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The Logical Analysis of Continuous, Non-linear Biochemical Control Networks

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We propose a mapping to study the qualitative properties of continuous biochemical control networks which are invariant to the parameters used to describe the networks but depend only on the logical structure of the networks. For the networks, we are able to place a lower limit on the number of steady states and strong restrictions on the phase relations between components on cycles and transients. The logical structure and the dynamical behavior for a number of simple systems of biological interest, the feedback (predator–prey) oscillator, the bistable switch, the phase dependent switch, are discussed. We discuss the possibility that these techniques may be extended to study the dynamics of large many component systems.

1. Logical vs. Continuous Models and Biological Observables

So far it has proved impossible to develop general techniques which may be applied to find the asymptotic behavior of complex chemical systems. Despite the difficulties a number of important properties of complex chemical kinetic schemes have been demonstrated. They include the following.

(i) Oscillations cannot occur for reaction schemes involving only monomolecular reactions (Bak, 1963).
(ii) Oscillations can appear only far from equilibrium where the Onsager reciprocity relations no longer hold (Prigogine, 1961).
(iii) Some chemical reactions occurring in the homogeneous phase are

unstable with respect to small perturbations of concentration and evolve to an inhomogeneous asymptotic state (Turing, 1952).

In addition to these general results a number of specific chemical reaction schemes have been proposed and analyzed which display the interesting properties of oscillation (Lotka, 1920; Higgins, 1967), multiple steady states (Spangler & Snell, 1961), hysteresis (Edelstein, 1970) and spatially periodic asymptotic states (Turing, 1952; Prigogine & Nicolis, 1971).

Despite these important advances, the rudimentary nature of our knowledge about the properties of non-linear differential equations necessitates analyzing each reaction separately using cumbersome methods which have proved impractical to apply to all but the simplest systems. The limitations which appear to be inherent in the techniques of non-linear analysis make it imperative that alternate methods be developed which may be used to give at least qualitative information about the dynamic behavior of complex chemical systems.

In chemical kinetics progress has been made by studying chemical concentrations as a function of time. The kinetic equations and mechanisms of a large number of simple chemical reactions have been determined. Application of classical techniques to the study of simple enzyme reactions (Atkinson, 1966) as well as the gene control systems (Yagil & Yagil, 1971) has proved to be fruitful. However, the extremely low cellular concentrations of critical compounds as well as the need for monitoring the concentration of a large number of components as a function of time, will pose substantial difficulties to future investigators of the properties of complex chemical systems, such as are found in biochemical control networks.

However, a number of global biological observables, which have been experimentally studied may also be subject to theoretical analysis. They include the following.

(i) Localization of activity—although cells undergo large reversible modulations of both morphology and enzyme composition during their lifetime, they may in general be readily classified as belonging to one of a small number of different cell types which are found in a given organism.

(ii) The number of cell types—the number of cell types of all organisms of a given species are the same. The number of cell types apparently increases as the number of genes of organisms increases.

(iii) Phase relationships between components-activated puffing patterns of genes have been observed for a variety of cells in fruitflies. Different cell types have different characteristic puffing patterns (Berendes, 1968).

(iv) Limited potential for differentiation—in development in animals there is only a limited time during which the path of differentiation of a given cell may be altered by manipulation. There are also only a limited number of cell types to which any particular cell can be transformed. Similarly in transdetermination studies on fruitflies it has been observed that imaginal disks for different structures may be subject only to a limited number of transitions, e.g., a haltere disk can form a wing but not an eye (Gehring, 1968).

The global, topological nature of these observables apparently places their study outside of the range of classical chemical kinetics. A theory which has been developed explicitly to study these observables has recently been proposed by Kauffman (1969, 1971*a*,*b*). The assumption is made that key elements (genes) in the biochemical control networks of cells may be modeled as switches and that each component realizes a randomly chosen Boolean function on two or three randomly chosen inputs. If the assumption that an asymptotic state of the discrete switching network corresponds to a cell type of an organism is made, the same properties listed above can be studied for these model networks. Good qualitative agreement has been found between the global properties of the model networks and the corresponding properties of cells.

The assumption, that genes may be idealized as switches, invariably strikes those with a firm foundation in chemical thermodynamics as wrong. Chemical reactions at the metabolic level do not disobey the laws of thermodynamics. However, as a consequence of the cooperative interactions of key control elements (e.g. enzymes, repressors), the coupling of energy rich compounds into reactions and large displacement from thermodynamic equilibrium, many important reactions appear *in vivo* to be nearly irreversible and to follow a sigmoidal rate dependence on the concentration of key metabolites in the cell (Monod & Jacob, 1961; Atkinson, 1966). This behavior is well documented both in enzyme (Monod, Wyman & Changeux, 1965) and gene (Yagil & Yagil, 1971) control systems.

The extent to which continuous biochemical networks of cooperative components may be modeled by logical networks has not been thoroughly explored. Sugita (1963) has presented logical equivalents of some simple networks proposed by Monod & Jacob (1961). However, he left largely unexplored the possibility that differences might arise between the behavior of the logical and continuous systems and also did not investigate the extensions of the logical descriptions to more complex systems of many elements. Rosen (1968*a*) and Arbib (1966) have discussed some formal similarities between the properties of continuous and discrete biochemical

networks. Other literature has been thoroughly reviewed by Rosen (1968*b*).

In a recent study we explored the properties of some simple networks of catalytic components which were localized in compartments and produced diffusible chemicals which controlled the activities of other components via a sigmoidal control function (Glass & Kauffman, 1972). One of the principle findings of that work was that the qualitative behavior of the systems was largely independent of the precise form of the sigmoidal control function. For example, for a simple network corresponding to a feedback inhibition loop it was found that neither the period nor the phase relationships of the oscillation of the components was markedly altered by a change of the control functions from the discontinuous Heaviside step function to the more realistic continuous Hill function. This observation strongly suggests that switching networks may be used to study continuous networks which model realistic biological control systems.

In the present work we explore the relationships which exist between a class of non-linear equations representing biochemical control networks and homologous switching networks. After developing the equations which describe these homologous systems in section 2, we present a mapping in section 3 which allows us to study qualitative features of the continuous system without explicitly solving the equations of motion. In section 4 and 5 we discuss the application of these techniques to some simple biological systems and indicate how information may be determined about the important biological observables we have discussed above, the number of asymptotic states, phase relationships between components and transitions between asymptotic states. Our analytical results are presented in the Appendices in which we develop some formal qualitative properties of continuous systems which realize Heaviside functions on their inputs.

2. Continuous Biochemical Networks and their Discrete Homologues

We are interested in studying the dynamics of arbitrarily cross coupled biochemical networks in which the rates of synthesis of key metabolites are regulated by the concentrations of control molecules in the medium. Further, although synthesis is localized, key metabolites are assumed to undergo transport through the system as well as modification by biochemical transformation. We have proposed (Glass & Kauffman, 1972) that these systems may be represented by a series of coupled non-linear equations in which we explicitly consider (i) regulation of synthesis of key metabolites by non-linear control functions, called F_k, (ii) exponential decay of key metabolites at a rate proportional to their concentration and (iii) transport of metabolites by

diffusion. If we assume that there are N components in a spatially linear system of M compartments, we find

$$\frac{dX_k(J)}{dt} = \delta_{P_k J}\lambda_k F_k[X_i(J), X_j(J)] - \gamma_k X_k(J) + D_k \times [X_k(J-1) - 2X_k(J) + X_k(J+1)],$$
$$J = 1, M,$$
$$k = 1, N, \tag{1}$$

where $X_k(J)$ is the concentration of the kth component in the Jth compartment; λ_k, γ_k and D_k are the production, decay and diffusion constants, respectively, for the kth component; P_k is the compartment in which synthesis of the kth component is localized; the delta function $\delta_{i,j}$ is one if $i = j$ and is zero otherwise; and the boundary conditions

$$X_k(0) = X_k(1),$$
$$X_k(M+1) = X_k(M), \qquad k = 1, N \tag{2}$$

ensure that there is no flow out of the system.

We have found that when the control functions, F_k, are continuous homologues of logical control functions that some qualitative properties of equation (1) may be studied without solving the equations explicitly. In logical switching networks time is quantized and the state of each component is 0 or 1 at each time. The state of any component may be determined if the states of its inputs and if the logical function which it realizes on these inputs are known. Let us consider which logical functions are appropriate to represent observed biochemical control relationships.

In the studies of the regulation of the synthesis of inducible enzymes, for example, β-galactosidase, the enzyme is normally produced at a low basal rate. As the concentration of the inducer, in this case a metabolic derivative lactose, is increased through some critical concentration range, the synthesis of the enzyme rapidly increases to a maximal rate (Yagil & Yagil, 1971). The resulting curve is characteristically S-shaped having distinct upper and lower asymptotes and an intermediate region of rapid rate change. We may generalize this rate dependence in terms of a bounded, monotonic, sigmoidal function,

$$\frac{dX_2}{dt} = S(X_1), \qquad 0 \leqslant S(X_1) \leqslant 1. \tag{3}$$

where we have called β-galactosidase, X_2, and lactose, X_1. A functional form which has often been found to adequately represent the experimental data is the Hill function,

$$S(X) = \frac{X^n}{X^n + \theta_i^n}, \qquad n \geqslant 2, \tag{4}$$

where n is the Hill coefficient and θ_i is a threshold parameter for regulation of synthesis of the ith component. Regulatory functions of this type are suitable for substitution for F_κ in equation (1). The relationship X_1 induces X_2 is schematically represented in Fig. 1(a). In Fig. 1(b) we display the truth table for the discrete system which corresponds to induction, if X_1 is 1 at time τ, X_2 will be 1 at time $\tau+1$, and if X_1 is 0 at time τ, X_2 will be 0 at $\tau+1$. In similar fashion, the relationship X_1 represses (inhibits) the production of X_2 can be represented in terms of the generalized function

$$\frac{dX_2}{dt} = 1-S(X_1). \tag{5}$$

The corresponding diagrammatic and discrete functions are given in Figs 1(c), (d).

We determine the dynamic behavior of discrete systems and their continuous homologues in the following way. We first choose a logical structure

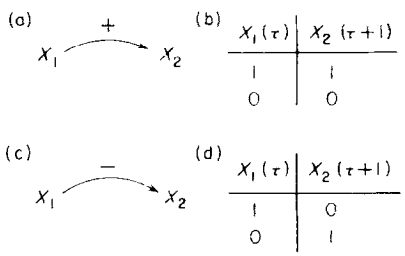

FIG. 1. The diagrammatic (a) and truth table (b) representations of the relationship X_1 activates X_2. The diagrammatic (c) and truth table (d) representations of the relationship X_1 inhibits X_2.

for a system. This logical structure may be simply represented diagrammatically using the symbols from Fig. 1. We then write down the switching network corresponding to this structure. The complete behavior of the switching network starting from any initial state may then be determined. The equation for the homologous continuous system may then be written by specifying F in equation (1) using equations (3) and (5). After selection of an appropriate expression for the sigmoidal function for example, equation (4), and the parameters in equation (1), the continuous equation is solved on a digital computer. In choosing the parameters for equation (1) the only requirements which we make are:

(a) the functions $S(X)$ must be sigmoidal; they must be monotonic and have distinct upper and lower asymptotes;

(b) the parameters must be chosen so that if a chemical is produced at either its maximal or basal rate the target control function must be either on its upper or lower asymptote.

To make comparisons between the continuous and switching networks we define the state of a component in a continuous network to be 1 if its total concentration as a function of time is increasing, and 0 if it is decreasing. In what follows, when we refer to the *state of a continuous system* we refer to the binary discrete state specified in this way, by examining the first derivatives of each component. A set of concentrations for which the first derivatives of each component of a continuous system are zero, will be called a *steady state*.

3. The Mapping

There is an important difference between the dynamic behavior of a continuous differential system and its discrete homologue. In a discrete system, time is quantized and there is *no* restriction on the Hamming distance (see Appendix A) between two consecutive states. For a continuous system, however, in which there are finite time lags due to diffusion, and thresholds, production and decay constants vary for different components, only the state of one component as measured by its first derivative will be expected to change at any given time. The length of time between changes of state may vary, but the Hamming distance between consecutive states is, in general, 1.

We have found from computer simulations that the homologous discrete network for a given continuous system imposes severe restrictions on these consecutive states which may be summarized by the following rule.

> Determine the derivative state of a continuous system. The next consecutive state in the continuous system lies on a shortest path (see Appendix A) between the first state and the next state to which the discrete homologue goes when placed in the homologous first state.

This rule, together with the observation that consecutive states in continuous networks have a Hamming distance of 1, makes the following construction of practical importance in determining the qualitative dynamics of a continuous system if its discrete homologue is known.

A Boolean system of N components has 2^N different states. For $N = 2, 3, 4$ we can map these states onto a torus so that each state appears only once and lies next to the N states which differ from it by a Hamming distance of 1. In Figs 2(a), (b), (c) we give a representation of the toroidal map on the plane

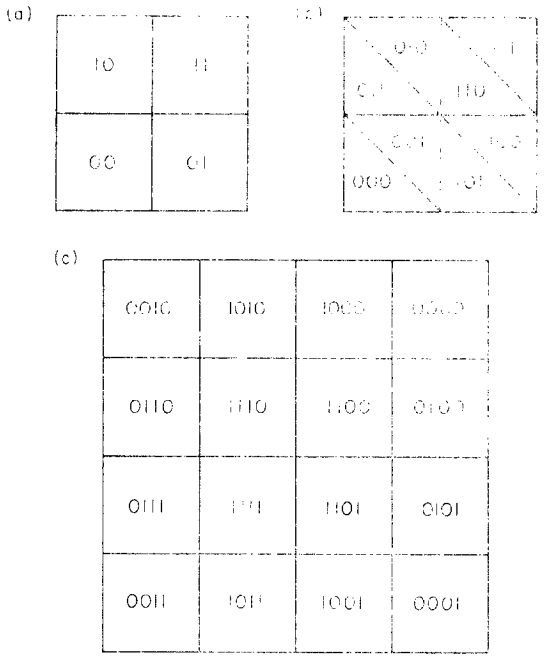

FIG. 2. A representation of the toroidal map on a plane for two-, three- and four-component networks, (a), (b), and (c) respectively. To reconstruct the toroidal map, we first join opposite edges of each square to form a cylinder, and then join the open ends of the cylinder to form a torus.

for 2, 3 and 4 component networks respectively. In order to reconstruct the toroidal map we first join opposite edges of each square in Fig. 2 to form a cylinder, and then join the open ends of the cylinder to form a torus. To study the qualitative behavior of a continuous system we may proceed as follows. We determine the truth table for the homologous switching network. For each discrete state of the continuous net we then draw an arrow to all neighboring states lying on the shortest paths to the next discrete state predicted by the homologous switching net. Provided no component is an input to itself (note that the condition of no self-input refers to the discrete system and does not preclude exponential decay in the continuous system) in the resulting mapping each edge in the unit cell will be crossed by only one arrow. The qualitative dynamics of 2, 3, and 4 component systems which are homologous to switching networks can in this way be represented. In the remainder of this paper we consider the interpretation of the resulting toroidal maps for some simple systems of biological interest.

4. Logical Analysis of Continuous Biochemical Dynamical Systems

(A) TWO COMPONENT SYSTEMS

Given the constraint that no component realizes a function on itself there are only two, two component discrete systems which have interesting (multiple steady states or oscillations) dynamic behavior.

The biochemical switch

One of these, the biochemical switch (trigger) has often been discussed (Delbrück, 1949; Monod & Jacob, 1961; Sugita, 1963; Simon, 1965; Grigorev, Polyakova & Chernavskii, 1967; Babloyantz & Nicolis, 1972) as a possible mechanism for cellular differentiation. We assume that there are two components, both of which mutually inhibit the production of the other. In Fig. 3, we give the diagrammatic representation for this network as well as the associated truth table, with its asymptotic states. There are three asymptotic states, the steady states 10,01 as well as the oscillatory state ($11 \rightarrow 00 \rightarrow 11 \rightarrow \ldots$). From a consideration of the truth table alone, there is no *a priori* way of determining if oscillation would be expected to arise in the continuous system. We use the rules of section 3 to construct the toroidal map for this system (Fig. 3(c)). The steady states from the logical system appear as stable states, whereas the states of the oscillation can be recognized as unstable states, they can never be re-entered once they have been left (cf. discussion in Sugita, 1963). Using the rules developed in section 2 we may write down the dynamical equations for this logical network in a homogeneous medium, with

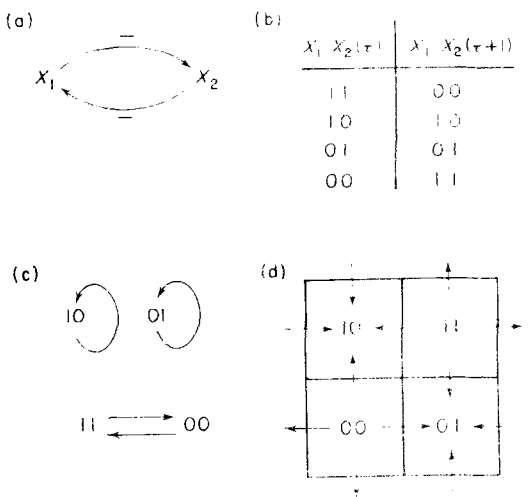

FIG. 3. The diagrammatic structure (a), truth table (b), asymptotic states (c), and toroidal map (d) for the biochemical switch.

Hill function control. Assuming all constants for the components are equal we find the equations

$$\frac{dX_1}{dt} = \lambda \frac{\theta^n}{\theta^n + X_2^n} - \gamma X_1,$$
$$\frac{dX_2}{dt} = \lambda \frac{\theta^n}{\theta^n + X_1^n} - \gamma X_2. \tag{6}$$

Although in the discrete system, X_1 realizes a logical function on X_2, and X_2 realizes a logical function on X_1, in the continuous homologue, both X_1 and X_2 undergo exponential decay as well as non-linear synthesis at a rate determined by the other's concentration. For $n = 1$, it may easily be determined (Simon, 1965) that these equations have a single steady state. For $n = 2$, provided $\lambda/\gamma > 2\theta$, there will be three steady states (Grigorev, Polyakova & Chernavskii, 1967). For example, in Fig. 4, we present a

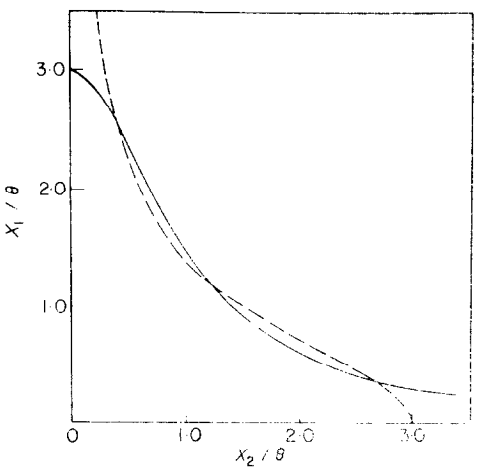

FIG. 4. A graphical solution for the steady states of equation (6), using the values of the parameters given in the text.

graphical solution for the steady states of equation (6) for $n = 2$, $\lambda/\gamma = 3\theta$. The state given by $X_1 = X_2 = 1{\cdot}21\theta$ is unstable, a saddle point, and the dynamical system will evolve to one of the other steady states given by $X_1 = 0{\cdot}38\theta$, $X_2 = 2{\cdot}62\theta$ or $X_1 = 2{\cdot}62\theta$, $X_2 = 0{\cdot}38\theta$, which are stable. These two *stable* states correspond to the steady states which were found in the discrete system and the toroidal map. This requires some elaboration, since at the steady state itself, the first derivatives of the concentrations are zero and it is impossible, using the first derivative rules we have presented, to assign any discrete state to a steady state of a continuous system. However,

we note that if the initial concentrations in the continuous system are different from their steady-state values, but fall within the concentration range spanned by the autonomous system (here $0{\cdot}38\theta$–$2{\cdot}62\theta$), then the steady states will be approached by a transient in which the concentration of a component is decreasing if the value of the component in the steady state of the discrete system is 0, and will be increasing if the value of the component is 1. Consequently, our first derivative rule is well defined arbitrarily close to the steady state. At the steady state itself, the first derivatives of all components vanish, and the concentration of a component will be maximum (minimum) if the corresponding steady state in the discrete system is 1(0). The asymptotic approach to the steady state can be seen in Figs 7(b), 9(a), 9(b) in the examples we discuss below. The two conditions on the parameters of equation (6) needed to give two steady states, $n = 2$, $\lambda/\gamma > 2\theta$ should be compared with the conditions on the parameters which we discuss at the end of section 2. The general conditions correspond to clearly defined mathematical limits in the analysis of equation (6). Precise analysis of the critical points of the equations of motion of these systems becomes hopelessly tedious for slightly more complex systems. However, our finding for this system, that steady states in the discrete system give stable steady states in the continuous homologues appears to hold regardless of the complexity of the network (see Appendix B).

The feedback inhibitor oscillator

The other two component system which has interesting dynamical behavior has often been proposed in slightly modified forms as an underlying mechanism for generating metabolic oscillations (Monod & Jacob, 1961; Sugita, 1963; Landahl, 1969; Walter, 1970), particularly in systems in which it is known that the end product of a synthetic network inhibits, via feedback to some early step, its own synthesis. In this system X_1, induces the production of X_2 and conversely, X_2 represses the production of X_1. In Fig. 5, we give the diagrammatic representation of this network as well as the associated truth table. There is only one asymptotic state in the discrete system an oscillation through all the states of the system. In the toroidal map (Fig. 5(d)), the oscillation remains intact, and no additional features appear. This should be compared with the result for the previous system in which an oscillation appeared in the discrete system, but not on the toroidal map. We have analyzed in some detail (Glass & Kauffman, 1972) the coupled continuous equations of the form of equation (1) for this system. For a homogeneous system the oscillation does not exist in the continuous system and there is a single asymptotic steady state. However, for a system in which the synthetic sites are *spatially separated*, a single asymptotic cycle, a stable limit cycle, is found

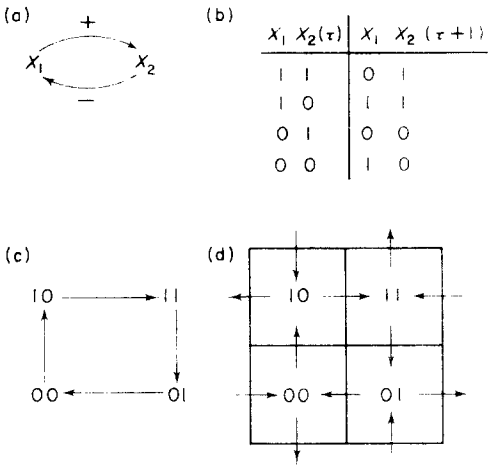

FIG. 5. The diagrammatic structure (a), truth table (b), asymptotic cycle (c), and toroidal map (d) for the feedback inhibitor oscillator.

for some values of the parameters. Although the waveform of this oscillation may be perturbed both by changes in control functions and distance between catalytic sites, the phase relationships of the components of the system remain invariant to these modifications and the continuous system cycles through the states ($10 \rightarrow 11 \rightarrow 01 \rightarrow 00 \rightarrow 10 \rightarrow \ldots$) just as in toroidal map, and the discrete system. To predict, using analytic techniques, which dynamic systems display limit cycle oscillations is always a non-trivial task (Davis, 1962). However, the conclusion from the study of the feedback oscillator, that if there is a cycle in a continuous network there will be a closed cyclic path on the toroidal map in which there are the same phase relationships between consecutive states as in the continuous system, appears to be generally valid, even for more complex systems.

(B) MULTICOMPONENT SYSTEMS

The number of different dynamical systems which can be built from biochemical components multiplies rapidly as the number of components of the system increases. To illustrate the application of these techniques to complex systems we analyze the dynamics of two multicomponent systems of biological interest.

A three component network–A phase dependent switch

We have previously investigated the dynamic behavior of the three component network represented diagrammatically in Fig. 6(a). For some con-

figurations of the components two asymptotic states may be found, one a steady state and the other a limit cycle oscillation. In Figs 6(b), (c), (d), we give the truth table, the asymptotic states of the discrete network, as well as the toroidal map for this system. We may immediately confirm the observations of the previous section; the steady state of the discrete system is present in the continuous network, and the oscillation which is found in the continuous network (Fig. 7(a)) (101 → 100 → 110 → 010 → 000 → 001 → 101 ...) forms a closed cyclic path on the toroidal map. It is interesting to note that the cyclic path on the toroidal map which the continuous system realizes does not pass through the state (111) even though this state is on the asymptotic cycle of the discrete system. Since there are two asymptotic states in this network external perturbations to the system can induce transitions between the states. We have studied the effect of introducing X_3 to the compartment in which X_2 is synthesized both as a function of the amount of X_3 introduced to the system, and the phase during the oscillation at which the perturbation is applied (Glass & Kauffman, 1972). Small perturbations have little effect on the oscillation whereas a large perturbation can induce a transition at any phase of the oscillation. Can the logical analysis presented here offer any

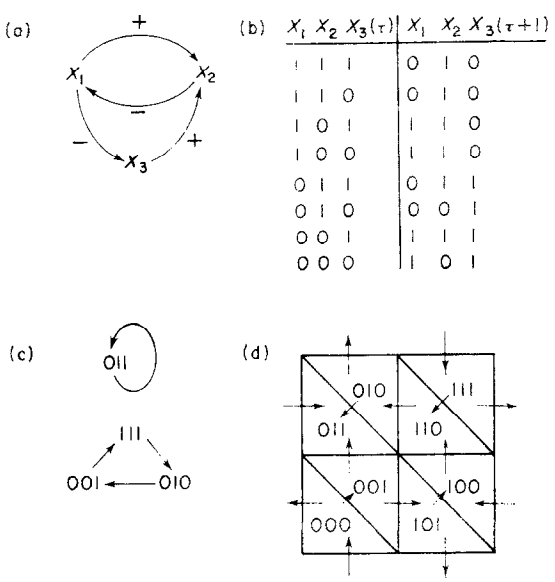

FIG. 6. The diagrammatic structure (a), truth table (b), asymptotic states (c), and toroidal map (d) for the three-component phase dependent switch.

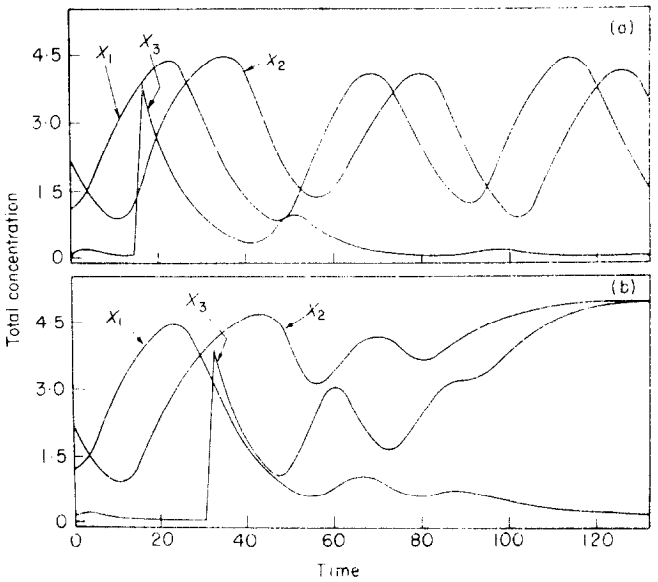

FIG. 7. Perturbation of the phase-dependent switch by adding component X_3 for two consecutive time iterations to the compartment in which X_2 is located at state 110 for Fig. 7(a) and 010 for Fig. 7(b). The parameters used in Glass& Kauffman (1972) were substituted into equations (1)–(5). The parameters are $\lambda = 0{\cdot}5$, $\gamma = 0{\cdot}1$, $D = 0{\cdot}4$, $\theta_1 = \theta_2 = 0{\cdot}4$, $\theta_3 = 0{\cdot}2$, $P_1 = 1, P_2 = 4, P_3 = 2$, M = 4, n = 6.

clue to the phase at which the network is *most* succeptible to external perturbation for some perturbation of intermediate magnitude? The perturbation corresponds to switching the state of component X_3 to 1 on the continuous state cycle. If this perturbation is applied to the state (010) the new state derived is (011) which is the asymptotically stable state. In Fig. 7 we show the effect of perturbations at two different phases in the continuous oscillation. When the perturbation was applied at (010) (Fig. 7(b)) a transition to state (011) after a rather lengthy transient was observed. The same perturbation given during state (110) (Fig. 7(a)) has little effect and the limit cycle oscillation is re-established. The phase of the oscillation during which the system is most liable to undergo transitions to the steady state under the perturbation described in the text, is apparently given by logical analysis presented here. The reader might like to confirm that the transitions on the transients after perturbation follow allowed paths on the toroidal map.

A four component network–A switch with intermediates

A trigger scheme similar to the two component scheme discussed previously

but in which X_1 and X_3 do not inhibit each other directly, but rather through the intermediates X_2 and X_4 (see Monod & Jacob, 1961), is depicted in Fig. 8, along with the associated truth tables and asymptotic states of the discrete system. We have examined the behavior of the continuous equations

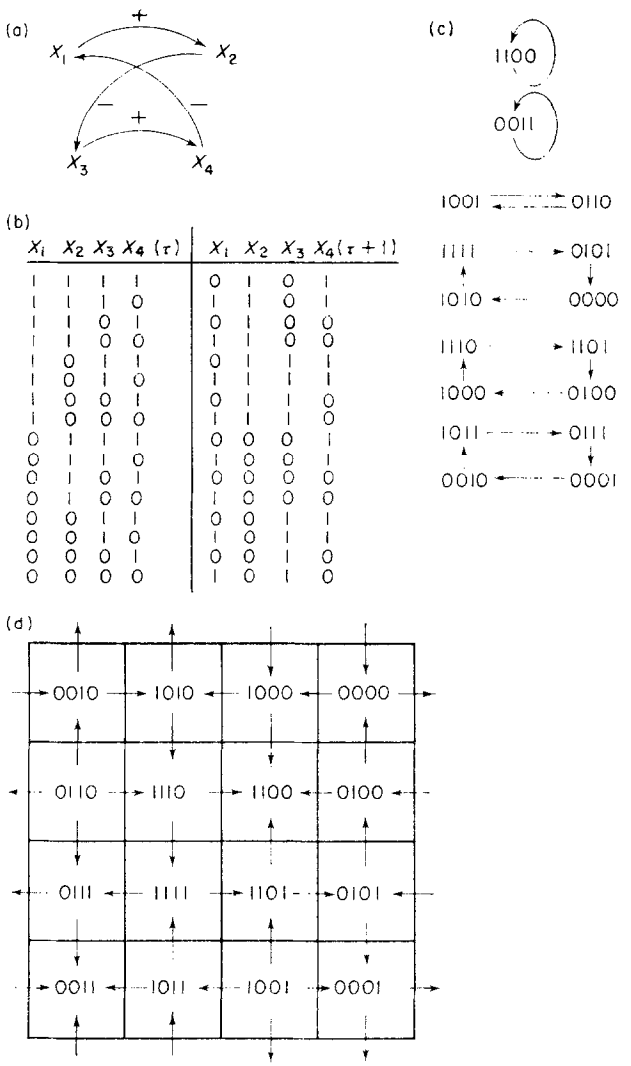

FIG. 8. The diagrammatic structure (a), truth table (b), asymptotic states (c), and toroidal map (d) for the four-component bistable switch with intermediates.

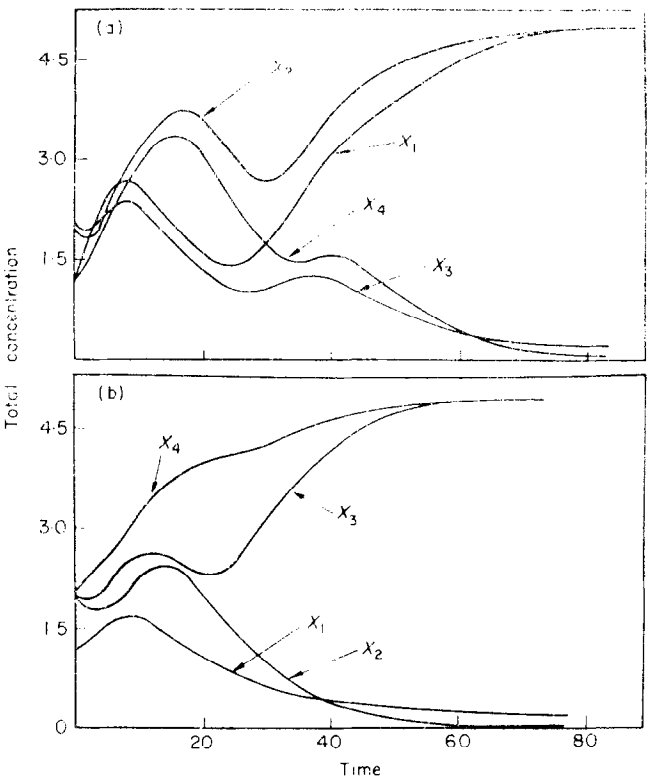

FIG. 9. The evolution of the four-component system to the state 1100 in Fig. 9(a), and 0011 in Fig. 9(b) from two different, initially homogeneous, concentration states.

(using equations (1)–(5)) for this system in 4 compartments with the parameters for all components equal and given by

$$\begin{aligned} \lambda &= 0{\cdot}5, & P_1 &= 2, \\ \gamma &= 0{\cdot}1, & P_2 &= 1, \\ D &= 0{\cdot}4, & P_3 &= 3, \\ \theta &= 0{\cdot}4, & P_4 &= 4, \\ n &= 4. \end{aligned}$$

This specifies 16 coupled non-linear equations. We have only found two asymptotic states for this system corresponding to the two steady states in the discrete system and the toroidal map. Transients to these steady states starting from initial homogeneous concentrations of molecules are shown in

Fig. 9. Writing down the consecutive states of the continuous system we find the transient passing through the states

$$1001 \rightarrow 1011 \rightarrow 1111 \rightarrow 0111 \rightarrow 0101 \rightarrow 0001 \rightarrow 0011$$

for Fig. 9(a) and the states

$$1001 \rightarrow (1011) \rightarrow 1111 \rightarrow (1101) \rightarrow 0101 \rightarrow 0100 \rightarrow$$
$$0000 \rightarrow 1000 \rightarrow 1010 \rightarrow 1110 \rightarrow 1111 \rightarrow 1101 \rightarrow 1100$$

for Fig. 9(b). The states in parentheses here are not actually observed because of the symmetries of the initial states and the coarseness of the time iteration. If the reader traces out these transients on the toroidal map he will note that all transitions are consistent with those given on the map. Further, although both transients pass through the states (1111) and (0101) the transitions into and out of these states are different. The steady states in the continuous system appear as *extremal* steady states in which the synthetic rates of components are either near their maximal or minimal rates. The transients to these states on the toroidal map appear as unstable counterclockwise rotations into the stable steady states. We have not been able to find any stable oscillations for this system.

5. Discussion

In section 3 we have proposed a mapping which allows us to compare certain qualitative features of the dynamics of some continuous biochemical control networks and their discrete homologues. Although an analytical proof of our findings has been found only for a restricted subclass of the continuous dynamical systems (homogeneous, one-input systems, with Heaviside control, see Appendix B) in which we are interested, the results of the previous section indicate that our techniques are more general than we have so far been able to prove. The findings of the previous section may be summarized.

(i) For each steady state of a discrete system, there will be a corresponding steady state in the homologous continuous system which is asymptotically approached at long times.
(ii) Any oscillatory state in the continuous system will have a closed cyclic path on the toroidal map.
(iii) Transients in the continuous system follow transitions consistent with the mapping.

At the moment it is still not clear if this mapping will allow us to determine all the major qualitative features of the continuous system (critical points, separatrices) once its logical structure is known. However, it is clear that the

following properties can *not* be determined from the mapping, at least in all cases.

(i) The stability of cycles—not all closed cyclic paths in the toroidal mapping represent stable limit cycle oscillations. In the feedback inhibitor, for example, the oscillation decays to a stable focus for homogeneous systems. Closed cyclic paths may also represent oscillations around an unstable focus in the continuous system.

(ii) A determination of all steady states of the continuous systems—in the feedback inhibitor a steady state may exist, which is not represented as a steady state in the mapping.

(iii) The determination of the next state of the continuous system if its present state is known—although the mapping provides restrictions on this transition, it does *not*, in most cases allow us to definitely predict the next consecutive state.

The global properties of multicomponent Boolean switching networks have been studied in considerable detail (Kauffman, 1969, 1971*a*,*b*). An underlying assumption in this work was that the qualitative features of the discrete systems which were being studied, would be reproduced if a continuous system made of real biochemicals were built. The present work gives strong circumstantial evidence that this is the case. Further studies of the correspondence between the properties of discrete and continuous systems for many component systems will certainly be of interest. The present techniques will also become unwieldy for systems in which the number of components is larger than five or six, and appropriate generalizations must be found.

The rules for constructing the toroidal map are strictly valid (see Appendix B) only for networks in which each component has a single input and realizes a Heaviside function. Despite these limitations, we feel the techniques developed here will prove a valuable analytical tool to compare the qualitative features of complex equations and the biological system they are supposed to represent. The examples given in Glass & Kauffman (1972) in which qualitative behaviors of continuous systems were unchanged when Heaviside functions were relaxed to Hill functions, suggest the toroidal map captures the structurally stable aspects (see Appendix B), (Thom, 1970) of behavior which are insensitive to the exact form of the sigmoidal function used.

The limitation of strict validity of the mapping to single input networks may not be a great limitation. First, the mapping works for at least some multi-input nets, for example the three component net depicted in Fig. 6. Second, in much larger nets of biologic interest, for example, nets with two or three control inputs per variable (Kauffman, 1969, 1971*a*,*b*) the nets commonly possess asymptotic states in which very few elements change, and these

commonly form single input subsystems whose remaining input variables are constant. An example is given in Table 1. In Table 1 we give the structure of a

TABLE 1

The logical structure of the 50-component, two-input, randomly-constructed Boolean network described in the text

Component	Input 1	Input 2	Function	Steady-state value	Component	Input 1	Input 2	Function	Steady-state value
1	18	11	0101	1	26	24	27	1000	0
2	9	12	1100	0	27	46	36	0000	0
3	21	48	0111	1	28	9	29	1001	0
4	49	11	0110	0	29	46	15	1001	1
5	29	24	0000	0	30	32	7	1011	0
6	32	29	1110	1	31	3	12	1101	1
7	38	24	0001	0	32	42	49	1001	1
8	47	24	0110	0	33	16	13	1010	1
9	18	48	1000	0	34	34	35	0011	1,0
10	29	39	0100	1	35	7	31	1001	0
11	29	23	0110	0	36	21	47	1101	1
12	33	41	0110	0	37	2	34	1111	1
13	50	32	1011	1	38	43	3	1011	1
14	41	12	0101	1	39	33	19	0001	0
15	9	49	0101	1	40	19	43	0011	0
16	10	25	0111	1	41	19	31	1010	1
17	6	16	1110	1	42	18	35	0000	0
18	45	29	0000	0	43	49	33	0000	0
19	22	23	1111	1	44	11	22	0111	1
20	49	34	0110	0,1	45	32	46	0111	0
21	18	49	1100	0	46	40	25	1001	1
22	34	18	0001	1,0	47	43	38	0101	0
23	5	16	1111	1	48	39	44	0001	0
24	4	36	0001	0	49	16	2	1000	0
25	32	19	0111	0	50	21	10	0001	0

randomly constructed, two input Boolean switching network of fifty components. For each component we designate the Boolean function realized on the inputs in a standard way; for example, the truth table for component X_1 is,

X_{18}	X_{11}	(τ)	X_1	$(\tau+1)$
1	1		0	
1	0		1	
0	1		0	
0	0		1	

The network has a cyclic asymptotic state in which all the variables are constant except three, X_{20}, X_{22}, X_{34}, which oscillate through two states. In the constant background provided by all the other 47 components remaining at their asymptotic values, these three components form the one-input switching network which is indicated in Fig. 10. Here, since X_{34} realizes a function on itself, in the toroidal mapping (Fig. 10(d)) edges may be crossed by arrows from two directions corresponding to the oscillation of X_{34} between the states 1 and 0. Since no possible phase relation of the three oscillating

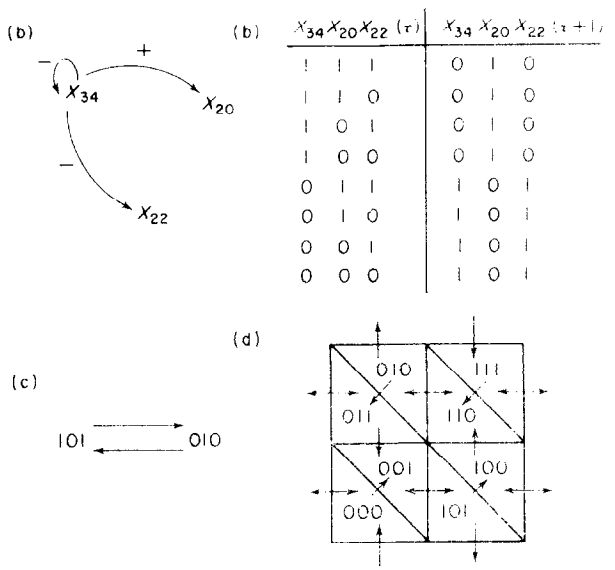

FIG. 10. The diagrammatic structure (a), truth table (b), asymptotic states (c) and toroidal map (d) for the three-component switching network embedded in the 50-component network discussed in the text.

variables can perturb the remaining 47 from their steady state, in continuous realizations of this network we would anticipate a steady state of the 47 components in which dynamically interesting and perhaps functionally important behavior of the three oscillating components is embedded. We may thus reduce the study of dynamics of the system of 2^{50} states, to a problem of the dynamics of 2^3 states, a much more manageable problem for which the toroidal map will be useful. The extension of this analysis to an arbitrary network of biological interest will only be successful if a decomposition of the network into an active subsystem embedded in a constant background can be found. Although there is no way of determining *a priori* if such a decomposition will hold, the conjecture that restricted regions of the

genome play key roles in differentiation is attractive. The dynamics of these regions and the subsequent regulation of differentiation could perhaps be studied using the techniques applied here. For systems in which there are more than four active components it is impossible to find a toroidal mapping such as we have presented here. However, since discrete Boolean systems can be mapped onto a hypercube it can be shown using graph theoretic techniques (Harary, 1969) that a mapping similar to the one presented here can be accomplished on a surface of genus,

$$\gamma = 1+(n-4)2^{n-3},$$

where n is the number of components and the genus, γ, is the number of "handles" which must be added to a sphere to form the surface. This extension will be pursued in future work.

Limitations of the mapping due to strict validity only for one input functions may prove unimportant for a deeper reason. The mapping attempts to provide qualitative information, not by performing the integrations which are so unwieldy in complex non-linear systems, but by providing an ambivalent mapping from the current (derivative) state of a continuous net to *all* the next states it might go to. If the mapping *does* contain all possible next states for each state of the continuous system, then any ergodic sets in the mapping, must contain the asymptotic (sets of) states in the continuous net. It may be hoped that such ergodic sets, their sizes, the numbers of them and relations between them, would contain the qualitative information most insensitive to details of parameter values, and of most significance to the study of biological problems. The 50 component network discussed above, contains the 8 state ergodic set shown in Fig. 10(d) with the other 47 components constant at their steady state values listed in Table 1.

When the mapping described in the text is invalid, (not all transitions are specified for some multi-input systems) it may be possible to extend it to add the extra next states. As long as the mapping is from one initial state to few subsequent ones, it should still be useful. Extending the mapping for particular classes of multiple input functions of biological interest, for example the "forcible" functions (Kauffman, 1971*a*,*b*) should be possible.

Another interesting theoretical problem is to generalize the equations for which this mapping is of value. For example, it is clear that the same *logical* relationship which drives the feedback inhibitor oscillations also drives the predator–prey, Volterra–Lotka oscillation (Goel, Maitra & Montroll, 1971). However, the classical equation which has been proposed for this oscillation can not immediately be cast in the form of equation (2). How can one write down all the equations which have the same qualitative dynamics as the predator–prey, feedback–inhibitor oscillation?

The theory we discuss here should have application to experimental work in two ways. It should be possible to determine the logical structures of interesting biological and chemical oscillatory phenomena, eclosion rhythm (Winfree, 1970), gene puffing patterns (Berendes, 1968) and oscillating reactions (Zaikin & Zhabotinsky, 1970). Hopefully a knowledge of the logical structures of these systems would facilitate interpretation in terms of plausible mechanisms.

Since the work of Monod & Jacob (1961), there have been many proposals that chemical networks can and some day will be synthesized in the laboratory which are capable of performing predetermined logical functions. From the analysis here, it is clear that in the synthesis of these networks, explicit consideration will have to be taken of the continuous properties of these networks. Chemical automata can be built, but the architect must be careful to ensure that the desired behavior will in fact, be realized in the real continuous system.

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Appendix A

Consider two discrete states of the same number of components. The number of loci in which these two states differ from each other is called the *Hamming distance.* We wish to convert the first state to the second state in a number of discrete steps in which we change only one component in each step. If the Hamming distance between 2 states is n, then the minimum number of steps needed to make this conversion is also n, and any sequence of n steps which accomplishes this transition will be called a *shortest path* between the two states. Since the first step of a shortest path can be made in n ways, the second in $(n-1)$ ways and so forth, there are n factorial shortest paths between 2 states which have a Hamming distance, n. Each of the shortest paths, passes through $(n-1)$ of the (2^n-2) states which lie on the shortest paths between the two initial states. The sum of the Hamming distances from any state lying on a shortest path between two given states, to each of these states, is equal to the Hamming distance between the two given states. Conversely, a state is on a shortest path between two given states, if the sum of the Hamming distances to the two states from the initial state, is equal to the Hamming distance between the two given states.

Appendix B

A dynamical system is said to be *structurally stable* if for a sufficiently small perturbation of the equations of motion of the system, the system remains topologically isomorphic to the unperturbed system (see Thom, 1970). In the

preceding we have demonstrated a number of topological properties of dynamic systems given by equation (1) which are apparently dependent only on the logical structure of the system but not on the choice of parameters of the system. Further we have indicated how these properties may be studied using the toroidal mapping described in section 3. A formal justification for the mapping as well as a definite statement concerning the limits of the mapping, have not been offered. In this Appendix we offer two proofs of the properties of homogeneous systems in which the control of synthesis is mediated by Heaviside step functions. Since the properties we study have been observed to be insensitive to the precise form of the control function as well as the structure of the system the proofs are expected to hold for a large class of homologous systems. Since we cannot specify this class of systems we offer these proofs to make plausible our conclusions concerning the observed properties of continuous biochemical networks and their homologues. To help eliminate ambiguity in the proofs we designate all Boolean variables by a tilde ($\sim$).

(A) ASYMPTOTIC STEADY STATES

For each steady state in a logical system we specify concentrations in the continuous homologue, in terms of the production and decay parameters describing the continuous system. We then show that the concentrations we have specified represent a stable steady state of the continuous system.

$\tilde{\mathbf{X}}(\tau)$ is a state vector of the logical variables $\tilde{1}$, $\tilde{0}$, at time τ. The kth component of this vector is determined by the logical structure of the network

$$\tilde{X}_k(\tau+1) = L_k\{\tilde{\mathbf{X}}(\tau)\}, \tag{A1}$$

where L_k is a logical operator. The operators L_k, $k = 1, N$ where N is the number of components in the system completely determines the behavior of the discrete system.

$\mathbf{X}$ is the state vector of a homologous continuous network. Call θ_k the threshold concentrations of all targets of the kth component.

$$\delta < \theta_k \tag{A2}$$

where δ is a finite positive real number. Define an operation, M

$$\tilde{\mathbf{X}}^M = M\mathbf{X} \tag{A3}$$

where the kth component of the vector $\tilde{\mathbf{X}}^M$ is given by

$$\tilde{X}_k^M = \tilde{1} \quad \text{if} \quad X_k > \theta_k, \tag{A4}$$

$$\tilde{X}_k^M = \tilde{0} \quad \text{if} \quad X_k < \theta_k. \tag{A5}$$

We now define function H_k, homologous to the logical net so that

$$\text{Iff } L_k\{M\mathbf{X}\} = \tilde{1} \quad \text{then} \quad H_k\{\mathbf{X}\} = 1 \tag{A6}$$

and

$$\text{Iff } L_k\{M\mathbf{X}\} = \tilde{0} \qquad \text{then} \qquad H_k\{\mathbf{X}\} = 0. \tag{A7}$$

For the case where L_k is a function of a single locus of the logical vector $\mathbf{X}$, the function H_k can be written in terms of the Heaviside step function on the homologous continuous variable. It is for this case where the toroidal mapping described in section 3 is strictly valid (see the following proof). For cases where L_k is a function of several loci, H_k will not always have a simple or unique representation and the limitations on the phase relationships between consecutive states derived from the toroidal mapping will not apply. We anticipate that only when the logical functions are restricted to the monotonic functions (Newman & Rice, 1971) will strong constraints on the phase relationships of the continuous systems be observed. Let us consider the biochemical network

$$\frac{\mathrm{d}X_k}{\mathrm{d}t} = \lambda_k H_k\{\mathbf{X}\} - \gamma_k X_k, \tag{A8}$$

where λ_k, γ_k are production and decay constants, respectively of the kth component and

$$\frac{\lambda_k}{\gamma_k} > \theta_k + \varepsilon, \tag{A9}$$

where ε is a finite positive number. If we limit the concentration of X_k so that

$$0 < X_k < \frac{\lambda_k}{\gamma_k}$$

and consider a closed system from equation (A8), we find that,

$$\text{if } H_k\{\mathbf{X}\} = 1, \qquad \text{then} \qquad \frac{\mathrm{d}X_k}{\mathrm{d}t} \geqslant 0 \tag{A10}$$

and

$$\text{if } H_k\{\mathbf{X}\} = 0, \qquad \text{then} \qquad \frac{\mathrm{d}X_k}{\mathrm{d}t} \leqslant 0. \tag{A11}$$

Now assume there is a steady state in the logical net. That is,

$$\tilde{X}_k(\tau) = \tilde{X}_k(\tau+1) = L_k\{\tilde{X}(\tau)\}, \qquad k = 1, N. \tag{A12}$$

Associate with this state, a concentration state in the continuous network in the following way

$$\text{If } \tilde{X}_k = \tilde{1}, \qquad \text{then} \qquad X_k = \lambda_k/\gamma_k. \tag{A13}$$

$$\text{If } \tilde{X}_k = \tilde{0}, \qquad \text{then} \qquad X_k = 0, \tag{A14}$$

$$k = 1, N.$$

Let us consider an arbitrary component, X_j, whose concentration we assume

to be λ_j/γ_j. Since the maximum concentration of X_j, equation (A8) is λ_j/γ_j, then

$$\frac{dX_j}{dt} \leqslant 0. \tag{A15}$$

However, since $H_j\{\mathbf{X}\} = 1$ from equation (A6), we find, equation (A9)

$$\frac{dX_j}{dt} \geqslant 0. \tag{A16}$$

Equations (A15) and (A16) can both be true only if

$$\frac{dX_j}{dt} = 0. \tag{A17}$$

In a similar fashion we can show that if initially $X_j = 0$, its first derivative would have also been 0. Consequently the state assigned in equations (A13) and (A14) is a steady state, since the first derivatives of all the components are 0.

We may determine the stability of this steady state by linearizing equation (A7) around the concentration defined in equations (A13) and (A14). From the statements immediately preceeding equation (A2) and equation (A9) we see that all the off diagonal elements of this stability matrix must be 0, since no infinitesimal perturbation to any of the components will alter the rates of production of its targets. The exponential decay terms in equation (A8) insure that all diagonal elements will be negative. Therefore, the eigenvalues of the matrix are all negative, and the state defined is asymptotically stable.

(B) PHASE RELATIONSHIPS OF COMPONENTS

We now confine our attention to networks in which each component receives inputs from only one other component in the system, and in which the thresholds for the targets of any one component are all equal. From equation (A8) we confirm that the discrete state $\tilde{\mathbf{X}}(\tau_1)$ found from the operation

$$\tilde{\mathbf{X}}(\tau_1) = L\{M\mathbf{X}(t_1)\} \tag{A18}$$

is the same state obtained by assigning the Boolean variable $\tilde{1}$ to all variables whose first derivative is increasing and $\tilde{0}$ to all variables whose first derivative is decreasing. These relationships are depicted in Fig. 11. The state $\tilde{\mathbf{X}}(\tau_1 + 1)$ is the subsequent discrete state in this Boolean system.

Now consider some time $t_2 > t_1$ at which a single component which had previously been above (or below) its threshold at t_1, has passed its threshold and is below (or above) this value. Define the states $\mathbf{X}(t_2)$, $\tilde{\mathbf{X}}^M(\tau_2)$, $\tilde{\mathbf{X}}(\tau_2)$ in an analogous way to the states in Fig. 11. By definition $\tilde{\mathbf{X}}^M(\tau_2)$ differs from $\tilde{\mathbf{X}}^M(\tau_1)$ in only one, say the jth locus. In the jth locus $\tilde{\mathbf{X}}^M(\tau_2)$ must have the

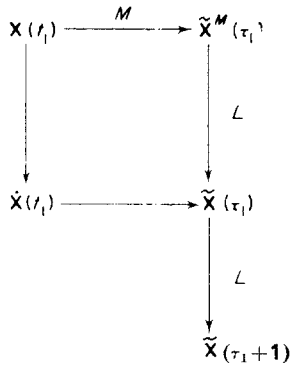

FIG. 11. The relationships between the states $\mathbf{X}(t_1)$, $\dot{\mathbf{X}}(t_1)$, $\tilde{\mathbf{X}}^M(\tau_1)$, $\mathbf{X}(\tau_1)$, $\mathbf{X}(\tau_1+1)$. See Appendix B for details.

same value as $\tilde{\mathbf{X}}(\tau_1)$. If a value was above (or below) threshold its first derivative must have been decreasing (increasing) in order for it to pass the threshold.

The states $\tilde{\mathbf{X}}(\tau_1)$, $\tilde{\mathbf{X}}(\tau_2)$ are derived by applying the same operations, L, to two states which differ in only the jth locus. They will therefore only differ from each other in the loci which are the targets of the jth locus. If there are k targets of the jth locus the Hamming distance between $\tilde{\mathbf{X}}(\tau_2)$ and $\tilde{\mathbf{X}}(\tau_1)$ will therefore be k. Now since the jth locus is the same in $\tilde{\mathbf{X}}(\tau_1)$ and $\tilde{\mathbf{X}}^M(\tau_2)$ the targets of this locus will be the same in the derived states, namely $\tilde{\mathbf{X}}(\tau_1+1)$ and $\tilde{\mathbf{X}}(\tau_2)$, respectively. If the Hamming distance between $\tilde{\mathbf{X}}(\tau_1)$ and $\tilde{\mathbf{X}}(\tau_1+1)$ is n, the Hamming distance between $\tilde{\mathbf{X}}(\tau_2)$ and $\tilde{\mathbf{X}}(\tau_1+1)$ will be $n-k$. Since the sum of the Hamming distances from $\tilde{\mathbf{X}}(\tau_2)$ to $\tilde{\mathbf{X}}(\tau_1)$ and $\tilde{\mathbf{X}}(\tau_1+1)$, is equal to the Hamming distance from $\tilde{\mathbf{X}}(\tau_1)$ to $\tilde{\mathbf{X}}(\tau_1+1)$, $\tilde{\mathbf{X}}(\tau_2)$ lies on the shortest path between these states (see Appendix A).