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Instability and Dynamic Pattern in Cellular Networks

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Dynamic instability of reaction and transport processes in groups of intercommunicating cells can lead to pattern formation and periodic oscillations. Turing's 1952 analysis suggests a more general theory. A powerful new method for analyzing onset of instability in arbitrary networks of compartments or model cells is developed. Network structure is found to influence interaction of intracellular chemical reactions and intercellular transfers, and thereby the stability of uniform stationary states of the network. With the theory, effects of changes in network topology can be treated systematically. Several regular planar and polyhedral networks provide illustrations. Influences of boundary conditions and intercellular permeabilities on patterns of instability are illustrated in simple networks. Non-linear aspects of instability are not treated.

1. Introduction

To discover and elucidate the physicochemical mechanisms that govern spatial differentiation and pattern formation in multicellular organisms is a major challenge in developmental biology. In passing from unicellular to multicellular systems one encounters a new level of organization and complexity, a level stemming directly from cell-to-cell interactions of various types. Through physical contact, cells may exert forces on one another and thereby deform one another, altering shapes and sizes directly and metabolic and other aspects less directly, perhaps. Through chemical contact cells may interchange one or more of their constituents, thereby altering metabolic states. That is, chemical transformations taking place within individual cells may be influenced by mass transport between cells, and thereby differences, less or more regular, in cell composition may be established. Inherent in this type of dynamic interaction are possibilities of natural instabilities that may

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lead to spatial organization and temporal oscillations within groups of cells—the genesis of form and even rhythm. These possibilities we focus on here, taking chemical interactions for our model even though there are, of course, other modes of intercellular communication.

Ultimately the functioning, metabolic and other, of a truly multicellular organism must depend as much on the spatial organization and interconnections within the aggregate as on the nature of the individual cells. One way in which this may come about is made clear by the theory developed here. Its potential utility is much more extensive, however, because it is a basic theory pertaining to any network of compartments with internal transformation processes connected by diffusion-like transport paths that have limited conductance.

Nearly a score of years ago the Cambridge mathematician Alan Turing (1952) proposed to explain the origin of patterns in groups of cells by means of a model which is conceptually simple, although to analyze its operation requires some mathematical sophistication. Unquestionably overidealized from many biological points of view, the model illustrated a principle that may very well be at work in reality, and for that reason Turing's ideas would seem to warrant far more attention than they have received since his untimely death in 1954. In the model, chemical substances Turing called "morphogens" diffuse between contiguous cells and react together within the cells. Turing hypothesized that differentiation or preferential growth occurs in the cells in which certain of the morphogens accumulate. He was able to show that random disturbances can trigger instability in an isolated ring of cells, each communicating equally well with its two immediate neighbors, and that nonuniform distributions of two or three morphogens can be maintained by interference between the intracellular chemical reaction and the intercellular diffusion. Some of the concentration distributions are quite regular around the ring, and the end result according to Turing's hypothesis would be a well-defined spatial pattern of growth or differentiation.

If so simple a model yields such suggestive results, what might be learned from even slightly more realistic versions? What would be the consequences of interchange between cells and extracellular fluid? Of one cell communicating with more than two others? Of communication through more than one kind of connection? Of more than three chemical substances in the reaction–diffusion system? How sensitive are instability and pattern formation to the arrangement, or topology, of intercellular connections in two- and three-dimensional networks? . . . To answer such questions efficiently one needs a theory formulated along the lines of Turing's analysis but of far broader scope. The generalization we develop here depends, as is so often the case, on mathematical abstraction.

The next section presents the equations of concentration change of any number of chemical substances in an arbitrary network, or lattice, of compartments or cells interconnected by semipermeable membranes [e.g. "junctional membranes" (Loewenstein, 1968; Furshpan & Potter, 1968; Payton, Bennett & Pappas, 1969; Spitzer, 1970)] or similar low-capacity diffusion paths [e.g. "cytoplasmic bridges" (Furshpan & Potter, 1968; Cone, 1969)], and bathed by liquid of substantially uniform and constant composition ("extracellular fluid"). We suppose that initially all the cells in the network are in the same chemical, dynamic steady state, but that either external or internal influences continually perturb the system, causing small fluctuations from the uniform set of states. Provided a fluctuation is sufficiently small a linearized version of the nonlinear equations of change is adequate to describe its evolution. After solving the linearized equations we can draw on previous work (Othmer & Scriven, 1969) to determine the conditions under which fluctuations are damped, are just sustained, or are amplified. The last case signals instability, which can lead to a non-uniform pattern of states in the network. Although linearized analysis yields some information about the scale and steadiness of patterns that can result, they are really controlled by non-linear dynamics, a circumstance examined in the sequel (Othmer & Scriven, manuscript in preparation).

In section 3 the stability analysis is reformulated in terms of matrix and operator theory, which leads to a unified treatment of transport and transformation in all networks, regular or not, and reveals an underlying structure. All of the interrelationships of cells in a network are embodied in a single abstract operator characteristic of the topology of that network, and any pattern on the network can be represented in terms of the eigenvectors of the operator.

The theory is applied in sections 4 and 5 to a number of examples: Turing's ring, open chains of cells, doubly periodic lattices of quadrangular cells and hexagonal cells, and several regular polyhedral lattices. Some effects of boundary conditions and intercellular permeabilities on stability behavior are examined, examples being given in the Appendix. Section 7 concludes the paper with observations on the significance of compartmentalization for transport processes and stability, on some of the ways the theory may be extended, and on its possible significance in relation to mechanisms of differentiation and development.

2. Stability Analysis

The model system consists of a connected network of N discrete, homogeneous compartments or cells, each containing a mixture of n chemically

reactive species that participate in the set of reactions of interest. That set includes r independent chemical reactions that can occur in each cell; moreover $r \leqslant n-1$. Every cell is in chemical contact with at least one other cell, i.e. no cell is isolated from the others so far as mass transfer is concerned. The entire network is immersed in a bath of uniform and constant composition (see Fig. 1). For simplicity the volumes of all the cells can be regarded as equal and constant, and so can the areas of mutual contact between pairs of cells.

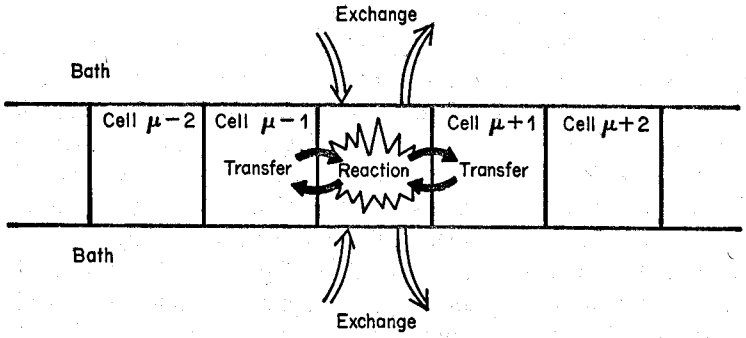

FIG. 1. One-dimensional prototype of the model system.

The concentration c_i^μ of the ith species in the μth cell can be altered by any of three processes: the substance can be produced or consumed by chemical reaction at a rate $R_i^\mu(c_j^\mu)$ that depends on composition within the cell; the substance can be exchanged with the bath at a rate $N_i^\mu(c_j^\mu, c_j^\circ)$ that depends on composition or chemical potential differences between the cell and the bath; and the substance can be transferred to or from contiguous cells at a net rate $J_i^\mu(c_j^\mu, c_j^\nu)$ that depends on composition differences between cells. The rate of accumulation is related to these rates by the equation of change:

$$\frac{dc_i^\mu}{dt} = J_i^\mu + N_i^\mu + R_i^\mu, \qquad \begin{cases} i = 1, 2, \ldots, n. \\ \mu = 1, 2, \ldots, N. \end{cases} \tag{1}$$

The Latin index ranges through the number of substances; the Greek index, through the number of cells, which like the substances must be labeled in some definite though arbitrary sequence for accounting purposes.

In virtue of the upcoming linearization of the equations of change it is really not necessary to choose particular constitutive relations for the transfer rates. Nevertheless we select simple linear relations for illustration. First is

$$J_i^\mu = \sum_\nu \sum_j D_{ij}^{\mu\nu}(c_j^\nu - c_j^\mu). \tag{2}$$

The index j ranges over all species whose concentration differences influence the transfer of the ith substance. The transfer coefficients $D_{ij}^{\mu\nu}$ are, in the simplest instance, independent of concentrations and equal between all pairs of contiguous cells, and so may be written D_{ij}. The index ν ranges over all cells in contact with the μth cell. In terms of the difference operator for the μth cell, so defined that†

$$\Delta^{\mu}c_j^{\mu} = \sum_{\nu}(c_j^{\mu} - c_j^{\nu}),$$

equation (2) takes the compact form

$$J_i^{\mu} = \sum_j D_{ij}\Delta^{\mu}c_j^{\mu}.$$

As indicated, the difference operator may differ from one cell to the next, as indeed it does in irregular lattices; even in regular lattices it is specific to the one under consideration. Examples are given below.

In the second place we select for exchange between the bath and the μth cell the relation

$$N_i^{\mu} = \sum_j H_{ij}(c_j^{\circ} - c_j^{\mu}),$$

where the transfer coefficients H_{ij} are already presumed to be the same for all cells. Concentrations in the bathing fluid are denoted by c_j°. The coefficients in both sets, D_{ij} and H_{ij}, are in the nature of permeabilities, i.e. of conductances of diffusion paths that have negligible capacity for accumulating the diffusing substances. As defined, all of the coefficients contain a ratio of transfer area to cell volume, but the area in D_{ij} may differ from that in H_{ij}. For this and other reasons D_{ij} is generally unequal to H_{ij}: the permeability of "junctional membrane" differs from that of "ordinary membrane".

With these constitutive relations and the simplification that the reaction rate laws are the same in all cells the equation of change (1) becomes

$$\frac{\mathrm{d}c_i^{\mu}}{\mathrm{d}t} = \sum_j D_{ij}\Delta^{\mu}c_j^{\mu} + \sum_j H_{ij}(c_j^{\circ} - c_j^{\mu}) + R_i(c_j^{\mu}). \tag{3}$$

Any uniform, non-equilibrium, stationary solution—any UNESS—is a non-trivial positive solution c_j^{s} of the steady-state equation,

$$0 = \sum_j H_{ij}(c_j^{\circ} - c_j^{s}) + R_i(c_j^{s}).$$

This always has a solution if the c_j° are regarded as variable parameters, as may be proved (Othmer, 1969). To examine the stability of any given UNESS with respect to infinitesimal concentration fluctuations we introduce

† Except for certain special cells that might exist, especially on the margin of a lattice, each cell is in chemical contact with more than one other cell, and the operator is in essence a second-difference operator.

series expansions about c_j^s and retain only those terms that are of first order in the concentration excursion from the stationary solution, $x_j^\mu \equiv c_j^\mu - c_j^s$. The resulting equation for fluctuations is

$$\frac{dx_i^\mu}{dt} = \sum_j D_{ij}\Delta^\mu x_j^\mu + \sum_j K_{ij}x_j^\mu, \qquad \begin{cases} i = 1, \ldots, n, \\ \mu = 1, \ldots, N, \end{cases}$$

where the pseudo-first-order rate constants of reaction *and* exchange are given by (cf. Gmitro & Scriven, 1966)

$$K_{ij} \equiv -H_{ij}(c_j^s) + \sum_k \left(\frac{\partial H_{ij}}{\partial c_k}\right)_{c_k = c_k^s} (c_k^\circ - c_k^s) + \left(\frac{\partial R_i}{\partial c_j}\right)_{c_j = c_j^s}.$$

The fluctuation equation can be put in compact matrix form by defining an excursion vector, a "transfer" matrix, and a "reaction" matrix (the latter representing reaction and exchange):

$$\mathbf{x}^\mu \equiv \begin{pmatrix} x_1^\mu \\ \cdot \\ \cdot \\ \cdot \\ x_n^\mu \end{pmatrix} \qquad \mathbf{D} \equiv \begin{bmatrix} D_{11} \ldots D_{1n} \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ D_{n1} \ldots D_{nn} \end{bmatrix}, \qquad \mathbf{K} \equiv \begin{bmatrix} K_{11} \ldots K_{1n} \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ K_{n1} \ldots K_{nn} \end{bmatrix}.$$

The linearized equations governing stability of a UNESS thus become a set of N matrix differential equations†

$$\frac{d\mathbf{x}^\mu}{dt} = \mathbf{D}\Delta^\mu \mathbf{x}^\mu + \mathbf{K}\mathbf{x}^\mu, \qquad \mu = 1, \ldots, N. \tag{4}$$

This set must be solved subject to an initial condition that represents an arbitrary cell-by-cell fluctuation in concentrations:

$$\mathbf{x}^\mu(0) = \mathbf{x}_0^\mu. \tag{5}$$

In the following section we formulate the problem in more abstract terms which lead to a powerful method of solving, in principle at the least, the set of equations for virtually any network of cells.

3. Reformulation

Equation (4) governs stability of a compartmentalized and discontinuous system. If the analogous equation for a continuous system were written, the Laplacian operator ∇^2 would appear in place of the difference operator Δ^μ and a function of the continuous position variable would take the place of $\mathbf{x}^\mu$, which is a function of cell-index, μ, a discrete variable. The continuous

† This happens to be the same as the set describing a closed system obeying linear reaction rate laws, in which case **K** is a matrix of true kinetic rate constants.

analog of (4) could be solved by separation of time and the position variable, the solution being represented in the form of a series expansion with respect to the infinite set of eigenfunctions of ∇^2 which is appropriate to the geometric configuration of the continuous system. We wish to solve equation (4) in similar fashion. Because no adequately general analog of the eigenfunction method appears to have been detailed before, we must digress.

It is plain that eigenfunctions of a difference operator are lattice functions, i.e. functions of the index μ, and it might be anticipated that for any finite lattice they constitute a finite set that depends on the connectedness of the lattice rather than on its configuration, i.e. on topology instead of geometry. But it is not clear, *a priori*, how to find the needed eigenfunctions and employ them to solve equation (4). We now show that by regarding the entire set of local operators Δ^μ for a lattice as an operator Δ on an abstract, linear space having as many dimensions as there are cells in the network, the eigenfunctions can be found by standard methods, for they are simply the eigenvectors of a particular matrix representation of the lattice operator Δ. Our formulation is new but follows logically from Friedman's (1956) abstract treatment of the method of separation of variables.

A number of definitions are relevant. Let $\mathscr{V}^n$ be an inner-product space with as many dimensions as there are chemical species in the system, and let its elements be vectors, or ordered lists, of n real-valued, smooth functions of time. Let $\mathscr{S}^N$ also be an inner-product space, one having as many dimensions as there are cells in the network, and let its elements be vectors of N complex numbers. The tensor product of a vector $\mathbf{u} = (u_1, \ldots, u_N)^\mathrm{T}$ from $\mathscr{S}^N$ and a vector $\mathbf{y} = (y_1, \ldots, y_n)^\mathrm{T}$ from $\mathscr{V}^n$ is defined by the formula (see, e.g., Halmos, 1958, § 52; note also that superscript T denotes transposition, which turns typographically convenient row vectors into ordinary column vectors)

$$\mathbf{u} \otimes \mathbf{y} \equiv (u_1 y_1, \ldots, u_1 y_n, \ldots, u_N y_1, \ldots, u_N y_n)^\mathrm{T}.$$

All such products together with linear combinations of them constitute the $N \times n$-dimensional space $\mathscr{S}^N \otimes \mathscr{V}^n$. In this space the inner product of any two ctors is defined by

$$\langle \mathbf{u} \otimes \mathbf{y}, \mathbf{v} \otimes \mathbf{z} \rangle \equiv \left(\sum_{i=1}^{N} u_i v_i^* \right) \left(\sum_{j=1}^{n} y_j z_j \right).$$

(The sums are recognizable as inner products in $\mathscr{S}^N$ and $\mathscr{V}^n$, respectively; the asterisk denotes the complex conjugate of v_i.) Important for our purposes is the fact that if a set of N vectors $\{\mathbf{v}_i\}$ is an orthonormal basis for $\mathscr{S}^N$, and a set of n vectors $\{\boldsymbol{\eta}_j\}$ is an orthonormal basis for $\mathscr{V}^n$, then the set of $N \times n$ vectors $\{\mathbf{v}_i \otimes \boldsymbol{\eta}_j\}$ is an orthonormal basis for $\mathscr{S}^N \otimes \mathscr{V}^n$ (see, e.g., Halmos, 1958, § 25). Consequently any vector $\mathbf{x}$ in $\mathscr{S}^N \otimes \mathscr{V}^n$ can be projected onto the

orthonormal basis in $\mathscr{S}^N$ to yield a set of vectors in $\mathscr{V}^n$ which are defined by

$$\mathbf{y}_k \equiv \sum_{j=1}^{n} \langle \mathbf{x}, \mathbf{v}_k \otimes \boldsymbol{\eta}_j \rangle \boldsymbol{\eta}_j,$$

and from which the original vector can be recovered as the sum of tensor products:

$$\mathbf{x} = \sum_{k=1}^{N} \mathbf{v}_k \otimes \mathbf{y}_k.$$

Moreover, the tensor product of an operator $\mathbf{R}$ on $\mathscr{S}^N$ and another operator $\mathbf{T}$ on $\mathscr{V}^n$ is defined to be the operator $\mathbf{R} \otimes \mathbf{T}$ whose action is given by (Halmos, 1958, § 52)

$$(\mathbf{R} \otimes \mathbf{T})\mathbf{x} = \sum_{i=1}^{N} \sum_{j=1}^{n} \langle \mathbf{x}, \mathbf{v}_i \otimes \boldsymbol{\eta}_j \rangle (\mathbf{R}\mathbf{v}_i) \otimes (\mathbf{T}\boldsymbol{\eta}_j).$$

(The inner products are recognizable as the components of $\mathbf{x}$ with respect to the tensor basis.) Every operator defined on a finite-dimensional space can be represented by a matrix (Halmos, 1958, § 38), a situation that suits our purposes. The direct, Kronecker, or tensor product of matrices is so defined as to be entirely consistent with the foregoing product of operators. If $\mathbf{R}$ is an $N \times N$ matrix and $\mathbf{T}$ an $n \times n$ one, their tensor product is an $Nn \times Nn$ one (Halmos, 1958, § 52; Bellman, 1960, § 12–5):

$$\mathbf{R} \otimes \mathbf{T} = \begin{bmatrix} R_{11}T \ldots R_{1N}T \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ \cdot \quad\quad \cdot \\ R_{N1}T \ldots R_{NN}T \end{bmatrix}.$$

If the set of vectors representing concentration excursions is written as a vector of vectors, i.e. a vector in $\mathscr{S}^N \otimes \mathscr{V}^n$,

$$\tilde{\mathbf{x}} \equiv \begin{pmatrix} \mathbf{x}^1 \\ \cdot \\ \cdot \\ \cdot \\ \mathbf{x}^N \end{pmatrix} = (\mathbf{x}_1^1, \ldots, \mathbf{x}_n^1, \ldots, \mathbf{x}_1^N, \ldots, \mathbf{x}_n^N)^{\mathrm{T}},$$

then all the foregoing definitions come to bear, because it is found that the equation set (4) becomes the simple matrix equation

$$\frac{d\tilde{\mathbf{x}}}{dt} = (\boldsymbol{\Delta} \otimes \mathbf{D})\tilde{\mathbf{x}} + (\mathbf{I}_N \otimes \mathbf{K})\tilde{\mathbf{x}}, \tag{6}$$

where $\boldsymbol{\Delta}$ is an $N \times N$ matrix which incorporates all of the local operators Δ^μ and represents a new operator in $\mathscr{S}^N$. Clearly the $n \times n$ matrices $\mathbf{D}$ and $\mathbf{K}$ represent operators in $\mathscr{V}^n$.

Inasmuch as the matrix Δ is found to depend solely on the connectedness or structure of the lattice it can be called the "structural matrix" of the given network. Because contact of one cell with another is a symmetric relationship Δ is necessarily a non-trivial, real, symmetric matrix† (thus it represents a self-adjoint operator) and it therefore has real eigenvalues α_i and a complete set of orthogonal eigenvectors $\mathbf{u}_j$ all satisfying the equation

$$\Delta \mathbf{u}_k = \alpha_k \mathbf{u}_k, \qquad k = 1, 2, \ldots N,$$

where α_k is the eigenvalue belonging to the eigenvector $\mathbf{u}_k$. The eigenvectors can be normalized to unity, i.e. $\mathbf{u}_k^{\mathrm{T}} \mathbf{u}_k^* = 1$, and are a highly apt choice as the orthonormal basis $\{v_i\}$ for $\mathscr{S}^N$. It is appropriate to call them "structural modes" of the network, for they too depend solely on lattice structure.

The initial condition (5) in full matrix form is $\tilde{\mathbf{x}}(0) = \tilde{\mathbf{x}}_0$, and because the vector $\tilde{\mathbf{x}}_0$ lies in $\mathscr{S}^N \otimes \mathscr{V}^n$ we see from the above that it can be expanded in the structural modes:

$$\tilde{\mathbf{x}}_0 = \sum_{k=1}^{N} \mathbf{u}_k \otimes \mathbf{y}_k^0, \qquad \mathbf{y}_k^0 \equiv \sum_{j=1}^{n} \langle \tilde{\mathbf{x}}_0, \mathbf{u}_k \otimes \boldsymbol{\eta}_j \rangle \boldsymbol{\eta}_j. \tag{7}$$

To bring our digression to a close we quote a theorem the proof of which has been given by Friedman (1956).

Theorem 1

Suppose that $\mathbf{T}^1, \ldots, \mathbf{T}^p$ are operators on $\mathscr{V}^n$ and $\mathbf{R}^1, \ldots, \mathbf{R}^p$ are self-adjoint operators on $\mathscr{S}^N$ and that the latter have a common spectral representation, that is, there exist a complete orthonormal set $\{\mathbf{u}_j\}$ in $\mathscr{S}^N$ and a set of real numbers α_{qk} such that $\mathbf{R}^q \mathbf{u}_k = \alpha_{qk} \mathbf{u}_k$ for $q = 1, \ldots, p$ and $k = 1, \ldots, N$. Let L be an operator in $\mathscr{S}^N \otimes \mathscr{V}^n$ defined by $L \equiv \mathbf{R}^1 \otimes \mathbf{T}^1 + \ldots + \mathbf{R}^p \otimes \mathbf{T}^p$. Then the equation $Lf = h$, where h is any element of $\mathscr{S}^N \otimes \mathscr{V}^n$, has a solution if the operators $(\alpha_{1k}\mathbf{T}^1 + \ldots \alpha_{pk}\mathbf{T}^p)^{-1}$ are uniformly bounded for all k, $k = 1, \ldots, N$. Moreover the solution is given by

$$f = \sum_{k=1}^{N} \mathbf{u}_k \otimes (\alpha_{1k}\mathbf{T}^1 + \ldots + \alpha_{pk}\mathbf{T}^p)^{-1} h_k$$

where

$$h_k \equiv \sum_{j=1}^{n} \langle h, \mathbf{u}_k \otimes \boldsymbol{\eta}_j \rangle \boldsymbol{\eta}_j.$$

Now by setting $f = \tilde{\mathbf{x}} - \tilde{\mathbf{x}}_0$, $h = (\mathbf{I}_N \otimes \mathbf{K})\tilde{\mathbf{x}}_0$, $\mathbf{T}^1 = [\mathbf{I}_n(\mathrm{d}/\mathrm{d}t) - \mathbf{K}]$, $\mathbf{T}^p = -\mathbf{D}$, $\mathbf{R}^1 = \mathbf{I}_N$, and $\mathbf{R}^p = \Delta$ we can apply the theorem, for all of the hypotheses

† Strictly, this is true only if the membrane permeabilities or conductances for each species are independent of the *direction* of transfer, a circumstance that may be more the exception than the rule in biological systems. If the two unidirectional permeabilities differ, the structural matrix is no longer symmetric; in fact, it may no longer be possible to distinguish $\mathbf{D}$ and Δ. Then the theory is mathematically more involved but admits an even richer variety of physical possibilities.

are met, as has been shown (Othmer, 1969). Inversion of the operators $\mathbf{I}_n(\mathrm{d}/\mathrm{d}t)-\mathbf{K}-\alpha_k\mathbf{D}$ is well known (Bellman, 1960, chaps 10 and 11). In this way the general solution of the stability problem (6) with (7) is found to be

$$\tilde{\mathbf{x}}(t) = \sum_{k=1}^{N} \mathbf{u}_k \otimes \mathrm{e}^{(\mathbf{K}+\alpha_k\mathbf{D})t}\mathbf{y}_k^\circ. \tag{8}$$

That this is the solution can be verified by direct substitution in (6). That it is analogous in form to the solution obtained by separation of variables in the continuous case is already evident but is brought out plainly in the next section.

The advantage of the abstract formulation is that the solution (8) to which it leads applies to any network of cells. In using (8) to determine stability behavior of a system with respect to infinitesimal concentration fluctuations about a UNESS the procedure is first, deduce the structural matrix of the network, $\mathbf{\Delta}$, and calculate its eigenvalues, α_k, and eigenvectors, $\mathbf{u}_k$; and second, for each of the N numbers, α_k, calculate the eigenvalues, λ_{kj}, of the reaction-and-transfer matrix, $\mathbf{K}+\alpha_k\mathbf{D}$. Then if all $N\times n$ eigenvalues, λ_{kj}, have negative real parts the exponential functions in (8) tend toward zero, i.e. all of the structural modes of *any* disturbance decay to zero, and so $\lim_{t\to\infty} \tilde{\mathbf{x}}(t) = 0$: the system is stable. If one or more of the eigenvalues, λ_{kj}, have positive real parts, the system is unstable, in the sense that at least one structural mode that may be and generally is present in random fluctuations is amplified exponentially in time, until its amplitude reaches proportions at which nonlinear effects come into play. If an eigenvalue has an imaginary part the amplitude of the corresponding structural mode may undergo damped or amplified oscillations and there is even a possibility that the system can propagate disturbances having the character of traveling waves (cf. Gmitro & Scriven, 1966; Othmer, 1969).

For each structural eigenvalue, α_k, the eigenvalues, λ_{kj}, are solutions of the nth order determinantal equation

$$|\mathbf{K}+\alpha_k\mathbf{D}-\lambda_k\mathbf{I}_n| = 0.$$

A thorough parametric analysis of the eigenvalues in the instance that α_k varies continuously and n equals two or three has been presented elsewhere (Othmer & Scriven, 1969). From the results the eigenvalues of $\mathbf{K}+\alpha_k\mathbf{D}$ may be obtained for any structural eigenvalue, α_k, when only two or three chemical substances participate in the system of reaction and transfer.†

† Analyses of the stability of Turing's and related reaction schemes have been reported by Prigogine and coworkers [*J. chem. Phys.* **46**, 3542 (1967); *J. chem. Phys.* **48**, 1695 (1968); *International Conference on Theoretical Physics and Biology* (M. Marois, ed.) pp. 23–52, (1969)]. They confirm that the postulates of irreversible thermodynamics are consistent with unstable solutions Turing found, and that diffusion can play an essential role.

In the next section we apply our general solution and the procedure outlined here to the relatively simple case of one-dimensional networks. Thereafter we treat a number of two-dimensional networks, with results that are interesting in their own right, besides illustrating the power of the theory.

4. One-dimensional Lattices

The first example is a ring of N cells, each in contact with two neighbors, as diagrammed in Fig. 2. This is one instance of a periodic, one-dimensional network with a period of N, i.e. $\mathbf{x}^{\mu+N} = \mathbf{x}^{\mu}$. The compartmentalized disk of Fig. 2 is another example. In such networks $\Delta^{\mu}\mathbf{x}^{\mu} = \mathbf{x}^{\mu+1} - 2\mathbf{x}^{\mu} + \mathbf{x}^{\mu-1}$ for

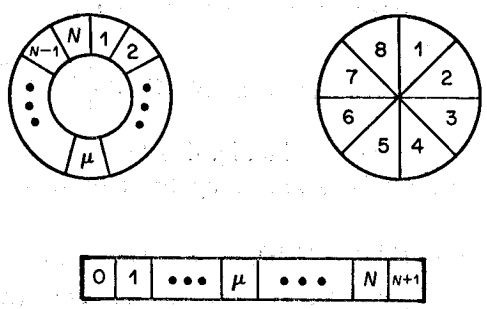

FIG. 2. Examples of one-dimensional networks.

all μ, 1 to N. Consequently, the matrix representation of the structural matrix is†

$$\Delta = \begin{bmatrix} -2 & 1 & 0 & 0 & 0 & \dots & 0 & 1 \\ 1 & -2 & 1 & 0 & 0 & \dots & 0 & 0 \\ 0 & 1 & -2 & 1 & 0 & \dots & 0 & 0 \\ \cdot & & & & & & & \cdot \\ \cdot & & & & & & & \cdot \\ \cdot & & & & & & & \cdot \\ 1 & 0 & 0 & 0 & 0 & \dots & 1 & -2 \end{bmatrix}.$$

This is a special type of matrix called a circulant matrix, the eigenvalues and eigenvectors of which are well known (e.g. Bellman, 1960, § 12.15). The

† Both rows and columns are indexed consecutively by cell number; i.e. this is the matrix representation of the operator Δ with respect to the canonical basis $\{e_j = (0, \dots, 0, \overset{j}{1}, 0, \dots, 0); j = 1, \dots, N\}$. All subsequent representations by structural matrices are also with respect to canonical bases.

eigenvalues are

$$\alpha_k = -2+\rho_k+\rho_k^{-1} = -4\sin^2(\pi k/N), \qquad k = 1, \ldots, N,$$

where ρ_k is the primitive root of unity given by $\rho_k \equiv \exp(2\pi ik/N)$; these eigenvalues are degenerate, for, as may be readily verified, $\alpha_{N-k} = \alpha_k$. The corresponding eigenvectors in normalized form are

$$\mathbf{u}_k = \sqrt{1/N}(1, \rho_k, \rho_k^2, \ldots, \rho_k^{N-1}), \qquad k = 1, \ldots, N.$$

These are complex-valued but there is a fully equivalent set of real-valued eigenvectors:

$$\left.\begin{aligned}\mathbf{u}_k &= \sqrt{2/N}(1, \cos 2\pi k/N, \ldots, \cos 2\pi k(N-1)/N)\\ \mathbf{u}_{N-k} &= \sqrt{2/N}(0, \sin 2\pi k/N, \ldots, \sin 2\pi k(N-1)/N)\end{aligned}\right\}\begin{cases}k = 1, \ldots, N/2-1\\ \qquad\qquad \text{if } N \text{ even.}\\ k = 1, \ldots, (N-1)/2\\ \qquad\qquad \text{if } N \text{ odd.}\end{cases}$$

$$\mathbf{u}_k = \sqrt{2/N}(1, \cos 2\pi k/N, \ldots, \cos 2\pi k(N-1)/N)\begin{cases}k = N/2, N \text{ if } N \text{ even.}\\ k = N \text{ if } N \text{ odd.}\end{cases}$$

(the set for $N = 4$ may be seen in Fig. 4 below). Stability in a periodic, one-dimensional network is governed by the eigenvalues, λ_{kj}, of the N matrices

$$\mathbf{K}-(4\sin^2 \pi k/N)\mathbf{D}, \qquad k = 1, \ldots, N.$$

Because the α_k are degenerate, as noted above, only $(N+2)/2$ of these matrices are distinct when N is even and only $(N+1)/2$ are distinct when N is odd. Consequently each degenerate eigenvalue is shared by two eigenvectors, i.e. two distinct structural modes display the same time course, including rate of exponential damping or amplification. Their stability behavior is therefore identical. In the complex-valued set the degenerate pairs of modes are complex conjugates; in the real-valued set they are complementary odd and even functions, being sines and cosines, respectively, of the same arguments.

From equation (8) the vector of concentration excursions in the μth cell is found to be

$$\mathbf{x}^\mu(t) = \frac{1}{N}\sum_{k=1}^{N} e^{2\pi i(\mu-1)k/N} e^{(\mathbf{K}+\alpha_k\mathbf{D})t}\left(\sum_{\nu=1}^{N} e^{-2\pi i(\nu-1)k/N}\mathbf{x}_o^\nu\right). \tag{9}$$

This formula is analogous to that obtained by separation of variables and the eigenfunction method in the case of a *continuous*, one-dimensional system. The term in large parentheses is simply the kth Fourier coefficient in the eigenfunction expansion of the initial condition; the factor $\exp(2\pi i(\mu-1)k/N)$ is the value of the kth eigenfunction or Fourier component at the μth cell (μ being analogous to position in the continuous system); the remaining factor

is the time course of the kth eigenfunction; and the concentration excursion is a linear combination, a superposition of normal modes, or structural modes all evaluated at the cell in question.

In comparison with Turing's 1952 assault on two and three "morphogens" in a closed ring of cells the theory brought to bear here may seem of unnecessarily large caliber, but it does expose an important distinction between structural and dynamic aspects, it can extract solutions for (linearized) systems of many more morphogens, and it is the only systematic method available for attacking two- and three-dimensional lattices of any but the simplest regular connectivity.

In a periodic, one-dimensional lattice that departs *slightly* from a UNESS the variation of concentrations from cell to cell is determined by the factor $\exp(2\pi i(\mu-1)k/N)$ in equation (9) and is therefore periodic, or wave-like, along the lattice. The maximum wavelength corresponds to $k = N$. In this mode there is no variation from cell to cell at any time: they all share the same excursion, whether it is damped or amplified. When N is even the minimum possible wavelength, or repeat interval on the lattice, is two and corresponds to $k = N/2$. In this mode the concentration excursions in adjacent cells are equal in magnitude but opposite in sign or, if the perturbations have traveling-wave character, the adjacent cells are out of phase by half a time-period. Interestingly, the minimum possible wavelength is quite different when N is odd. If N is a prime number *all* of the structural modes are of the maximum wavelength, i.e. the only periodicity is that of the lattice itself. If N is a composite odd number the minimum possible wavelength is the smallest divisor of N; for example, if $N = 15$, the repeat unit consists of three successive cells each with a different concentration excursion, this mode corresponding to $k = 5$.

If the eigenvalues of $\mathbf{K}+\alpha_k\mathbf{D}$ include a complex one, say $\lambda_{kj} = \lambda_r+i\lambda_i$ (and necessarily its complex conjugate $\lambda_r-i\lambda_i$ too), then time-dependent, oscillatory normal modes can be identified in equation (9). These can be organized either as standing waves, $\cos(\lambda_i t)\exp(i2\pi k\mu/N)$, or as traveling waves, $\exp i(2\pi k\mu/N-\lambda_i t)$ and $\exp i(2\pi k\mu/N+\lambda_i t)$, the latter moving through the lattice at a speed of $\lambda_i N/2\pi k$ cells per second. Whether standing or progressing, the waves are unstable and amplifying, stable and attenuating, or marginally stable with unchanging amplitude, according as λ_r is positive, negative, or zero, respectively. In all cases the concentrations in the cells, except any nodal cells in standing wave patterns, oscillate rhythmically in time, with radial frequency λ_i. This may be regarded as a natural "chemical frequency". The appearance of standing or progressive waves depends on phase differences from cell to cell, differences that are governed by boundary conditions and initial conditions on the network.

The wavelengths of structural modes depend solely on topological features of the network and are not influenced by the dynamic processes occurring within individual cells.†

However, the time course of each mode is determined by the corresponding eigenvalues, λ_{kj}, of the matrix $\mathbf{K}+\alpha_k\mathbf{D}$. Thus network structure influences the dynamic processes through the structural eigenvalues, α_k. Structure and dynamics together determine which modes are unstable and, of those, which are amplified most rapidly. In initially random fluctuations the most rapidly amplified modes are the ones most likely to dominate a new, non-uniform dynamic state to which instability leads through non-linear effects. It must be emphasized that the present analysis of linearized equations of change is but the first step toward determining the entire time course of concentration fluctuations in unstable systems. Such things as multiple stationary states, limit cycles, and almost periodic behavior can only be uncovered by non-linear analysis.

In the preceding example of the ring of cells the periodicity condition $\mathbf{x}^{\mu+N} = \mathbf{x}^{\mu}$ had to be satisfied. On an open, one-dimensional network of the sort indicated in Fig. 2 it is necessary to satisfy two end conditions instead. Different types of end conditions result in different structural matrices and in different sets of eigenvalues, α_k, and structural modes $\mathbf{u}_k$. For example, if the concentrations were all held fixed in the end cells of an $N+2-$ cell string the end conditions on excursions would be $\mathbf{x}^0 = \mathbf{x}^{N+1} = 0$ and the $N \times N$ structural matrix would be

$$\Delta = \begin{bmatrix} -2 & 1 & 0 & 0 & \dots & 0 & 0 \\ 1 & -2 & 1 & 0 & \dots & 0 & 0 \\ 0 & 1 & -2 & 1 & \dots & 0 & 0 \\ \cdot & & & & & & \cdot \\ \cdot & & & & & & \cdot \\ \cdot & & & & & & \cdot \\ 0 & 0 & 0 & 0 & \dots & 1 & -2 \end{bmatrix}.$$

This is a pseudo-circulant matrix, with well-known eigenvalues and eigenvectors. If transfer beyond cells 1 and N were prevented it would be as though the concentrations in cell 0 were always matched to those in cell 1, and those in cell $N+1$ to those in cell N; the end conditions would be $\mathbf{x}^0 = \mathbf{x}^1$ and $\mathbf{x}^N = \mathbf{x}^{N+1}$ and the structural matrix would be slightly different. Yet another slightly different structural matrix would arise if concentration were

† This is true only so long as all cells and junctions share the same constitutive relations for reactions and transfers, respectively. If they do not, formidable complications arise which are akin to those in continuous systems with spatially-dependent coefficients.

fixed at one end and transfer beyond the other end were prevented. Results for the four types of end conditions are summarized in Table 1.†

It is obvious that end conditions, or boundary conditions, can have significant influence on the structural modes of a system and, through the structural eigenvalues, on stability behavior. This influence is illustrated in

TABLE 1

Structural eigenvalues, α_k, and structural modes, $\mathbf{u}_k$, of one-dimensional networks with various end conditions

End Condition	$\sin^{-1}\sqrt{-\alpha_k/4}$	$\mathbf{u}_{kl}$, $\mathbf{u}_k = (\mathbf{u}_{k1}, \mathbf{u}_{k2}, \ldots, \mathbf{u}_{kN})^T$
Periodic	$\pi k/N$	$\sqrt{1/N}\exp(2\pi ik(l-1)/N)$‡
Fixed concentrations	$\pi k/2(N+1)$	$\sqrt{2/(N+1)}\sin[\pi kl/(N+1)]$
No transfer	$\pi k/2N$	$\dfrac{\sin[\pi kl/N]-\sin[\pi k(l-1)/N]}{\sqrt{2N}\lvert\sin[\pi k/2N]\rvert}$
	0 when $k = N$	$\sqrt{1/N}$ when $k = N$
Mixed	$\pi(k-\frac{1}{2})/(2N+1)$	$\sqrt{2/(N+\frac{1}{2})}\sin[\pi(k-\frac{1}{2})l/(N+\frac{1}{2})]$

‡ Real-valued modes are given in the text.

Appendix A below by a particular example involving only two participating chemical substances in a four-cell chain. The effect of a deviant permeability of the junction between one pair of cells can be illustrated simply with a short, one-dimensional array, and this too is done in the Appendix. The example confirms the expectation that stability in a network can be under the control of junctional transport mechanisms.

5. Two-dimensional Lattices

Despite their simplicity one-dimensional lattices lack certain structural features that may well be important in networks of living cells. A suspension of tissue culture cells if allowed to reaggregate on a surface generally begins

† Not included here is the slightly more complicated end condition of the "third kind", i.e. a linear combination of fixed-concentration and fixed-flux conditions, amounting to an end cell connected by a junction of special permeability.

assembling into a two-dimensional rather than a one-dimensional network; this is especially true of epitheliocytes (Willmer, 1958; Lucey & Curtis, 1959). Single sheet systems of cells, as in epidermis and epithelium, are featured in D'Arcy Thompson's (1942) classic review, which also covers a number of regular three-dimensional assemblies, as occur in parenchymatous tissues and early stages of embryonic development. The number of ways of arranging communication links between cells jumps farther as one passes from one- to

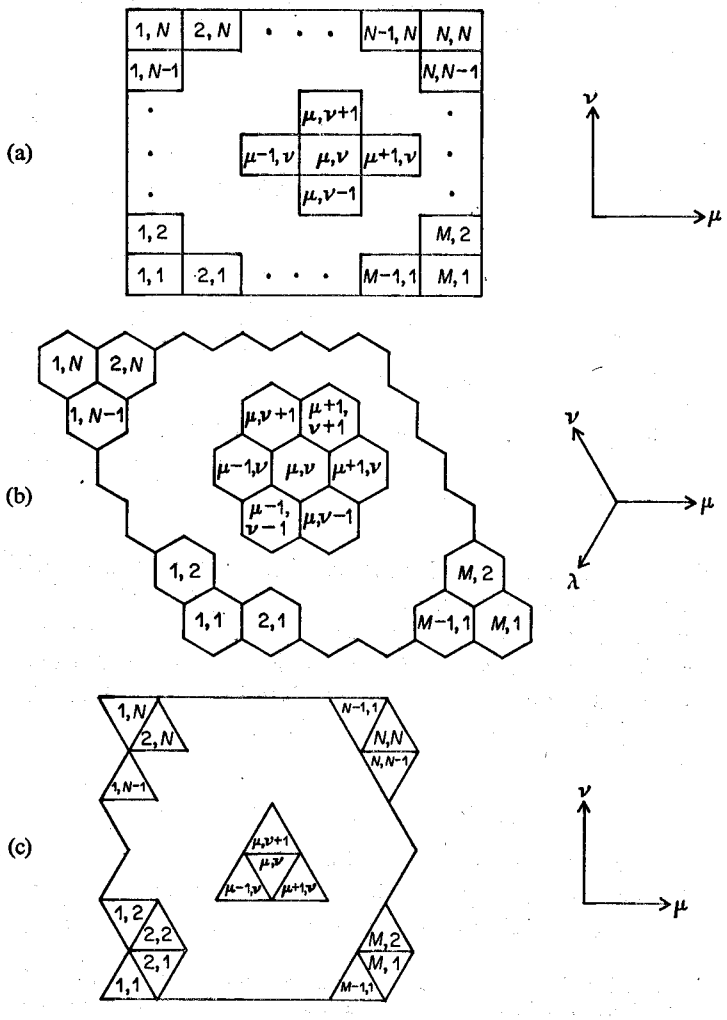

FIG. 3. Examples of regular two-dimensional networks.

two-dimensional lattices than as one passes on to three-dimensional networks. The character of the latter is foretold by analysis of two-dimensional systems, which are less tedious to treat.

There are regular, two-dimensional lattices which can be analyzed with straightforward extensions of the theory as it applies to cases in the preceding section. Simplest is a doubly periodic network in which each cell contacts four neighbors. The situation is represented in Fig. 3(a). Each cell is conveniently indexed by a pair of coordinate integers (μ, ν); the period is then M in μ and N in ν, i.e. $\mathbf{x}^{\mu+M,\nu} = \mathbf{x}^{\mu,\nu} = \mathbf{x}^{\mu,\nu+N}$. The difference operator is defined by

$$\Delta^{\mu,\nu}\mathbf{x}^{\mu,\nu} = \mathbf{x}^{\mu+1,\nu} - 2\mathbf{x}^{\mu,\nu} + \mathbf{x}^{\mu-1,\nu} + \mathbf{x}^{\mu,\nu+1} - 2\mathbf{x}^{\mu,\nu} + \mathbf{x}^{\mu,\nu-1}.$$

But this is just the sum of the operators (defined in section 3) for the two separate directions, i.e. $\Delta^{\mu+\nu} = \Delta^{\mu} + \Delta^{\nu}$; consequently the structural matrix can be composed from those for rings of length M and N, respectively, as defined at equation (9):

$$\Delta = \Delta_N \otimes \mathbf{I}_M + \mathbf{I}_N \otimes \Delta_M.$$

$\mathbf{I}_N$ and $\mathbf{I}_M$ are the $N \times N$ and $M \times M$ unit matrices, respectively; in the next equation $\mathbf{I}_{NM} = \mathbf{I}_N \otimes \mathbf{I}_M$ is the $NM \times NM$ one. Equation (6) governing stability of a UNESS becomes

$$\frac{d\tilde{\mathbf{x}}}{dt} = [(\Delta_N \otimes \mathbf{I}_M + \mathbf{I}_N \otimes \Delta_M) \otimes \mathbf{D}]\tilde{\mathbf{x}} + (\mathbf{I}_{NM} \otimes \mathbf{K})\tilde{\mathbf{x}} \tag{10}$$

with $\tilde{\mathbf{x}}(0) = \tilde{\mathbf{x}}_0$.

This equation is readily solved with the aid of a result proved by Friedman (1961).

Theorem 2

Let

$$C \equiv \sum_{q=1}^{p} \mathbf{A}_q \otimes \mathbf{B}_q,$$

where $\mathbf{A}_1, \ldots, \mathbf{A}_p$ are $N \times N$ matrices and $\mathbf{B}_1, \ldots, \mathbf{B}_p$ are $M \times M$ matrices. Suppose the $\mathbf{A}_q$ are all simultaneously diagonizable and let $\mathbf{v}$ be an eigenvector of the set, i.e., $\mathbf{A}_q\mathbf{v} = \alpha_q\mathbf{v}$, $q = 1, \ldots, p$. If there exist a vector $\mathbf{w}$ and a number λ such that $(\alpha_1\mathbf{B}_1 + \ldots + \alpha_p\mathbf{B}_p)\mathbf{w} = \lambda\mathbf{w}$, then λ is an eigenvalue and $\mathbf{v} \otimes \mathbf{w}$ an eigenvector of the $NM \times NM$ matrix $\mathbf{C}$, i.e. $\mathbf{C}(\mathbf{v} \otimes \mathbf{w}) = \lambda\mathbf{v} \otimes \mathbf{w}$.

It follows immediately that here the eigenvalues of Δ are sums of those of

Δ_N and Δ_M. Thus the structural eigenvalues and structural modes are

$$\alpha_{kl} = \alpha_k + \alpha_l = -4[\sin^2(\pi k/M) + \sin^2(\pi l/N)] \tag{11}$$

$$\mathbf{u}_{kl} = \mathbf{u}_k \otimes \mathbf{u}_l = \sqrt{1/MN}(1, \rho_k, \ldots, \rho_k^{M-1}) \otimes (1, \sigma_l, \ldots, \sigma_l^{N-1}), \begin{cases} k = 1, \ldots, M, \\ l = 1, \ldots, N, \end{cases}$$

where $\rho_k \equiv \exp(2\pi ik/M)$ and $\sigma_l \equiv \exp(2\pi il/N)$.

Stability behavior is therefore governed by the eigenvalues of the MN matrices

$$\mathbf{K} - 4[\sin^2(\pi k/M) + \sin^2(\pi l/N)]\mathbf{D}.$$

The vector of concentration excursions in the (μ,ν)th cell is

$$\mathbf{x}^{\mu,\nu}(t) = \frac{1}{MN} \sum_k \sum_l e^{2\pi i[(\mu-1)k/M + (\nu-1)l/N]} e^{(\mathbf{K} + \alpha_{kl}\mathbf{D})t} \cdot \left(\sum_\gamma \sum_\delta e^{-2\pi i[(\gamma-1)k/M + (\delta-1)l/N]} \mathbf{x}_0^{\gamma,\delta} \right).$$

In essence this is the double Fourier series representation of the solution of (10). Because the structural modes are doubly periodic the discussion in section 4 about maximum and minimum wavelengths in one-dimensional networks pertains separately to each coordinate direction. In addition the structural modes may contain definite lines of cells in which the excursions are maximal or minimal, and nodal lines along which the excursions vanish, so that the modes are genuinely two-dimensional patterns. By way of example the 16 periodic structural modes of a 4×4 lattice are diagrammed in Fig. 4.

Minor variations on the foregoing case are easily dealt with. First, the intercellular transfer coefficients D_{ij} may be different in the two directions of cell–cell contact. If we let $\mathbf{D}'$ be the $n \times n$ matrix of transfer coefficients D'_{ij} in the μ-direction and $\mathbf{D}''$ be that of coefficients D''_{ij} in the ν-direction, then instead of (10) we have

$$\frac{d\tilde{\mathbf{x}}}{dt} = [(\Delta_N \otimes \mathbf{I}_M) \otimes \mathbf{D}'' + (\mathbf{I}_N \otimes \Delta_M) \otimes \mathbf{D}']\tilde{\mathbf{x}} + (\mathbf{I}_{NM} \otimes \mathbf{K})\tilde{\mathbf{x}}. \tag{12}$$

This is analogous to the equation for a continuous system in which the diffusion–coefficient tensor is anisotropic. From the preceding paragraph it can be seen that the structural modes are still given by (11), i.e. they are the same whether transfer coefficients are the same in both lattice directions [equation (10)] or different [equation (12)]. But of course the network structure, or topology, is the same in both cases. In the latter case, however, *stability* is governed by the eigenvalues of the new matrices.

$$\mathbf{K} + \alpha_k \mathbf{D}' + \alpha_l \mathbf{D}'' = \mathbf{K} - 4[\sin^2(\pi k/M)]\mathbf{D}' - 4[\sin^2(\pi l/N)]\mathbf{D}''.$$

In this way the anisotropy manifests itself in dynamic behavior of the network. Other variations of the basic case arise when the end conditions on any

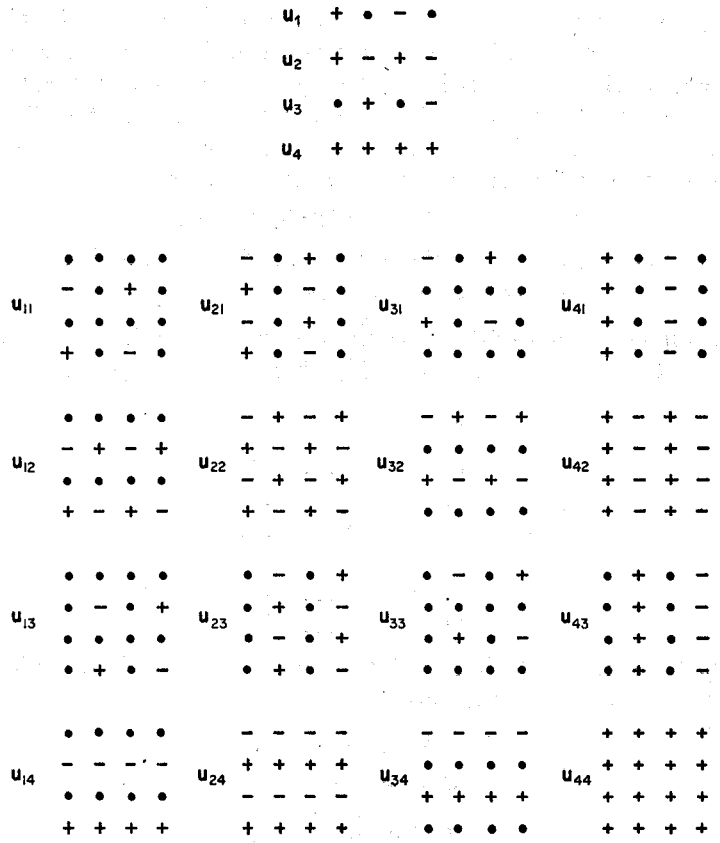

FIG. 4. Periodic structural modes of a 4-cell ring and of a 4×4-cell torus ($\mathbf{u}_{ij} \equiv \mathbf{u}_i \otimes \mathbf{u}_j$). Key: $+ = +1$, $- = -1$, $\bullet = 0$. The modes shown are not normalized.

or all boundaries of a rectangular network with connectivity four are altered. Structural eigenvalues and modes can be found by taking sums and tensor products, respectively, of those listed in Table 1.

In close-packed planar arrays of nearly identical objects there is often a strong tendency for each to be surrounded by and in contact with not four but six others. The corresponding regular network has a connectivity of six and can be represented by a hexagonal lattice, as in Fig. 3(b). This provides an instructive contrast to the lattices just considered.

Though triangular coordinates (λ, μ, ν) seem natural for labeling cells, one of them is redundant—any point in a plane can be located by two coordinates. We again retain coordinate integers (μ, ν) but now associate them with two directions at 120° to each other [see Fig. 3(b)]. For simplicity

we restrict consideration to a network periodic in both μ and ν with the same period N; it follows that it must have a period of $2N$ in the direction of λ.

A "hexagonal" network is just a "tetragonal" network so modified that the connectivity is six instead of four. One accordingly expects both likenesses and differences in their stability and pattern-forming tendencies. The difference operator for the "hexagonal" network is defined by

$$\Delta^{\mu+\nu}\mathbf{x}^{\mu,\nu} = \Delta^{\mu}\mathbf{x}^{\mu,\nu} + \Delta^{\nu}\mathbf{x}^{\mu,\nu} + \Delta^{\mu\nu}\mathbf{x}^{\mu,\nu},$$

where the new operator, stemming from the added connectivity, is given by

$$\Delta^{\mu\nu}\mathbf{x}^{\mu,\nu} \equiv \mathbf{x}^{\mu+1,\nu+1} - 2\mathbf{x}^{\mu,\nu} + \mathbf{x}^{\mu-1,\nu-1}.$$

The corresponding part of the structural matrix of the entire network can be written as $\mathbf{J}_N \otimes \mathbf{J}_N - 2\mathbf{I}_N \otimes \mathbf{I}_N + \mathbf{J}_N^{\mathrm{T}} \otimes \mathbf{J}_N^{\mathrm{T}}$, where $\mathbf{J}_N$ is the cyclic permutation matrix,

$$\mathbf{J}_N \equiv \begin{bmatrix} 0 & 1 & 0 & \dots & 0 \\ 0 & 0 & 1 & \dots & 0 \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ 0 & 0 & 0 & \dots & 1 \\ 1 & 0 & 0 & \dots & 0 \end{bmatrix}.$$

The structural modes $\mathbf{u}_{kl}$ of an $N \times N$ "tetragonal" network [cf. equation (11)] are found to satisfy the equations

$$(\mathbf{J}_N \otimes \mathbf{J}_N)\mathbf{u}_{kl} = \rho_k \rho_l \mathbf{u}_{kl},$$
$$(\mathbf{I}_N \otimes \mathbf{I}_N)\mathbf{u}_{kl} = \mathbf{u}_{kl},$$
$$(\mathbf{J}_N^{\mathrm{T}} \otimes \mathbf{J}_N^{\mathrm{T}})\mathbf{u}_{kl} = \frac{1}{\rho_k \rho_l}\mathbf{u}_{kl}.$$

Therefore they are also the structural modes of the $N \times N$ "hexagonal" network, i.e. eigenvectors of the structural matrix

$$\Delta_N \otimes \mathbf{I}_N + \mathbf{I}_N \otimes \Delta_N + \mathbf{J}_N \otimes \mathbf{J}_N - 2\mathbf{I}_N \otimes \mathbf{I}_N + \mathbf{J}_N^{\mathrm{T}} \otimes \mathbf{J}_N^{\mathrm{T}}.$$

However, as is easily shown, the structural eigenvalues of the $N \times N$ "hexagonal" network are

$$\alpha_{kl} = -4[\sin^2(\pi k/N) + \sin^2(\pi l/N) + \sin^2(\pi(k+l)/N)].$$

These differ in the third term from the structural eigenvalues of the "tetragonal" network. Consequently stability behavior, which is determined by the eigenvalues of the N^2 matrices

$$\mathbf{K} - 4[\sin^2(\pi k/N) + \sin^2(\pi l/N) + \sin^2(\pi(k+l)/N)]\mathbf{D},$$

is different. The differences between the two networks lie not in the structural modes *per se*, but rather in the dynamical behavior of each mode. Most of

the modes $\mathbf{u}_{kl}$ (except $\mathbf{u}_{NN}$, which is indifferent to network topology, and the $N-1$ modes of the form $\mathbf{u}_{k,\,N-k}$) may be stable in the "hexagonal" network and unstable in the "tetragonal" network, or *vice versa.*

Besides the "hexagonal" and "tetragonal" networks there is only one other that corresponds to perfectly regular planar arrays: it is the "triangular" network, in which each cell is in contact with just three of its neighbors, as indicated in Fig. 3(c). There are new features in the structural matrix, and its eigenvectors are not those of the "hexagonal" and "tetragonal" networks, although some resemblances show through; the eigenvalues, however, are quite different (Othmer, 1969). The distinctive structural modes and stability behavior of the "triangular" network highlight how important connectivity in a network can be.

6. Regular Polyhedral Lattices

In certain simple multicellular organisms and in early stages of development of others, cell groupings are to be seen which are more or less spherical, or are at least topologically equivalent to a sphere, or rather to some polyhedral network (cf. Thompson, 1942). The doubly periodic, two-dimensional lattices of the preceding section can be neatly wrapped on a torus but not on a sphere, which, being topologically of another genus, warrants separate attention. If we restrict consideration to regular polyhedral networks on the sphere there are just five, corresponding to the five Platonic solids, as shown in Fig. 5.† We present results for "the tetrahedral", "cubic", and "octahedral" lattices only; the somewhat bulkier results for "dodecahedral" and "icosahedral" lattices have also been obtained (Othmer, 1969).

It must be emphasized that in no spherical lattice, whether regular or not, are there any edges at which to impose end conditions or boundary conditions, and so for a given lattice there is but one set of structural eigenvalues and structural modes.

The structural matrices, as may be confirmed from Fig. 5, are:

$$\text{tetrahedral, } \Delta = \mathbf{I}_2 \otimes \begin{bmatrix} -3 & 1 \\ 1 & -3 \end{bmatrix} + \mathbf{J}_2 \otimes \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix};$$

$$\text{cubic, } \Delta = -4\mathbf{I}_3 \otimes \mathbf{I}_2 + (\mathbf{J}_3 + \mathbf{J}_3^{\mathrm{T}}) \otimes \begin{bmatrix} 1 & 1 \\ 1 & 1 \end{bmatrix};$$

$$\text{octahedral, } \Delta = \mathbf{I}_4 \otimes \begin{bmatrix} -3 & 1 \\ 1 & -3 \end{bmatrix} + (\mathbf{J}_4 + \mathbf{J}_4^{\mathrm{T}}) \otimes \mathbf{I}_2.$$

† Note that the missing number in each of the Schlegel diagrams is the number of the face through which all the others are projected.

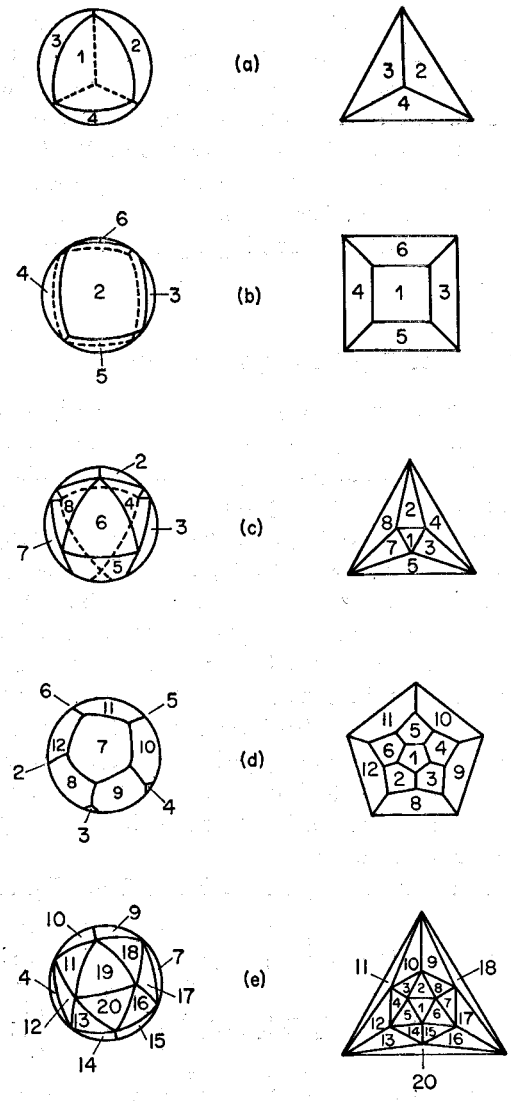

FIG. 5. Regular polyhedral networks. Spherical polyhedra and corresponding Schlegel diagrams. (a) Tetrahedral, (b) cubic, (c) octahedral, (d) dodecahedral, (e) icosahedral networks.

The structural eigenvalues and, in unnormalized form, the real-valued structural modes are:

Tetrahedral lattice

Eigenvalue α_k		−4	−4	−4	0
	$\mathbf{u}_{k1}$	1	1	0	1
Eigenvector	$\mathbf{u}_{k2}$	0	−1	1	1
components	$\mathbf{u}_{k3}$	−1	1	0	1
	$\mathbf{u}_{k4}$	0	−1	−1	1

Cubic lattice

Eigenvalue α_k		−6	−6	−4	−4	−4	0
	$\mathbf{u}_{k1}$	1	0	1	0	1	1
	$\mathbf{u}_{k2}$	1	0	−1	0	−1	1
Eigenvector	$\mathbf{u}_{k3}$	0	1	1	1	0	1
components	$\mathbf{u}_{k4}$	0	1	−1	−1	0	1
	$\mathbf{u}_{k5}$	−1	−1	0	1	1	1
	$\mathbf{u}_{k6}$	−1	−1	0	−1	−1	1

Octahedral lattice

Eigenvalue α_k		−6	−4	−4	−4	−2	−2	−2	0
	$\mathbf{u}_{k1}$	1	1	0	1	0	1	1	1
	$\mathbf{u}_{k2}$	−1	−1	0	1	0	1	−1	1
	$\mathbf{u}_{k3}$	−1	0	1	−1	1	0	1	1
Eigenvector	$\mathbf{u}_{k4}$	1	−1	−1	−1	1	0	−1	1
components	$\mathbf{u}_{k5}$	1	−1	0	1	0	−1	1	1
	$\mathbf{u}_{k6}$	−1	1	0	1	0	−1	−1	1
	$\mathbf{u}_{k7}$	−1	0	−1	−1	−1	0	1	1
	$\mathbf{u}_{k8}$	−1	0	1	−1	−1	0	−1	1

Though these tabulations are for the cell labelings in Fig. 5, relabeling of the cells leaves the eigenvalues unchanged and merely permutes the components of any given eigenvector.

The degeneracy of most of the structural modes, i.e. the fact that more than one correspond to the same eigenvalue, implies that so long as the dynamic behavior is close enough to being linear that equation (6) applies, modes

having different spatial patterns follow the same time course. For example, if a tetrahedral lattice were unstable at $\alpha_k = -4$, then from any initially random fluctuation three structural modes would be amplified with the same exponential growth rate. Which, if any, of the modes is selected for dominance by the dynamics of the system can be detected only by nonlinear analysis.

7. Discussion

Multicellular systems seldom show even topological regularity that comes anywhere close to that of the foregoing examples—rings with connectivity two, sheets with connectivity four or six (or three), closed shells with connectivity three or four (or five, in the dodecahedral lattice). However, the power of the theory developed here extends to irregular networks as well. Mathematically the additional difficulty in treating irregular networks is strictly a matter of computing eigenvalues and eigenvectors of structural matrices that are not as simple as those in the examples here. Simplicity of a matrix is largely a matter of pattern in its entries: that mathematical structure of the matrix array reflects directly the topological structure of the cell network is both satisfying and suggestive, from a theoretical point of view. Many ramifications remain to be studied. For one, it is natural to ask, what are the consequences of a local imperfection in an otherwise perfect lattice, e.g. a four-connected and two neighboring seven-connected cells in the middle of a hexagonal lattice?

The role of boundary conditions is an important one, as the simple example in Appendix A details and as such work as that of Loewenstein & Penn (1967) testifies. Apart from periodicity conditions, boundary conditions represent prescribed interaction of a system with its immediate surroundings —and inclusions too—whose states are largely independent of the state of the system itself. Whether they are on the edges or in the interior of a network, any cells displaying a marked degree of autonomy with respect to the bulk of the network can be regarded as boundary cells and modeled mathematically by boundary conditions. The example treated in Appendix A barely opens the subject.

Appendix B shows how changes in permeability between two cells of a three-cell network can turn a stable system into an unstable one, or *vice versa.* The result is indicative of what may be expected of larger networks. That changes in permeabilities of junctional membranes have pronounced effects on cell differentiation has been suggested on other grounds, of course (cf. Loewenstein, 1968; Furshpan & Potter, 1968). The approach laid out here may provide a rigorously logical framework within which such biological phenomena can be analyzed.

Yet another ramification is oscillatory instability and the associated standing waves and traveling waves in networks, an aspect barely touched on in section 4, in connection with one-dimensional systems, though oscillations and wave phenomena are every bit as possible in principle in two- and three-dimensional lattices. As pointed out elsewhere (Gmitro & Scriven, 1966), steady-state systems of reaction and diffusion or diffusion-like transport can be arranged to propagate chemical signals as waves traveling at speeds far faster than allowed by transport alone. Chemical concentration waves could provide large numbers of parallel signal-transmission channels between cells in a network.

A regular cellular array represents a homogeneously compartmentalized system, which is perhaps the simplest example of pre-existing pattern in an otherwise uniform system. The results reported above thus shed light on the effect of pre-existing pattern on spontaneously developing pattern and rhythm, which is one of the basic problems posed by Gmitro & Scriven (1966).

The theory, by separating the structural aspects from the dynamical aspects of the stability problem, reveals how network topology can influence patterns of instability and wave propagation in any sort of network. It also brings out the influence of end conditions in an open chain of cells, edge conditions in an open sheet of cells, outer surface conditions in a three-dimensional aggregation of cells. The matrix methods to which the theory leads can be readily applied to a wide variety of dynamical processes on open networks governed by linear, differential–difference equations.

Perhaps the most profound conclusion that can be drawn from the theory in its present state of development and application is the following. Compartmentalization signifies partitioning of what might have been a continuous system into regions of comparatively rapid transport, enclosed by envelopes having high, and perhaps highly localized, resistance to transport of certain things. Thus compartmentalization can replace diffusion-control of transport processes by control at membranes, interfaces, and transport bridges. Transport can thus be made to depend far more on *number of compartments* to be traversed in a network than on *distance* to be traversed or size of compartments: localized transport routes can be established as particular *sequences of compartments* within the network; and in these ways transport processes can be made to depend on *topological* rather than metrical features of the network. That is, compartment size and shape and connector configuration can give way to *numbers* and *relationships* of cells and connectors, and to the performance of the latter as transport paths. The tyranny of random-walk diffusion processes, so slow and given to isotropy, can be broken. For example, in place of concentration *gradient* comes concentration

difference; that is, concentration change per cell replaces concentration change per unit length. It is not necessarily true that, as has been asserted, Turing's old two-morphogen model would predict that different patterns should arise if particular stages of differentiation occurred in embryos of different sizes.

And when there are transformation processes occurring within the compartments as well as transport processes between them and exchange processes with a surrounding bath, the potentialities of compartmentalization are multiplied, particularly in regard to instabilities, spatial patterns, temporal oscillations and wave propagation—as the body of the paper illustrates. In a network of cells, such phenomena may come under the control of junctional membranes and cytoplasmic bridges, intracellular metabolic processes and control mechanisms, and concentration levels in whatever fluid bathes exposed outer surfaces of the cells.

As in Loewenstein's (1968) categorization, cells may communicate with each other in three ways: (i) directly through intervening membrane if they are in contact; (ii) along lateral surface membranes provided they belong to a connected clump of cells; and (iii) indirectly via intercellular fluids bathing the cells which, in this case, need not belong to the same clump (clearly the three ways are not mutually exclusive). While we have focused here on direct contact and the simplest sort of bath, the theory can be augmented to account for communication along connected membranes. Gradients and transients in the bathing fluid can be incorporated too, at the expense of added mathematical complexities. More importantly, the theory encompasses stability of *non*-uniform, non-equilibrium stationary states on networks: examples will be found in the sequel (Othmer & Scriven, manuscript in preparation). Thus the means are at hand for analyzing instabilities of stationary states in which various cells of a network are in different chemical, dynamic steady-states (or maybe only states that are almost steady). Such differentiation might have originally arisen by non-linear locking-in of a pattern triggered by instability of an initially uniform system as it developed under internal controls that alter reaction rate constants and membrane permeabilities. The differentiated network may function as an inducing field or pre-pattern as the internal controls go on to develop a new competence for instability and transition to yet another non-uniform set of dynamic states, which might act as pre-pattern for the next round. With the theory one can envision a succession of instabilities leading to successively more complex dynamic structures in a developing, compartmentalized system.

Possibly this sort of theory will lead toward the science of self-organization and dynamic morphology envisioned by P. Weiss (1962). Perhaps there is here something of the mathematical theory of epigenesis and development which

Waddington (1962) had hoped to find in the still evolving theory of chemical reactors—another field of interacting reaction and transport processes, where multiple dynamic states are commonplace and their stability or instability is crucial.

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APPENDIX A

Influence of Boundary Conditions on Stability Behavior

Consider two one-dimensional networks of four cells each, one with periodic end conditions, the other transferring nothing across its ends. From Table 1 the structural eigenvalues and modes are as follow:

End conditions		Periodic	No flux
Structural eigenvalues	α_1	-2	$-2+\sqrt{2}$
	α_2	-4	-2
	α_3	-2	$-2-\sqrt{2}$
	α_4	0	0
Structural modes	u_1	$(1, 0, -1, 0)/\sqrt{2}$	$\left(\frac{\sqrt{2}}{2}, \frac{2-\sqrt{2}}{2}, \frac{\sqrt{2}-2}{2}, \frac{-\sqrt{2}}{2}\right)\Big/\sqrt{4-2\sqrt{2}}$
	u_2	$(1, -1, 1, -1)/2$	$(1, -1, -1, 1)/2$
	u_3	$(0, 1, 0, -1)/\sqrt{2}$	$\left(\frac{\sqrt{2}}{2}, \frac{-2-\sqrt{2}}{2}, \frac{2+\sqrt{2}}{2}, \frac{-\sqrt{2}}{2}\right)\Big/\sqrt{4+2\sqrt{2}}$
	u_4	$(1, 1, 1, 1)/2$	$(1, 1, 1, 1)/2$

Cell-to-cell variation in each of these modes is indicated in Fig. A1. Though the curves there are continuous, only the values at integral l have meaning.

In order to illustrate the influence of the end conditions on stability behavior let us now suppose that there are two chemical substances which actively participate in the system of reaction and transfer and that at UNESS conditions the effective kinetic coefficients and transfer coefficients happen to satisfy the five equations,

$$K_{11}+K_{22} = -4\zeta,$$
$$K_{11}K_{22}-K_{12}K_{21} = 2\zeta^2,$$
$$D_{11}+D_{22} = \zeta,$$
$$D_{11}D_{22}-D_{12}D_{21} = \zeta^2/8,$$
$$K_{11}D_{11}+K_{12}D_{21}+K_{21}D_{12}+K_{22}D_{22} = -\zeta^2(5+\varepsilon),$$

and the two inequalities,

$$\zeta > 0, \qquad \varepsilon > 0.$$

ε is taken to be a small number. If the elements of **K** and **D** satisfy the foregoing, the 2×2 matrix **K** has two eigenvalues, $-(2\pm\sqrt{2})\,\zeta$, both negative; and the 2×2 matrix **D** has two eigenvalues, $(1\pm\sqrt{2}/2)\,\zeta/2$, both positive. Thus the system would be stable were there solely reaction and exchange, or were there only transfer between cells. Both eigenvalues of the actual stability matrix, $\mathbf{K}+\alpha\mathbf{D}$, are negative except in the neighborhood of $\alpha = -4$, where the larger turns positive (the eigenvalues are $-4(1\pm\sqrt{1+\varepsilon/4})\zeta$: thus the width of the interval over which the larger is positive can be made arbitrarily small by decreasing ε). So with periodic end conditions the structural mode u_2, which has the shortest possible wavelength and corres-

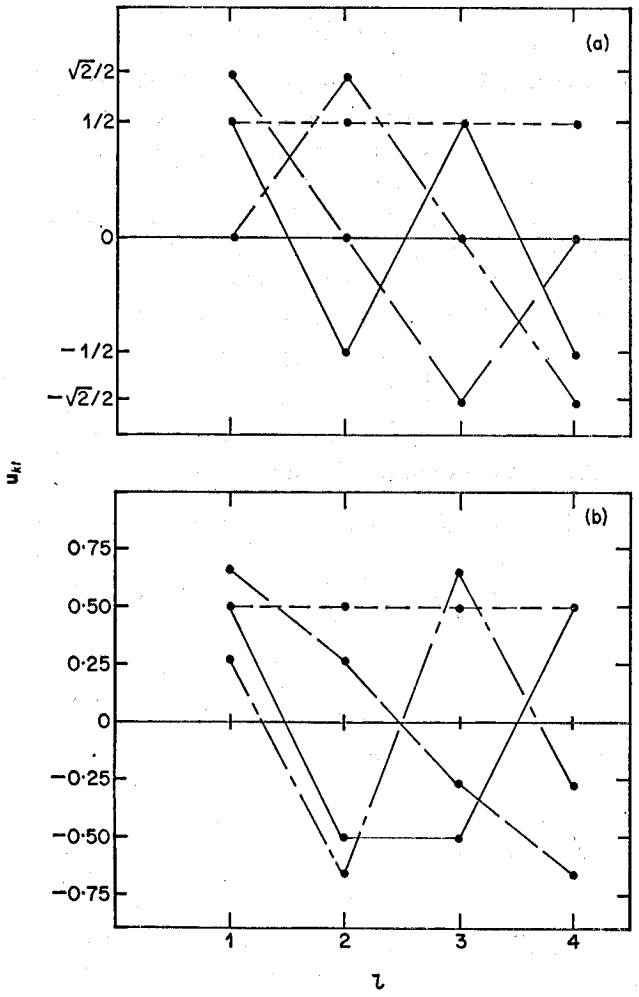

FIG. A1. Cell-to-cell variation in four-cell, one-dimensional networks: (a) periodic end conditions; (b) zero-flux end conditions. Key: — —, u_1; ——, u_2; — — —, u_3; - - - -, u_4.

ponds to $\alpha_2 = -4$, is unstable. In contrast, with no transfer across the ends of the string of cells none of the structural modes are unstable, i.e. the system is stable, even with respect to fluctuations of the shortest possible wavelength. Were the kinetic and transfer coefficients altered in a certain way, the stability behavior of the two systems could be interchanged. Thus boundary conditions can control stability behavior.

APPENDIX B

Influence of Intercell Permeability on Stability Behavior

Consider a one-dimensional network of just three cells, with no transfer across its ends, i.e. the only transfer is that between cells 1 and 2 and between cells 2 and 3. Suppose the matrices of transfer coefficients are **D** and $\delta\mathbf{D}$, respectively, where $0 \leq \delta \leq 1$; when $\delta = 0$ the membrane between cells 2 and 3 is impermeable and when $\delta = 1$ it has the same permeability as that between cells 1 and 2 (the same situation could arise by addition of cell 3 to an existing two-cell network). The structural matrix is

$$\Delta = \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1-\delta & \delta \\ 0 & \delta & -\delta \end{bmatrix}.$$

The structural eigenvalues and modes are

$$\alpha_1, \alpha_2 = -1-\delta \pm \sqrt{1-\delta+\delta^2}, \qquad \alpha_3 = 0$$

$$u_1, u_2 = \left(\frac{1}{1+\alpha_k}, 1, \frac{\delta}{\delta+\alpha_k}\right)/a_k, \qquad u_3 = (1, 1, 1)/\sqrt{3}$$

where

$$a_k \equiv \sqrt{\left(\frac{\delta}{\delta+\alpha_k}\right)^2 + 1 + \left(\frac{1}{1+\alpha_k}\right)^2}.$$

The two limiting cases are as follows:

Permeability ratio		$\delta = 0$	$\delta = 1$
Structural eigenvalues	α_1	0	-1
	α_2	-2	-3
	α_3	0	0
Structural modes	u_1	$(1, 1, 0)/\sqrt{2}$	$(1, 0, -1)/\sqrt{2}$
	u_2	$(1, -1, 0)/\sqrt{2}$	$(-1, 2, -1)/\sqrt{6}$
	u_3	$(1, 1, 1)/\sqrt{3}$	$(1, 1, 1)/\sqrt{3}$

In the first case the isolation of cell 3 is manifest. Both sets of structural modes are orthonormal bases for $\mathscr{S}^3$ and so any pattern, any combination of cell-to-cell variations, can be represented whether or not cell 3 is coupled in with cells 1 and 2. However, the structural eigenvalues α_k are different, the pair $(0, -2)$ metamorphosing into $(-1, -3)$ as δ passes from 0 to 1. Therefore

a given perturbation may die out in one network yet grow in the other, depending on the permeability ratio δ and the values of the kinetic and transfer coefficients in the matrices **K** and **D**. A UNESS on the three-cell network may be stable when cell 3 communicates poorly with cell 2 but may become unstable when communication opens up.