

ON THE THEORY OF THE GENERATION OF STABLE INHOMOGENEOUS STATES  
IN HOMOGENEOUS MIXTURES

W.Th.Hermens, P.W.Hemker\* and H.C.Hemker

Laboratory of Cardiovascular Biochemistry, University Hospital,  
Leiden, The Netherlands\* Mathematical Centre, 2e Boerhaavestraat 49, Amsterdam, The  
Netherlands

## INTRODUCTION

One of the important questions arising from the problem of the evolution of living matter is how to give a physical-chemical description of the development of structure in an originally homogeneous 'soup' of chemical components.

In his paper 1) 'The chemical basis of morphogenesis' (1952) A.M.Turing was the first to succeed in describing such a system, starting from generally accepted principles such as the law of mass action for chemical reactions and the Fick law for diffusion. He considered a mixture of chemical reactants taking part in a (complicated) set of chemical reactions and diffusion and showed the existence of a homogeneous stationary state in which any fluctuation could initiate the generation of an inhomogeneous state (structure). Only in the last few years have such systems actually been realized in the laboratory 2), 3), 4).

Such a Turing system is quite different from the (Lotka) systems described much earlier, in which chemical oscillations occur in time. In contrast to a Lotka system a Turing system becomes spatially inhomogeneous and need not show variations in time (stable system).

Until recently, the thermodynamic theory of Turing systems was quite obscure. Thermodynamic equilibrium implies the disappearance of concentration gradients, whereas a Turing system develops from a homogeneous state to a state in which concentration gradients exist and are maintained. Moreover, the appearance of a structure is entropy-lowering, and at first sight this seems difficult to reconcile with the second law of thermodynamics, which states that the local entropy production is always positive. Not long ago, however, Glansdorff and Prigogine 5) developed the thermodynamic theory of irreversible processes far from equilibrium. They showed that the stationary states of a Turing system can only be maintained at the cost of dissipated energy (dissipative structures) and by exchange of matter and entropy with the surroundings. The chemical reactions proceeding in a Turing system give a production of heat that more than balances the entropy loss due to the arising structure. To keep the system at constant temperature for example, the heat has to be transported to the outside world, resulting in an entropy loss. In this way one ends up with a lower entropy than in the homogeneous state. This situation can be compared to the condensation of a vapour in a vessel kept at constant temperature, but in the theory of dissipative structures no phase transitions are considered.

Glansdorff and Prigogine also developed a stability criterion which they called the general evolution criterion. For a given set of chemical reactions, this criterion is useful for investigation of the stability of a stationary state.

All Turing systems described so far start with ad hoc chemical reaction schemes. At the moment, no general theory establishing the reaction kinetics needed for such systems is available. The only feature mentioned is that autocatalytic or cross catalytic reactions seem indispensable. If such a theory existed, a systematic search for chemical reactions satisfying the kinetic requirements would become possible.

At the FEBS congress in 1971 we reported 6), for the simplest case of only one reactant with variable concentration (one-morphogen system), a mathematical criterion that must be fulfilled if the system is to have an inhomogeneous stationary state. The simplest reaction scheme satisfying this criterion was set up. As a first result, the important role of zeroth-order (Michaelis-Menten type) enzyme kinetics, already present in the original Turing scheme, was understood. In this paper, we shall therefore start with a systematic analysis of zeroth-order, first-order, and second-order chemical reaction kinetics for a one-morphogen system.

To qualify for a Turing system, the existence of an unstable homogeneous stationary state as well as a stable inhomogeneous stationary state must be demonstrated. Moreover, the class of perturbations for which the system will actually reach this latter state must be determined.

According to the above standard, we show in the first part of this paper that *no physically realistic one-morphogen Turing system is possible*. In the second part of this paper, we apply the above mentioned general evolution criterion to the case of second-order chemical kinetics in a one-morphogen system. It is shown that there is a discrepancy between the results obtained in this way and the results of mathematical analysis. In the last section we investigate a possible explanation of this discrepancy by applying the general evolution criterion to a three-morphogen system.

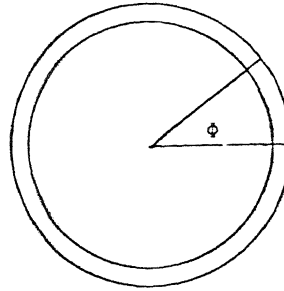
## MATHEMATICAL ANALYSIS

### 1) Zeroth-order chemical kinetics

Following Turing's example, we consider a one-dimensional system consisting of a closed ring of tissue (fig. 1). We assume the system to be filled with a dilute mixture of chemical components. The simplest kinetic equation conceivable for a single substance  $X(t, \phi)$  taking part in chemical reactions as well as diffusion is:

$$\frac{\partial X(t, \phi)}{\partial t} = c + D \frac{\partial^2 X(t, \phi)}{\partial \phi^2} \quad (1)$$

Fig. 1: One-dimensional ring of tissue. Concentrations in the ring are functions of time  $t$  and angle  $\phi$ .



The lefthand side of equation (1) denotes the change in time of  $X$  for a fixed point  $\phi$ , whereas the righthand side shows that this change is due to diffusion ( $D$ =diffusion constant of substance  $X$ ) and to chemical removal or formation of  $X$  with a constant velocity  $c$ , which is independent of the concentration  $X$ . It should be stressed that it is particularly in biochemistry that zeroth-order reaction kinetics often occur. This is due to the very familiar type of Michaelis-Menten enzyme kinetics:



in which the substrate  $X$  is enzymatically converted into the product  $P$ . When the enzyme is added at  $t=0$ , such a system will often, after a short initial phase, be in the so called Briggs-Haldane approximation with maximal reaction rate, in which the enzyme is saturated with substrate and the over-all break down rate is only dependent on the reaction constant  $k_3$  and concentration of complex  $C$ . By making  $X$  the product of reaction (2), one can also obtain a positive value for  $c$  in equation (1).

It might be argued that if reactions of type (2) are to be used to obtain spatial inhomogeneities, this could also be the case for the concentration  $C$  of complex, so we would no longer be allowed to assume a constant reaction rate. In reality, however, the enzyme (and complex) molecules are often very large as compared to the substrate molecules. This means that their diffusibilities can easily differ by several orders of magnitude (7), in which case we could for a certain period of time safely assume the concentration of  $C$  to remain homogeneous even if  $X$  is no longer so.

#### Homogeneous stationary state

A solution of the kinetic equation (1) not depending on time will be denoted  $x(\phi)$ . If, in addition,  $x$  is also not dependent upon space, we have a homogeneous stationary state denoted by  $\bar{x}$ .

Since we have  $\frac{\partial \bar{x}}{\partial t} = \frac{\partial^2 \bar{x}}{\partial \phi^2} = 0$ , we conclude from (1) that no ho-

homogeneous stationary state is feasible in this case.

Inhomogeneous stationary state

From equation (1) we find that  $x(\phi)$  satisfies:

$$0 = c + D \frac{d^2 x(\phi)}{d\phi^2} \quad (3)$$

$$\text{with the solution: } x(\phi) = -\frac{c}{2D} \phi^2 + k_1 \phi + k_2, \quad (4)$$

where  $k_1$  and  $k_2$  are arbitrary constants.

$$\begin{aligned} &\text{Using the time-independent boundary conditions} \\ &x(0) = x(2\pi) = \text{constant}, \quad (5) \\ &\text{we obtain:} \end{aligned}$$

$$k_1 = \frac{c\pi}{D}$$

while the extremum occurs for  $\phi = \pi$ .

Because negative values for the concentration  $x$  are excluded we obtain from (4) and (6) the following condition for  $k_2$ :

$$\begin{aligned} c > 0 : k_2 &> 0 \\ c < 0 : k_2 &> -\frac{c}{2D} \pi^2 \end{aligned} \quad (7)$$

The physical picture of this inhomogeneous stationary state is clear: throughout the ring there is a constant breakdown ( $c < 0$ ) or formation ( $c > 0$ ) of substance  $X$ , and this effect is balanced by a diffusional transport due to the existent concentration gradient. In order to maintain the boundary conditions (5) there should be an external supply ( $c < 0$ ) or drain ( $c > 0$ ) in the point  $\phi = 0$ . This could better be visualized by considering, instead of the ring, a situation as pictures in fig. 2.

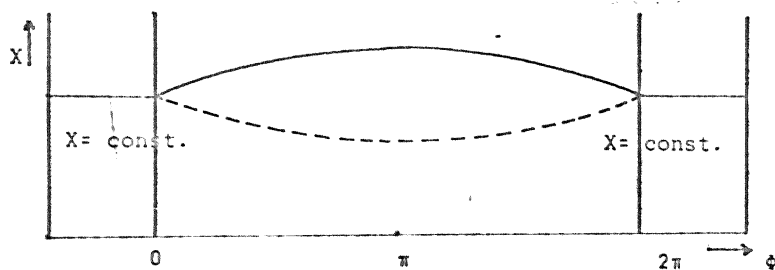


Fig. 2: Inhomogeneous stationary state in a vessel in which a zeroth-order chemical reaction takes place and which is bordered on both sides by reservoirs containing fixed concentrations of  $X$ .

To investigate the stability of the solution (4), we consider the time-evolution of a small perturbation  $v(t, \phi)$  of  $x(\phi)$ . Substituting  $X(t, \phi) = x(\phi) + v(t, \phi)$  into (1) and using also (3), we obtain

$$\frac{\partial v(t, \phi)}{\partial t} = D \frac{\partial^2 v(t, \phi)}{\partial \phi^2}$$

This equation has the solution:

$$v(t, \phi) = C_{\eta} \exp(-\eta^2 D t) \exp(i \eta \phi), \quad (9)$$

where  $\eta$  and  $C_{\eta}$  can take arbitrary real or complex values and are determined by the boundary conditions. From the condition (5) we find:

$$v(t, 0) = v(t, 2\pi) = 0 \quad (10)$$

Condition (10) shows that only purely periodic spatial perturbations are allowed. So  $\eta$  can only take real values. In that case, by adding complex conjugate solutions we write (9) as the following expression:

$$v(t, \phi) = 2 \{ \text{Re}(C_{\eta}) \cos \eta \phi - \text{Im}(C_{\eta}) \sin \eta \phi \} \exp(-\omega_{\eta} t) \quad (11)$$

with  $\omega_{\eta} = \eta^2 D > 0$

So the perturbation  $v(t, \phi)$  will be damped out in the course of time, showing that we have a stable state.

Although we have a stable inhomogeneous state, the absence of a homogeneous stationary state shows that according to the definition given in the introduction, no one-morphogen Turing system can be realized with zeroth-order chemical kinetics.

## 2) First-order chemical kinetics

We now consider a kinetic equation which is one step more complicated than equation (1):

$$\frac{\partial X(t, \phi)}{\partial t} = bX(t, \phi) + c + D \frac{\partial^2 X(t, \phi)}{\partial \phi^2} \quad (12)$$

The first term on the righthand side corresponds for  $b < 0$  to a simple random breakdown of  $X$ :



The condition  $b > 0$  is, however, more restrictive, because here we need an autocatalytic reaction:



For a large excess of A, the reaction rate will be linear in X. Again it should be noted that it is especially in biochemistry that many examples of this type of reaction are found.

#### Homogeneous stationary state

From equation (12) we obtain by putting  $X(t, \phi) = \bar{x}$ :

$$\bar{x} = -\frac{c}{b} \quad (15)$$

We find that a homogeneous stationary state exists, provided that c and b have opposite signs. To investigate the stability of this solution we consider again a small perturbation  $v(t, \phi)$  of  $\bar{x}$  and obtain from (12):

$$\frac{\partial v}{\partial t} = bv + D \frac{\partial^2 v}{\partial \phi^2} \quad (16)$$

with the solution

$$v(t, \phi) = \sum_{\eta} C_{\eta} \exp(-\omega t) \cdot \exp(i\eta \phi) \quad \text{with } \omega = \eta^2 D - b \quad (17)$$

As before, we conclude from the boundary condition (5) that  $\eta$  must be real. From (17) we find in that case that for  $b > 0$  those perturbations satisfying  $\eta^2 D < b$  will be amplified, showing that we have an unstable state. For  $b < 0$ , however, each perturbation will be damped, so we have a stable state.

Together with the condition of opposite signs of b and c, we obtain the stability conditions:

$$\begin{array}{ll} b < 0 & c > 0 \quad \text{stable state,} \\ b > 0 & c < 0 \quad \text{unstable state.} \end{array} \quad (8)$$

Again the physical picture is clear: as long as we have a constant formation of X balanced by a breakdown proportional to X, a fluctuation in X will be leveled. If, however, we have a constant removal balanced by an autocatalytic formation of X, any fluctuation will be amplified.

Here we have an example of the fact that autocatalytic reactions introduce the possibility of the existence of unstable homogeneous stationary states.

#### Inhomogeneous stationary state

From equation (12) we find that  $x(\phi)$  must satisfy:

$$0 = bx(\phi) + c + D \frac{d^2 x(\phi)}{d\phi^2} \quad (19)$$

with the solution

$$x(\phi) = C \exp(i\sqrt{(b/D)}\phi) - \frac{c}{b} \quad (20)$$

For a given ratio  $b/D$  this expression will generally not satisfy the boundary condition (5), so we have no stationary inhomogeneous state in this case.

Again we find that no one-morphogen Turing system is possible for first-order chemical reaction kinetics.

It should be stressed here that in the literature a criterion often used for a morphogenetic system is the existence of an unstable homogeneous state in which any fluctuation will cause the system to develop to an inhomogeneous (structured) state. In this restricted sense a morphogenetic system can be realized with linear chemical reaction kinetics.

### 3) Second-order chemical kinetics

In this section we analyse the second-order kinetic equation

$$\frac{\partial X(t, \phi)}{\partial t} = a X^2(t, \phi) + b X(t, \phi) + c + D \frac{\partial^2 X(t, \phi)}{\partial \phi^2} \quad (21)$$

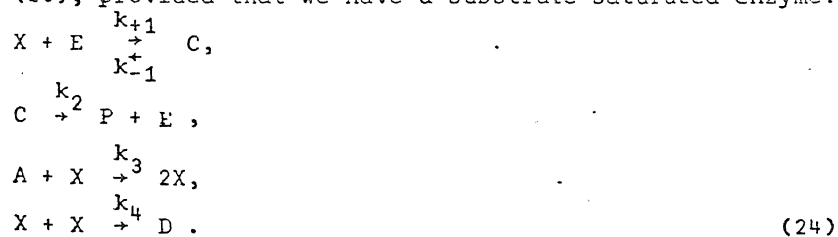
The first term on the righthand side can only be realized for  $a < 0$ , for example by a simple dimerization reaction:



As we will show below, a stationary inhomogeneous state is only feasible if we have:

$$a < 0, b > 0, \text{ and } c < 0 \quad (23)$$

Combining the reactions given in the preceding sections, the following set of chemical reactions would satisfy (21) and (23), provided that we have a substrate-saturated enzyme:



### Homogeneous stationary state

From equation (21) we obtain for  $X(t, \phi) = \bar{x}$ :

$$\bar{x} = \frac{b \pm \sqrt{b^2 - 4ac}}{-2a} \quad (25)$$

From (23) and (25) we find that two different homogeneous stationary states are possible if the following condition is satisfied:

$$b^2 > 4ac.$$

For the reaction scheme (24) this is equivalent to:

$$(k_3A)^2 > 4k_2k_4C. \quad (26)$$

To determine the stability of these states, we substitute into (21) again a solution of the form:

$$X(t, \phi) = \bar{X} + v(t, \phi) \quad |v/\bar{X}| \ll 1,$$

and obtain after linearization, i.e. neglecting terms containing  $v^2$ :

$$\frac{\partial v}{\partial t} = (2a\bar{X} + b)v + D \frac{\partial^2 v}{\partial \phi^2} \quad (27)$$

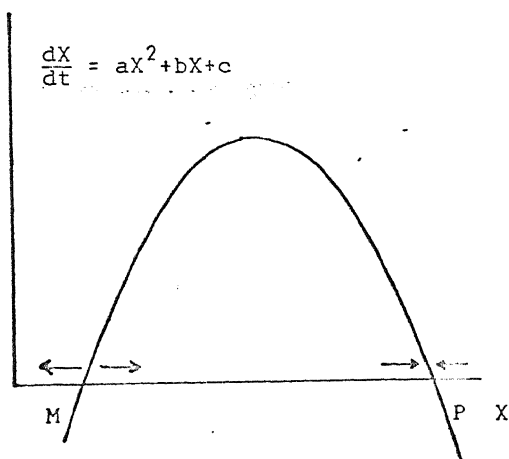
This equation has the solution (17) where  $\omega$  is given by:

$$\omega = 2a\bar{X} + b - \eta^2 D = \pm \sqrt{(b - 4ac) - \eta^2 D}. \quad (28)$$

From this equation we find that the plus sign in (25) corresponds to a stable state ( $\omega < 0$ ), whereas the minus sign denotes an unstable state (there are values of  $\eta$  for which we have  $\omega > 0$ ).

This is illustrated in fig. 3, where  $dX/dt$  is plotted versus  $X$  for a homogeneous mixture. Conditions (23) have been used here. The two stationary points ( $dX/dt=0$ ) are the points P and M.

Fig. 3: Stationary states in a homogeneous mixture for second-order chemical kinetics.



It is easily verified that the sign of  $dX/dt$  corresponding to a perturbation of  $X$  is such that in P the system will be driven back, whereas the perturbation in M will be amplified.



## Inhomogeneous stationary state

Equation (21) reduces in this case to:

$$0 = ax^2 + bx + c + D \frac{d^2x}{d\phi^2} \quad (29)$$

To obtain conditions (23) for the coefficients  $a$ ,  $b$ , and  $c$ , we study the properties of  $dx/d\phi$ . Multiplying (29) by  $dx/d\phi$  and integrating with respect to  $\phi$ , we obtain

$$k = g(x) + \frac{1}{2}D(dx/d\phi)^2 \quad (30)$$

$$\text{where } g(x) = \frac{1}{3}ax^3 + \frac{1}{2}bx^2 + cx \quad (31)$$

and  $k$  is an arbitrary constant.

From (30) we find:

$$\frac{dx}{d\phi} = \pm \sqrt{(2/D) \cdot (k - g(x))} \quad (32)$$

In fig. 4 we have plotted  $g(x)$  and  $dx/d\phi$  as functions of  $x$ , making use of our chemical condition  $a < 0$ .

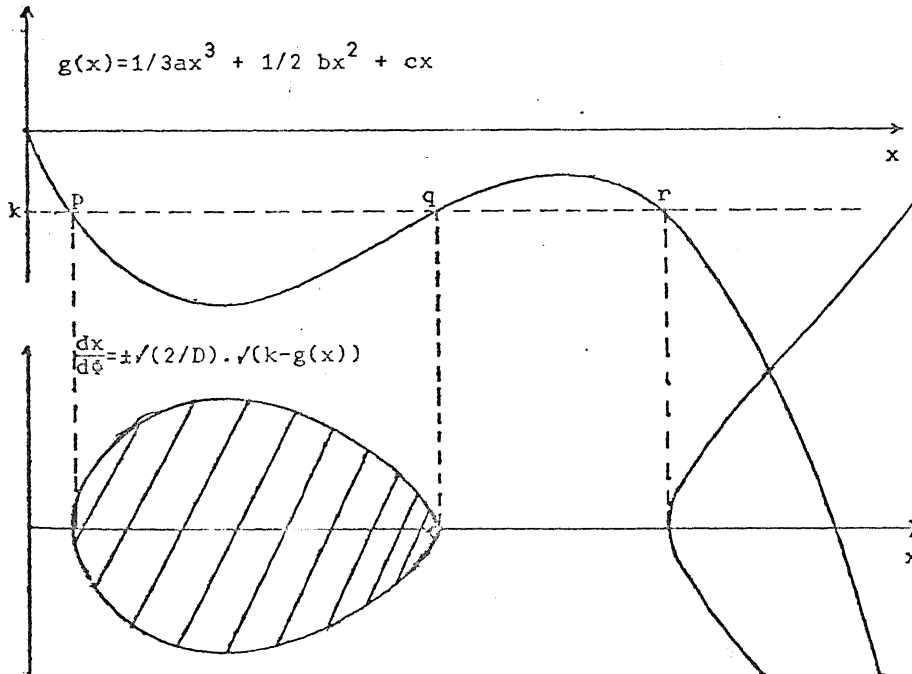


Fig. 4: Inhomogeneous stationary state for a system with second order chemical reaction kinetics. There is a  $k$ -dependent region in which the concentration gradient is bounded.

The path enclosing the dashed region in fig. 4 represents a periodic solution in the ring. To obtain the total period of this solution we solve  $\phi$  as a function of  $x$  from equation (32) obtaining

$$\phi = \pm \sqrt{(D/2)} \cdot \int_{x_0}^x \frac{d\xi}{\sqrt{(k-g(\xi))}} \quad (33)$$

The total period then becomes

$$\phi_{\text{tot}} = 2\sqrt{(D/2)} \cdot \int_p^q \frac{d\xi}{\sqrt{(k-g(\xi))}} \quad (34)$$

The condition that the solution fits into the ring is expressed as:

$$n\phi_{\text{tot}} = 2\pi \quad (n=\text{integer}) \quad (35)$$

This condition fixes the possible, discrete values of the constant  $k$ .

From fig. 4 we find the obvious physical condition that the equation  $k-g(x)=0$  should have three real roots. Also, the relative minimum of  $g(x)$  should be reached for positive value of  $x$ . This amounts to saying that both roots of the equation:  $g'(x) = ax^2 + bx + c = 0$

must be positive. In that case the product and the sum of these roots are also positive, leading to the conditions:

$$-\frac{b}{a} > 0, \quad \frac{c}{a} > 0. \quad (36)$$

From (36) and  $a < 0$  we find conditions (23).

We still have to find the solution  $x(\phi)$  that is the inverse of equation (33). To this end we write (33) in the form:

$$\phi = \pm \sqrt{(D/2)} \cdot \int_p^x \frac{d\xi}{\sqrt{((-a/3)(\xi-p)(\xi-q)(\xi-r))}} \quad (37)$$

Introducing new variables:

$$\begin{aligned} \xi &= (q-p) \sin^2 \psi + p & 0 < \psi &\leq \pi/2 \\ (q-p)/(r-p) &= m^2 \end{aligned} \quad (38)$$

we find from equation (37):

$$\phi(\psi_x) = \pm \sqrt{(6D/(-a)(r-p))} \cdot \int_0^{\psi_x} \frac{d\psi}{\sqrt{(1-m^2 \sin^2 \psi)}} \quad (39)$$

with the following relation between  $x$  and  $\Psi_x$ :

$$x = (q-p)\sin^2\Psi_x + p \quad (40)$$

The Jacobian elliptic function  $u(\Psi)$  is defined by (cf. 8)):

$$u(\Psi) = \int_0^\Psi \frac{d\theta}{\sqrt{(1-m^2\sin^2\theta)}}, \quad (41)$$

where the angle  $\Psi$  is called the amplitude:

$$\Psi = \text{am } u. \quad (42)$$

The elliptical sine (notation:  $\text{sn}$ ) is defined by:

$$\text{sn } u = \sin(\text{am } u) = \sin \Psi. \quad (43)$$

From equations (39), (40), and (41), and (43) we obtain the solution  $x(\phi)$ :

$$x(\phi) = (q-p)\text{sn}^2\{(\sqrt{-a/6D})(r-p)\phi\} + p. \quad (44)$$

We now investigate the stability of this stationary state. As before, we obtain by substituting a solution consisting of a small perturbation  $v(t, \phi)$  of  $x(\phi)$  into (21):

$$\frac{\partial v(t, \phi)}{\partial t} = (2ax(\phi) + b)v(t, \phi) + D \frac{\partial^2 v(t, \phi)}{\partial \phi^2}; \quad (45)$$

where  $x(\phi)$  is given by expression (44).

Instead of trying to find the general solution of equation (45) we use the so-called normal mode procedure 9); that is we consider perturbations  $v(t, \phi)$  of the form (47). By substituting a single component of this series into (45), we find:

$$\omega = 2ax(\phi) + b - \eta^2 D. \quad (46)$$

Solution (44) will be unstable if for some values of  $x$  and  $\eta$  we have  $\omega > 0$ . The value  $\tilde{x}$  of  $x$  at which the second derivative of  $g(x)$  (cf. 31)) vanishes, satisfies the equation:

$$g''(\tilde{x}) = 2a\tilde{x} + b = 0. \quad (47)$$

As we see from fig. 4, however, there are values of  $x$  (for instance  $x=p$ ) for which we have:

$$x < \tilde{x}$$

From (46), (47), and (48), we conclude that  $\omega > 0$ , so we have an unstable state. This result implies that no one-morphogen Turing system can exist for second-order chemical kinetics. Because in normal chemistry the probability of trimolecular

collisions is negligible, there is no need to consider reaction schemes of third or higher order. Therefore, we reach the conclusion that a one-morphogen Turing system is not possible.

#### THERMODYNAMICAL ANALYSIS

##### The general evolution criterion

The thermodynamic description of irreversible phenomena such as chemical reactions and diffusion, was restricted until recently to the so-called linear range, i.e. one had to assume linear relations between the irreversible fluxes, such as the chemical reaction rate and the diffusion flow, and the corresponding thermodynamic forces, such as the chemical affinity and the concentration gradient. (One should not confuse this thermodynamic linear range with the linear reaction kinetics previously introduced).

In the last few years, however, Glansdorff and Prigogine 5) developed their evolution criterion, which is valid in the general case, i.e. also for the non-linear thermodynamical range. The validity of this criterion depends on the assumption of 'local equilibrium', i.e. one assumes that the local form of the thermodynamic Gibbs relation still holds. This assumption can give rise to criticism, because it can be concluded from kinetic theories, such as those based on the equation of Boltzmann, that the local Gibbs relation is only valid in the linear thermodynamic range. (cf. ref. 10, Ch. IX). However, chemical reactions form an exception to this rule, as shown by Ross and Mazur 11), who proved that even for a non-linear relation between the chemical reaction rate and the chemical affinity the so-called bilinear expression for the local entropy production, which is a consequence of the Gibbs relation, remains valid. This fact makes the application of the general evolution criterion to systems in which the only non-linear transport phenomenon is a chemical reaction, particularly interesting. Since we assumed the linear Fick law for diffusion and the law of mass action for chemical reactions, the latter being non-linear except for situations very close to equilibrium, our system meets these conditions.

The above-mentioned bilinear form of the local entropy production  $\sigma$  for a system in which irreversible phenomena occur is written:

$$\sigma = dS/dt = - \sum_i J_i X_i \geq 0, \quad (49)$$

where  $S(t, \phi)$  is the local entropy,  $J_i(t, \phi)$  is a local irreversible flux, and  $X_i(t, \phi)$  the corresponding local thermodynamic force. The inequality in relation (49) is the local formulation of the second law of thermodynamics.

Integrating expression (49) over the total volume  $V$  of the system, one obtains the entropy production  $P$ :

$$P = \int_V \sigma dV \geq 0. \quad (50)$$

Within the linear range one can show that the entropy production will always diminish during the evolution of the system and reach a minimum for a stationary state (minimum entropy production theorem):

$$dP/dt \leq 0, \quad (51)$$

where the equality sign holds for a stationary state.

Relations (50) and (51) imply that the stationary state must be stable. This situation can be described by the conditions:

$$\delta P = \int_V - \sum_i (j_i \delta x_i + x_i \delta j_i) dV = 0 \quad (52)$$

and

$$\delta^2 P = \int_V - \sum_i (\delta j_i \delta x_i) dV > 0, \quad (53)$$

where  $j_i$  and  $x_i$  are the fluxes and forces in the stationary state, and  $\delta j_i$ ,  $\delta x_i$  are perturbations compatible with the kinetic equations of the system.

The stability condition (53) expresses the fact that within the linear range no physically realistic perturbations of a stationary state can develop, so that the condition (51) of diminishing entropy production is satisfied.

The minimum entropy production theorem leads to the conclusion that from a morphogenetic point of view, the linear domain of thermodynamics is of no interest, because the homogeneous stationary state, if it exists, is always stable. This means that any thermodynamic description of morphogenetic systems is inevitably a description of phenomena in the non-linear region.

In this non-linear region the inequality (50) is no longer valid. However, the general evolution criterion states that the change in the forces  $X_i$  is always such as to lower the value of the entropy production:

$$d_x P/dt = \int_V - \sum_i (J_i \cdot \frac{dX_i}{dt}) dV \leq 0, \quad (54)$$

where the equality sign holds for a stationary state.

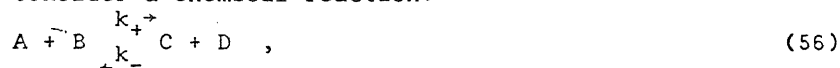
For a stationary state  $x_i, j_i$  we again obtain a stability condition:

$$\delta_x^2 P = \int_V - \sum_i (\delta j_i \delta x_i) dV > 0 \quad (55)$$

where  $\delta x_i$  are perturbations in the thermodynamic forces compatible with the kinetic equations of the system, and  $\delta j_i$  are the resulting perturbations in the irreversible fluxes.

The quantity  $\delta_x^2 P$  is called the excess entropy production.

For a system in which chemical reactions and diffusion are the only transport processes, the fluxes and thermodynamic forces appearing in the bilinear expression of the entropy production are defined as follows. Consider a chemical reaction:



where A, B, C, and D are the reactant concentrations. The chemical reaction rate  $J_r$  is defined by the law of mass action:

$$J_r = k_+ AB - k_- CD, \quad (57)$$

and the thermodynamical force of a chemical reaction by 10):

$$X_r = A/T \quad (58)$$

The chemical affinity A in expression (58) is defined by:

$$A = \mu_C + \mu_D - \mu_A - \mu_B, \quad (59)$$

where  $\mu_i$  stand for the chemical potential of species i.

For a dilute mixture under constant pressure we have the following relation between the chemical potential  $\mu_i$  and the concentration  $c_i$ :

$$\mu_i = RT \ln c_i + \mu_i^\circ \quad (60)$$

The standard chemical potential  $\mu_i^\circ$  in this expression is only dependent upon temperature T. R is the gas constant. For chemical equilibrium, we have  $J_r = A = 0$ . Using this, we find from equations (57), (59), and (60):

$$\mu_C^\circ + \mu_D^\circ - \mu_A^\circ - \mu_B^\circ = RT \ln(k_-/k_+).$$

From this equality we find, using also (58), (59), and (60):

$$X_r = \frac{A}{T} = R \ln(k_- CD / k_+ AB) \quad (61)$$

For vanishing inverse reaction constant  $k_-$  the affinity becomes infinite. In order to give a thermodynamic description of reactions such as the last three in scheme (24), we therefore assume that the reverse reaction constants do not vanish but are several orders of magnitude smaller than the forward reaction constants.

The thermodynamic force of diffusion in expression (44) is defined by:

$$X_d = \nabla(\mu/T) \equiv \frac{\partial}{\partial \phi}(\mu/T) \quad (62)$$

and the diffusion flow by:

$$J_d = -D X_d. \quad (63)$$

Application to the one-morphogen system

We now apply the general evolution criterion to the reaction scheme (24). The assumption of constant concentration of complex C (substrate-saturated enzyme) has as a consequence that no perturbations  $\delta C$  compatible with the kinetic equations are possible. The same holds true for perturbations  $\delta A$ , since reaction (14) will only result in a first-order formation of X if we assume the concentration A constant. There is also in this case no free enzyme ( $E^0$ ), so a perturbation  $\delta X$  will not cause a change in the reaction rate of the first reaction of scheme (24). Consequently only the last two chemical reactions of (24) will contribute in the chemical part of the excess entropy production. Using expressions (57) and (61), we obtain from these reactions for a perturbation  $\delta \bar{X}$  of the homogeneous stationary state  $\bar{X}$ :

$$-\sum \delta J_r \delta(A/T) = R(4k_4 \bar{X} - k_3 A) \frac{(\delta \bar{X})^2}{\bar{X}}. \quad (64)$$

To calculate the effect of diffusion, we again use the normal mode procedure. Substituting a perturbation of the type:

$$X = \bar{X} + \delta \bar{X} = \bar{X} + \xi \exp(\omega t) \cdot \exp(i n \phi) \quad |\xi/\bar{X}| \ll 1, n \text{ real},$$

and using also (63), (62), and (60) for the contribution to the excess entropy production due to diffusion (the complex conjugate is indicated by a bar), we obtain:

$$-\delta J_d \delta X_d = D(\delta X_d)(\overline{\delta X_d}) = D R^2 n^2 \frac{(\delta \bar{X})^2}{\bar{X}^2}. \quad (65)$$

As we see, this contribution is positive. This indicates that diffusion has a stabilizing effect, which is a general feature. On the other hand, without diffusion no inhomogeneous stationary state would be possible.

Adding the contributions due to chemical reactions and to diffusion, we obtain the following expression for the excess entropy production, dropping the integral signs:

$$\delta_x^2 P = R(4k_4 \bar{x} - k_3 A + D\eta^2 R/\bar{x}) \frac{(\delta \bar{x})^2}{\bar{x}} \quad (66)$$

As we see, the only negative, i.e. destabilizing, contribution comes from the autocatalytic reaction. We have an unstable homogeneous stationary state when the following inequality is satisfied:

$$4k_4 \bar{x} - k_3 A + D\eta^2 R/\bar{x} \leq 0 \quad \text{or} \quad k_3 A - 4k_4 \bar{x} > 0. \quad (67)$$

From (21), (24), and (25), we find:

$$\bar{x} = \frac{k_3 A \pm \sqrt{(k_3 A)^2 - 4k_2 k_4 C}}{2k_4} \quad (68)$$

Substituting (68) into (67) we obtain:

$$k_3 A \pm 2/\{(k_3 A)^2 - 4k_2 k_4 C\} < 0 \quad (69)$$

For the plus sign in (69), the inequality is clearly impossible. So this must be a stable state, which is in agreement with our mathematical analysis. However, for the minus sign the inequality (69) will only be satisfied, i.e. we will only have an unstable state, if:

$$4k_2 k_4 C < \frac{1}{4}(k_3 A)^2. \quad (70)$$

This condition is more restrictive than the previously obtained condition (26). Here, we have a discrepancy between the results obtained by mathematical analysis and by the present application of the general evolution criterion.

For  $\frac{1}{4}(k_3 A)^2 < 4k_2 k_4 C < (k_3 A)^2$  we would incorrectly conclude from the general evolution criterion that both stationary states are stable. In the next section a possible explanation of this discrepancy is investigated.

#### Application to the three-morphogen system

In accordance with our assumption of a substrate-saturated enzymatic reaction, in the derivation given above, we neglected the fluctuations in the concentrations of enzyme E and complex C. Since, however, the general evolution criterion

describes essentially the evolution of fluctuations, it could be argued that in doing so we restrict the class of allowed perturbations in a critical way. This means that the general expression for  $\delta_x^2 P$  obtained when fluctuations in E

and C are included would not approach to expression (66) in the limit of a substrate-saturated enzymatic reaction. In the following we will show that this is not the case. To do so, we now also allow for fluctuations  $\delta C$  and  $\delta E$ ; that is, we drop the assumption of constant concentration of complex C. The reaction scheme (24) in that case describes a three-morphogen system with the following kinetic equations:



$$\begin{aligned}
\frac{\partial X}{\partial t} &= -k_{+1}XE + k_{-1}C + k_3AX - k_4X^2 + D_x \frac{\partial^2 X}{\partial \phi^2}, \\
\frac{\partial C}{\partial t} &= -(k_{-1} + k_2)C + k_{+1}XE + D_c \frac{\partial^2 C}{\partial \phi^2}, \\
\frac{\partial E}{\partial t} &= (k_{-1} + k_2)C - k_{+1}XE + D_e \frac{\partial^2 E}{\partial \phi^2}.
\end{aligned} \tag{71}$$

We introduce the symbol  $S$  for the sum of the concentrations of enzyme and complex:

$$S = E + C. \tag{72}$$

From equations (71) and (72), we obtain:

$$\begin{aligned}
\frac{\partial X}{\partial t} &= -k_{+1}X(S-C) + k_{-1}C + k_3AX - k_4X^2 + D_x \frac{\partial^2 X}{\partial \phi^2}, \\
\frac{\partial C}{\partial t} &= k_{+1}X(S-C) - (k_{-1} + k_2)C + D_c \frac{\partial^2 C}{\partial \phi^2}, \\
\frac{\partial S}{\partial t} &= (D_c - D_e) \frac{\partial^2 C}{\partial \phi^2} + D_e \frac{\partial^2 S}{\partial \phi^2}.
\end{aligned} \tag{73}$$

To find the stationary homogeneous states, we notice that every solution  $\bar{s}$  satisfies equation (73). This leaves us with  $\bar{s}$  as an arbitrary parameter in the following equations for  $\bar{x}$  and  $\bar{c}$ :

$$\begin{aligned}
0 &= -k_{+1}X(\bar{s} - \bar{c}) + k_{-1}\bar{c} + k_3A\bar{x} - k_4\bar{x}^2, \\
0 &= k_{+1}\bar{x}(\bar{s} - \bar{c}) - (k_{-1} + k_2)\bar{c}.
\end{aligned} \tag{74}$$

From equations (74) we find the homogeneous stationary states:

$$\bar{x} = \frac{k_3A - k_4K_m \pm \sqrt{(k_3A + k_4K_m)^2 - 4k_2k_4\bar{s}}}{2k_4} \tag{75}$$

$$\bar{c} = \frac{\bar{x} \cdot \bar{s}}{K_m + \bar{x}} \tag{76}$$

with  $K_m \equiv (k_{-1} + k_2)/k_{+1}$  (Michaelis constant).

Equation (75) has different real positive solutions when the following conditions are satisfied:

$$\begin{aligned}
(k_3A + k_4K_m)^2 &> 4k_2k_4\bar{s}, \\
k_3A &> k_4K_m, \\
k_2\bar{s} &> k_3K_mA.
\end{aligned} \tag{77}$$

From equation (76) we find that in the limit:

$$\frac{K_m}{\bar{X}} \rightarrow 0 \quad (78)$$

we obtain

$$\bar{c} = \bar{s} \quad (79)$$

In other words, we have a substrate-saturated enzyme. In that case, we also find that (75) reduced to expression (68) and the first condition (77) to condition (26). The remaining conditions (77) are automatically satisfied in this case. We now calculate the excess entropy production in the same way as before, but this time also allowing for fluctuations  $\delta S$  and  $\delta C$ . For the critical chemical contribution to  $\delta^2 P$ , we find:

$$\begin{aligned} -\delta J_r \delta(A/T) = & R\{4k_4 \bar{x} - k_3 A - k_{+1}(\bar{s} - \bar{c})\} \frac{(\delta \bar{x})^2}{\bar{x}} - \frac{Rk_{+1} \bar{x}}{\bar{s} - \bar{c}} (\delta \bar{s})^2 - \\ & \frac{R\bar{s}(k_{+1} \bar{x} + k_{-1} + k_2 \bar{c})}{\bar{c}(\bar{s} - \bar{c})} (\delta \bar{c})^2 + \frac{R\{k_{+1} \bar{x}(\bar{c} + \bar{s}) + k_{-1} \bar{c}\}}{\bar{x} \bar{c}} \delta \bar{x} \delta \bar{c} + \\ & \frac{R\{k_{+1} \bar{x}(\bar{s} + \bar{c}) + k_{-1} \bar{c}\}}{\bar{c}(\bar{s} - \bar{c})} \delta \bar{s} \delta \bar{c} - 2Rk_{+1} \delta \bar{x} \delta \bar{s} \end{aligned} \quad (80)$$

To draw any conclusion from this equation, we must have relations between the fluctuations  $\delta \bar{x}$ ,  $\delta \bar{c}$ , and  $\delta \bar{s}$ . Again, we use the fact that to become macroscopically important, the fluctuations must be such that the kinetic equations (73) are satisfied. We use the normal-mode procedure by taking the following expressions for  $X$ ,  $E$ , and  $S$ :

$$\begin{aligned} X &= \bar{x} + \xi \cdot \exp(\omega t) \cdot \exp(i\eta\phi), \\ C &= \bar{c} + \gamma \cdot \exp(\omega t) \cdot \exp(i\eta\phi), \\ S &= \bar{s} + \sigma \cdot \exp(\omega t) \cdot \exp(i\eta\phi), \end{aligned} \quad (81)$$

where the perturbations satisfy the following conditions:

$$|\xi/\bar{x}| \ll |\gamma/\bar{c}| \ll |\sigma/\bar{s}| \ll 1.$$

Substitution of (81) into equations (73) gives us after linearization for the critical value  $\omega=0$ , a set of equations from which we find the following relations between  $\xi$ ,  $\gamma$ , and  $\sigma$ :

$$\gamma = \{(\bar{s} - \bar{c})/(\bar{x} D_c / D_e + K_m + \eta^2 D_c / k_{+1})\} \xi \quad (82)$$

$$\sigma = \{(D_e - D_c)/D_e\} \gamma \quad (83)$$

From these equations we find that in the limit of a substrate-saturated enzymatic reaction, that is when conditions (78) and (79) are satisfied,  $\gamma$  and  $\sigma$  vanish, which means that no fluctuations in  $C$  and  $S$  such that the kinetic equations hold, are possible.

Also, by substitution of relations (82) and (83) into (80) we find that the chemical part in the excess entropy production reduces in the limit (78) to expression (66). Consequently the discrepancy in the result obtained from the general evolution criterion, remains unsolved. Further investigation of this point is needed.

From the first part of this paper we conclude that to find the minimal requirements for a Turing system, we have to study two-morphogen systems. This considerably wider field is presently studied.

The authors gratefully acknowledge the stimulating discussions with the members of the group for biomathematics from the Mathematical Centre in Amsterdam.

#### REFERENCES

1. Turing, A.M., Phil.Trans.Roy.Soc. London B 237 (1952) 37.
2. Herschkowitz, M., Compt.Rend., 270, Série C, (1970) 1049.
3. Zaikin, A. and Zhabotinsky, A., Nature 225, 535, (1970).
4. Winfree, A.T., Science 175, 634, (1972).
5. Glansdorff, P. and Prigogine, I., Thermodynamic theory of structure, stability and fluctuations. Wiley, London, 1971.
6. Hemker, P.W., Hermens, W.Th. and Hemker, H.C., F.E.B.S. Abstracts, no. 217 and 218, (1971).
7. Schultze, H.E. and Heremans, J.F., Molecular biology of human proteins. Elsevier, Amsterdam, 1966.
8. Abramowitz, M. and Segun, I., Handbook of mathematical functions, p. 569, Dover, New York, 1968.
9. Chandrasekhar, S., Hydrodynamic and hydromagnetic stability, Clarendon Press, Oxford, 1961.
10. Groot, S.R. de and Mazur, P., Non-equilibrium thermodynamics, North Holland, Amsterdam, 1962.
11. Ross, J. and Mazur, P., J.Chem. Phys. 35, 634, (1972).