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CURRENT PROBLEMS IN PATTERN FORMATION

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I. INTRODUCTION

One of the oldest and most intriguing problems in theoretical biology concerns the origin of spatial pattern in developing systems. In Wolpert's formulation [1], the problem is that of 'assigning specific states to an ensemble of cells, whose initial states are relatively similar, such that the resulting ensemble of states forms a well-defined spatial pattern'. The first significant mathematical analysis of this problem was done by Turing [2], who originated what is currently called the reaction-diffusion theory of pattern formation. Turing's theory is built around the remarkable fact, which he first proved, that a spatially uniform stationary state of a reacting mixture can be unstable to spatially nonuniform disturbances if reaction and diffusion interact appropriately. The theory then envisions that as certain slowly-varying kinetic or transport coefficients cross critical (bifurcation) values, the uniform state loses stability and a spatially nonuniform state emerges. Depending on the nature of the instability, the resulting nonuniform state may be steady or time dependent. If one or more of the chemicals in this spatially nonuniform concentration pattern activates transcription of a gene that codes for a key enzyme or structural protein, a nonuniform pattern of cell differentiation will result. Such an interpretation

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has been used to explain the origin of insect bristle patterns [3].

In the past decade there has been a renewal of interest in the theoretical aspects of Turing's theory, stimulated in part by Gmitro and Scriven's work [4], and in part by the wide variety of spatio-temporal patterns observed in the Belousov-Zhabotinskii reaction [5]. Linear stability analysis, the first step in any analysis of pattern formation, has been worked out in detail for two- and three-component systems with arbitrary kinetic mechanisms and diffusion matrices [6]. Such analysis is useful in studying the onset of instability and for gaining insight into how the different types of instability are produced by the interaction of reaction and diffusion. However, to predict what spatial pattern ultimately evolves from an instability requires a nonlinear analysis, and here one cannot expect results of the scope available in the linear theory [7]. Certainly more analysis of specific kinetic mechanisms, such as that done in [8], is needed before any general conclusions on the evolution of systems near their bifurcation points emerge. When parameters are far from their bifurcation values, there is usually no alternative to numerical solution of the governing equations. An indication of the complexity of patterns possible in reaction-diffusion systems is given by the computational results of Gierer and Meinhardt [9].

The outlines of a comprehensive nonlinear theory of pattern formation from steady uniform states are emerging from analyses such as the foregoing and those done in the context of ecological problems [10]. Some important problems that remain are the following.

(1) Linear stability analysis answers the question of how reaction, diffusion and system geometry or topology interact to produce instability. A complete nonlinear theory should predict, for example, how these factors govern the direction and stability of the bifurcating solutions. One general result will be given in a later section.

(2) Closely related to the preceding point are the problems of bifurcation from multiple eigenvalues and of secondary bifurcation when there is a pair of nearly-degenerate eigenvalues [11]. These in turn are related to the problem of pattern selection when more than one type of pattern is predicted by linear analysis [12]. Results for these problems will illustrate how a succession of instabilities can be used to generate increasingly complex spatial patterns.

(3) In Turing's work and virtually all subsequent work, the only mode of transport considered is diffusion. Furthermore, the model systems all deal with structureless, tightly coupled cells or their continuum analogs. Some future work should be directed toward (a) the analysis of other modes of transport, (b) more realistic models of cell and tissue structure [13], and (c) networks of cells that communicate only indirectly via the external medium.

While significant progress has been made toward predicting the emergence of spatial pattern from uniform steady states, the theory is still in its infancy when the underlying chemical dynamics are time-periodic. Even linear analysis of the interaction between reaction and transport is difficult in such cases, because the equations are non-autonomous and the periodic solution is rarely known in analytical form. Nonetheless, the following examples suggest that the problem is of sufficient importance to warrant detailed investigation.

It is widely recognized that biological systems are periodic at virtually every level of organization from the sub-cellular to the organismic level. The most carefully studied biochemical oscillations are the glycolytic oscillations in yeast cells [14], observable in single cells, in intact cell suspensions where cells communicate indirectly, and in cell-free extract. There is as yet no

direct evidence that links glycolytic oscillations with any morphogenetically significant process, but many have speculated that similar oscillations may be important in circadian rhythms [15]. Populations of interacting oscillators have been studied in this context [16].

The second class of examples in which the dynamics are time-periodic comprises multinucleate cells obtained by fusion [17], the true slime molds such as Physarum polycephalum [18], and the colonial fungi [19,20]. In multinucleate cells and in Physarum, the underlying periodicity is that of the mitotic cycle,while fungi undergo a more complicated life cycle that involves differentiation. It has been observed that in Physarum the contents of fused plasmodia are mixed by a combination of diffusion and cytoplasmic streaming and that nuclei in a fused plasmodium divide synchronously provided the diameter is less than about 15 cm [21]. In larger plasmodia, propagating waves of mitosis can occur. As the paper by Kauffman in this volume shows, this system is an ideal one for testing hypotheses about the basis for the periodicity in the mitotic cycle.

More complicated spatial patterns are often observed in various fungi. These include concentric rings and spirals [20] very similar to those observed in the Belousov-Zhabotinskii reaction. In the ascomycete Chaetomium robustum, the alternating light and dark zones seen in a growing colony are transparent zones of scantily branched hyphae alternating with dense zones of intense ramification. In relatively small colonies these dense zones are very uniform around the circumference, which indicates a high degree of synchrony between adjacent hyphae, at least in the latter stage of the life cycle. The mode of communication between hyphae is not yet known, but a plausible hypothesis is that synchronization is maintained by transport of some 'messenger' molecule. The conjecture that transport is by diffusion is supported by the fact that the dense zones become increasingly irregular as the colony expands [20]. Moreover, it has not been established that the life cycle is controlled by a biochemical oscillator but it could well be. Granting this,

the observed patterns are amenable to analysis using a reaction-transport model. A highly-simplified model, which nonetheless gives rise to spatial patterns similar to those observed, is due to Pavlidis [22].

In addition to the experimental examples, there are numerous theoretical models in which interaction between cells with periodic dynamics plays a role. Waddington [23] suggested that entrainment of non-oscillatory cells by oscillatory cells could constitute a mechanism of tissue induction in embryology. Goodwin and Cohen [24] have constructed a model of development in which oscillatory cells in a developing tissue are entrained to the frequency of localized pacemakers by periodically-propagating waves. The local phase difference between a fast wave and a slow wave provides positional information and thereby governs local differentiation.

Burton and Canham [25] have recently proposed a model for contact inhibition of cell division based on biochemical oscillators coupled by diffusion. This model stems from the observations by Lowenstein and Kanno [26] that intercellular communication in certain tumor cells is very slight compared with communication in their normal counterparts. The main hypotheses of the Burton-Canham model are that there is a key substance involved in contact inhibition that diffuses between cells and whose concentration within each cell oscillates harmonically in time, with a period that varies from cell to cell. They propose that contact inhibition results when cells communicate freely and the level of the key substance remains below a threshold for initiation of the mitotic cycle. Their major result is that the amplitude of the oscillation can be suppressed by virtue of coupling between neighboring cells. Despite its apparent success, several criticisms of the model can be raised. Firstly, cellular communication has no affect on the dynamics of the oscillator but merely provides for leakage of the key substance between neighboring cells. This forces the authors to assume that all cells have different frequencies so as to preclude synchronization. Secondly, it is generally true

that only small-amplitude oscillations are satisfactorily approximated by sinusoids and as a result, the control system proposed is very sensitive to small concentration changes. Both of these criticisms can be vitiated by postulating that the transported species is directly involved in the dynamics of the underlying oscillator, as Kauffman and Wille [27] assume in their model of mitosis. Nonetheless the fundamental idea of Burton and Canham is attractive and warrants further investigation.

The common features of most of the foregoing experimental examples and theoretical models are (i) the dynamics of individual 'units' are periodic or capable of being entrained by a periodic signal and (ii) the units are coupled, either directly via diffusion or active transport across cell membranes, tight junctions or within a plasmodium, or indirectly, by transport of a chemical species through an external medium in contact with all cells. In all the examples one can ask (i) under what conditions do the coupled units synchronize into a single collective mode and (ii) what properties of the internal (biochemical) dynamics and of the coupling produce nonuniform spatio-temporal patterns? These questions are addressed in the following sections.

The outline for the remainder of the paper is as follows. Section II describes the general hypotheses on the chemical kinetics. Because the analysis and exposition is simplest when there are only two chemical species, we restrict attention to this case. Most of the results are extendable to systems involving more species at the expense of added algebraic detail. In Section III we use a continuum model of coupled cells to derive conditions on kinetic and transport parameters that provide the answer to the first question of the preceding paragraph. The conditions are stringent and will generally be met only in small systems. Weaker conditions will suffice to guarantee that a system in a spatially-uniform periodic state remains there in the face of small random concentration disturbances. These are derived in Section IV. A partial answer to the

second question posed is given in Section V and several possible topics for further investigation are suggested in the concluding section.

II. THE CHEMICAL KINETICS

No specific kinetic mechanism involving the two active species will be postulated here; instead we simply assume that the kinetics are described by a smooth nonlinear function $R(c,p)$ and that the equation of change for a uniform system is

$$\frac{dc}{dt} = R(c,p). \tag{1}$$

Here

$$c = \begin{pmatrix} c_1 \\ c_2 \end{pmatrix} \qquad R(c,p) = \begin{pmatrix} R_1(c_1,c_2,p) \\ R_2(c_1,c_2,p) \end{pmatrix}$$

and p is a positive scalar parameter. This parameter might, for instance, be the concentration of a slowly-varying substrate. Throughout we assume that R is such that the solution of (1) for positive initial values remains non-negative and bounded for all $t>0$. In addition to these standard assumptions, we make the following hypotheses about R.

(H1) For every $p>0$ there is a unique solution c^* of $R(c,p)=0$.

(H2) Let K be the matrix of the linearization of (1) around c^*, viz.

$$\frac{d\zeta}{dt} = K\zeta$$

$$\zeta \equiv c-c^* \qquad K_{ij} \equiv \left(\frac{\partial R_i}{\partial c_j}\right)_{c_j=c_j^*}$$

There are two parameter values p_0 and p_1, $0<p_0<p_1$, such that both eigenvalues of K have non-negative real parts for $p_0 \leq p \leq p_1$. The eigenvalues are complex conjugates, $\alpha \pm i\beta$, in some neighborhood of p_0 and in some neighborhood of p_1. At p_0

$$\alpha(p_0) = 0, \qquad \frac{d\alpha}{dp}(p_0) > 0$$

while at p_1

$$\alpha(p_1) = 0, \qquad \frac{d\alpha}{dp}(p_1) < 0,$$

(H3) For any $p\varepsilon(p_0,p_1)$ there exists a unique periodic solution that is globally asymptotically stable, but for the steady state c^*. There are no periodic solutions for any other value of p.

It follows from (H2), (H3) and the Hopf theorem [28] that a stable periodic solution emerges at p_0 and disappears at p_1 as p increases through these values. It can happen that $p_1=\infty$.

The matrix K will have a pair of complex conjugate eigenvalues with non-negative real part if and only if

$$\text{tr } K = k_{11} + k_{22} \geq 0$$

and

$$(\text{tr } K)^2 - 4 \det K = (k_{11}-k_{22})^2 + 4 k_{12}k_{21} < 0.$$

The first condition requires that at least one of the species be self-activating since at least one k_{ii} must be positive. We shall specify that species 1 is self-inhibiting for all p and so k_{11} is always negative. Therefore species 2 must be self-activating near p_0 and p_1 and in fact, it follows from (H2) that k_{22} must be positive for all p in an interval $[\hat{p}_0,\hat{p}_1]$ that contains $[p_0,p_1]$. We can allow $\hat{p}_0=0$ and $\hat{p}_1=\infty$. To satisfy the second condition it is necessary that $k_{12}k_{21}<0$. This means that the mutual interaction of 1 and 2 near p_0 and p_1 must either be that 1 activates 2 and 2 inhibits 1 or, that 1 inhibits 2 and 2 activates 1. One model reaction mechanism that fulfills all the above hypotheses is the mechanism proposed by Zhabotinskii, et. al. [29] for the Belousov-Zhabotinskii reaction. This model is analyzed in [30]. A somewhat simpler scheme that also has these properties arises from models for glycolytic oscillations [31].

III. GLOBAL STABILITY OF UNIFORM SOLUTIONS

Now suppose that we have N identical cells, in each of which the kinetics for the two species of interest are as just described. Further, suppose that the cells are submerged in a bath and that both species can be exchanged between cells. This exchange can occur in one of two ways. In the first, communication between cells is indirect in that each cell exchanges material only with the extracellular milieu. This mode is used in yeast cell suspensions and presumably in many other similar situations. We shall not pursue an analysis of indirect communication here; suffice it to say that when individual cells are oscillatory, ($p\varepsilon[p_0,p_1]$), the oscillations can be suppressed <u>in vivo</u> by making the volume of the extracellular compartment large enough. Such a density effect is observed in glycolytic oscillations [32].

The alternate mode of chemical communication, which we assume prevails, is via intercellular junctions that can be formed upon cell-to-cell contact. These tight junctions can pass molecules of $\sim 10^3$ MW [33] and therefore, metabolites and other substances that may exert control over cellular activities can readily diffuse from cell to cell. Individual cells in an aggregate can be connected to one or more other cells and different cells can have a different number of connections. This leads to a rich variety of topologically and dynamically distinguishable networks [34], but for simplicity we regard the entire aggregate as a continuum contained in a two-dimensional region Ω. When $p\varepsilon[p_0,p_1]$, one has a continuum of chemical oscillators, linearly coupled by diffusion.

The governing equation for the system is

$$\begin{aligned} &\frac{\partial c}{\partial t} = D\Delta c + R(c) && \text{in } \Omega \\ &c(\underset{\sim}{r},0) = c_0(\underset{\sim}{r}) \\ &\underset{\sim}{n}\cdot\nabla c = 0 && \text{on } \partial\Omega. \end{aligned} \tag{2}$$

The diffusivities are positive constants and D is diagonal. Δ is the Laplacian for the domain Ω and $\underset{\sim}{n}$ is the unit outward normal. The fact that D is positive definite, combined with the hypotheses on the kinetics, ensures that the solution of (2) is componentwise nonnegative for all time. We assume that it is smooth and bounded pointwise in space as well, without elaborating the conditions that guarantee this.

Were $R(c)\equiv 0$, diffusion would always smooth out initial nonuniformities in concentration and this is the case in a reacting system as well, provided the diffusivities are sufficiently large. The following result formalizes this contention by giving conditions under which the system always evolves to a uniform state.

THEOREM. Let

$$\hat{k} \equiv \max_{c} \left|\left|\frac{\partial R}{\partial c}\right|\right|$$

and let μ_1 be the smallest non-zero eigenvalue of the scalar problem

$$\Delta u + \mu u = 0 \qquad \text{in } \Omega$$

$$\underset{\sim}{n}\cdot\nabla u = 0 \qquad \text{on } \partial\Omega.$$

If

$$\min_{i} (D_i)\mu_1 > \hat{k}, \tag{3}$$

then all spatial nonuniformities decay exponentially in time.

To derive this result, write the solution of (2) as

$$c(\underset{\sim}{r},t) = \bar{c}(t) + \phi(\underset{\sim}{r},t)$$

$$= Pc(\underset{\sim}{r},t) + Qc(\underset{\sim}{r},t)$$

where $\int_{\Omega} \phi_i \, d\Omega = 0.$

The operators P and $Q \equiv I-P$ are projections in the appropriate Hilbert space. Equation (2) can then be written

$$\frac{\partial(\bar{c}+\phi)}{\partial t} = D\Delta(\bar{c}+\phi) + R(\bar{c}+\phi)$$

and by operating on this with P and Q it follows that

$$\frac{\partial \bar{c}}{\partial t} = PD\Delta\phi + PR(\bar{c}+\phi)$$

$$\frac{\partial \phi}{\partial t} = QD\Delta\phi + QR(\bar{c}+\phi).$$

The object is to show that

$$|\phi| \equiv \left(\int_\Omega ||\phi||^2 \, d\Omega\right)^{1/2} = \left(\int_\Omega \langle\phi,\phi\rangle \, d\Omega\right)^{1/2}$$

tends to zero as $t\to\infty$ when (3) holds. Here and hereafter $||\cdot||$ and $\langle,\rangle$ denote the Euclidean norm and inner product, respectively.

Analysis of the various terms in the ϕ equation leads to the inequality

$$\frac{d}{dt}|\phi|^2 \leq -\lambda_1|\phi|^2 + \hat{k}|\phi|^2$$

where

$$\lambda_1 \equiv \min_{\substack{|u|=1 \\ \int_\Omega u d\Omega = 0}} \int_\Omega \langle \nabla u, D\nabla u\rangle \, d\Omega.$$

Therefore, if $\lambda_1 > \hat{k}$, $|\phi|^2 \to 0$ exponentially in t. The constant λ_1 is the smallest non-zero eigenvalue of the vector equation

$$D\Delta v + \lambda v = 0$$

$$\underset{\sim}{n}\cdot\nabla u = 0$$

and so

$$\lambda_1 = \min_i (D_i)\mu_1$$

where μ_1 is the smallest non-zero eigenvalue of the scalar equation

$$\Delta u + \mu u = 0$$
$$\underset{\sim}{n}\cdot\nabla u = 0 . \qquad (4)$$

This proves the result.

The parameter $\hat{k}$ is a global measure of the sensitivity of the reaction rate to concentration changes. Its reciprocal is the shortest kinetic relaxation time in the system. For some kinetic mechanisms, such as the control mechanisms for inducible or repressible enzymes, estimates of $\hat{k}$ are readily made without knowing a priori bounds on the concentrations. The quantity $[\min_i (D_i)\mu_1]^{-1}$ is the longest relaxation time for diffusion, and if this is less than the shortest kinetic relaxation time, all spatial nonuniformities decay to zero. As a result, (3) is sufficient to ensure that there are no nonuniform steady states. Furthermore, even though these conclusions are derived for zero-flux boundary conditions, they hold for other boundary conditions whenever the eigenvalue problem corresponding to (4) has a zero eigenvalue*.

It should be noted that nothing has been said concerning the nature of the uniform solution. If p is in the range for which (1) has a globally stable steady state, then solutions of (2) always relax to this steady state when (3) holds. When $p\varepsilon[p_0,p_1]$, (1) has a globally attracting periodic solution, and the solution of (2) ultimately approaches this solution. The latter case is of interest here because then (3) provides a sufficient condition for a globally-synchronized oscillation, regardless of the initial conditions. A criterion such as this should be of interest in any problem dealing with populations of coupled oscillators. Different modes of coupling will naturally

*The dimensionless number $\hat{k}/(\min D_i)\mu_1$ is a Thiele modulus in chemical engineering terminology. For an interesting discussion of the role played by transport limitations in cellular processes see [35].

require a somewhat different analysis and lead to different criteria for synchronization.

To estimate when condition (3) is met, consider a one-dimensional system of length L. The smallest non-zero eigenvalue is $\mu_1=\pi^2/4L^2$ and a typical value for $\hat{k}$, the pseudo first-order kinetic constant, is 10^{-1} sec^{-1}. Therefore, (3) is satisfied if $\min (D_i)>\sim.04L^2$. If L is 10μ (a typical cell diameter), then the smallest D_i must be larger than $\sim 4.0\times10^{-8}$ cm^2/sec. This is well within the range of diffusivities for small molecules *in vitro*. If the kinetically active species can freely pass through the junctions, it is to be expected that two cells coupled out of phase will ultimately synchronize. However, when 10 cells are joined in a line, $\min D_i$ must be greater than $\sim 4.0\times10^{-6}$ cm^2/sec and this is already near the upper limit of diffusivities *in vitro*. Of course, the choice of $\hat{k}$ is crucial in these estimates and if the kinetic relaxation time is much longer, as it probably would be in the case of a biochemical oscillation that controls mitosis, the diffusivities can be correspondingly smaller or the lengths correspondingly greater.

IV. LOCAL STABILITY OF PERIODIC SOLUTIONS

The condition that guarantees ultimate synchronization starting from arbitrary initial conditions is a stringent one and can only be met in small systems or when reaction rates are low. At the other extreme in initial conditions, it is clear that a system initially in a uniform periodic state would remain there, but for the inevitable small concentration disturbances. The object here is to find conditions under which these small disturbances decay. The results will partially answer the question of when an artifically-synchronized aggregate of cells will maintain its synchrony. Such questions arise naturally in studies of circadian rhythms.

Let $\Phi(t)$ be the periodic solution of (1) and let T be its period. Write

$$c(\underset{\sim}{r},t) = \Phi(t) + \sum_k y_k(t)u_k(\underset{\sim}{r})$$

where $y_k(t)$ is the amplitude vector for the $k^{\underline{th}}$ eigenfunction $u_k(\underset{\sim}{r})$ of the Laplacian. For small-amplitude disturbances of Φ, (2) can be linearized in y_k and the result is the set of amplitude equations

$$\frac{dy_k}{dt} = (K(t)-\mu_k D)y_k. \qquad (5)$$

Here the matrix $K(t)$ is T-periodic: $K(t+T)=K(t)$. The solution for y_k can be written

$$y_k(t) = \Theta_k(t)y_k(0).$$

The matrix $\Theta_k(t)$ is that fundamental matrix of (5) for which $\Theta_k(0)=I$ [36]. Evidently the $\Theta_k(t)$ govern the evolution of the amplitude in the initial disturbance. If $k=0$ corresponds to the zero eigenvalue of the Laplacian, then it follows from the hypotheses on the kinetics that $\Theta_0(T)$ has one eigenvalue equal to one and one eigenvalue less than 1.

The periodic solution $\Phi(t)$ will be an orbitally asymptotically stable solution of (2) if both eigenvalues of $\Theta_k(T)$ are less than one in modulus, or equivalently, if the spectral radius $\rho(\Theta_k(T))$ is less than 1, for all $k\geq 1$. Since $\rho(\Theta_k(T)) \leq ||\Theta_k(T)||$, [37], it suffices to make $||\Theta_k(T)|| < 1$ for $k\geq 1$.

Define

$$\delta = \max_i D_i \qquad D^* = D-\delta I$$

and write the equation for $\Theta_k(t)$ as

$$\frac{d\Theta_k}{dt} = (K(t)-\mu_k D)\Theta_k = (K(t)-\mu_k\delta I-\mu_k D^*)\Theta_k.$$

Let $\Omega_k(t)$ be defined by

$$\Theta_k(t) = e^{-\mu_k\delta t}\,\Theta_0(t)\Omega_k(t)$$

and Ω_k will satisfy

$$\frac{d\Omega_k}{dt} = -\mu_k(\theta_0^{-1}(t)D^*\theta_0(t))\Omega_k.$$

By converting this to an integral equation and applying Gronwall's inequality [36] to the equation for the norm, it is found that

$$||\Omega_k(T)|| \leq e^{\mu_k \int_0^T ||\theta_0^{-1}(\tau)D^*\theta_0(\tau)||d\tau}.$$

Therefore, $||\theta_k(T)|| < 1$ for $k \geq 1$ if

$$\delta T > \int_0^T ||\theta_0^{-1}(\tau)D^*\theta_0(\tau)||d\tau$$

and this in turn is satisfied if

$$\frac{||D^*||}{\delta} < \frac{1}{\max\limits_{t\in[0,T]} \{||\theta_0^{-1}(t)||\cdot||\theta_0(t)||\}}. \tag{6}$$

Since no use was made in deriving this of the facts that there are only two species and that D is a diagonal matrix, the result is true in general. It is noteworthy that this condition is independent of the eigenvalue μ_k and therefore of geometric factors, in contrast to the condition at (3) for global stability. This is because only ratios of diffusivities enter here. On the other hand, the kinetics enter in a more complicated manner than previously.

The Euclidean norm of the two by two matrix D^* is $|D_2-D_1|$ and consequently the preceding can be rearranged to read

$$\frac{\chi-1}{\chi} < \frac{D_2}{D_1} < \frac{\chi}{\chi-1} \tag{7}$$

where

$$\chi \equiv \max_{t\in[0,T]} [||\theta_0^{-1}||\cdot||\theta_0(t)||].$$

χ is never less than 1. The analog of (7) for an n-component system is

$$\frac{\min D_j}{\max D_j} > \frac{\chi - 1}{\chi} .$$

In any event, the conclusion is that the uniform periodic solution is stable with respect to small disturbances if the diffusivities are not too different.

That the diffusivities are not too different is also a sufficient condition for the absence of 'synergistic' or 'diffusive' instabilities of the uniform steady state [6]. A more precise relation between stability of the steady state and stability of the periodic solution is established by the following result.

THEOREM. Suppose that for $k \geq 1$, the matrix $K-\mu_k D$, corresponding to linearization around the uniform steady state, has only eigenvalues with negative real parts. Then uniform periodic solutions of sufficiently small amplitude are orbitally asymptotically stable.

To prove this, consider the amplitude equations for $k \geq 1$:

$$\frac{dy_k}{dt} = (K(t)-\mu_k D)y_k$$

$$= (K-\mu_k D)y_k + (K(t)-K)y_k,$$

and write the solution as

$$y_k(t)=e^{(K-\mu_k D)t} y_k(0) + \int_0^t e^{(K-\mu_k D)(t-\tau)} (K(\tau)-K)y_k(\tau)d\tau.$$

By hypothesis, $K-\mu_k D$ has only eigenvalues with negative real parts so we can find positive constants γ_k and Ω_k such that

$$||e^{(K-\mu_k D)t}|| \leq \Omega_k e^{-\gamma_k t}.$$

It follows that

$$||y_k(T)|| \leq e^{(\Omega_k \tau-\gamma_k)T} ||y_k(0)||$$

where

$$\tau \equiv \max_{[0,T]} ||K(t)-K||.$$

The conclusion follows provided

$$\tau < \min_{k\geq 1} (\frac{\gamma_k}{\Omega_k})$$

and, because $\tau \to 0$ with the amplitude of the periodic solution, this will be satisfied if the amplitude is sufficiently small. This is certainly true sufficiently near the bifurcation points p_0 and p_1.

The correspondence between stability of the steady state and stability of the periodic solution, both with respect to nonuniform disturbances, naturally breaks down for large solutions. Nonetheless, one can sometimes still check stability of the periodic solution without computing the Floquet multipliers, as the following result shows.

THEOREM. Let Γ be an annular neighborhood of the periodic solution and suppose that the matrix $Z \equiv \frac{1}{2}(K+K^T)-\mu_k D$ has only negative eigenvalues for $c \in \Gamma$. Then $\Phi(t)$ is orbitally asymptotically stable.

This follows directly from the estimate [37] $||y_k(t)|| \leq ||y_k(0)|| \exp\{\int_0^t \lambda^Z_{max}\, d\tau\}$. To apply it, one needs an estimate of the location of $\Phi(t)$. This is sometimes easy to obtain, particularly for relaxation oscillations.

V. SECONDARY BIFURCATIONS OF NONUNIFORM PERIODIC SOLUTIONS

Aside from the special case in which the diffusivities are equal and diffusion can never lead to destabilization of a uniform periodic solution, it is impossible to analyze destabilization of periodic solutions in the generality possible for steady states. However, one result of the preceding section is that stability of small-amplitude periodic solutions goes hand in hand with stability of the

steady state with respect to nonuniform disturbances, and therefore the latter question should be addressed when p is near a bifurcation point. This requires analysis of the eigenvalues of the pencil of matrices $K-\mu_k D$, $k\geq 1$. When K and D are 2×2 these eigenvalues are

$$\lambda_{\pm} = \frac{1}{2}\left(\mathrm{Tr}K-\mu_k \mathrm{Tr}D \pm \sqrt{\Delta(\mu_k)}\right)$$

where the discriminant Δ is given by

$$\Delta(\mu_k)=(D_2-D_1)^2\mu_k^2 + 2(k_{11}-k_{22})(D_2-D_1)\mu_k + (k_{11}-k_{22})^2 + 4k_{12}k_{21}.$$

For the present we take p near p_0 or p_1 and therefore can assume that the constant term in Δ is negative. It follows that the eigenvalues are always complex for small μ_k and real for large μ_k. If both diffusivities are strictly positive the eigenvalues are negative for large μ_k and there is at most a finite number of μ_k's for which either eigenvalue has a positive real part.

The following discussion will be simplified if we restrict attention to a system in which there are only two Fourier modes. Therefore we drop the continuum description temporarily and focus on a system of just two coupled cells. In this case one mode is uniform and the other is nonuniform; these have eigenvalue 0 and 2, respectively [34]. Moreover, we shall assume that $\Delta(2)>0$, as the other case is uninteresting. As a result, there are only five distinct types of λ vs μ_k diagrams in which at least one eigenvalue has a zero real part, as shown in Figure 1.

Which one of these obtains depends on the relationship between the parameters p, D_1 and D_2. Certainly $p=p_0$ or $p=p_1$ in (b), (c) and (e) because bifurcation of uniform periodic solutions is independent of D_1 and D_2.

The loci along which there is a zero real eigenvalue at $\mu_k=2$ are given by

$$J \equiv \det(K-2D) = 4D_1D_2-2(k_{11}D_2+k_{22}D_1) + \det K = 0.$$

The zero eigenvalue is simple, and therefore, in every case except possibly (e), a nonuniform steady state bifurcates from the uniform steady state when parameters cross the

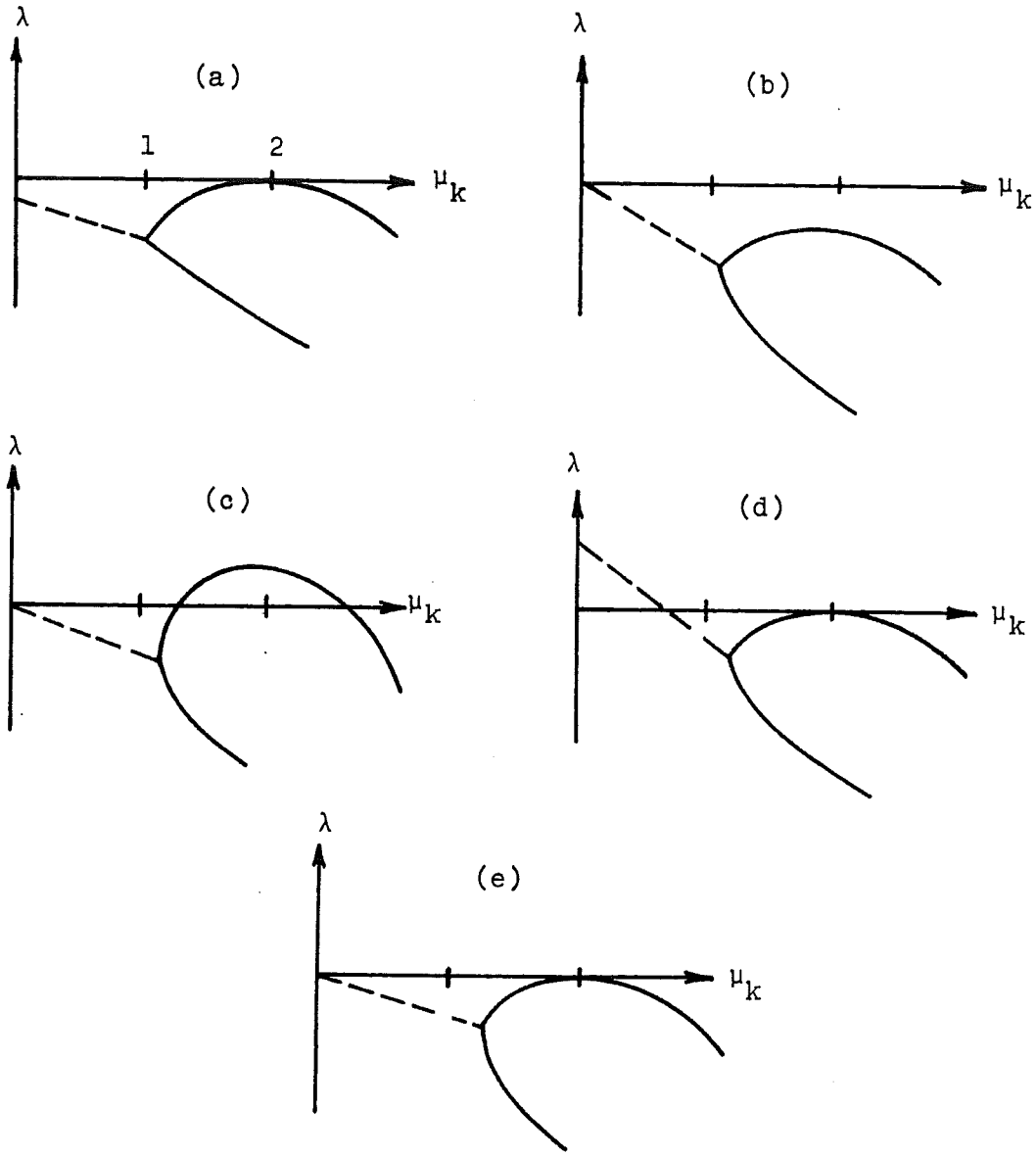

Figure 1. Eigenvalue vs μ_k diagrams when one eigenvalue has a zero real part. ----: Real part of a complex eigenvalue, ——— real eigenvalue. Only the values at $\mu_k=0$ and 2 have meaning in the present context.

locus $J=0$. At any fixed p the kinetic coefficients are fixed and this locus is a hyperbola in the D_1-D_2 plane, as shown in Figure 2. In the continuum case there is a countable number of such curves, the $k^{\underline{th}}$ having D_1-intercept $\det K/\mu_k k_{22}$ and horizontal asymptote k_{22}/μ_k. How these

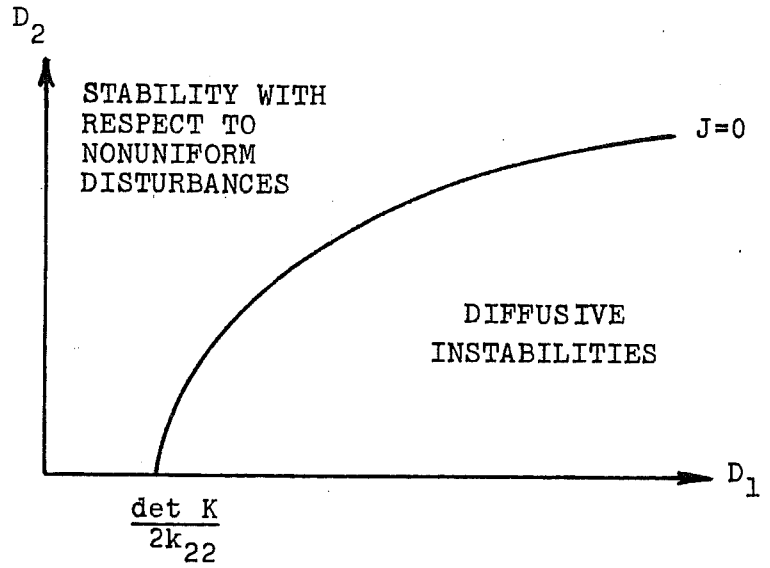

Figure 2

curves vary with p depends on $\det K$ and k_{22}; if for instance $\det K/\mu_k k_{22}$ has a single turning point for $p\varepsilon[\hat{p}_0,\hat{p}_1]$ then we have the following picture for a fixed non-zero μ_k. When there are a finite or countable number of positive μ_k there is a second family of loci, parameterized by μ_k. The ZZKK mechanism [29] for the Zhabotinskii-Belousov reaction leads to a diagram like that shown.

A Hopf bifurcation of a uniform periodic solution always occurs upon crossing the lines $p=p_0$ and $p=p_1$, and by hypothesis the periodic solution exists only for $p\varepsilon[p_0,p_1]$ and is stable with respect to uniform disturbances. Diffusion does not affect these bifurcations when zero-flux boundary conditions are imposed. Now suppose that D_2 is fixed and let D_1^* be the intersection of the corresponding $J=0$ locus with the line $p=p_0$ (Figure 3). If $D_1>D_1^*$, then as p increases, the uniform steady state loses stability with respect to the nonuniform disturbance at some $\tilde{p}<p_0$. At this p-value the applicable $\lambda-\mu_k$ diagram is Figure 1(a) and a nonuniform steady state bifurcates from the uniform steady state. One can show that with zero-flux (or periodic) boundary conditions the bifurcating solution

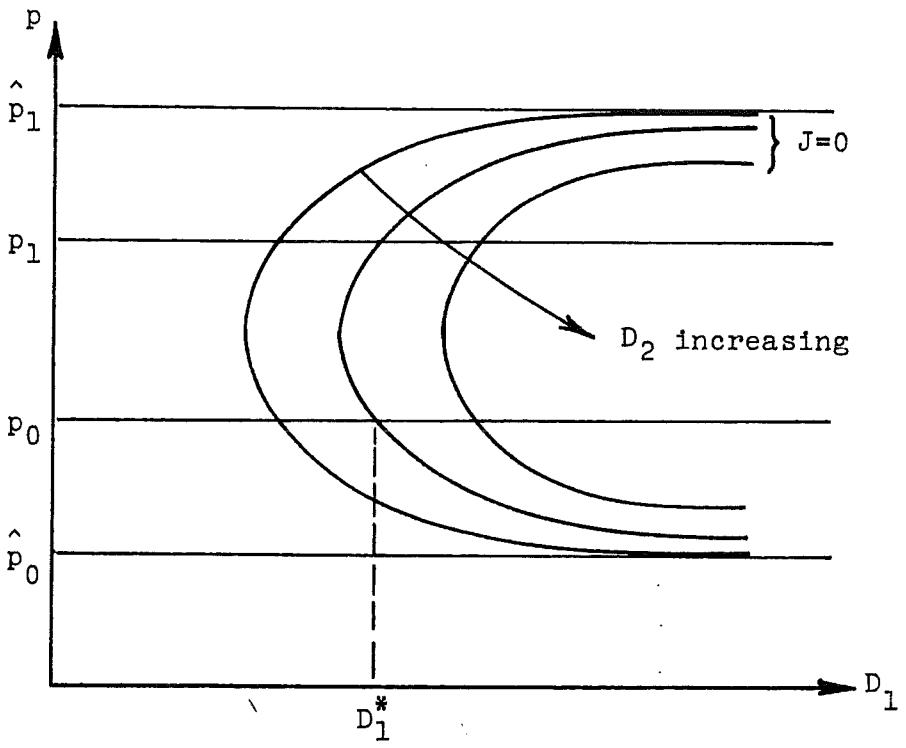

Figure 3. The loci J=0 for variable D_2. The lines $p=\hat{p}_0$ and $p=\hat{p}_1$ are the loci $k_{22}=0$; $\hat{p}_0=0$ and $\hat{p}_1=\infty$ are allowed.

exists on only one side of the bifurcation point. Let us suppose that for (D_1,p) near (D_1^*,p_0) the bifurcation is supercritical; then the bifurcating solution is stable. At $p=p_0$ the periodic solution emerges and at this point the appropriate λ-μ_k diagram is Figure 1(c). Note that the periodic solution is unstable for p near p_0, because the nonuniform mode grows in time.

If $D_1-D_1^*$ is small and positive, the amplitude of the nonuniform solution is small when p is near p_0 and the linearization of the nonlinear equations along the bifurcating branch has a pair of complex conjugate eigenvalues near those of K. By making $D_1-D_1^*$ sufficiently small, one can ensure that there is a point p^* near p_0 at which this complex pair of eigenvalues has a zero real

part. At this point a secondary bifurcation occurs and a stable nonuniform periodic solution bifurcates supercritically. If it happens that $p^* > p_0$, the amplitude vs p diagram is as shown in Figure 4. Some computational results that correspond to this case are given in [38].

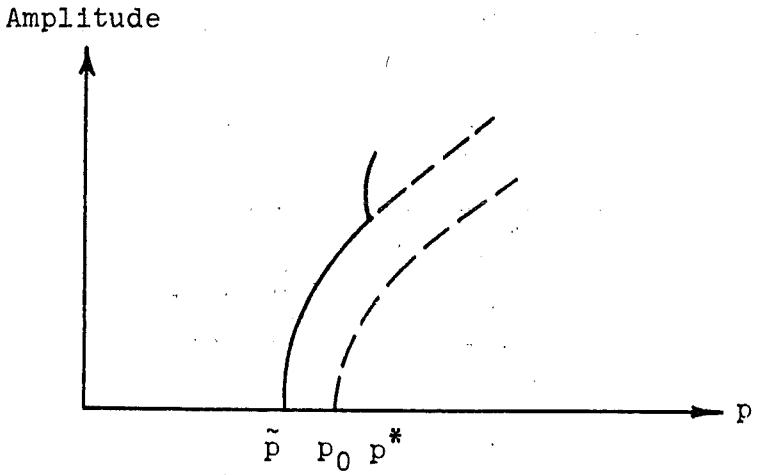

Figure 4. The amplitude vs p diagram when the uniform solution first becomes unstable to nonuniform disturbances Solid lines: stable solutions;dashed lines: unstable solutions. The mirror image steady state, in which the cells are interchanged, is omitted.

Let us summarize the preceding. When $\tilde{p}-p$ is small and positive, the linearization around the uniform solution has two real negative eigenvalues and a complex conjugate pair with negative real part. As p crosses $\tilde{p}$, one real eigenvalue crosses the imaginary axis and a nonuniform solution emerges, while at $p=p_0$, the real part of the complex pair crosses the imaginary axis and a uniform periodic solution emerges. At $\hat{p}$, a nonuniform periodic solution emerges from the nonuniform steady state. A qualitative understanding of the corresponding changes in the phase portrait can be gotten from the following three-dimensional section of the four-dimensional phase space. This cross-section omits the direction corresponding to the real eigenvalue that always remains negative. Distance along the vertical axis corresponds to the amplitude of the

nonuniform mode.

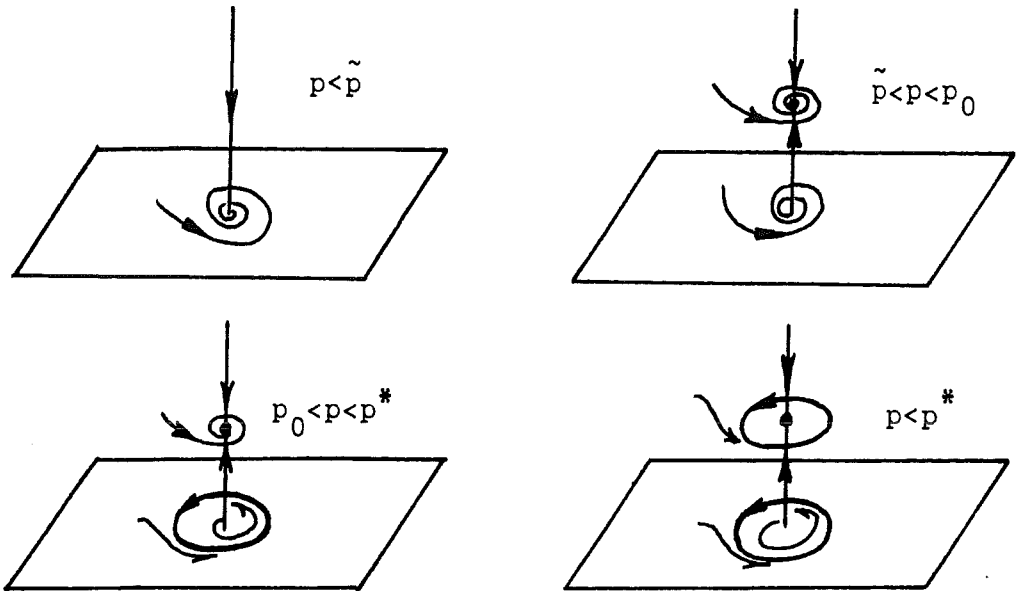

Figure 5. A three-dimensional section of phase space. The mirror image steady state is omitted.

The order in which the bifurcations occur is reversed from the preceding when $D_1-D_1^*$ is small and negative. The uniform periodic solution is now stable and the bifurcating nonuniform steady state is unstable. It is to be expected that if $D_1-D_1^*$ is small enough there will be a point on the branch of uniform periodic solutions at which an eigenvalue of $\theta_1(T)$ crosses the unit circle. It can be shown by using Abel's formula [36] that in a two-component reacting system with a stable periodic solution, the eigenvalues of $\theta_k(T)$, $k\geq 1$, can only cross the unit circle at ± 1. Therefore, when bifurcation of a new periodic solution occurs, it must have period close to T or $2T$ near the bifurcation point. Now the amplitude vs p diagram is as shown in Figure 6.

This picture has been confirmed by numerical integration of the equations for the ZZKK mechanism; the details will be reported elsewhere.

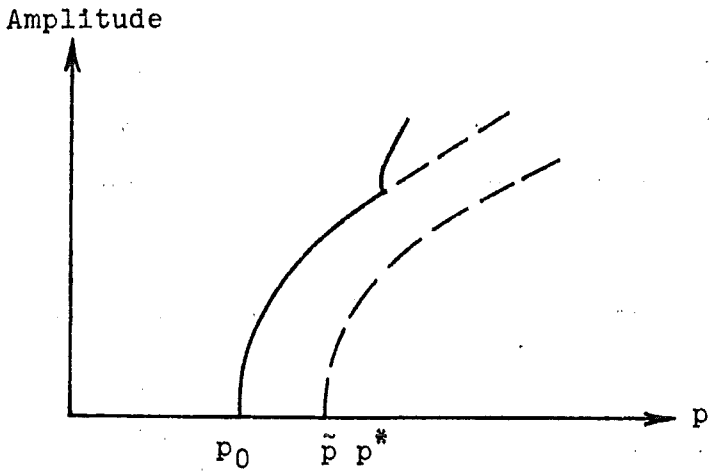

Figure 6. The analog of Figure 4 when bifurcation of the periodic solution precedes bifurcation of the nonuniform steady state.

As D_1 varies in a neighborhood of D_1^*, a curve is generated in the D_1p-plane along which the nonuniform periodic solutions emerge. As D_2 varies, this curve generates a bifurcation surface. As a result, the nonuniform solutions exist in some open set of parameter values. In the continuum case there is the further possibility that the nonuniform periodic solutions become unstable and a tertiary branching of another periodic solution occurs. The complete picture has not been worked out for even the simplest case of one nonuniform mode, but it should be evident by now that the kind of destabilization of a uniform periodic solution that we have described is a robust phenomenon that must be considered whenever the self-activating species is also the slower diffusing species.

VI. CONCLUSION

It is interesting, in light of the experimental evidence linking the absence of intercellular communication with abnormal growth [26], that the asynchronous states found here exist only when the diffusivities are sufficiently

different. As intercellular exchange of the self-activating species increases, the spatially nonuniform solutions disappear, leaving only the synchronous oscillation. One is tempted to identify the 'resting' state of the cellular network with this uniform state and mitotic activity with a nonuniform state, in direct contrast to Burton and Canham's interpretation. In this vein, it is noteworthy that the uniform periodic solution only exists when there is no flux across the boundary of the network or when the network is one unit is a repeated pattern. Interruption of intercellular communication, for example by wounding, destroys the uniform state and might thereby stimulate cell division.

The nonuniform periodic solutions we have described represent standing oscillations, in contrast to the propagating waves that are found in infinite domains [39]. In the two-cell case studied here, the cells are 180° out of phase in the nonuniform mode, but more complicated spatial patterns of phase relationships can arise when more cells are present. These patterns provide the time-dependent analog of the stationary morphogentic 'maps' discussed in the Introduction. How nonuniform patterns of phase can be translated into a spatio-temporal framework for gene activation is discussed elsewhere [40].

It has been assumed throughout that all parameters in the chemical kinetics are spatially uniform and as a result, the period of oscillation is uniform throughout the system. Because of cell-to-cell variations in enzyme and substrate concentrations, this will not be true in reality. Nonetheless, if the spatial variation in the period is small one may still expect to find a spatially-synchronized oscillation under conditions similar to those given here. This has been established for a two-cell system [41] but apparently not for a continuum description of a multicellular system. Recent experiments using the Belousov-Zhabotinskii reaction in coupled stirred reactors illustrate synchronization at a common frequency for small frequency differences and subharmonic synchronization for large frequency differences [42].

REFERENCES

1. L. Wolpert, 'Positional Information and the Spatial Pattern of Cellular Differentiation', J. Theor. Biol., 25, 1 (1969).

2. A.M. Turing, 'The Chemical Basis of Morphogenesis', Phil. Trans. Roy. Soc., B237, 37 (1952).

3. J. Maynard-Smith and K.C. Sondhi, 'The Arrangement of Bristles in Drosophila', J. Embry. Exp. Morph., 9, 661 (1961).

4. J.I. Gmitro and L.E. Scriven, 'A Physicochemical Basis for Pattern and Rhythm', in Intracellular Transport (K.B. Warren, ed.) Academic Press (1966).

5. A.N. Zaiken and A.M. Zhabotinsky, 'Concentration Wave Propagation in Two-dimensional Liquid-phase Self-oscillating System', Nature, 225, 535 (1970).

A.T. Winfree, 'Spiral Waves of Chemical Activity', Science, 175, 634 (1972).

J.A. DeSimone, D.L. Beil and L.E. Scriven, 'Ferroin-Colloidion Membranes: Dynamic Concentration Patterns in Planar Membranes', Science, 180, 946 (1973).

N. Kopell and L.N. Howard, 'Pattern Formation in the Belousov Reaction', Lects. on Math. in the Life Sciences, 7, 201, (1974).

6. H.G. Othmer and L.E. Scriven, 'Interactions of Reaction and Diffusion in Open Systems', Ind. Eng. Chem. Fund. 8, 302 (1969).

L.A. Segel and J.L. Jackson, 'Dissipative Structure: An Explanation and an Ecological Example', J. Theor. Biol. 37, 545, (1972).

7. C. Georgakis and R.L. Sani, 'On the Stability of the Steady State in Systems of Coupled Diffusion and Reaction', Arch. Rat. Mech. Anal., 52, 266 (1973).

8. J.F.G. Auchmuty and G. Nicolis, 'Bifurcation Analysis of Nonlinear Reaction - Diffusion Equations', Bull. Math. Biology, 37, 323, (1975).

9. A. Gierer and H. Meinhardt, 'A Theory of Biological Pattern Formation', Kybernetik, 12, 30 (1972).

10. S.A. Levin, 'Spatial Patterning and the Structure of Ecological Communities', Pages 1-36 in S.A. Levin, ed. Lectures on Mathematics in the Life Sciences, Vol. 8: Some Mathematical Questions in Biology VII (1976).

L.A. Segel and S.A. Levin, 'Application of Nonlinear Stability Theory to the Study of the Effect of Diffusion on Predator-Prey Interactions', in Topics in Statistical Mechanics and Biophysics: A memorial to Julius L. Jackson, AIP Conference Proceedings, No. 27 (in press).

11. T.J. Mahar and B.J. Matkowsky, 'A Model Biochemical Reaction Exhibiting Secondary Bifurcation', preprint.

12. H.G. Othmer and L.E. Scriven, 'Nonlinear Aspects of Dynamic Pattern in Cellular Networks', J. Theor. Biol., 43, 87 (1974).

13. H.G. Othmer, 'Nonlinear Wave Propagation in Reacting Systems', J. Math. Biology, 2, 133 (1975).

14. E.K. Pye, 'Biochemical Mechanisms Underlying the Metabolic Oscillations in Yeast', Can. Jour. Bot., 47, 271 (1969).

15. T. Pavlidis and W. Kauzmann, 'Toward a Quantitative Biochemical Model for Circadian Oscillators', Arch. Biochem. Biophys., 132, 338 (1969).

E.K. Pye, 'Periodicities in Intermediary Metabolism', in Biochronometry, Friday Harbor Symposium, 623 (1971).

16. A.T. Winfree, 'Biological Rhythms and the Behavior of Populations of Coupled Oscillators', J. Theor. Biol., 16, 15 (1967).

T. Pavlidis, 'Populations of Biochemical Oscillators as Circadian Clocks', J. Theor. Biol., 33, 319 (1971).

17. A. Westerfeld and M.A. Freeke, 'Cell Cycle of Multinucleate Cells after Cell Fusion', Exp. Cell Res., 65, 140 (1971).

18. H.P. Rusch, W. Sachsenmaier, K. Behrens, and V. Gruter, 'Synchronization of Mitosis by the Fusion of the Plasmodia of Physarum polycephalum', J. Cell. Biol., 31, 204 (1966).

19. K. Esser, 'Genetic and Biochemical Analysis of Rhythmically Growing Mycelia in the Ascomycete Podospora anserina', J. Int. Cycle Res., 3, 123 (1972).

20. G. Kraepelin and G. Franck, 'Self-synchronization in Yeast and Other Fungi', Int. Jour. Chronobiol., 1, 163 (1973).

21. E. Guttes, S. Guttes and H.P. Rusch, 'Morphological Observations on Growth and Differentiation of Physarum polycephalum Grown in Pure Culture', Dev. Biol., 3, 588 (1961).

22. T. Pavlidis, Biological Oscillators: Their Mathematical Analysis, Academic Press (1973).

23. C.H. Waddington, 'Autogenous Cellular Periodicities as (a) Temporal Templates and (b) the Basis of "Morphogentic Field"', J. Theor. Biol., 8, 367 (1965).

24. B. Goodwin and M. Cohen, 'A Phase-shift Model for the Spatial and Temporal Organization of Developing Systems', J. Theor. Biol., 25, 49 (1969).

25. A.C. Burton and P.B. Canham, 'The Behavior of Coupled Biochemical Oscillators as a Model of Contact Inhibition of Cellular Division', J. Theor. Biol., 39, 555 (1973).

26. W.R. Loewenstein and Y. Kanno, 'Intercellular Communication and the Control of Tissue Growth. Lack of Communication between Cancer Cells', Nature, 209, 1248 (1966).

27. S. Kauffman and J.J. Wille, 'The Mitotic Oscillator in Physarum polycephalum', J. Theor. Biol., 55, 47 (1975).

28. D. Ruelle and F. Takens, 'On the Nature of Turbulence', Comm. Math. Phys., 20, 167 (1971).

29. A.M. Zhabotinskii, A.N. Zaiken, M.D. Korzukhin and G.P. Kreitser, 'Mathematical Model of a Self-oscillating Chemical Reaction', Kinet. Cat., 12, 516 (1971).

30. H.G. Othmer, 'On the Temporal Characteristics of a Model for the Zhabotinskii-Belousov Reaction', Math. Biosci, 24, 205 (1975).

31. J. Aldrige and H.G. Othmer, in preparation.

32. J. Aldridge and E.K. Pye, 'Cell Density Dependence of Oscillatory Metabolism', Nature, 259, 670 (1976).

33. W.R. Loewenstein, 'Emergence of Order in Tissues and Organs', Dev. Biol. Supp., 2, 151 (1968).

34. H.G. Othmer and L.E. Scriven, 'Instability and Dynamic Pattern in Cellular Networks', J. Theor. Biol., 32, 507 (1971).

35. P. Weisz, 'Diffusion and Chemical Transformation', Science, 179, 433 (1973).

36. F. Brauer and J. Nohel, Qualitative Theory of Ordinary Differential Equations, W.A. Benjamin, Inc. (1969).

37. J.L. Daleckiĭ and M.G. Krein, Stability of Solutions of Differential Equations in Banach Space, Am. Math. Soc. (1974).

38. J. Tyson and S. Kauffman, 'Control of Mitosis by a Continuous Biochemical Oscillation', J. Math. Biol., 1, 289 (1975).

39. N. Kopell and L.N. Howard, 'Plane Wave Solutions to Reaction-Diffusion Equations', Stud. Appld. Math., 52, 291, 1973.

P. Ortoleva and J. Ross, 'On a Variety of Wave Phenomena in Chemical Reactions', J. Chem. Phys., 60, 5090, 1974.

40. Z. Hejnowicz, 'A Model for Morphogenetic Map and Clock', J. Theor. Biol., 54, 345 (1975).

41. V. Torre, 'Synchronization of Non-linear Biochemical Oscillators Coupled by Diffusion', Biol. Cyber., 17, 137 (1975).

42. M. Marek and E. Svobokova, 'Nonlinear Phenomena in Oscillatory Systems of Homogeneous Reactions-Experimental Observations', Biophys. Chem., 3, 263 (1975).

M. Marek and I. Stuchl, 'Synchronization in Two Interacting Oscillatory Systems', Biophys. Chem. 3, 241 (1975).

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