibition networks can be represented by continuous equations of the form (2.1). For example, in neurobiology it has been proposed that 'sequential disinhibition' may underlie oscillatory mechanisms (Kling and Szekely, 1968; Friesen, Poon and Stent, 1976). Continuous and PL equations analogous to those given here for feedback inhibition, have been proposed for networks displaying 'sequential disinhibition' (Glass and Pasternack, 1978).

3. State Transition Diagrams for the PL Equations

Our theorem is applicable to (2.6) with the following two modifications.

i) The production constants λ_i can depend on the Boolean vector $\tilde{y}$.
ii) $\gamma_1 = \gamma_2 = \cdots = \gamma_N$.

For convenience, we translate the coordinate system so that the intersection of the N threshold hyperplanes, defined by $y_i = \theta_i$, $i = 1, 2, \ldots, N$, is defined as the origin of N-dimensional Euclidean space in the transformed variables. For each i, let

$$x_i = y_i - \theta_i \tag{3.1}$$

and define the corresponding Boolean variable by

$$\tilde{x}_i = 1 \quad \text{if } x_i > 0, \qquad \tilde{x}_i = 0 \quad \text{if } x_i < 0. \tag{3.2}$$

The PL differential equations of present interest are given by

$$\frac{dx_i}{dt} = \Lambda_i(\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_N) - x_i, \quad i = 1, 2, \ldots, N, \tag{3.3}$$

where Λ_i is nowhere zero and for each i the sign of $\Lambda_i(\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_N)$ is independent of x_i.

A curve in N-dimensional phase space $(x_1(t), x_2(t), \ldots, x_N(t))$ is a *solution* to (3.3) provided that the $x_i(t)$ are continuous, piecewise differentiable and satisfy (3.3) whenever all $x_i \neq 0$. A *non-singular solution* is one in which for each i, $x_i(t) = 0$ only at isolated values of t. A non-singular solution defines an oriented curve in N-dimensional phase space called a *trajectory*. We are only concerned with non-singular solutions.

A local solution of (3.3) originating at a point $(c_1, c_2, \ldots, c_N)$ not on a coordinate hyperplane is given by

$$x_i(t) = \lambda_i + (c_i - \lambda_i)\, e^{-t}, \quad i = 1, 2, \ldots, N, \tag{3.4}$$

where

$$\lambda_i = \Lambda_i(\tilde{c}_1, \tilde{c}_2, \ldots, \tilde{c}_N), \quad i = 1, 2, \ldots, N \tag{3.5}$$

with $\tilde{c}_i$ being the Boolean variable associated to c_i by (3.2). The solution given by (3.4) is a straight line originating at $(c_1, c_2, \ldots, c_N)$ and directed toward $(\lambda_1, \lambda_2, \ldots, \lambda_N)$. All the local solutions to (3.3) in a given orthant are straight lines directed to the same point, called a *focal point*.

Equation (3.4) defines the solution portrait in N-space off the coordinate hyperplanes. To extend the solution portrait to the coordinate hyperplanes maximally extend the local solutions given by (3.4). In general, the solutions will have 'corners' (i.e., be non-differentiable) at the coordinate hyperplanes.

Using the assumption that the sign of $\Lambda_i(x_1, x_2, \ldots, x_N)$ is independent of x_i, it will now be shown that the trajectories are well defined at points of the coordinate hyperplanes where only one coordinate is zero. Each of the 2^N orthants of N-space can be labelled by a Boolean N-tuple $\mathcal{O}(a_1, a_2, \ldots, a_N)$ with $a_i = 1$ if the ith variable is positive and $a_i = 0$ if it is negative. Let $S(a_1, \ldots, a_{i-1}, *, a_{i+1}, \ldots, a_N)$ be the open subset of the hyperplane $x_i = 0$ given by

$$\tilde{x}_1 = a_1, \tilde{x}_2 = a_2, \ldots, \tilde{x}_{i-1} = a_{i-1}, \tilde{x}_{i+1} = a_{i+1}, \ldots, \tilde{x}_N = a_N. \tag{3.6}$$

We will refer to $S(a_1, \ldots, a_{i-1}, *, a_{i+1}, \ldots, a_N)$ as the '*open common boundary*' between the neighboring orthants $\mathcal{O}(a_1, \ldots, a_{i-1}, 0, a_{i+1}, \ldots, a_N)$ and $\mathcal{O}(a_1, \ldots, a_{i-1}, 1, a_{i+1}, \ldots, a_N)$. Since Λ_i is nowhere zero solution curves approach an open common boundary transversely. Trajectories from the same orthant cannot intersect at an open common boundary point and thus, to show that two trajectories from different orthants can be patched together at an open common boundary point it is sufficient to show that at the boundary point, an orientation for the trajectory can be defined. This is guaranteed by the assumption that the sign of $\Lambda_i(\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_N)$ is independent of $\tilde{x}_i$. In fact, if the sign of Λ_i is positive in $\mathcal{O}(a_1, \ldots, a_{i-1}, 0, a_{i+1}, \ldots, a_N)$ and $\mathcal{O}(a_1, \ldots, a_{i-1}, 1, a_{i+1}, \ldots, a_N)$ the trajectories flow across $S(a_1, \ldots, a_{i-1}, *, a_{i+1}, \ldots, a_N)$ from $\mathcal{O}(a_1, \ldots, a_{i-1}, 0, a_{i+1}, \ldots, a_N)$ to $\mathcal{O}(a_1, \ldots, a_{i-1}, 1, a_{i+1}, \ldots, a_N)$, and flow is in the opposite direction if the sign of Λ_i is negative in both orthants.

It is a consequence of this discussion that the flow defined by (3.3) can be represented by an N-cube with directed edges. To each orthant $\mathcal{O}(a_1, \ldots, a_N)$ is associated the vertex $(a_1, \ldots, a_N)$ of an N-cube and to each open common boundary of two orthants is associated the connecting edge on the N-cube; the edge is directed according to the direction of flow across the boundary. The N-cube with directed edges is called the *state transition diagram* for the system (3.3).

For example, the following equation models a simple feedback inhibition system,

$$\begin{aligned} \frac{dx_1}{dt} &= (-1 + 2\bar{\tilde{x}}_N) - x_N, \\ \frac{dx_i}{dt} &= (-1 + 2\bar{\tilde{x}}_{i-1}) - x_i, \quad i = 2, 3, \ldots, N, \end{aligned} \tag{3.7}$$

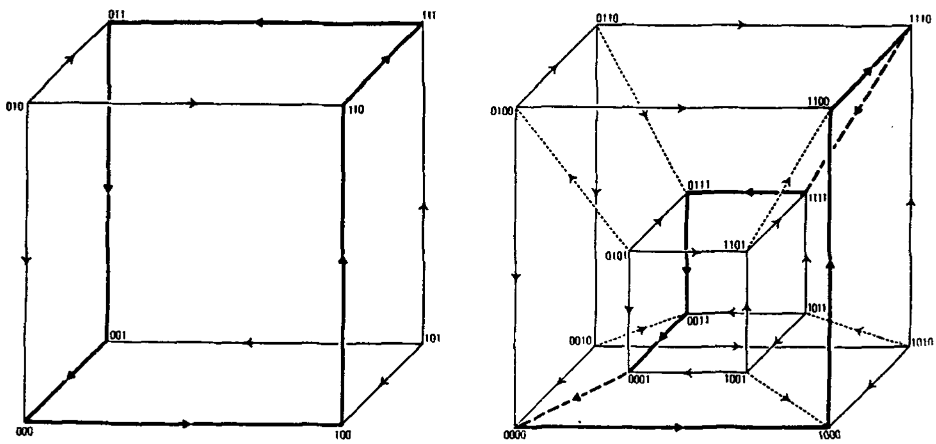
where $\bar{0} = 1$ and $\bar{1} = 0$ (compare with (2.8)). The state transition diagrams for (3.7) with $N = 3, 4$ are shown in Figure 1.

4. Limit Cycles in the PL Equations

In the theory of ordinary differential equation, a *stable limit cycle* is a periodic solution to a differential equation which has the property that the trajectory through every point in phase space sufficiently close to the closed curve defined by the periodic solution approaches that closed curve asymptotically as $t \to \infty$ (Hirsch and Smale, 1974). The analogue of a stable limit cycle is now defined for a cycle on an N-cube with directed edges.

Definitions

i) A *cycle* on an N-cube with directed edges is a finite sequence of vertices, each vertex sharing a common edge with the preceding and succeeding vertex in sequence and no vertex appears more than once in the sequence except the first



(a) (b)

Fig. 1a and b. The state transition diagrams for Eq. (3.7) with **a)** $N = 3$, **b)** $N = 4$. The cyclic attractor is indicated by heavy edges

and last vertices are identical. The edge between successive vertices is directed from one to the next in sequence.

ii) A vertex, not on a given cycle, which shares a common edge with a vertex of the cycle is *adjacent* to the cycle.

iii) A *cyclic attractor* is a cycle for which there are $(N - 2)$ vertices adjacent to each vertex of the cycle and the edge(s) from each adjacent vertex to the cycle is (are) directed toward the cycle.

iv) An *N-dimensional cyclic attractor* is a cyclic attractor on an N-cube which is not contained on any lower dimensional sub-cube.

The main result of this paper is the following theorem which gives a complete classification of the topological features of the flow defined by (3.3) through the orthants of phase space associated to an N-dimensional cyclic attractor in the state transition diagram.

Theorem. *Given an N-dimensional system of Equations* (3.3) *in which the state transition diagram has an N-dimensional cyclic attractor, then one of the following two situations holds:*

1) *There is a stable limit cycle in phase space which passes through the orthants in the same sequence and order as the cyclic attractor in the state transition diagram. The trajectories through the points of orthants represented by vertices of the cyclic attractor and the points of boundaries represented by edges of the cyclic attractor asymptotically approach the limit cycle as $t \to \infty$.*
2) *The trajectories through the points of orthants and boundaries represented by the cyclic attractor asymptotically approach the origin as $t \to \infty$.*

The two cases can be distinguished by determining if the leading eigenvalue of a positive matrix associated with the PL equations is greater than 1.

The strategy of the proof is as follows. Let S_i be any open common boundary represented by an edge of the cyclic attractor. Then we will show that the PL equations define a map $h_i: S_i \to S_i$. h_i is called the *Poincaré map* or *return map*. A *fixed point* of the Poincaré map, $v^* \in S_i$ is defined by

$$h_i(v^*) = v^*. \tag{4.1}$$

Fixed points of the Poincaré map correspond to limit cycles in the PL equations (Hirsch and Smale, 1974). For a point $z \in S_i$, consider the sequence z, $h_i(z)$, $h_i^2(z) = h_i(h_i(z)), \ldots, h_i^n(z) = h_i(h_i^{n-1}(z))$. Then the theorem can be proved once we show that for all $z \in S_i$ either

$$\text{i)} \lim_{n\to\infty} h_i^n(z) = v^*,$$

or (4.2)

$$\text{ii)} \lim_{n\to\infty} h_i^n(z) = 0.$$

In the first case, all trajectories asymptotically approach a limit cycle which passes through $v^* \in S_i$. In the second case, all trajectories asymptotically approach the origin. We prove the theorem by first explicitly computing the form of the Poincaré map for the flows defined by the cyclic attractors in the PL equations, and then by demonstrating that the properties of the Poincaré map under iteration are as given above. The details of the proof are given in the Appendix.

5. Examples

In this section we present three examples to show that stable limit cycle oscillations exist in all dimensions $N \geqslant 2$, and to explicitly compute the limit cycle in selected cases. As we shown in the Appendix for (3.3) with an N-dimensional cyclic attractor, the Poincaré map for the flow in regions of phase space corresponding to the cyclic attractor contains an $(N - 1) \times (N - 1)$ positive matrix, A. If the leading eigenvalue, ρ, of A is greater than 1, stable limit cycles are found. We omit most computations.

Example 1. The 2-dimensional cyclic attractor.

This is the system found for feedback inhibition in 2 dimensions. A verbal description of this system is x_1 stimulates the production of x_2 and x_2 inhibits the production of x_1. Thus, the system is analogous to a predator–prey system where x_1 is the prey and x_2 is the predator. A 2-dimensional vector field is shown in Figure 2. Assume the Λ_i in the four quadrants are assigned the values

$\tilde{x}_1$	$\tilde{x}_2$	Λ_1	Λ_2
1	1	a_4	b_4
1	0	a_3	b_3
0	1	a_1	b_1
0	0	a_2	b_2

Then it can be easily shown that the positive x_1 axis is mapped into itself by the map

$$h_1(x_1) = \frac{\rho x_1}{1 + r x_1}, \tag{5.1}$$

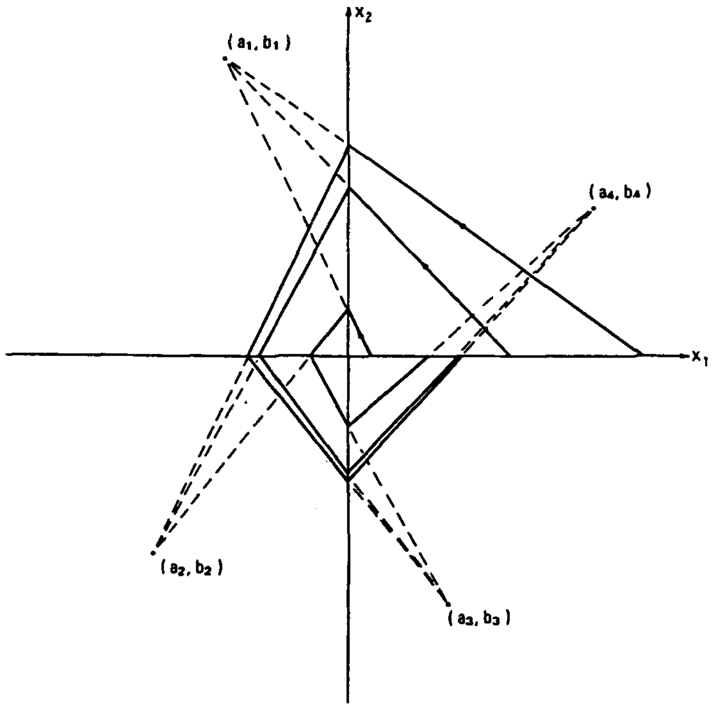


Fig. 2. A geometrical construction of the flow for the two dimensional cyclic attractor of Eq. (3.3). For this case all trajectories tend to a stable limit cycle oscillation (see Example 1, Section 5)

where

$$\rho = \frac{a_4 b_3 a_2 b_1}{b_4 a_3 b_2 a_1}, \tag{5.2}$$

$$r = \frac{1}{a_1} + \frac{b_1}{a_1 b_2} + \frac{b_1 a_2}{a_1 b_2 a_3} + \frac{b_1 a_2 b_3}{a_1 b_2 a_3 b_4}.$$

It is well known (May, 1975) and also follows from the results in the Appendix, that

$$\begin{aligned} &\lim_{n\to\infty} h_1^n(x_1) = 0, \quad \text{for } \rho \leqslant 1, \\ &\lim_{n\to\infty} h_1^n(x_1) = \frac{\rho - 1}{r}, \quad \text{for } \rho > 1, \end{aligned} \tag{5.3}$$

for all $x_1 > 0$. Thus the number $(a_4 b_3 a_2 b_1)/(b_4 a_3 b_2 a_1)$ can be used to classify the flow into two topologically distinct phase portraits. This result is reminiscent of results found in many other studies of non-linear oscillations in two dimensions (May, 1972; Hirsch and Smale, 1974).

Example 2. A choice of focal points generating limit cycles for $N \geqslant 3$.

Assume all the focal points for the flows in (3.3) lie on the '*unit N-cube*' of side length 2 whose vertices are at the coordinates $(w_1, w_2, \ldots, w_N)$, where $w_i = \pm 1$

for $i = 1, 2, \ldots, N$. Consider any system (3.3) with an N-dimensional cyclic attractor $N \geqslant 3$. In this particular case, it is not difficult to show that each entry a_{ij} of the matrix A of the Poincaré map (see Appendix, Equation (A.1)) is a positive integer. Define

$$S_i = \sum_{k=1}^{N-1} a_{ik}, \quad s = \min S_i, S = \max S_i; \tag{5.4}$$

then the dominant eigenvalue, ρ, satisfies (Gantmacher, 1959)

$$s \leqslant \rho \leqslant S \tag{5.5}$$

so that $\rho > 1$. Consequently, from the theorem, for each case there is a stable limit cycle oscillation. This conclusion appeared as a conjecture in earlier publications (Glass, 1977a, 1977b; Glass and Pasternack, 1978).

Remark. Counting only those cyclic attractors which are different under the symmetry of the N-cube there is one 3-dimensional cyclic attractor, three 4-dimensional cyclic attractors, and eighteen 5-dimensional cyclic attractors (Glass, 1977b). The period for the limit cycle associated with each of these cyclic attractors for the set of focal points described in this section was numerically computed by Glass (1977b).

Example 3. Equation (3.7).

Here we consider the N-dimensional cyclic attractor for feedback inhibition systems with N-variables when the focal points are on the unit N-cube. The equation corresponding to this case is given in (3.7). From Example 2 we know that there is a unique stable limit cycle for $N \geqslant 3$. The focal points are located in the orthants (cf. Figure 1) $(1, 1, 1, \ldots, 1, 1) \to (0, 1, 1, \ldots, 1, 1) \to (0, 0, 1, \ldots, 1, 1) \to \cdots \to (0, 0, 0, \ldots, 0, 0) \to (1, 0, 0, \ldots, 0, 0) \to (1, 1, 0, \ldots, 0, 0) \to \cdots \to (1, 1, 1, \ldots, 1, 1) \to \cdots$. Equation (3.7) displays a $2N$-fold symmetry generated by the map

$$(x_1, x_2, \ldots, x_N) \to (-x_N, x_1, \ldots, x_{N-1}). \tag{5.6}$$

From (A.3)

$$f_1(x_1, x_2, \ldots, x_{N-1}, 0) = (0, x_1 + x_2, \ldots, x_1 + x_{N-1}, x_1)/(1 + x_1). \tag{5.7}$$

Using the symmetry (5.6) a point $(u_1, u_2, \ldots, u_{N-1}, 0)$ satisfying

$$f_1(u_1, u_2, \ldots, u_{N-1}, 0) = (0, u_1, u_2, \ldots, u_{N-1}) \tag{5.8}$$

is a fixed point of the return map h_1.

The solution of (5.7) and (5.8) is

$$u_1 = (1 - R_m)/R_m, \tag{5.9}$$

$$u_i = \left(1 - \sum_{j=1}^{i-1} R_m^j\right)(1 - R_m)R_m^{-i}, \quad i = 2, 3, \ldots, m - 1,$$

$$u_m = 1 - R_m,$$

where $m = N - 1$ and R_m is the unique root of the equation

$$0 = \sum_{j=1}^{m} z^j - 1, \quad 0 < z < 1. \tag{5.10}$$

Remark. This example is closely related to two classic applications of linear algebra. The matrix A of the linear fractional map defined by (5.7) (see Appendix) is related to the Leslie matrices of population biology (Leslie, 1945; Busher, 1972). In fact, for this case the matrix A is the transpose of a Leslie matrix in which all non-zero elements are 1. In addition, R_m is related to the ratio of successive terms of a generalized Fibonacci series. If an *mth order Fibonacci series* is defined recursively by the formula

$$\beta_n = \beta_{n-1} + \beta_{n-2} + \cdots + \beta_{n-m} \tag{5.11}$$

then

$$R_m = \lim_{n \to \infty} \frac{\beta_{n-1}}{\beta_n}. \tag{5.12}$$

Using (5.9) and (5.12) the coordinates of a point on the stable limit cycle of (3.7) can be readily computed on a hand calculator. For example, for $N = 3$, the cycle passes through the point (0.618, 0.382, 0), and for $N = 4$, the cycle passes through the point (0.839, 0.704, 0.456, 0). These values were found numerically by direct integration of (3.7) well before the analytic results were available (Glass, 1977a, 1977b).

6. Discussion

In the preceding sections we have demonstrated asymptotic stability over a well defined domain in phase space for limit cycle oscillations in PL equations (3.3). We believe these results are of interest, both from a mathematical and biological perspective.

For small amplitude limit cycles, numerous results concerning existence and stability have been derived by bifurcation analysis (Marsden and McCracken, 1977). However, there are only a few demonstrations of uniqueness and stability for large amplitude limit cycles in non-linear differential equations in more than two dimensions (Smale, 1974; May and Leonard, 1975; Glass, 1977a; Hastings, 1977). Moreover, topological approaches using the Brouwer fixed point theorem have only succeeded in demonstrating existence of cyclic solutions (Tyson, 1975; Hastings, Tyson and Webster, 1977; Hsü, 1977). As a result of our choice of the PL equations, we have been able to compute explicitly the form of the Poincaré map, and this enabled us to prove our theorem. To our knowledge, this work constitutes the first proof of large amplitude stable limit cycles in a class of differential equations in N-dimensions. The next step is to show stability and uniqueness of the limit cycle oscillations in continuous equations which approximate the PL equations (Glass and Pasternack, 1978). Generalizations of Perron's theorem (see Appendix) (Lee, 1972) may be valuable in extending our results.

We believe that it makes sense to try to classify dynamics of complex biological systems, and further that the properties of the PL equations are sufficiently broad, that a great many biological systems can be identified with one or another of the classes of the PL equations (Glass and Kauffman, 1973; Glass, 1975b; Glass and Pasternack, 1978). Knowledge of the qualitative dynamics of the PL equations is necessary to identify particular biological examples. Our proof of limit cycles in a broad class of the PL equations provides a context for detailed analysis of the interactions which lead to oscillations in complex biological control systems.

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Appendix—Proof of the Theorem

The proof relies on our ability to compute the Poincaré map for flows in PL equations whose state transition diagrams contain a cyclic attractor. As we will show, the Poincaré map is of the form

$$f(z) = \frac{Az}{1 + \langle \phi, z \rangle} \tag{A.1}$$

where A is an $(N-1) \times (N-1)$ matrix, ϕ is an $(N-1)$ vector, z is a point on an open common boundary, and the symbol $\langle \ , \ \rangle$ represents the inner product of two vectors. The map defined in (A.1) is called a *linear fractional map.* Linear fractional maps are commonly used in complex analysis (Marsden, 1973). The composition of two linear fractional maps is again a linear fractional map.

In Proposition 1 we show that for the Poincaré maps of interest herein, A can be taken to be a positive matrix (all elements of a *positive matrix* are positive real numbers) and the vector ϕ is a non-negative vector with at least one non-zero component. The Perron theorem states that for a positive matrix, the largest or dominant eigenvalue is positive, and associated to this eigenvalue is an eigenvector all of whose components can be taken positively (Gantmacher, 1959; Bellman, 1970). Furthermore, there is no other linearly independent eigenvector in the non-negative orthant. Proposition 2 utilizes the Perron theorem to establish the limiting behavior of the Poincaré maps.

The Form of the Poincaré Map

Let $\mathcal{O}_1, \mathcal{O}_2, \ldots, \mathcal{O}_L$ be the open orthants corresponding to the vertices of a cyclic attractor in the state transition diagram (see p. 212). Each orthant has N neighboring orthants and for each $\mathcal{O}_j$, $j = 1, 2, \ldots, L$ the flow is directed into the orthant from $(N-1)$ of its neighboring orthants and out of the orthant through its common boundary with $\mathcal{O}_{j+1}$; the focal point for $\mathcal{O}_L$ is in $\mathcal{O}_1$.

Let S_1 be the open common boundary of $\mathcal{O}_L$ and $\mathcal{O}_1$, and S_j be the open common boundary of $\mathcal{O}_{j-1}$ and $\mathcal{O}_j$. By relabelling the axes we may assume that the sequence $\mathcal{O}_L, \mathcal{O}_1, \mathcal{O}_2$ corresponds to the sequence of vertices $(1, 1, 1, \ldots, 1, 0), (1, 1, 1, \ldots, 1, 1)$, $(0, 1, 1, \ldots, 1, 1)$. From (3.4) it follows that a trajectory through $(x_1, x_2, \ldots, x_N) \in \mathcal{O}_1$ intersects S_2 at

$$x_1' = 0,$$

$$x_i' = \frac{\lambda_i x_1 - \lambda_1 x_i}{x_1 - \lambda_1} \quad \text{for } i = 2, 3, \ldots, N, \tag{A.2}$$

where $\lambda_i = \Lambda_i(1, 1, \ldots, 1)$ for $i = 1, 2, \ldots, N$. Since $(\lambda_1, \lambda_2, \ldots, \lambda_N) \in \mathcal{O}_2$, $\lambda_1 < 0$ and $\lambda_i > 0$ for $i = 2, 3, \ldots, N$ and (A.2) can be rewritten

$$x_1' = 0$$

$$x_i' = \frac{|\lambda_i/\lambda_1|\,|x_1| + |x_i|}{|x_1/\lambda_1| + 1}. \tag{A.3}$$

Equation (A.3) applies equally well for points $(x_1, x_2, \ldots, x_{N-1}, 0) \in S_1$. Thus all trajectories in $\mathcal{O}_1$ and all trajectories passing through S_1 intersect S_2.

The map $f_1\colon S_1 \to S_2$ defined by (A.3) is of the form shown in (A.1), where $z = (x_1, x_2, \ldots, x_{N-1})$ is a $(N-1)$ vector giving the coordinates on the hyperplane $x_N = 0$, A is a $(N-1) \times (N-1)$ matrix and ϕ is a $(N-1)$ vector. A and ϕ are given by

$$A = \begin{pmatrix} |\lambda_2/\lambda_1| & 1 & 0 & \cdots & 0 \\ |\lambda_3/\lambda_1| & 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & & \\ |\lambda_{N-1}/\lambda_1| & 0 & 0 & \cdots & 1 \\ |\lambda_N/\lambda_1| & 0 & 0 & \cdots & 0 \end{pmatrix}, \tag{A.4}$$

$$\phi = (1/|\lambda_1|, 0, 0, \ldots, 0).$$

The map f_1 gives the coordinates of $f_1(z)$ with respect to the basis $(0, 1, 0, 0, \ldots, 0)$, $(0, 0, 1, \ldots, 0), \ldots, (0, 0, 0, \ldots, 1)$ of the coordinate hyperplane $x_1 = 0$.

In a similar manner, trajectories through S_2 intersect S_3 and so on with trajectories through S_L intersecting S_1. Let f_1 map S_1 into S_2 according to formula (A.2), let f_j map S_j to S_{j+1} analogously for $j = 2, 3, \ldots, L-1$ and f_L map S_L to S_1. The Poincaré map h_1 is given by the composition of the f_i

$$h_1 = f_L \circ f_{L-1} \circ \cdots \circ f_2 \circ f_1. \tag{A.5}$$

Each of the maps f_j, $j = 2, 3, \ldots, L$ can also be written in the form of (A.1) by suitable choice of bases for each of the common boundaries S_j. Since the composition of linear fractional maps is a linear fractional map, h_1 is of the form shown in (A.1).

Proposition 1. *For a point $z \in S_1$, the Poincaré map h_1 is of the form shown in* (A.1) *where A is a positive $(N-1) \times (N-1)$ matrix and ϕ is a non-negative non-zero vector.*

Proof of Proposition 1. First observe that each map f_i in (A.5) defines a linear fractional map with domain the $(N - 1)$ dimensional coordinate hyperplane containing S_i. Let $e_1 = (1, 0, \ldots, 0)$, $e_2 = (0, 1, \ldots, 0), \ldots, e_{N-1} = (0, 0, \ldots, 1, 0)$ be a basis for the hyperplane $x_N = 0$. From (A.2),

$$f_1(e_1) = (0, |\lambda_2|, |\lambda_3|, \ldots, |\lambda_N|)/(1 + |\lambda_1|), \tag{A.6}$$

$$f_1(e_i) = e_i \quad \text{for } i = 2, 3, \ldots, N - 1.$$

It is seen that $f_1(e_1) \in S_2$ and since f_i maps S_i into S_{i+1} it follows from (A.5) that $h_1(e_1) \in S_1$. This means that $h_1(e_1)$ has positive coordinates with respect to the above basis for S_1. Thus the first column of the matrix A has only positive entries.

For some integer $q = 2, 3, \ldots, N - 1$ S_3 is contained in the coordinate hyperplane $x_q = 0$. It follows that $f_2(f_1(e_q)) \in S_3$. Again, since f_j: $S_j \to S_{j+1}$ it can be concluded from (A.5) that $h_1(e_q) \in S_1$, and $h_1(e_q)$ has positive coordinates with respect to the above basis for S_1. Thus the qth column of A has only positive entries.

Continuing in this way, it follows that all the columns of A have only positive entries because the cyclic attractor is N-dimensional and thus for each $i = 1, 2, \ldots, N - 1$, $h_1(e_i)$ has positive coordinates with respect to the above basis for S_1. Further, since the formula for the maps f_j are analogous to (A.4), ϕ is a non-zero, non-negative vector. (Q.E.D. Proposition 1.)

Proposition 2. *Consider the map in* (A.1) *where A is a positive matrix and ϕ is a non-negative vector with at least one non-zero component. Let ρ be the dominant eigenvalue of A with associated positive eigenvector v. Then, for all non-zero z in the non-negative orthant*

$$\lim_{n \to \infty} f^n(z) = \alpha v \tag{A.7}$$

where

$$\begin{aligned} &\text{i)}\ \ \alpha = 0 \quad \textit{for } \rho \leqslant 1, \\ &\text{ii)}\ \ \alpha = \frac{\rho - 1}{\langle \phi, v \rangle} \quad \textit{for } \rho > 1. \end{aligned} \tag{A.8}$$

Proof of Proposition 2. By iterating the map f, one computes

$$f^n(z) = \frac{A^n(z)}{1 + \langle \phi, z + A(z) + \cdots + A^{n-1}(z) \rangle}. \tag{A.9}$$

It is an immediate consequence of Perron's Theorem that A/ρ is similar to a stochastic matrix (Gantmacher, 1959) and that consequently (Gantmacher, 1959; Bellman, 1970)

$$M = \lim_{n \to \infty} \frac{A^n}{\rho^n} \tag{A.10}$$

where M is a matrix all of whose columns are scalar multiples of the eigenvector v. From (A.1) we have

$$f^n(z) = \frac{A^n(z)/\rho^n}{(1 + \langle \phi, z + A(z) + \cdots + A^{n-1}(z) \rangle)/\rho^n}. \tag{A.11}$$

Case 1, $\rho < 1$

The numerator of (A.11) is finite and the denominator is infinite as $n \to \infty$. Thus

$$\lim_{n\to\infty} f^n(z) = 0. \tag{A.12}$$

Case 2, $\rho = 1$

Here $\langle \phi, z + A(z) + \cdots + A^{n-1}(z)\rangle$ is infinite as $n \to \infty$ and (A.12) holds in this case.

Case 3, $\rho > 1$

Define $S_n(z)$ and $\sigma_n(z)$ by the following,

$$S_n(z) = \frac{z}{\rho^n} + \frac{A(z)}{\rho^n} + \frac{A^2(z)}{\rho^n} + \cdots + \frac{A^{n-1}(z)}{\rho^n}, \tag{A.13}$$

$$\sigma_n(z) = \frac{z}{\rho^n} + \frac{M(z)}{\rho^{n-1}} + \frac{M(z)}{\rho^{n-2}} + \cdots + \frac{M(z)}{\rho}, \tag{A.14}$$

where M is defined in (A.10). Substituting (A.13) into (A.11) gives

$$f^n(z) = \frac{A^n(z)/\rho^n}{\rho^{-n} + \langle \phi, S_n(z)\rangle}. \tag{A.15}$$

From (A.10) for each z,

$$\lim_{n\to\infty} A^n(z)/\rho^n - M(z) = 0. \tag{A.16}$$

Using (A.16) together with the fact that

$$\lim_{n\to\infty} \frac{n}{\rho^n} = 0 \quad \text{for } \rho > 1, \tag{A.17}$$

it is not difficult to verify that

$$\lim_{n\to\infty} S_n(z) - \sigma_n(z) = 0 \quad \text{for all } z. \tag{A.18}$$

By factoring out $M(z)$ and summing the geometric series

$$\lim_{n\to\infty} \sigma_n(z) = \frac{M(z)}{\rho - 1} \quad \text{for all } z. \tag{A.19}$$

(A.18) and (A.19) imply that

$$\lim_{n\to\infty} S_n(z) = \frac{M(z)}{\rho - 1} \quad \text{for each } z. \tag{A.20}$$

Taking the limit in (A.15) yields

$$\lim_{n\to\infty} f^n(z) = \frac{(\rho - 1)M(z)}{\langle \phi, M(z)\rangle} \quad \text{for each } z. \tag{A.21}$$

For z a non-negative vector, $z \neq 0$, $M(z) = \beta v$ for some non-zero scalar β. Thus

$$\lim_{n\to\infty} f^n(z) = \frac{(\rho - 1)v}{\langle \phi, v\rangle} \tag{A.22}$$

for all non-negative vectors z, $z \neq 0$. (Q.E.D. Proposition 2.)

From Proposition 2 it follows that $f(z)$ has a non-zero fixed point if and only if $\rho > 1$. In this case, the iterates of f approach the non-zero fixed point. If f has no non-zero fixed point, the iterates of f approach zero.

To complete the proof, it is now observed that if v^* is a non-zero fixed point of the return map h_1, then $f_j \circ f_{j-1} \circ \cdots \circ f_1(v^*)$ is a non-zero fixed point of the return map h_j. Similarly, if any h_j has a non-zero fixed point then h_1 has a non-zero fixed point. Thus, all the return maps $h_j, j = 1, 2, \ldots, L$ have a non-zero fixed point or none of them do. In the former case, the flow exhibits a cycle which passes through the fixed points of the return maps h_j.

The trajectories in each orthant $\mathcal{O}_j, j = 1, 2, \ldots, L$ are straight lines which intersect the boundary S_{j+1} in finite time and the theorem therefore follows from the limiting property of iterations of the return maps. (Q.E.D. Theorem.)

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