Bulletin of Mathematical Biology Vol. 48, No. 2, pp. 213–228, 1986.
Printed in Great Britain.

0092-8240/86$3.00 + 0.00
Pergamon Press Ltd.

PATTERN FORMATION AND MORPHOGENESIS: A REACTION–DIFFUSION MODEL

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The problem of cellular differentiation and consequent pattern generation during embryonic development has been mathematically investigated with the help of a reaction–diffusion model. It is by now a well-recognized fact that diffusion of micromolecules (through intercellular gap junctions), which is dependent on the spatial parameter (r), serve the purpose of 'positional information' for differentiation. Based on this principle the present model has been constructed by coupling the Goodwin-type equations for RNA and protein synthesis with the diffusion process. The homogeneous Goodwin system can exhibit stable periodic solution if the value of the cooperativity as measured by the Hill coefficient (ρ) is greater than 8, which is not biologically realistic. In the present work it has been observed that inclusion of a negative cross-diffusion can drive the system into local instability for any value of ρ and thus a time-periodic spatial solution is possible around the unstable local equilibrium, eventually leading to a definite pattern formation. Inclusion of a negative cross-diffusion thus makes the system biologically realistic. The cross-diffusion can also give rise to a stationary wave-like dissipative structure.

1. Introduction. The origin of biological form and pattern generation is a problem which still poses a challenge to the biologist. According to molecular biology, an organism starts its life as a single cell. Every detail of every phase of development is coded in the genetic material of this single cell and embryological development proceeds with almost clocklike regularity. It is still not clear how the genetic information induces the formation of a pattern of differentiated cells. Some additional information is provided by the interaction of developing cells with their environment, generally termed positional information, and the information is coded in the form of concentration gradients before the actual differentiation. In the latter stage differentiation occurs according to the assigned positional information (Wolpert, 1969; Lewis *et al.*, 1977).

The general model of RNA and protein synthesis using the feedback mechanism in which the end product acts as a repressor molecule was discussed by Goodwin (1963, 1965), Griffith (1968), Walter (1970), Rapp (1975a, b, 1976), Tyson and Othmer (1977), Murray (1977) and others. Griffith found that the model system possesses a periodic behaviour provided the cooperativity of the repressor metabolite as measured by the Hill coefficient (ρ) exceeds a high value, namely $\rho > 8$, which is biologically not

realistic. Generalizing the Goodwin model by extending the chain length Tyson and Othmer (1977) showed that if $\rho < \sec^n(\pi/n)$, where n is the length of the chains, there can be no sustained oscillation. Since biologically $\rho \leqslant 4$, it is deduced that a feedback network with chain length exceeding 5 can exhibit such phenomena. It is observed that the model developed by Tapaswi and Bhattacharya (1981) for transcription and translation during embryogenesis can exhibit a stable periodic solution when $\rho > 4$. Including time-delay, a small value of ρ indicates sustained oscillation (Tapaswi, 1982). Turing (1952), and Othmer and Scriven (1971) showed that the instabilities of the uniform state may arise from the interaction of reaction and transport (involving large distance) and these instabilities may lead to non-uniform spatio-temporal concentration patterns. The role of transport in the dynamics of a control circuit assuming that species involved in a feedback loop are free to diffuse through a three-dimensional region with no flux boundary condition, was studied by Othmer (1977). He showed that if the steady state is stable to non-uniform disturbances, then all small amplitude uniform periodic solutions are asymptotically stable. He conjectured that spatially uniform periodic solutions in a repressible system are asymptotically stable, and non-uniform spatial patterns with diffusive transport may not be possible with single loop feedback circuits. In coupled circuits involving 10 enzymes, each of whose products may activate or inhibit the other and diffuses through the compartment, it was shown by Glass and Kauffman (1972) and Glass and Perez (1974) that for appropriate diffusivities and decay rates the system could exhibit sustained oscillation where the diffusion matrix is diagonal. This 10-enzyme system becomes more relevant if one considers gene control where an enzyme may be looked upon as operator for a structural gene and others localized in the cytoplasm. It is generally believed that when transport is an important mechanism many simple schemes in non-uniform systems can mimic the behaviour of a complicated network in uniform systems.

One of the most successful concepts in the studies of morphogenesis is the morphogenetic field, a kind of prepattern formed by concentration gradients of 'morphogens' which serve the purpose of positional information and trigger cell differentiation and localization. Most of the studies in morphogenesis and pattern formation have been based on the models developed by Lefever and Prigogine (1968) and Gierer and Meinhardt (1972, 1974) who have followed the concept of Turing (1952) and utilized the general principle of lateral inhibition giving rise to a morphogenetic field. Important contributions to the further analysis of these models have been made by Martinez (1972), Babloyantz and Hiernaux (1975), Nicolis and Auchmuty (1974), Auchmuty and Nicolis (1975, 1976), Granero *et al.* (1977), Haken and Olbrich (1978), Erneux *et al.* (1978), Berding and Haken

(1982), and others. Much work has been done on a model biochemical reaction called the Brusselator and the idea of structures arising out of instabilities in succession has been developed by a number of authors (Auchmuty and Nicolis, 1975; Boa and Cohen, 1976; Maher and Matkawsky, 1977). An excellent discussion on self-organization and dissipative structures can be found in Nicolis and Prigogine (1977).

In the present paper we are considering a generalized reaction–diffusion model including diagonal and non-diagonal diffusion. Taking into account the transport mechanism namely, self- and cross-diffusion in a three-component system the non-uniform model is constructed. The main idea is to investigate whether inclusion or diffusion terms in a stable uniform system can drive it into instability and give rise to a dissipative structure (stable wave-like pattern) leading to morphogenetic pattern generation. A spatio-temporal solution of the model around the equilibrium point has also been obtained to exhibit the formation of the earliest layers of the differentiation process, i.e. generation of endoderm, mesoderm and ectoderm, during embryogenesis.

2. The Homogeneous System (Without Diffusion). The Goodwin model of synthesis of mRNA(X), protein (Y) and repressor molecules (Z) is given by the following:

$$\begin{aligned} \dot{X} &= \frac{\alpha_x}{1 + hZ^\rho} - \beta_x X \\ \dot{Y} &= \alpha_y X - \beta_y Y \\ \dot{Z} &= \alpha_z Y - \beta_z Z \end{aligned} \tag{1}$$

where $\dot{}$ denotes the derivatives with respect to time; α_x, α_y, α_z, and h are the rate constants associated with the reactions; β_x, β_y, β_z are the rate constants of degradation and ρ is the Hill coefficient, indicating the number of repressor molecules essential for cooperative inhibition of the regulatory system. The system (1) has a unique steady state which is globally stable. Griffith (1968) has shown that for $\rho > 8$, there exists positive bifurcation values of the parameters such that the steady state is unstable, and by Hopf bifurcation theorem it was proved by Tyson (1975), Murray (1977) and others that the system possesses a small amplitude limit cycle solution in the vicinity of the bifurcation values. In an extended and modified model of RNA and protein synthesis by Tapaswi and Bhattacharya (1981) the result was improved so that the system could possess a limit cycle solution for $\rho > 4$. But a more realistic value of ρ is either 1 or 2. We now construct a model taking into account the transport phenomena. Since information transfer at the cellular level from cell to cell occurs by the process of diffusion of some micromolecules rather than macromolecules of RNA and

protein, the most efficient and flexible system of transmitting chemical information from one part of a biological system to another is to couple the chemical reaction and biologically accepted transport mechanism. The possibility of the reaction–diffusion mechanism giving rise to spatially inhomogeneous structures in a finite, closed domain with zero flux of the species at the boundary is of fundamental importance in developmental biology. It is observed that diffusion-driven instability gives rise to dissipative structures, i.e. stable, spatially heterogeneous structures.

3. General Reaction–Diffusion Model. Let us first consider a general reaction–diffusion system involving mRNA(x), regulator (which is itself a protein enzyme or a product of protein) y and morphogen (z) taking into account the self-diffusion and cross-diffusion terms in the rate of formation of the regulator and morphogen. One can then write the following kinetic equations in dimensionless form with zero flux boundary conditions.

$$\begin{aligned} \frac{\delta x}{\delta t} &= \frac{1}{1+y^{\rho}} - \gamma_1 x \\ \frac{\delta y}{\delta t} &= x - \gamma_2 y + D_{22}\Delta^2 y + D_{23}\Delta^2 z \\ \frac{\delta z}{\delta t} &= y - \gamma_3 z + D_{33}\Delta^2 z + D_{32}\Delta^2 y \end{aligned} \tag{2}$$

where Δ^2 is the Laplacian (diffusion) operator and

$$\left.\frac{\delta x}{\delta r}\right|_{r=0,L} = \left.\frac{\delta y}{\delta r}\right|_{r=0,L} = \left.\frac{\delta z}{\delta r}\right|_{r=0,L} = 0 \quad (0 \leqslant r \leqslant L). \tag{3}$$

The system can be conveniently expressed in the matrix form

$$\frac{\delta \mathbf{w}}{\delta t} = f(\mathbf{w}) + D\Delta^2 \mathbf{w} \tag{4}$$

$$\mathbf{n}\cdot\Delta\mathbf{w} = 0 \quad \text{on} \quad \delta B \tag{5}$$

where

$$\mathbf{w} = \mathbf{w}(t, x, y, z)$$

$$f(\mathbf{w}) = \begin{bmatrix} -\gamma_1 x + \dfrac{1}{1+y^{\rho}} \\ x - \gamma_2 y \\ y - \gamma_3 z \end{bmatrix} \qquad D = \begin{bmatrix} 0 & 0 & 0 \\ 0 & D_{22} & D_{23} \\ 0 & D_{32} & D_{33} \end{bmatrix}$$

δB is the boundary of the finite domain B and n is the outward drawn unit normal to δB. Expression (5) denotes zero flux boundary condition.

For small perturbations from the steady state (x_0, y_0, z_0)

$$x = x_0 + a$$
$$y = y_0 + b$$
$$z = z_0 + c$$

the linearized equations for the small perturbations a, b and c, neglecting terms of second degree, become

$$\frac{\delta a}{\delta t} = -\gamma_1 a - kb$$

$$\frac{\delta b}{\delta t} = a - \gamma_2 b + D_{22}\Delta^2 b + D_{23}\Delta^2 c \tag{6}$$

$$\frac{\delta c}{\delta t} = b - \gamma_3 c + D_{33}\Delta^2 c + D_{32}\Delta^2 b$$

where $k = \rho y_0^{\rho-1}(1 + y_0^{\rho})^{-2}$.

The three eigenvalues $\lambda_{1,2,3}$ are given by the characteristic equation:

$$p_3\lambda^3 + p_2\lambda^2 + p_1\lambda + p_0 = 0 \tag{7}$$

where

$$p_0 = \gamma_1\gamma_2\gamma_3 + \gamma_1\gamma_3 m^2 D_{22} + m^2 D_{33}(\gamma_1\gamma_2 + k) + m^2\gamma_1 D_{23}(1 - m^2 D_{32}) + \gamma_1 m^4 D_{22} D_{33} + k\gamma_3$$

$$p_1 = \gamma_1\gamma_2 + \gamma_2\gamma_3 + \gamma_3\gamma_1 + m^2 D_{22}(\gamma_1 + \gamma_3) + m^2 D_{33}(\gamma_1 + \gamma_2) + m^2 D_{23}(1 - m^2 D_{32}) + m^4 D_{22} D_{33} + k$$

$$p_2 = \gamma_1 + \gamma_2 + \gamma_3 + m^2(D_{22} + D_{33})$$

and

$$p_3 = 1$$

with m as the basic wavenumber.

The eigenvalues govern the time evolution of perturbation. If any of the values of λ has a positive real part, the system is unstable with respect to the pattern size $2\pi/m$. If λ is complex with a positive real part, the system shows oscillatory instability with a frequency equal to the imaginary part divided by 2π.

When the system is homogeneous, $D_{22} = D_{23} = D_{33} = D_{32} = 0$, the Routh-Hurwitz condition for stability of the system is

$$(\gamma_1\gamma_2 + \gamma_2\gamma_3 + \gamma_1\gamma_3 + k)(\gamma_1 + \gamma_2 + \gamma_3) - (\gamma_1\gamma_2\gamma_3 + k\gamma_3) > 0$$

i.e.

$$(\gamma_1 + \gamma_3)(\gamma_2 + \gamma_3) + k > 0$$

which is always satisfied since γ_1, γ_2, γ_3 and k are positive and hence the system is uniformly stable.

In the absence of cross-diffusion, $D_{23} = D_{32} = 0$, the system is stable if

$$(\gamma_1 + \gamma_3 + m^2D_{33})(\gamma_2 + \gamma_3 + m^2D_{22} + m^2D_{33}) + k > 0. \tag{8}$$

Since $D_{22} > 0$ and $D_{33} > 0$ the above condition is always satisfied and hence the system in this case is also stable. Thus a dissipative structure is not possible without cross-diffusion.

4. Reaction-Diffusion Model of Morphogenesis and Pattern Generation. We shall construct here a simple model of morphogenesis and pattern generation during embryogenesis including a cross-diffusion term in the rate equation of the regulator enzyme y and a self-diffusion term in that of the morphogen z. The self-diffusion of z produces a morphogenetic gradient which serves the purpose of positional information and the cross-diffusion term specifies the reaction of the regulator enzyme to this gradient.

In this section it is shown that inclusion of a negative cross-diffusion can maintain wave-like solutions and give rise to a dissipative structure within a realistic value of the cooperativity ρ.

Let us consider a simple system where the regulator enzyme y diffuses only under the concentration gradient of the morphogen z and z diffuses under its own concentration gradient.

$$\begin{aligned}
\frac{\delta x}{\delta t} &= \frac{1}{1 + y^\rho} - \gamma_1 x \\
\frac{\delta y}{\delta t} &= x - \gamma_2 y + D_{23}\Delta^2 z \\
\frac{\delta z}{\delta t} &= y - \gamma_3 z + D_{33}\Delta^2 z \\
0 \leqslant r \leqslant L, \quad &\left(\frac{\delta z}{\delta r}\right)_0 = \left(\frac{\delta z}{\delta r}\right)_L = 0.
\end{aligned} \tag{9}$$

From the linear stability analysis the characteristic equation is given by

$$p_3\lambda^3 + p_2\lambda^2 + p_1\lambda + p_0 = 0 \tag{10}$$

where

$$\begin{aligned} p_3 &= 1 \\ p_2 &= \gamma_1 + \gamma_2 + \gamma_3 + m^2 D_{33} \\ p_1 &= \gamma_1\gamma_2 + \gamma_2\gamma_3 + \gamma_1\gamma_3 + m^2 D_{33}(\gamma_1 + \gamma_2) + m^2 D_{23} + k \end{aligned} \tag{11}$$

and

$$p_0 = \gamma_1\gamma_2\gamma_3 + m^2 D_{33}(\gamma_1\gamma_2 + k) + \gamma_1 m^2 D_{23} + k\gamma_3.$$

When

$$p_1 > 0 \quad \text{and} \quad p_0 < 0,$$

that is

$$-\frac{\gamma_1\gamma_2 + \gamma_2\gamma_3 + \gamma_1\gamma_3 + k}{D_{23} + (\gamma_1 + \gamma_2)D_{33}} < m^2 < -\frac{\gamma_1\gamma_2\gamma_3 + k\gamma_3}{\gamma_1 D_{23} + (\gamma_1\gamma_2 + k)D_{33}} \tag{12}$$

the homogeneous steady-state solution undergoes a non-oscillatory instability with respect to inhomogeneous perturbation and the system may evolve toward a dissipative (regular spatially ordered) structure.

Since m^2, γ_1, γ_2, γ_3, k and D_{33} are all positive, it is evident from the relation (12) that D_{23} must be negative in order to achieve dissipative structure.

The Routh–Hurwitz criterion for oscillatory instability (through complex roots) requires

$$\begin{aligned} D_{23} \leqslant -\frac{\gamma_1 + \gamma_2}{m^2(\gamma_2 + \gamma_3 + m^2 D_{33})} \\ \times [(\gamma_1 + \gamma_3)(\gamma_2 + \gamma_3) + m^2 D_{33}(\gamma_1 + \gamma_2 + 2\gamma_3 + m^2 D_{33}) + k]. \end{aligned} \tag{13}$$

Thus in this case also, D_{23} is negative. Hence in order to achieve a dissipative structure either through oscillatory instabilities or non-oscillatory instabilities, the cross-diffusion coefficient must be negative. In other words, inclusion of negative cross-diffusion in the system leads to a dissipative (spatial) structure.

In the special case when

$$p_0 = 0 \quad \text{and} \quad p_1 > 0,$$

that is

$$m^2 = -\frac{\gamma_1\gamma_2\gamma_3 + k\gamma_3}{\gamma_1 D_{23} + (\gamma_1\gamma_2 + k)D_{33}} \tag{14}$$

where

$$-(\gamma_1 + \gamma_2) < D_{23}/D_{33} < -[\gamma_2 + (k/\gamma_1)] \tag{15}$$

the dominant eigenvalue is $\lambda_1 = 0$, and a stationary dissipative structure evolves. The computer solution for infinite time where $\delta x/\delta t = \delta y/\delta t = \delta z/\delta t = 0$ is given in Fig. 1. The numerical analyses were carried by a fourth-order Runge–Kutta method. The rate constants are $\gamma_1 = \gamma_2 = \gamma_3 = 1$, $D_{33} = 1$, $D_{23} = -1.403$. The initial conditions are spatially uniform: $x(0) = x_0 = 0.615$, $y(0) = y_0 = 0.615$ and $z(0) = z_0 - 0.05 = 0.565$.

The number of unit cells is $n = 30$ which can be calculated in advance from (14).

$$m^2 = 361$$

$$n = \frac{2Lm}{2\pi} = \frac{10 \times 19}{2\pi} = 30$$

$2L = 10$ is the length of the system.

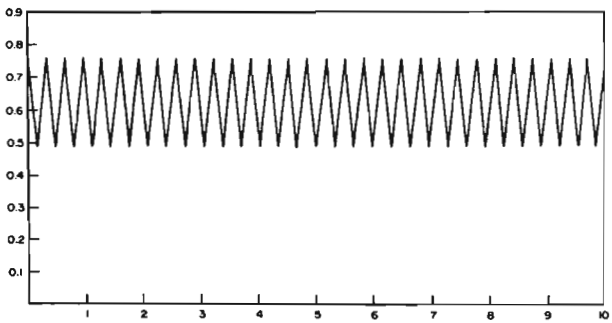

Figure 1. Stationary spatial pattern formation for the diffusive epigenetic system (9) with negative cross-diffusion.

$$\gamma_1 = \gamma_2 = \gamma_3 = 1, \quad D_{33} = 1, \quad D_{23} = -1.403.$$

Initial concentrations:

$$x(0) = x_{ss} = 0.615, \quad y(0) = y_{ss} = 0.615, \quad z(0) = z_{ss} - 0.05 = 0.565$$

$n = 30$ unit cells.

4. Pattern Formation During Embryonic Development. In this section we shall verify the applicability of the above model as expressed by (9) in investigating the mechanism of spatial pattern formation during early embryonic development. The primary layers of differentiation evolve as early as the gastrula stage. The layers from within outwards in a spherical mass of embryonic cells are arranged as (i) endoderm, (ii) mesoderm and (iii) ectoderm. In order to study the evolution of these concentric circular layers the geometry of interest is a circular membrane which represents the cross section of the spherical embryonic mass at about the gastrula stage.

The linearized form of the problem (9) with zero flux boundary condition is

$$\frac{\delta \mathbf{u}}{\delta t} = M\mathbf{u} + D\Delta^2\mathbf{u}, \quad \mathbf{n}\cdot\Delta\mathbf{u} = 0, \quad r \in B \tag{16}$$

where

$$\mathbf{u} = \begin{bmatrix} x - x_0 \\ y - y_0 \\ z - z_0 \end{bmatrix}, \quad M = \begin{bmatrix} -\gamma_1 & -k & 0 \\ 1 & -\gamma_2 & 0 \\ 0 & 1 & -\gamma_3 \end{bmatrix}$$

$$D = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & D_{23} \\ 0 & 0 & D_{33} \end{bmatrix}.$$

Let the eigenvalues for the geometry of interest be m^2, that is the eigenvalues of

$$\Delta^2\mathbf{u} + m^2\mathbf{u} = 0, \quad \mathbf{n}\cdot\Delta\mathbf{u} = 0, \quad r \in \mathrm{B}. \tag{17}$$

The characteristic equation of (16) has already been obtained in Section 3 [equation (10)]. The necessary and sufficient conditions for diffusion-driven instability of the system (9) are

$$p_i > 0, \quad i = 0, 1, 2, 3 \tag{18}$$

and

$$p_1 p_2 - p_0 p_3 < 0$$

that is, when

$$\mu > \frac{f}{m^2 D_{33}}[h + m^2 D_{33} + k/(g + m^2 D_{33})] \tag{19}$$

where

$$\mu = -\frac{D_{23}}{D_{33}} > 0 \quad (\text{since } D_{23} < 0)$$

$$f = \gamma_1 + \gamma_2, \quad g = \gamma_2 + \gamma_3, \quad h = \gamma_1 + \gamma_3$$

and

$$D_{33} > \frac{1}{m^2}[kf/(k - \gamma_1^2) - g] \tag{20}$$

with respect to the eigenvalues $m^2 > 0$ given by

$$H(m^2) = m^4 D_{33}^2(f - \mu) + m^2 D_{33}[f(h + g) - g\mu] + f(gh + k) < 0. \tag{21}$$

This determines a critical $\mu = \mu_c$, such that $H(m^2) = 0$ for some $m^2 > 0$. Bifurcating stable time-periodic spatial solution corresponding to the eigenvalues $\lambda = \pm i\omega$ evolves when

$$\mu = \mu_c = \frac{f}{m^2 D_{33}}[h + m^2 D_{33} + k/(g + m^2 D_{33})] \tag{22}$$

with respect to the eigenvalues m^2 given by

$$\frac{1}{D_{33}}[kf/(k - \gamma_1^2) - g] < m^2 = \frac{1}{2D_{33}(\mu - f)}\{[fh - g(\mu - f)] + [(fh - g(\mu - f))^2 + 4f(\mu - f)(k + hg)]^{1/2}\}. \tag{23}$$

A direct measure of the scale are the parameters D_{33} and $\mu = |D_{23}/D_{33}|$ which for given geometries has a critical bifurcation value μ_c for the existence of spatial structures, all other parameters f, g, h, k being kept fixed. In the following part of this section it will be seen that they play a critical role in maintaining the size invariance of the pattern, that is, the property of the same pattern forming in large and small embryos.

For the geometry of a circular membrane of radius r the eigenvalue problem in the coordinate system r, θ is

$$\frac{\delta^2 \mathbf{u}}{\delta r^2} + \frac{1}{r}\frac{\delta \mathbf{u}}{\delta r} + \frac{1}{r^2}\frac{\delta \mathbf{u}}{\delta \theta^2} + m^2 \mathbf{u} = 0 \tag{24}$$

with

$$\mathbf{n} \cdot \Delta \mathbf{u} = 0, \quad r \in \mathrm{B}.$$

We shall consider the solutions $\mathbf{u}(r)$ of this equation which are radially symmetric, that is which do not depend on θ.

Then equation (24) reduces to the form

$$\frac{\delta^2 \mathbf{u}}{\delta r^2} + \frac{1}{r}\frac{\delta \mathbf{u}}{\delta r} + m^2 \mathbf{u} = 0. \tag{25}$$

Since the membrane has a fixed boundary $r = R$, we have the boundary condition

$$\mathbf{u}(R, t) = 0 \quad \text{for all} \quad t \geqslant 0 \tag{26}$$

Let

$$s = mr, \quad \text{i.e. } \mathrm{d}s = m\mathrm{d}r,$$

then (25) becomes

$$\frac{\delta^2 \mathbf{u}}{\delta s^2} + \frac{1}{s}\frac{\delta \mathbf{u}}{\delta s} + \mathbf{u} = 0. \tag{27}$$

This is a Bessel's equation of order $\nu = 0$. Then the solutions of (25) are of the form

$$\mathbf{u}_n(r) = J_0(m_n r), \quad n = 1, 2, \ldots. \tag{28}$$

Where $J_0(m_n r)$ is the Bessel's function of the first kind and $m_n = \alpha_n/R$, α_n $(n = 1, 2, \ldots)$ being the positive zeros of J_0.

The bifurcating time-periodic spatial solutions of the system corresponding to the eigenvalues $\lambda = \pm i\omega_n$ are then

$$\mathbf{U}_n(r, t) = (a_n \cos\omega_n t + b_n \sin\omega_n t) J_0(m_n r) \tag{29}$$

where $n = 1, 2, \ldots$, and a_n and b_n are constants.

With m^2 from (28) the relation (23) becomes, after simplification

$$-D_{23} = \frac{fR^2}{\alpha_n^2}\left[h + \frac{D_{33}\alpha_n^2}{R^2} + \frac{kR^2}{R^2 g + D_{33}\alpha_n^2}\right], \quad (D_{23} < 0) \tag{30}$$

where

$$D_{33} > \frac{R^2}{\alpha_n^2}[kf/(k - \gamma_1^2) - g] \tag{31}$$

In this relation we have α_n $(n = 1, 2, \ldots)$ as constants and R, the radius, is different for different embryos necessitating different values of D_{23} and D_{33} in each case in order to satisfy (30). Similar patterns will be formed in different sizes of the embryos the radius of each of which lie in the domain as prescribed by (30).

Size invariance of the pattern is controlled by the parameters D_{23} and D_{33}, that is, the cross-diffusion coefficient and self-diffusion coefficient, respectively.

The solution (29) thus resembles the solutions of vibration of a circular

membrane of radius R with frequency $\omega_n/2\pi$ cycles per unit time. For each n the expression (29) is a mode or eigensolution and represents a specific spatial pattern. For $n = 3$ so that $\alpha_n = 8.6537$, the solution is as shown in Fig. 2. The circular membrane represents the central cross-section of the spherical mass of embryonic cells. The figure shows how the pattern is generated. The base line through the smooth curve corresponds to the homogeneous steady-state value y_0 of y. The wavy pattern of solution of y acts by switching on and off particular genes. For example, the large amplitude wave above the base line ($y \gg y_0$) switches on the genes the activities of which result in the formation of the endoderm in the area bounded by the innermost circle (innermost nodal line). Similarly the small amplitude wave below the base line ($y < y_0$) is responsible for the formation of mesoderm in the area bounded by the intermediate circle and that above the base line ($y > y_0$) stimulates the generation of the outermost layer namely, ectoderm.

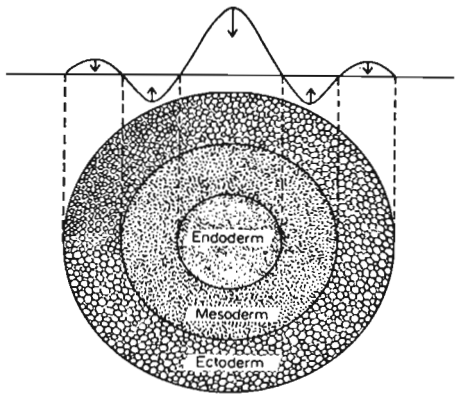

Figure 2. Bifurcating time-periodic spatial solution leading to the formation of the earliest occurring patterns—endoderm, mesoderm and ectoderm, at about the gastrula stage of the embryonic development. The straight line through the wavy curve is the base line which represents the steady-state value y_0 of the morphogenetic regulator y. The central large deflection of y above y_0 ($y \gg y_0$) switches on the genes responsible for the formation of the endodermal tissues the downward deflection ($y < y_0$) on both sides of the former switches on the genes corresponding to the mesodermal tissues. The small deflection above the base line ($y > y_0$) on the extreme sides switches on the genes corresponding to the ectodermal tissues as depicted in the circular membrane below the curve. When one set of switches are 'put on' the remaining sets of switches remain in the 'off' position.

It may be assumed that when one set of genes is switched on by its corresponding threshold value of y (greater than or less than y_0) the other sets of genes remain switched off, that is inactive.

5. Discussion. That negative cross-diffusion plays an important role in achieving a dissipative structure for the model system (9) has been shown in this paper. Jorne (1977) has shown that the diffusive Lotka–Volterra mechanism can give rise to a stationary dissipative structure by the inclusion of a negative cross-diffusion coefficient. For the diffusive epigenetic system (9) investigated here we find that by inclusion of a negative cross-diffusion the system evolves into such a stationary dissipative structure. The system also undergoes a time-periodic spatial structure if the cross-diffusion coefficient is negative.

The term negative cross-diffusion coefficient implies active counter transport. In self-diffusion, that is, passive transport, the diffusion coefficient is always positive and the diffusive substance moves from a higher to a lower concentration. In active transport the diffusion coefficient is negative and the diffusion takes place in the opposite direction, that is, from a lower to a higher concentration of the same substance. We have used the term cross-diffusion with negative diffusion coefficient to imply active counter transport, that is, the diffusive substance moves towards a higher concentration of another substance.

Active transport is one of the most important features of life processes. It resolves the contradiction between the preservation of spatial heterogeneity and metabolism—the exchange of matter and energy with the surrounding medium. The source of free energy for active transport is ATP (Volkenshtein, 1983).

An increased concentration of K^+ ions and a decreased concentration of Na^+ ions inside the cell are determined by the active membrane transport which proceeds against the electrochemical gradient. The movement of amino acids into the cells also takes place by the active transport process.

The examples of negative cross-diffusion or active counter transport are not rare in biology. Studies on squid axon reveal that calcium can cross the axon membrane by counter transport with sodium, and high internal sodium concentrations would be expected to increase calcium influx (Marchbanks, 1970). Counter transport of methionine by histidine in brain cells has been shown by Nakamura (1963). Jorne (1975) has shown that negative cross-diffusion coefficients are possible in electrolytic solutions, since ions diffuse interactably and are influenced by the resulting diffusion potential.

Like active transport, active counter transport also may take place in two ways: in the simple case the diffusive substance diffuses itself to the opposite side of the cell membrane against the concentration gradient of another

substance. In the other case the substance to be transported, being immobile itself, cannot diffuse except by forming a complex with a carrier molecule which can cross the membrane to the opposite side having a higher concentration of another substance. The substrate–carrier complex then breaks down and the free carrier molecules return to the original side to carry more substrate molecules to the opposite side of the membrane.

The particular example given in this paper represents the second case of active counter transport as discussed above. The regulator enzyme y which is a product of mRNA and which is simultaneously a regulator of mRNA synthesis, is itself immobile but forms a complex with the molecules of the morphogen z which acts as a carrier. The enzyme–morphogen complex in a cell then diffuses towards its neighbouring cell having a higher concentration of z ($D_{23} < 0$). After reaching the neighbouring cell the complex then breaks down and the free z molecules return to the original cell (with passive diffusion) to carry more molecules of y by the same process of active counter transport, that is, by a cross-diffusion with negative diffusion coefficient. However, z diffuses under its own gradient which plays the role of positional information.

The scheme as mentioned above is merely a postulation and like the morphogenetic gradient theory the particular negative cross-diffusion mechanism acting during early embryogenesis as postulated has not yet obtained any experimental support by laboratory findings. But, that negative cross-diffusion, that is, active counter transport, plays an important role in pattern generation during embryonic development has been shown in this paper.

It has also been demonstrated that the pattern is size invariant, that is, the property of the same pattern formation in large and small embryos is maintained. This size invariance can be achieved by controlling the parameters D_{23} and D_{33}, that is, the cross-diffusion coefficient and the self-diffusion coefficient respectively.

It has been experimentally observed that the earliest layers of differentiated cells, endoderm, mesoderm and ectoderm, can be traced in the gastrula stage of embryogenesis. It is shown in this paper that the concentration of the regulator enzyme y varies spatially corresponding to the morphogenetic gradient established by z and the different sets of genes the activities of which are responsible for the formation of different types of tissues are activated at different ranges of concentration (threshold concentration) of the regulator y as depicted in Fig. 2.

We wish to thank Mr S. K. Dey, Indian Statistical Institute, Calcutta for drawing the figures for this manuscript.

LITERATURE

Auchmuty, J. F. G. and G. Nicolis. 1975. "Bifurcation Analysis of Nonlinear Reaction-Diffusion Equations—I: Evolution Equations and the Steady State Solutions." *Bull. math. Biol.* **37**, 323–365.

—— and ——. 1976. "Bifurcation Analysis of Nonlinear Reaction-Diffusion Equations —III: Chemical Oscillations." *Bull. math. Biol.* **38**, 325–350.

Babloyantz, A. and J. Hiernaux. 1975. "Models for Cell Differentiation and Generation of Polarity in Diffusion Governed Morphogenetic Fields." *Bull. math. Biol.* **37**, 637–657.

Berding, C. and H. Haken. 1982. "Pattern Formation in Morphogenesis." *J. math. Biol.* **14**, 133–151.

Boa, J. A. and D. S. Cohen. 1976. "Bifurcations of Localized Disturbances in a Model Biochemical Reaction." *SIAM J. appl. Math.* **30**, 123–135.

Erneux, T., J. Hiernaux and G. Nicolis. 1978. "Turing's Theory in Morphogenesis." *Bull. math. Biol.* **40**, 771–789.

Gierer, A. and H. Meinhardt. 1972. "A Theory of Biological Pattern Formation." *Kybernetik* **12**, 30–39.

—— and ——. 1974. "Applications of a Theory of Biological Pattern Formation Based on Lateral Inhibition." *J. Cell. Sci.* **15**, 321–376.

Glass, L. and S. A. Kauffman. 1972. "Co-operative Components, Spatial Localization and Oscillatory Cellular Dynamics." *J. theor. Biol.* **34**, 219–237.

—— and R. Perez. 1974. "Limit Cycle Oscillations in Compartmental Chemical Systems." *J. chem. Phys.* **61**, 5242–5239.

Goodwin, B. C. 1963. *Temporal Organization in Cells*. New York: Academic Press.

——. 1965. "Oscillatory Behaviour in Enzymatic Control Processes." *Adv. Enzyme Regulat.* **3**, 425–438.

Granero, M. I., A. Porati and D. Zanacca. 1977. "Bifurcation Analysis of Pattern Formation in a Diffusion Governed Morphogenetic Field." *J. math. Biol.* **4**, 21–27.

Griffith, J. S. 1968. "Mathematics of Cellular Control Process I: Negative Feedback to One Gene." *J. theor. Biol.* **20**, 202–208.

Haken, H. and H. Olbrich. 1978. "Analytical Treatment of Pattern Formation in the Gierer-Meinhardt Model of Morphogenesis." *J. math. Biol.* **6**, 317–331.

Jorne, J. 1975. "Negative Ionic Cross Diffusion Coefficient in Electrolytic Solution." *J. theor. Biol.* **55**, 529–532.

——. 1977. "The Diffusive Lotka-Volterra Oscillating System." *J. theor. Biol.* **65**, 133–139.

Lefever, R. and I. Prigogine. 1968. "Symmetry-Breaking Instabilities in Dissipative System—II." *J. chem. Phys.* **48**, 1695–1700.

Lewis, J., J. M. W. Slack and L. Wolpert. 1977. "Thresholds in Development." *J. theor. Biol.* **65**, 579–590.

Mahar, T. J. and B. J. Matkawsky. 1977. "A Model Biochemical Reaction Exhibiting Secondary Bifurcation." *SIAM J. appl. Math.* **32**, 394–404.

Marchbansk, R. M. 1970. "Ion Transport and Metabolism in Brain." In *Membranes and Ion Transport*, Vol. 2, E. Bittar (Ed.). New York: Wiley.

Martinez, H. M. 1972. "Morphogenesis and Chemical Dissipative Structures: a Computer Simulated Case Study." *J. theor. Biol.* **36**, 479–501.

Murray, J. D. 1977. *Lectures on Nonlinear-Differential-Equation Models in Biology*. Oxford: Oxford University Press.

Nakamura, R. 1963. "The Transport of Histidine and Methionine in Rat Brain Slices." *J. Biochem.* **53**, 314–332.

Nicolis, G. and J. F. G. Auchmuty. 1974. "Dissipative Structures, Catastrophes and Pattern Formation: a Bifurcation Analysis." *Proc. natn. Acad. Sci. U.S.A.* **71**, 2748–2751.

Nicolis, G. and I. Prigogine. 1977. *Self Organization in Non-Equilibrium Systems*. New York: Wiley.

Othmer, H. C. 1977. "Current Theories of Pattern Formation." In *Lectures on Mathematics in Life Sciences*, S. Levin (Ed.), Vol. 9, pp. 55–86. American Mathematical Association.

—— and L. E. Scriven. 1971. "Instability and Dynamic Pattern in Cellular Networks." *J. theor. Biol.* **32**, 507–537.

Rapp, P. E. 1975a. "Biochemical Oscillators—a Search Procedure." *Mathl Biosci.* **23**, 289–303.

——. 1975b. "A Theoretical Investigation of a Large Class of Biochemical Oscillators." *Mathl Biosci.* **25**, 165–188.

——. 1976. "Mathematical Techniques for the Study of Oscillations in Biochemical Control Loops." *Bull. Inst. Math. Applics.* **12**, 11–21.

Tapaswi, P. K. 1982. "Time Lags in an Extended Mathematical Model of Transcription and Translation During Embryogenesis." *Cybernetica* **25**, 151–162.

—— and P. Bhattacharya. 1981. "An Extended Mathematical Model of Transcription and Translation during Embryogenesis." *Cybernetica* **24**, 61–84.

Turing, A. M. 1952. "The Chemical Basis of Morphogenesis." *Phil. Trans. R. Soc.* **B237**, 37–72.

Tyson, J. J. 1975. "On the Existence of Oscillatory Solutions in Negative Feedback Cellular Control Processes." *J. math. Biol.* **1**, 311–315.

—— and H. G. Othmer. 1977. "The Dynamics of Feedback Control Circuits in Biochemical Pathways." In *Progress of Theoretical Biology*, Vol. 5. New York: Academic Press.

Volkenshtein, M. V. 1983. *Biophysics*. Moscow: MIR Publishers.

Waddington, C. H. 1956. *Principles of Embryology*. London: George Allen & Unwin.

Walter, C. F. 1970. "The Occurrence and Significance of Limit Cycle Behaviour in Controlled Biochemical Systems." *J. theor Biol.* **27**, 259–272.

Wolpert, L. 1969. "Positional Information and the Spatial Pattern of Cellular Differentiation." *J. theor. Biol.* **25**, 1–47.

RECEIVED 2-4-85
REVISED 10-18-85