Dissipative structures in a two-cell system: Numerical and experimental approaches

(bifurcations/compartmentation/hysteresis/thylakoids)

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ABSTRACT It has been shown that the coupling between the photoreduction of the oxidized form of dichloroindophenol (an artificial electron acceptor) by thylakoids and the incident light intensity can lead to the appearance of multiple steady states when the system is operated under open conditions. In the present work, a numerical study and experimental evidence are presented on the occurrence of dissipative structures in an arrangement of two continuously stirred tank reactors with mutual mass exchange of dichloroindophenol through an inert membrane. The stable spatial structures are generated by the creation of transient internal and external asymmetries. A nontrivial hysteresis effect between symmetric and asymmetric stable steady states has been observed.

The use of well-stirred compartments to model cells rests on the assumption that transport into the cell is membrane-limited (1, 2). The validity of this assumption appears reasonable for a broad class of solutes. Thus, groups of intercommunicating compartments with internal mass exchange and nonlinear reactions are widely used as models for cell growth in morphogenesis, multicellular-organism differentiation, and numerous physiological and pharmacokinetic systems. In particular, they emphasize the influence of mass transport between neighboring cells (or compartments) or between cells and the surrounding medium on the biochemical processes taking place within each individual cell.

In 1952, Turing (3) presented a theoretical description that dealt with the connection between fictitious autocatalytic chemical reactions and compartmentation. It was pointed out that the interaction of diffusive transport and nonlinear chemical kinetics can lead to instabilities of a concentration field, with the result that initially homogeneous domains develop regional nonuniformities. The idea that chemical reaction and diffusion can give rise to stable spatial patterns was further developed by Prigogine and co-workers (4–6). Scriven and co-workers (7–9) and Martinez and Baer (10, 11) have extended and generalized the work of Turing and thus far have analyzed the onset to instability in arbitrary networks of compartments. The coexistence of multiple stationary solutions with properties similar to those described by Martinez and Scriven has been found also in distributed reaction–diffusion systems (12–16).

Few models are concerned with enzyme kinetics that often exhibit important nonlinearities and play an important role in biological processes. Bunow and Colton (17) have modeled the collective kinetic behavior of a linear array of cells containing a substrate-inhibited enzyme in which each cell is considered as a well-stirred compartment surrounded by a semipermeable membrane. Over prescribed ranges of reservoir concentrations, multiple stable steady states can occur, some of which are characterized by asymmetric pro-

files of concentration and reaction rate across the array. Similar results were obtained by Aris and Keller (18) and Bailey and Luss (19) for the case of a porous membrane with an active layer of enzyme on each surface; this system obeys substrate inhibition kinetics.

From the experimental point of view, only one system has been reported in which stable asymmetric profiles in an arrangement of interconnecting cells have been observed (20). The chemical system under study was the Belousov–Zhabotinsky reaction (21). Various combinations of upper and lower stationary states in individual cells were observed. The study of the coupling between a photochemical reaction, the reduction of dichloroindophenol (DCIPox) by thylakoids, and the excitation light intensity has been reported (22). The existence of multiple steady states in a single open reactor has been shown experimentally. This present paper deals with the numerical study and the experimental observation of nonmonotonic concentration profiles in two identical, coupled open reactors with mutual diffusion through an inert membrane. The asymmetric steady states are experimentally obtained by applying transient variations on the parameters of the system.

MATERIALS AND METHODS

Preparation of Thylakoids. Thylakoids were prepared from fresh lettuce (Lactuca sativa, var. romaine) in a sorbitol buffer (pH 7.6) by the procedure of Epel and Neumann (23).

Immobilization Procedure. The immobilization process, based on a method using a calcium alginate gel (24), was modified to obtain films rather than beads. The thylakoid suspension was mixed with an equal volume of a 4% sodium alginate solution in sorbitol buffer (330 mM, pH 7.6). Aliquots were spread out on nylon nets and pressed between two glass plates. Each membrane was frozen at −20°C for 15 min, then immersed in a cold solution of 0.1 M CaCl₂, and finally stored at +4°C.

Immobilized Photosystem Activity Measurements. The photosystem activity was determined by following the photoreduction of DCIPox. The overall Hill reaction is represented as

$$2H_2O + 2DCIPox_{\text{thylakoids}} \overset{4hv}{\longrightarrow} 2DCIP_{\text{red}} + O_2.$$ 

Under our experimental conditions, the DCIPox exhibits an absorption peak centered at 600 nm (ε₀₀₀ = 17.9 mM⁻¹·cm⁻¹). No detectable absorbance of the reduced form (DCIPred) is measured for wavelengths between 500 and 800 nm. All measurements were carried out in a modified extraction buffer with no phosphate, 50 mM CaCl₂, and 5 mM MgCl₂.

Abbreviations: DCIP, dichloroindophenol; CSTR, continuously stirred tank reactor; bp, bifurcation points; lp, limit points.
Absorbed; thus, light (600 koid membranes DCIPOX solution branes) both coefficient, through which reactors (R tank provides light thylakoid bilized membranes). Both concentrations are filled with DCIPoX at concentration A0 and emptied (A + AH2) at the same flow rate, D. The outlet solution absorbance is recorded at either 600 or 670 nm depending on the concentration of DCIPoX. Each cell is illuminated with a red light (600 nm < λ < 800 nm) at intensity I0. When traversing the DCIPoX solution (depth X = 2.5 cm), a part of the incident light is absorbed; thus, the actual light intensity impinging upon the thylakoid membranes equals I, with I < I0. N2, bubbling nitrogen.

**Experimental Device.** The experimental system, described in Fig. 1, is composed of two identical continuously stirred tank reactors (CSTRs) containing the DCIPoX substrate solution connected by an inactive cellophane membrane. Immobilized thylakoid membranes are fixed at the bottom of each of the two CSTRs. The incidence of illumination is normal with respect to the immobilized thylakoids, and the source consists of an incandescent halogen lamp (100 W, 12 V; Spindler and Hoyer, K. G.). Two filters are placed between the light source and the thermostated reactors: an IR filter that absorbs radiation beyond 800 nm and a red filter that provides a sufficient cutoff of radiation below 580 nm. Under these conditions, the light intensity is initially absorbed by the DCIPoX in the solution, according to a classical Beer–Lambert relationship. The resultant light intensity at the membrane–solution interface excites the thylakoid system, leading to the reduction of the DCIPoX. The outlet DCIPoX concentration is followed spectrophotometrically (Beckman DBT) at two wavelengths: 600 nm for DCIPoX concentrations between 0 and 0.01 mM and 670 nm (ε670 = 9.9 mM⁻¹ cm⁻¹) for DCIPoX concentrations between 0.01 mM and 0.20 mM. All experiments were carried out at 23°C under continuous rapid stirring and nitrogen bubbling to avoid spontaneous reoxidation of DCIPoX by oxygen.

**Numerical Methods.** All the parameter-dependence diagrams presented in this work were obtained by using the software package AUTO, a program for the automatic bifurcation analysis of autonomous systems (25).

**DESCRIPTION OF THE MODEL**

**Derivation of the Reaction Term Under Closed Conditions.** The rate of photoreduction of DCIPoX without absorption of light has been studied as a function of both the DCIPoX concentration, A, and the light intensity, at the surface of the thylakoid membrane, I. The global behavior of the system can be expressed by the following phenomenological reaction rate (26), which accounts for the observed experimental data

\[
f(A, I) = V \cdot IA^3/(K_1^3A + IA^3 + K_2I^3),
\]

where \( V \) denotes the activity at saturating \( A \) and \( I \). The constants \( K_a \) and \( K_i \) refer to the concentration and intensity, respectively, for which the activity is half-maximal. The best fits in a nonlinear regression analysis were obtained for \( K_a \) and \( K_i \) equal to 1.4 mM and 35 W·m⁻², respectively. At this stage, it is noteworthy that no mechanistic interpretation at either molecular or electronic level is intended. In this case, as the light is not passing through the DCIPoX solution, the intensity, \( I \), is equal to the incident intensity, \( I_0 \). In contrast, when the light is traversing the solution before impinging upon the membrane, the two intensities are related through the Beer–Lambert law

\[
I(X) = I_0 \exp(-\varepsilon X A),
\]

in which \( \varepsilon \) is the effective absorption coefficient, \( X \) is the thickness of the solution traversed, and \( A \) is the concentration of the light-absorbing molecule. However, this law is only valid under conditions where the incident light is monochromatic and collimated. In our experimental situation, this condition is obviously not valid, as the system is illuminated with wavelengths between 600 and 800 nm. Nevertheless, a satisfactory approximation could be obtained by using the sum of two exponential terms.

\[
I = I_0[a \exp(-\varepsilon_1 X A) + (1 - a) \exp(-\varepsilon_2 X A)],
\]

with \( a = 0.76 \), \( \varepsilon_1 = 13.2 \text{ mM}^{-1} \cdot \text{cm}^{-1} \) and \( \varepsilon_2 = 0.92 \text{ mM}^{-1} \cdot \text{cm}^{-1} \).

Finally, the expression of the photoreduction rate, \( f(A, I) \), is a combination of Eqs. 1 and 3.

**The Two-Cell System Under Open Conditions.** The evolution of the DCIPoX concentration, \( dA/dT \), in each compartment will be the result of (i) its flow in and out of the CSTR (Eq. 4), (ii) its diffusion across the membrane (Eq. 5), and (iii) its consumption by the reaction:

\[
\frac{dA_1}{dT}_{\text{flow}} = \frac{D}{v_r}(A_0 - A_1),
\]

where \( A_0 \) is the DCIPoX inlet concentration, \( D \) is the flow rate, and \( v_r \) is the volume of the cell.

Throughout the following treatment, subscripts 1 and 2 related to \( A \) (and \( I \)) will refer to cell 1 and 2, respectively.

\[
\frac{dA_1}{dT}_{\text{diff}} = \frac{D_A \Omega}{v_r} (A_2 - A_1),
\]

where \( D_A \) is the diffusion coefficient of species \( A \) across the membrane and \( \varepsilon \) and \( \Omega \) are the thickness and the surface area of the membrane separating the two cells, respectively. Thus,

\[
\frac{dA_1}{dT} = \frac{D}{v_r}(A_0 - A_1) + \frac{D_A \Omega}{v_r} (A_2 - A_1) - f(A_1, I).
\]

Similarly, the expression of \( dA_2/dT \) is obtained by permuting subscripts 1 and 2 in Eq. 6.

Finally, by using the following dimensionless variables

\[
t = T \frac{D}{v_r}, \quad a = \frac{A}{K_a}, \quad i = -\frac{I}{K_i}, \quad \text{and} \quad x = \varepsilon_1 K_a,
\]

the evolution of \( A \) in each compartment will be governed by the pair of differential equations:

\[
\begin{align*}
\frac{da}{dt} & = \frac{D}{v_r}(a_0 - a) + \frac{D_A \Omega}{v_r} (a_2 - a) - f(a, i), \\
\frac{di}{dt} & = \frac{D}{v_r}(-a + \alpha) + \frac{D_A \Omega}{v_r} (a_2 - a) - f(a, i).
\end{align*}
\]
\[ \frac{da_1}{dt} = (a_0 - a_1) + \lambda(a_2 - a_1) - \sigma f(a_1, i) = G(a_1, a_2) \]  
\[ \frac{da_2}{dt} = (a_0 - a_2) + \lambda(a_1 - a_2) - \sigma f(a_2, i) = H(a_1, a_2) \]

with
\[ f(a) = ai^3/(i^3 + a + ai^3) \]
and
\[ i = i_0 \text{exp}(-ax) + (1 - a) \text{exp}(-kx); \quad k = \frac{\varepsilon_2}{\varepsilon_1}. \]

In Eqs. 7 and 8,
\[ \lambda = \frac{D \Omega}{D\varepsilon} \quad \text{and} \quad \sigma = \frac{V \cdot \varepsilon_2}{K_a D}. \]

RESULTS AND DISCUSSION

Under steady-state conditions, for \( G(a_1, a_2) = H(a_1, a_2) = 0 \), a plot of \( a_1 \) (or conversely \( a_2 \), by symmetry arguments) as a function of \( a_0 \) is given in Fig. 2. The S-shaped primary curve (as indicated by "PC") contains two stable branches and a single unstable branch. The solutions along the primary curve are symmetric (i.e., \( a_1 = a_2 = a \)). Thus, these steady states are simply solutions of
\[ (a_0 - a) - \sigma f(a, i) = 0. \]

Consequently, the curve is invariant with respect to \( \lambda \), the ratio of transport terms for the system.

In this system of two coupled cells, for \( a_1 \) and \( a_2 \) concentrations not equal, the interesting new feature is the emergence of a secondary closed curve. This secondary curve intersects the primary curve at two bifurcation points (bps) located on the unstable branch of the primary curve (bp1 and bp2). This bifurcated curve consists of asymmetric solutions (\( a_1 \neq a_2 \) except at the bps) of which those portions of the curve between limit points (lps) lp1 and lp2 on the upper branch and lp3 to lp4 on the lower branch are stable. Consequently, given a particular value of \( a_0 \) (for example, \( a_0 = 0.134 \) for \( \lambda = 0.1 \) as indicated in Fig. 2), the system may exhibit four different stable solutions: two symmetric steady states (\( \triangle \) and \( \blacktriangle \) in Fig. 2), \( a_1 = a_2 \) located on the upper and lower stable branches of the primary curve and two asymmetric steady states (\( \square \) in Fig. 2). Although \( a_1 \) was chosen as the ordinate variable in Fig. 2, \( a_2 \) would likewise have been sufficient since the two asymmetric steady states are related through Eqs. 7 and 8. The same qualitative behavior was observed when \( i_0 \), rather than \( a_0 \), was taken as the control parameter.

As these asymmetric stable steady-states do not arise spontaneously in such a system, it was necessary to develop methods to reach these asymmetric states experimentally. We chose to focus on the variation of internal parameters (\( a_1 \) and \( a_2 \)) or on the perturbation of the external parameters of the system (\( a_0 \) and \( i_0 \)).

Perturbation of Internal Parameters. In this case, both cells are held at the same constant external concentration, \( a_0 \), and incident light intensity, \( i_0 \). The basins of attraction corresponding to each stable steady state, symmetric (\( \triangle \), \( \blacktriangle \)) or asymmetric (\( \square \)), can be determined by tracing the "nullclines" \( G(a_1, a_2) = H(a_1, a_2) = 0 \) in the phase plane, as shown in Fig. 3. Consequently, the choice of an initial \( (a_1, a_2) \) concentration couple will determine the final steady state of the system. By using this approach, asymmetric steady states are thus observed experimentally (Fig. 4).

\[ \frac{da_1}{dt} = (a_0 - a_1) + \lambda(a_2 - a_1) - \sigma f(a_1, i), i_0 = 0.91, \lambda = 0.10. \]

Heavy solid and dashed lines show the stable and unstable solutions of the primary curve (PC). Light solid and dashed lines show stable and unstable solutions of the secondary bifurcated (SC). That secondary curve intersects the primary one at two bps (\( \bullet \), bp1 and bp2). The portions of the secondary curve bounded by lps (\( \square \) lp1-lp2 and lp3-lp4 consist of stable asymmetric solutions. As an illustration, symmetric (\( \triangle \), \( \blacktriangle \)) steady states of the system are represented for the particular value of \( a_0 = 0.134 \) (vertical arrow on abscissa).

Perturbation of External Parameters. The initial conditions are such that the \( a_1 \) and \( a_2 \) concentration and the boundary conditions, \( a_0 \) and \( i_0 \), are symmetric. Our purpose is to observe the behavior of the system when a transient perturbation on either \( a_0 \) or \( i_0 \) is applied at one cell boundary. For this study, Eqs. 7 and 8 have been rewritten as follows:

\[ \frac{da_1}{dt} = (a_0 - a_1) + \lambda(a_2 - a_1) - \sigma f(a_1, i_0) \]

\[ \frac{da_2}{dt} = [(a_0 + \mu) - a_2] + \lambda(a_1 - a_2) - \sigma f[a_2, (i_0 + \xi)], \]

where \( \mu \) and \( \xi \) stand for the perturbations upon \( a_0 \) and \( i_0 \), respectively, and may take any positive or negative value such that the parameters retain physical significance. We consider the case where \( \xi = 0 \) and \( \mu \) is varied; all other parameters are held constant. The steady-state solutions of

\[ \text{FIG. 2. Steady-state solutions } a_1(a_2) \text{ of Eqs. 7 and 8 as a function of } a_0 \text{ for parameter values as follows: } \lambda = 7.1, i_0 = 0.91, \text{ and } \lambda = 0.10. \]

\[ \text{FIG. 3. Plot of the nullclines } G(a_1, a_2) = H(a_1, a_2) = 0 \text{ in the phase plane for } a_1 \text{ and } a_2 \text{ retaining physical significance. Domains } \text{(dashed area), } \text{ and } \text{ represent the basins of attraction corresponding to the stable asymmetric (c) and symmetric (\( \triangle \), \( \blacktriangle \)) steady states, respectively. The separatrices are symbolized by dotted curves. Parameter values are as follows: } \lambda = 0.1; \sigma = 7.1; a_0 = 0.143; \text{ and } i_0 = 0.91. \]
Fig. 4. Time evolution of concentrations of A in compartments 1 and 2 (A1 and A2), under symmetrical boundary conditions: the input concentrations and light intensities are $A_{1.0} = A_{2.0} = 0.2$ mM ($a_0 = 0.143$) and $I_{o,1} = I_{o,2} = 50$ W·m$^{-2}$ ($I_0 = 0.91$), respectively. The initial concentrations are 0.035 mM ($a_0 = 0.025$) in compartment 1 and 0.17 mM ($a_2 = 0.12$) in compartment 2. Under these conditions, the system reaches an asymmetrical steady state—that is, $A_1 = 0.035$ mM ($a_1 = 0.025$) and $A_2 = 0.162$ mM ($a_2 = 0.116$). The other parameter values are the same as in Fig. 3: $\sigma = 7.1$ and $\lambda = 0.1$.

Eqs. 10 and 11 with respect to $a_1$ and $a_2$ as a function of $\mu$ are given in Fig. 5. Each curve gives the steady-state concentration, $a$, in each compartment when one external concentration is varied ($\mu = a_2 - a_1$). Each curve is composed of nine branches, four of which are stable.

The stable steady-state solutions $a_1$ and $a_2$ corresponding to the unperturbed system are obtained for $\mu = 0$. The corresponding four solutions denoted as $S_1$, $B_1$, $B_2$, and $S_2$ are identical to those presented in Fig. 2 ($a_0 = 0.143$). Clearly, $S_1$ and $S_2$ are the stable upper and lower solutions ($a_1 = a_2$), while $B_1$ and $B_2$ are the stable structured (asymmetric) solutions ($a_1 \neq a_2$).

This diagram indicated how to vary $\mu$ in order for the system to reach $B_1$ and $B_2$ when starting from $S_1$ (or $S_2$): by decreasing $\mu$, $a_1$ (respectively, $a_2$) will decrease monotonically down to the limit points $C_1$ (respectively, $C_2$) ($\mu = 0$).

Fig. 5. Steady-state solutions $a_1$ and $a_2$ when $\mu$ is varied. Steady-state solutions $a_1$ and $a_2$ are represented by light (solid and dot-dashed) and heavy (solid and dashed) lines, respectively. The symmetrical steady states $S_1$ (a) and $S_2$ (b) and the asymmetrical ones $B_1$ and $B_2$ (c) are represented for $\mu = 0$—that is, for the unperturbed system. The path followed by the steady state $a_1$, when $\mu$ is consecutively decreased and increased, is symbolized by arrows: a complete hysteresis loop will be described by following first the path $S_1 \rightarrow C_1 \rightarrow D_1 \rightarrow B_1$ (asymmetrical steady state) and next $B_1 \rightarrow E_1 \rightarrow F_1 \rightarrow S_1$. Simultaneously, the steady state $a_2$ will follow the path $S_1 \rightarrow C_2 \rightarrow D_2 \rightarrow B_2 \rightarrow E_2 \rightarrow F_2 \rightarrow S_1$, in which $B_2$ is the asymmetrical steady-state associated to $B_1$.

Fig. 6. Time evolution of the DCIP$_{oa}$ concentrations in compartments 1 and 2 after a perturbation upon $A_1$ (Fig. 5) or $I_0$ at one cell boundary. (Upper) Prior to the perturbation, the input concentrations and light intensity are $A_{1.0} = A_{2.0} = 0.2$ mM ($a_0 = 0.143$) and $I_{o,1} = I_{o,2} = 50$ W·m$^{-2}$ ($I_0 = 0.91$), respectively. The initial concentrations within the cells are $A_1 = A_2 = 0.17$ mM ($a_1 = a_2 = 0.121$). Under these conditions, symmetrical steady-state concentrations are obtained, that is $A_1 = A_2 = 0.165$ mM ($a_1 = a_2 = 0.117$). This situation corresponds to the upper symmetrical solution of the system. Then, an external perturbation, $\mu$, is applied on compartment 1 (Arrow I) $A_{1.0}$ is decreased down to 0.1 mM ($\mu = -0.07$), $A_{2.0}$ remaining unmodified. Five hours later, the perturbation is removed (Arrow II). So, although the symmetry of external conditions is restored, steady asymmetric concentration between the two compartments may be observed: $A_1 = 0.04$ mM ($a_1 = 0.028$) and $A_2 = 0.165$ mM ($a_2 = 0.12$). (Lower) The initial boundary conditions are the same as in Upper. In that experiment, the initial concentrations in the two cells are $A_1 = A_2 = 0.035$ mM ($a_1 = a_2 = 0.025$). Parameters $\sigma$ and $\lambda$ have the respective values of $8.7$ and $0.11$. After the symmetrical steady-state concentrations (lower symmetrical solution of the system) are obtained ($A_1 = A_2 = 0.018$ mM; $a_1 = a_2 = 0.013$), illumination above compartment 1 is suppressed ($\xi = -0.9$). It is restored after about 6 hr ($\xi = 0$). As in the previous experiment, asymmetrical steady concentrations may be observed: $A_1 = 0.12$ mM ($a_1 = 0.086$) and $A_2 = 0.03$ mM ($a_2 = 0.021$). Let us remark that in these two experiments, the choice of either the upper or the lower symmetrical steady state is without any effect on the qualitative response of the perturbed system. The only consequence will be an alternation between asymmetric steady states $A_1$ and $A_2$. 

Beyond this critical value, $a_1$ and $a_2$ will jump simultaneously to the lower stable steady states $D_1$ and $D_2$, respectively. When $\mu$ will be returned to the value of zero, $a_1$ (respectively, $a_2$) follows to a steady state concentration defined by point $B_1$ (respectively, $B_2$).

An experiment carried out with the same parameter values as in Fig. 5 (that is, $\sigma = 7.8$, $\lambda = 0.1$, $a_0 = 0.113$, and $\Delta_0 = 0.91$) is shown in Fig. 6 Upper. The same qualitative behavior can be observed (Fig. 6 Lower) by varying the incident light intensity above one cell ($\mu = 0$; $-0.9 < \xi < 0$). In these two experiments, the creation of a sufficiently large external perturbation on one cell's boundary leads to the appearance of an asymmetry of the concentration between the two compartments. This asymmetry remains even after suppression of the perturbation.

The bifurcation diagram shown in Fig. 5 also suggests that the only way for the system to return to its initial symmetric state $S_1$ is to vary parameter $\mu$ so that $\mu > 0$, allowing $a_1$ and $a_2$ to exceed limit points $E_1$ and $E_2$ and to jump to points $F_1$ and $F_2$, respectively. It is possible, by then decreasing $\mu$ back to a value of 0, that $a_1$ and $a_2$ will return to point $S_1$.

The successive different steady states $(a_1$ and $a_2$) reached in each cell when $\mu$ is increased and decreased are of particular interest. In Fig. 7, the difference that exists between the concentrations of species A in each cell under steady-state concentration $(a_2 - a_1)$ is plotted versus the $\mu$ perturbation on the input concentration of cell 1. Letters $S$ through $F$ have the same meaning as in Fig. 5. A hysteresis loop ($C \rightarrow D \rightarrow E \rightarrow F$) is described when $\mu$ is decreased and then increased. Hence, the system may switch from a symmetric to an asymmetric situation. The steady states on both branches $(D \rightarrow E$ and $C \rightarrow F)$ are asymmetric, except for $\mu = 0$ on the lower branch (point $S$). Nevertheless, only the branch $D \rightarrow E$ is of a dissipative nature; the asymmetry between the two steady states $(a_1 \neq a_2)$ will be maintained, even if the symmetry of the boundary conditions is restored.

CONCLUSION

Our mathematical description, although related to a precise experimental situation, may be applied to various other physical arrangements without altering our conclusions regarding the existence of stable nonhomogeneous distributions of reactants.

In particular, our modeling is consistent with the descriptions and the conclusions drawn by Aris and Keller (18) and Bailey and Luss (19). In addition, a detailed analysis of bifurcation diagrams allowed us to partly answer the question asked by these authors and related to the transient conditions that may give rise to these stable asymmetrical steady states. In the fields of biochemistry, numerous mechanisms are capable of giving multiple steady states in diffusion-limited kinetics, primarily by the production or consumption of acids or bases combined with the sensitivity of enzymes to pH (27, 28) or by the reversible inhibition of enzyme activity by excess of substrate.

In the system presented here, the nonlinearity in the reaction term is not an intrinsic property of the reaction or the catalyst but arises from the association of two processes that, when taken separately, are monotonous functions of the concentrations of the reactants. Creel and Ross (29) and Zimmermann and Ross (30) have described photochemical systems qualitatively similar. Thus, the phenomena we describe in this paper are able to occur with many systems containing the same type of nonlinearities. Therefore, it is possible that the generation of sustained asymmetries is a general and widespread phenomenon, which may be involved (even partly) in various processes such as active transport, membrane permeability, and differentiation between subcellular compartments or cells.