An analysis is made of isothermal chemical reactions occurring in a volume bounded by a membrane and immersed in a reservoir of reactants and products at fixed concentrations. The permeability of the membrane to a given species is taken to be a function of the concentration of that species or of another species. This coupling between reaction and permeation provides feedback and, even for extremely simple (one or two step) reaction mechanisms, shows the following phenomena: (i) multiple steady states, some of which are stable, some unstable; (ii) hysteresis effects; (iii) reversible and irreversible transitions between stable branches of steady states induced by variation of one parameter and dependent on the values of other parameters; (iv) oscillatory temporal approach to a stable steady state; and (v) limit cycles. A method is given for predicting in certain limiting conditions the presence of unstable permeation–reaction feedback loops; the procedure is essentially independent of the details of the reaction mechanism. The theory is proposed as an appropriate construct for certain cellular phenomena, and as one example, is compared with some experiments on glycolysis in yeast cells.

1. Introduction

The presence of autocatalysis, product inhibition or other feedback mechanisms in chemical reactions far from equilibrium may, for given boundary conditions, give rise to a variety of oscillatory temporal behavior, multiple steady states and stable dissipative structures (Glansdorff & Prigogine, 1971; Higgins, 1967; Nicolis, 1971; Ortoleva & Ross, 1972a,b; Gilbert, Hahn, Ortoleva & Ross, 1972). Since some of these effects are also observed in biological systems (Hess & Boiteux, 1971; Azam & Kotyk, 1967) in which transport across a membrane may occur, it is the purpose of this article to investigate the role of variable permeability as a cause of such phenomena.
One aim of the work is the search for feedback mechanisms other than homogeneous catalysis.

Katchalsky & Spangler (1968) have shown the possibility of oscillations of a reacting system within a membrane due to a membrane phase transition with metastable regions. Rashevsky (1960) found multiple steady states in a system enclosed by a membrane for which the permeability of reactants was assumed to depend on the rate of reaction in the system. A model of coupled rate equations within a membrane, leading to a composition controlled permeability, has been proposed by Babloyantz & Sanglier to discuss multiple steady states and active transport in the all-or-none type transitions of β-galactosidase induction in *E. coli*. Molecular models for membranes including cooperative conformational changes were shown by Blumenthal, Changeux & Lefever (1970) to result in variable permeability and were used for a model of electrical excitation in which multiple steady states play a dominant role in the presence of concentration gradients across the membrane.

In this article we study various types of feedback mechanisms involving variable permeability, coupled with reactions which may or may not be stable, to determine the conditions for multiple steady states and their stability, oscillatory response to external probes, and, finally, the existence of stable limit cycles. Experimental evidence for the dependence of membrane permeability of one species on another species may be found for instance in Loewy & Siekevitz (1969); Davson & Danielli (1970); Azam & Kotyk (1969); Takeguchi & Nakagaki (1969). In our method of analysis we find that the extraction of conditions of instability dictated by the variable permeability is, in certain limits, insensitive to the assumed reaction mechanism. Although we discuss primarily the case of the control of the permeability for one species by another, the formulation of the theory includes self-induced control. Detailed study of two and three species systems is presented for the purpose of illustrating the general theory and demonstrating that the cited phenomena are possible with even the simplest one or two step reaction mechanisms with first- or second-order kinetics.

In section 2, we formulate the general problem, discuss the condition of material balance, and derive the stability criteria. The two-species system is treated in section 3, first in general and then for the reaction $S \rightarrow P$ within a membrane with a product controlled permeability of $S$. A simple graphical procedure determines both the location and stability of the steady states. Conditions are given for the absence of periodic behavior, and hysteresis effects are discussed. In section 4, we consider the Michaelis–Menten mechanism for the case of substrate permeability inhibited by product concentration, an example of physiological interest. We show the possibility of oscillatory regression to a stable steady state and, under certain conditions, the
existence of a limit cycle as confirmed by computer calculation. Finally, in section 5, we discuss the qualitative relation of the theory to several experiments on glycolysis.

2. Formulation of Theory

(A) RATE EQUATIONS AND MATERIAL BALANCE

Consider a set of isothermal chemical reactions taking place in a fixed volume $V$ surrounded by a membrane of surface area $a$. The reaction volume, enclosed in a membrane, is immersed in a homogenous medium with concentrations $n_1^n$, $n_2^n$, ..., independent of time. Inside the reaction volume the concentrations $n_i(t)$ are also uniform; diffusion is assumed to be sufficiently fast to minimize all concentration gradients.

The flux of species $i$ is in general driven by the composition (chemical potential) differences of all species across the membrane. For the purpose of a simple presentation of some instability phenomena, it suffices to take the flux $J_i$ of species $i$ across the membrane to be given by

$$J_i = h_i(n_i^n - n_i), \quad (1)$$

where $h_i$ is the permeability of species $i$, which we take to be a function of composition $(n, n^n)$. Therefore this flux is a non-linear function of concentration. The permeability may also depend on other variables $\zeta$ (membrane potential, temperature, pressure, light intensity, etc.). The rate of change of species in the system due to flux across the membrane is

$$\frac{dn_i}{dt} = h_i(n, n^n, \zeta)(n_i^n - n_i), \quad (2)$$

where $h_i$ is the permeability coefficient:

$$h_i = (a/V)\overline{h_i}. \quad (3)$$

In a more general treatment we would allow for cross effects coupling the flux of species $i$ to the concentration difference for species $j$. For simplicity we shall not consider this case here.

In the system there are $R$ reactions with rates $W^{(r)}(n, \zeta)$. Hence the total rate of change of species $i$ in the system is

$$\frac{dn_i}{dt} = \sum_{r=1}^{R} v_{ir} W^{(r)} + h_i(n_i^n - n_i), \quad (4)$$

where the stoichiometric coefficients $v_{ir}$ and the molecular weight $M_i$ obey the mass balance conditions for each reaction step $r$,

$$\sum_{i=1}^{s} v_{ir} M_i = 0. \quad (5)$$

Hence within the reaction volume the total mass density $m$ and its rate of
change are given by
\[ m = \sum_{i=1}^{s} n_i M_i, \quad (6) \]
\[ \frac{dm}{dt} = \sum_{i=1}^{s} h_i (n_i^* - n_i) M_i. \quad (7) \]

At steady state, denoted by the superscript \(^*\), \( dm/dt = 0 \), and the condition of material balance is
\[ \sum_{i=1}^{s} h_i^* (n_i^* - n_i^*) M_i = 0; \quad h_i^* = h_i(n^*), \quad (8) \]
where the sum effectively extends only over those species which may pass through the membrane. Note that we henceforth suppress the dependence of \( h \) on \( n^* \) and \( \zeta \). Steady states \( \{n_i^*\} \) of the system are specified not only by mass balance but also by the condition that the total rate of change \( dn_i/dt \) for all species \( i \) must vanish. Setting the right-hand side of equation (4) equal to zero we arrive at a set of non-linear algebraic equations for the \( \{n_i^*\} \) which may have one or multiple real solutions, corresponding to steady states which may or may not be stable. The system may oscillate around some of the steady states and may exhibit a stable limit cycle about some of the steady states.

**B) STABILITY ANALYSIS**

The stability of a given steady state to small perturbations may be determined by considering the behavior of the rate equations (4) linearized about that state. Thus if we write
\[ \delta n_i = n_i(t) - n_i^*, \quad (9) \]
\[ \Psi = \text{column} \left( \delta n_1, \delta n_2, \ldots, \delta n_s \right), \quad (10) \]
the equations linear in \( \Psi \) are
\[ \frac{d\Psi}{dt} = \Omega \Psi = (\Gamma - \mathbf{H} + \Delta) \Psi, \quad (11) \]
where the matrices \( \Gamma \), \( \mathbf{H} \) and \( \Delta \) are given by
\[ \Gamma_{ij} = \sum_r v_{ir}(\partial W^{(o)}/\partial n_r)^*, \]
\[ H_{ij} = h_i^* \delta_{ij}, \quad (12) \]
\[ \Delta_{ij} = (n_i^* - n_i^*) (\partial h_i/\partial n_j)^* \equiv (n_i^* - n_i^*) h_{ij}^*. \]

The stability of the steady state of the system is determined by the roots \( z \) of the characteristic equation of \( \Omega \)
\[ \det [\Omega - z] = 0. \quad (13) \]

In order to determine the stability of a given matrix \( \Omega \) one must either solve the characteristic equation directly or use the Routh–Hurwitz criteria
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(Graham & McRuer, 1961). We can localize the values of \( z \) according to Gersgorin’s theorem (Marcus & Minc, 1965) which states that the eigenvalues of an \( s \)-square matrix \( \Omega \) lie in that region of the complex plane which is the intersection of the union of all the discs

\[
|\Omega_{ii} - z| \leq \sum_{j \neq i} |\Omega_{ij}|, \quad i = 1, 2, \ldots, s, \tag{14a}
\]

and the union of all the discs

\[
|\Omega_{jj} - z| \leq \sum_{i \neq j} |\Omega_{ij}|, \quad j = 1, 2, \ldots, s. \tag{14b}
\]

Here \( \Omega_{ij} \) is the \((i,j)\) element of matrix \( \Omega \). From Gersgorin’s theorem we can see that if the coupling to the bath \( n^0 \) is very strong through the permeability, \( \eta_1 \) large, relative to reaction rates \( \Gamma_{ij} \) and permeability variation \( \Delta_{ij} \), then the state is always stable. Conversely if the permeability is low then the stability of a steady state will be determined by the reaction mechanisms and \( \Delta \).

(c) DIRECT REACTION–PERMEATION FEEDBACK LOOP

Let us consider a simple feedback loop involving only two species in a multispecies system. The \( h_i \) are all assumed constant except for \( h_1 \) which is taken to depend only on the concentration of species \( s \). If species 1 takes part in reactions directly effecting species \( s \), \( \Gamma_{s1} \neq 0 \), a simple feedback loop may be established. For this case we have

\[
\Delta_{ij} = (n_i^0 - n_f^0)(h_{is}^0 \delta_{ij} \delta_{js}) \equiv \lambda \delta_{ij} \delta_{js}. \tag{15}
\]

Consider conditions where the parameter \( \lambda \) is large. In this limit the solution to equation (13) can be written as a power series in \( \lambda z \),

\[
z = \lambda z \sum_{n=0}^{\infty} z_n / \lambda^{2n}, \tag{16}
\]

where \( z \) and \( z_n \) are to be determined by substitution of equation (16) into equation (13). As shown in Appendix A we obtain \( \alpha = 1/2 \), and

\[
z_0^{2/3}(z_0^2 - \Gamma_{s1}) = 0, \tag{17}
\]

for the case \( \Gamma_{s1} \neq 0 \) and \( \Delta_{s1} = 0 \) (direct reactive coupling). Thus there are \((s-2)\) bounded roots and two roots \( z_{\pm} \) whose asymptotic behavior is such that

\[
z_{\pm} \left[ z_{1-\infty} \right]^{1/2} = \pm [(n_i^0 - n_f^0)(h_{is}^0 \Gamma_{s1})]^{1/2}. \tag{18}
\]

It is obvious from equation (18) that asymptotically the steady state of the system is either unstable \((\lambda \Gamma_{s1} > 0; \Re z_{\pm} > 0)\), or that perturbations from it have an oscillatory character \((\lambda \Gamma_{s1} < 0; \Im z_{\pm} \neq 0)\). The dynamical feature of the oscillation can be seen in the following way: when \((n_i^0 - n_f^0) > 0\), \( h_{is}^0 < 0 \), and \( \Gamma_{s1} > 0 \), so that two roots \( z_{\pm} \) are pure imaginary, the formation of \( s \) due to a reaction involving 1 \((\Gamma_{s1} > 0)\) is followed by decreased permeation of 1 \((h_{is}^0 < 0)\), which in turn causes a decrease of \( s \), and this results in
an increased permeation of 1, thus completing the cycle. This dynamical loop is depicted in Fig. 1. For this oscillatory case instability may only be indicated in the higher order terms in the expansion (16). These terms involve indirect reactive coupling. The existence of such oscillatory instability may be accompanied by the presence of sustained oscillatory solutions of the full nonlinear equations (4) (see below for examples).

![Diagram of oscillatory loop](image)

**Fig. 1.** Oscillatory loop. Direct dynamical loop between species 1 and s for \( \Gamma_{s1} > 0 \), \( h_{1s} < 0 \), and \( (n_{s}^{i} - n_{s}^{f}) > 0 \). See equations (12) and (18).

The derivation of the properties of the roots, in particular, the extraction of the direct loop (1, s) is valid only in the limit of large \( \Gamma_{s1} \lambda \); otherwise indirect couplings become important.

Other mechanisms than \( \Gamma_{s1} \neq 0 \) may generate direct feedback loops: the flux of species s may be driven by the concentration difference of species 1, a possibility neglected in equation (1); the permeability of species s may depend on the concentration of species 1. In either case we get a direct feedback loop between 1 and s without reaction taking a direct role in the mechanism serving only to maintain the system far from equilibrium.

**D) SECOND-ORDER FEEDBACK LOOP**

Next we analyze the case \( \Gamma_{s1} = 0 \), that is the concentration of species s is not altered by reactions in which species 1 participates; s still controls the permeability of 1, \( \Delta_{1s} \neq 0 \), as above. Both species 1 and s take part in reactions affecting the other species in the system. Under these circumstances we obtain equation (16) with \( \alpha = 1/3 \), and

\[
z_{|\lambda| \to \infty} \Phi_{s1}(\Gamma) \lambda^{1/3} u_{1,2,3} \tag{19}
\]
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where $\Phi_{s1}$ is the sum of all indirect reactive coupling due to one intermediate species:

$$\Phi_{s1} = \sum_{j=2}^{s-1} \Gamma_{sj} \Gamma_{j1}. \quad (20)$$

The symbol $u_{1,2,3}$ denotes the three cube roots of ±1 for $\lambda \Phi_{s1} \geq 0$. For sufficiently large $\lambda$ the system is always unstable; for $\lambda \Phi_{s1} < 0$ the perturbations grow in an oscillatory manner, and the development of a stable limit cycle is likely (but not proven). The dynamical loop for non-oscillatory growth is shown in Fig. 2 in which the wavy lines indicate the indirect effect of species 1 on the production of the control species $s$ through one intermediate species ($\Phi_{s1} \neq 0, \Gamma_{s1} = 0$).

![Fig. 2. Non-oscillatory loop. Second-order dynamical loop with indirect reaction coupling via one intermediate species ($\Gamma_{s1} = 0$) for $\Phi_{s1} > 0$, $h_{s1} > 0$, and $(n_i - n_f) > 0$. See equations (12) and (20).](image)

(e) HIGHER ORDER FEEDBACK LOOPS

When more intermediate species are involved the analysis becomes somewhat lengthy but we may make some remarks on the general structure of the eigenvalues as a function of the number of intermediates. For $N$ intermediates equation (20) will be replaced by an analogous sum over products of $N$ matrix elements $\Gamma_{ij}$ whose indices trace a path of interaction from $s$ to 1 (for example $\Gamma_{s9} \Gamma_{92} \Gamma_{23} \Gamma_{31}$ would be a contribution to $\Phi_{s1}$ for three intermediates). The eigenvalues would be of a form similar to equation (19) involving, however, the $N+2$ roots of ±1 for $\Phi_{s1} \lambda \geq 0$. From the distribution of the $N+2$ roots of ±1 on the unit circle it is seen that for coupling only through one or more intermediates the steady state will always be
unstable if $\lambda$ is sufficiently large, i.e. if species 1 is sufficiently out of equilibrium with the environment, $|n_1^s - n_1^*| \gg 0$ and the permeability of species 1 depends sufficiently strongly on species $s$. More specifically, $\lambda$ must be large in comparison to the inverse times associated with reaction and permeation processes at constant permeability.

**(F) MULTIPLE CONNECTED LOOPS**

The possibilities for oscillations and monotonic instability increase rapidly with increasing coupling. For instance, let the permeability of species 1 and 2 be controlled by species $s$:

$$\Delta_1 = (n_1^0 - n_1^*)h_{1s}^*, \Delta_2 = (n_2^0 - n_2^*)h_{2s}^*.\tag{21}$$

Again let $\lambda$, defined now for species 1 and 2, be large. Then the eigenvalues are

$$z_{\pm} \sim \pm \left[ (n_1^0 - n_1^*)h_{1s}^* \Gamma_{s1} + (n_2^0 - n_2^*)h_{2s}^* \Gamma_{s2} \right]^{1/2}.\tag{21}$$

Now there are already several ways in which instability (Re $z_{\pm} > 0$) and oscillations (Im $z \neq 0$) may occur.

**(G) COMMENT**

It is seen from our analysis that the various instabilities discussed are strictly non-equilibrium phenomena. At equilibrium between the reaction volume and the environment $n_i^0 = n_i^*$ and thus $\Delta$ vanishes identically. If some species cannot exchange with the environment then there is the possibility for multiple steady states associated with the existence of metastable thermodynamic equilibrium states (such as supersaturation). These states will show an instability to a (seeding) perturbation. Such metastable thermodynamic branches of states are not considered here. We may thus state that the permeation–reaction instabilities are strictly non-equilibrium phenomena.

### 3. Reaction Systems with Two Species

In this section we discuss the stability of reaction systems with two species.

**(A) GENERAL**

Let the system consist of two species, $S$ and $P$, with an isomerization reaction

$$S \rightleftharpoons P.\tag{22}$$

The total reaction rate $W$ depends on $S$ and $P$:

$$W = W(S, P).\tag{23}$$
The rates of change of species $S$ and $P$ are then

$$\frac{dS}{dt} = -W + h_s(S^* - S), \quad (24)$$

$$\frac{dP}{dt} = W + h_p(P^* - P). \quad (25)$$

We assume for the steady state that $S^* > S^*$, $P^* < P^*$. Mass balance equation (8) at steady state yields

$$h_s(S^* - S^*) + h_p(P^* - P^*) = 0. \quad (26)$$

Linearization of the rate equations (24) and (25) about the steady state yields an equation of the form (11) with

$$\Omega_{ss} = -W_s + (S^* - S^*)h_s, \quad (27)$$

$$\Omega_{sp} = -W_p + (S^* - S^*)h_p, \quad (27)$$

$$\Omega_{ps} = W_s + (P^* - P^*)h_p, \quad (27)$$

$$\Omega_{pp} = W_p - h_p + (P^* - P^*)h_p, \quad (27)$$

where $W_s = \partial W/\partial S$ and $W_p = \partial W/\partial P$.

The eigenvalues of the matrix $\Omega$ show that if either of the two conditions

$$\text{Tr} (\Omega) > 0, \quad (28)$$

$$\text{det} (\Omega) < 0, \quad (29)$$

is satisfied then the steady state is unstable. Instability due to condition (28) may be attained by direct non-linearity in the permeabilities, $h_s^* > 0$ or $h_p^* < 0$, or by an unstable reaction mechanism $-W_s + W_p > 0$, or both. Instabilities associated with condition (29) are possible in the absence of direct non-linearity in the permeabilities if cross correlation, $h_s^* \neq 0$ or $h_p^* \neq 0$ occurs.

A simple geometric analysis of the stability of the steady states is useful. Let us denote the condition $(dS/dt) = 0$ by $I$ (input), $(dP/dt) = 0$ by $O$ (output), and the mass balance condition (26) by $B$. If $I$, $O$, and $B$ are plotted in the $S$–$P$ phase plane they will intersect at the steady states (only two of the three conditions are independent). The slopes of the three curves at the intersections, denoted by $\alpha_I$, $\alpha_O$, and $\alpha_B$ are

$$\alpha_I = -\Omega_{ss}/\Omega_{sp}, \quad \alpha_O = -\Omega_{ps}/\Omega_{pp}, \quad (30)$$

$$\alpha_B = -(\Omega_{ss} + \Omega_{ps})/(\Omega_{sp} + \Omega_{pp}), \quad (31)$$

with the elements of $\Omega$ given in equations (27). Now for example, let $\alpha_I > \alpha_O$; then with the auxiliary condition $\Omega_{sp}\Omega_{pp} \geq 0$, we have $\text{det} \Omega \geq 0$. Hence the relation between the slopes (and an auxiliary condition) determines graphically the stability of the steady state. Other cases of combinations of $I$ and $B$, and $O$ with $B$ are tabulated in Table 1 for convenience.
TABLE 1

Geometrical criteria for instability due to cross-correlation ($\text{Tr}(\Omega) < 0$ and $\det(\Omega) < 0$) with sufficient auxiliary conditions

<table>
<thead>
<tr>
<th>Geometrical criteria</th>
<th>Auxiliary conditions</th>
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</thead>
<tbody>
<tr>
<td>$a_1 &gt; a_0$</td>
<td>$\Omega_{sp}\Omega_{pp} &gt; 0$</td>
</tr>
<tr>
<td>$a_1 &lt; a_0$</td>
<td>$\Omega_{sp}\Omega_{pp} &lt; 0$</td>
</tr>
<tr>
<td>$a_1 &gt; a_s$</td>
<td>$\Omega_{sp}(\Omega_{sp} + \Omega_{pp}) &lt; 0$</td>
</tr>
<tr>
<td>$a_1 &lt; a_s$</td>
<td>$\Omega_{sp}(\Omega_{sp} + \Omega_{pp}) &gt; 0$</td>
</tr>
<tr>
<td>$a_0 &gt; a_s$</td>
<td>$\Omega_{pp}(\Omega_{sp} + \Omega_{pp}) &lt; 0$</td>
</tr>
<tr>
<td>$a_0 &lt; a_s$</td>
<td>$\Omega_{pp}(\Omega_{sp} + \Omega_{pp}) &gt; 0$</td>
</tr>
</tbody>
</table>

In a two species system, oscillatory instability of the steady state ($\text{Re} \, z > 0$, $\text{Im} \, z \neq 0$) requires $\text{Tr} \, (\Omega) > 0$, hence direct non-linear permeability or an unstable reaction mechanism.

(B) PERIODIC SOLUTIONS

For a general two species system it is easy to show that temporally periodic solutions to the kinetic equations (24) and (25) can only exist for either direct non-linear permeability, or oscillatory behavior in the chemical reaction mechanism, or the interplay of both. A negative criterion due to Bendixson (Minorsky, 1962) states that, in our notation, unless $\text{Tr} \, (\Omega) = \Omega_{ss} + \Omega_{pp}$ either changes sign or vanishes identically in a given region of the $(S, P)$ plane there can be no periodic solution to equations (24) and (25) in that region. In this discussion we have removed the restriction imposed on $\Omega$ in equations (27) that $S$ and $P$ be evaluated at a steady state.

From equations (27) we see that

$$\text{Tr} \, (\Omega) = -W_s + W_p - h_s - h_p + (S^* - S)h_{ss} + (P^* - P)h_{pp}. \quad (32)$$

The Bendixson negative criterion can therefore not be violated when both direct non-linear permeability and oscillatory behavior in the reaction mechanism are absent, the latter condition depending on whether $W_p - W_s$ itself can satisfy the criterion in the domain of $(S, P)$ space where periodic solutions are sought.

(C) PRODUCT-CONTROLLED PERMEABILITY

1. Multiple steady states

Let the permeability of species $S$ be a function of concentration of $P$, $h_s = h_s(P)$, and take $h_p$ to be constant. We take the simple isomerization $S \to P$ with $W = kS$. Since $\text{Tr} \, (\Omega) < 0$ there can be no periodic solutions
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for this system. Furthermore instability may be obtained only for det (Ω) < 0, which implies

\[ \lambda = (S^0 - S^*) h_* > (k + h_*)h_*/k. \] (33)

Some possible forms of \( h_* \) are shown in Fig. 3. We plot in Fig. 4 the \( I, O \) lines corresponding to \( h_*(P) \) of Fig. 3(a) (steady-state conditions obtained from

**Fig. 3.** Three examples of cross-correlation dependences of the permeability for \( S \) on \( P \). The functional form of the permeability is taken to be

\[ h_*(P) = h_*(\infty) + \frac{h_*(0) - h_*(\infty)}{1 + (P/\pi)^\gamma}. \]

- **Curve (a):** \( h_*(0) = 0.5, \ h_*(\infty) = 1.0, \ \pi = 20, \ \gamma = \infty. \)
- **Curve (b):** \( h_*(0) = 0.5, \ h_*(\infty) = 1.0, \ \pi = 20, \ \gamma = 10. \)
- **Curve (c):** \( h_*(0) = 10^{-3}, \ h_*(\infty) = 0.0, \ \pi = 0.3, \ \gamma = 100. \)

**Fig. 4.** Steady-state conditions in concentration phase space, plotted with input and output relations, for \( h_*(P) \) of Fig. 3(a) with \( k = 1, S^0 = 50, P^0 = 10, \) and \( h_* = \) constant = 2.
FIG. 5. Trajectories in the $S$–$P$ phase space obtained by solving the non-linear differential equation (24) numerically. Separatrix is indicated by a dotted line. Parameters are the same as in Fig. 4.

equations (24) and (25). Three steady states are possible for this system. We note that $\Omega_{sp}\Omega_{pp} < 0$; for steady states $1^*$ and $3^*$, $\alpha_f > \alpha_o$, and these states are stable, while for the steady state $2^*$, $\alpha_f < \alpha_o$, and the state is unstable.

In Fig. 5 we show the structure of $S$–$P$ phase space by giving trajectories, evaluated numerically, of the system for a variety of initial conditions. The system analyzed is one with a step-function permeability (Fig. 3(a)). We note that a "separatrix" divides the phase space into regions dominated either by the steady state $1^*$ or $3^*$. Any system prepared in region $I$ evolves to stable steady state $1^*$, and in region III, to $3^*$. For a general derivation of the equation of the separatrix for $s$ species see Appendix B.

2. Reversible transitions between stable states; hysteresis

It is interesting to see the behavior of the multiple steady states in terms of the external parameters, $S^o$ and $P^o$. By eliminating $S^*$ between the equations (24), (25) and (26), we have

$$P^* = P^o + kh^o_sS^o/[(k + h^o_s)h_p].$$ (34)

This relation between $P^*$ and $S^o$ yields a single straight line if $P^o$ is greater than the value $\pi$ of the $P$ concentration at which $h_s$ changes rapidly (see Fig. 3(a)); in that case there is only one branch of steady state in the system regardless of the value of $S^o$. If, however, we have $P^o < \pi$, then equation (34) yields multiple steady states if $S^o$ is in the interval $S_i^o \leq S^o \leq S_u^o$, where

$$S_i^o = (\pi - P^o)h_p[k + h^o_s(\infty)]/[kh^o_s(\infty)],$$

$$S_u^o = (\pi - P^o)h_p[k + h^o_s(0)]/[kh^o_s(0)].$$ (35)
These multiple steady states result in typical hysteresis as is seen in Fig. 6. If a system is prepared in the region $S^o < S_i^o (S^o > S_o^o)$ and $S^o$ is slowly increased (decreased), then the system makes a discontinuous transition $1^* \rightarrow 3^* (3^* \rightarrow 1^*)$ when $S^o$ passes beyond an upper (lower) critical value $S_i^o (S_o^o)$ (bypassing the unstable steady state $2^*$).

![Fig. 6. Branches of multiple steady states and hysteresis dependent on external substrate concentration $S^o$. Values of $S_i^o$ and $S_o^o$ are given in equation (35) and other parameters are the same as in Fig. 4.](image)

3. **Irreversible transitions between stable states**

The functional dependence of the steady states on the external concentration $P^o$ demonstrates three general types of behavior, including the important case of irreversible transitions. We denote the second term on the right hand side of equation (34) by $Q$, and distinguish three cases: (i) $Q(P = 0) < \pi$, $Q(P = \infty) < \pi$; there is hysteresis behavior analogous to the dependence of $P^*$ on $S^o$ (Fig. 7, case 1); (ii) $Q(P = 0) > \pi$, $Q(P = \infty) > \pi$; there is only one branch of steady states available to the system (Fig. 7, case 2); (iii) $Q(P = 0) < \pi$, $Q(P = \infty) > \pi$. As $P^o$ is increased from zero, a transition from the lowest to the uppermost branch occurs at a given value. If, however, the system is prepared in a steady state at $P^o$ exceeding that value and then $P^o$ is decreased the reverse transition does not occur (Fig. 7, case 3).

This analysis demonstrates that transitions from one branch of steady states to another may or may not be reversible with respect to variation of one parameter depending on the value of other parameters.
Fig. 7. Branches of multiple steady states and hysteresis dependent on external product concentration $P^o$. Three qualitatively different cases are considered, and obtained with the parameters: case 1, $S^o = 60$; case 2, $S^o = 130$; case 3, $S^o = 108$.
Other parameters are the same as in Fig. 4.

4. Michaelis–Menten Mechanism

We consider the Michaelis–Menten mechanism

\[ S + E \rightleftharpoons A, \]
\[ A \rightarrow P + E, \]

where $S$ is a reactant substrate, $P$ the product, $E$ an enzyme, and $A$ a complex. The membrane is taken to be impermeable to $E$ and $A$. Hence the total concentration of $E$ is constant.

\[ E + A = E_t \text{ (constant)}. \]

We take $S$, $A$, and $P$ to be the independent variables. The rate equations are

\[ \frac{dS}{dt} = -k_1(E_t - A)S + k_1A + h_1(S^o - S), \]
\[ \frac{dA}{dt} = k_1(E_t - A)S - (k_2 + k_3)A, \]
\[ \frac{dP}{dt} = k_3A + h_2(P^o - P). \]

Although this system appears to have a close relation to the simple $S \rightarrow P$ mechanism considered in section 3(c), its behavior may indeed be qualitatively very different.

We consider again the product controlled case $h_2 = h_2(P)$. $h_2 = $ constant. The steady states may be most easily obtained from the rate equations by eliminating $A^*$ in favor of $S^*$ and $P^*$. 
In general we may have one, two, or three steady states. For any one of these steady states, we consider the following limiting situation:

\[ S^\circ \to \infty, \quad h_s \to 0, \]
\[ |h_{sp}| \to \infty, \quad P^\circ, h_p, k_i \text{ non-zero, finite}. \]  

(40)

For this case we may use the analysis in section 2(c) (see equation (15)). Linearization of the rate equations about the steady state under consideration, \( \delta S(t) = S(t) - S^\circ \), etc., yields for the column vector of perturbations, \( \Psi = \text{column} (\delta S, \delta A, \delta P) \), an equation of the form (11). Keeping other parameters fixed we note that in the limit \( h_s \to 0 \), \( S^\circ \) is of the order of \( h_s \) and hence \( (S^\circ - S^\circ)h_{sp}^\circ \) becomes large as \( S^\circ \) is increased. Thus the parameter \( \lambda = (S^\circ - S^\circ)h_{sp}^\circ \) is large if \( S^\circ \) and \( h_{sp}^\circ \) are large. Since \( S \) does not take part in

![Fig. 8. Limit cycles in the phase space of the concentrations of \( S, A, P \) (arbitrary units) for the Michaelis-Menten mechanism with inhibitory membrane permeability (Fig. 3(c)). Numerical accuracy is good to \( 10^{-6} \). We used the values of the parameters \( k_1 = 0.2, \ k_2 = 0.01, \ k_3 = 0.1, \ E_c = 0.1, \ S^\circ = 10.0, \ P^\circ = 0.0, \ \pi = 0.3, \ h_p = 0.002, \ h_s = 10^{-5}[1+(P/p)^{100}]^{-1} \) (in arbitrary units). In each of the three cases we show the initial portions of a trajectory originating within the region defined by the limit cycle and of a trajectory originating from outside that region. The trajectories, terminated arbitrarily by broken lines, approach the limit cycle after about 30 oscillations.](image-url)
a reaction altering the concentration of $P$, the solution to the secular equation can be written as equation (19), and we obtain

$$z_{1,2,3} \rightarrow \infty \sim \left[k_1 k_3 E_i S^0/h_{sp}\right]^{1/3} u_{1,2,3},$$

(41)

where $u_{1,2,3}$ are the three cube roots of $h_{sp}/h_{sp}$ which lie on the unit circle. If $h_{sp} > 0$, then we have $u_{1,2,3} = 1, -1/2 \pm i\sqrt{3}/2$ and the steady state is unstable. This case is similar to the one discussed in section 3(c) 1. Graphical analysis shows that three steady states are possible.

If $h_{sp} < 0$, then there is only one steady state. The three roots are $u_{1,2,3} = -1, 1/2 \pm i\sqrt{3}/2$ and again the steady state is not stable. Since the instability is oscillatory, there is the suggestion of the development of a stable limit cycle. We have confirmed its existence with a computer solution of the non-linear equations (39). With a permeability $h_4(P)$ of the form shown in Fig. 3(c) the system attains a single unstable steady state and a limit cycle, Fig. 8. The stability of the cycle has been tested numerically with respect to a variety of perturbations.

For smaller values of $\lambda$ (i.e., decreasing the sharpness of the slope $h_{sp}$) the steady state becomes stable and the limit cycle is replaced by damped oscillations. (A similar comment holds for a decrease in $S^0$.)

5. Discussion

QUALITATIVE APPLICATION OF THEORY TO GLYCOLYSIS

The theory presented here is believed to have applications in biological systems where membrane transport of one species in a reaction mechanism is either a non-linear function of the concentration of that species or a function of concentration of another species in the mechanism. As an example, we review briefly some experiments and conclusions on glycolysis, a case studied extensively, and then, by qualitative comparison, show that the theory serves here as a useful construct.

(1) Damped oscillations of NADH (Ghosh & Chance, 1964; Pye, 1971; Becker & Betz, 1972) are observed in anaerobic glycolysis in yeast cells and (2) oscillations also occur in the glycolysis in cell free extracts (Betz & Sel’k’ov, 1965). It has been shown (Sols, 1966; Maitra, 1966; Gancedo, Gancedo & Sols, 1967) that (3) membrane transport is the rate limiting step in the sugar phosphorylation mechanism, and (4) this step is followed by a phosphorylation by hexokinase in a Michaelis–Menten mechanism. “Membrane transport is tightly coupled to hexokinase activity” (Becker & Betz, 1972). (5) “As a possible coupling factor between the phosphorylation step and transport, glucose-6-phosphate may be considered” (Becker & Betz, 1972). (6) The fact that the efflux of D-xylose from yeast cells decreases linearly
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with increasing concentration of glucose-6-phosphate within the cell provides further evidence for the dependence of membrane transport of one species on the concentration of another (Azam & Kotyk, 1969). (7) Limit cycle oscillations have been observed in glycolysis (Pye, 1971). (8) "... growth of baker's yeast on different mixtures of glucose and lactate produced cells in different metabolic states, different also in their sugar transport activity" (Azam & Kotyk, 1969).

The following mechanism is suggested then by the experiments on glycolysis in cells: the transport of glucose (or fructose) across the cell membrane, that is permeation of glucose, is followed by a Michaelis–Menten phosphorylation by hexokinase (3,4). The product, glucose-6-phosphate, inhibits the permeability of the membrane to glucose (5,6). This type of mechanism is similar to the one discussed in section 4. For that case the theory predicts, and provides for: damped oscillatory approach to a steady state (1), and limit cycle oscillations (7).

The mechanism for oscillations in cell extract (2) must differ from that in intact cells and some differences in observations have been noted (Pye, 1971; Betz & Sel'kov, 1965).

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REFERENCES
BABLYANTZ, A. & SANGLIER, M. To be published.
APPENDIX A

Determination of the Eigenvalues

The stability of the system (11) is determined by the characteristic equation (13). We take all $A_{ij}$ to be zero except $A_{1s} = (n^i_1 - n^i_0)h^i_s$ and write the characteristic equation in the form

$$0 = \det \begin{vmatrix} a_{11} - z & a_{12} & \ldots & a_{1s} + \lambda \\ a_{21} & a_{22} - z & \ldots & a_{2s} \\ \vdots & \vdots & \ddots & \vdots \\ a_{s1} & a_{s2} & \ldots & a_{ss} - z \end{vmatrix}. \quad (A1)$$

We want to solve this equation in the limit $\lambda \to \infty$, and expand the eigenvalue as in equation (16). Applying the Laplace expansion method to evaluate the determinant and keeping only those terms containing $\lambda$ and the highest powers of $z$, we approximate equation (A1) as

$$(-z)A_{11} + (-1)^{s+1} \lambda A_{1s} = 0, \quad (A2)$$

where $A_{ij}$ is the minor determinant of the $(i,j)$ entry of the matrix $[\Omega - z]$. For $A_{11}$ we have

$$A_{11} = (-z)^{s-1}[1 + O(1/z)]. \quad (A3)$$

The minor determinant $A_{1s}$ takes the form

$$A_{1s} = \begin{vmatrix} a_{21} & a_{22} - z & \ldots & a_{2s-1} \\ \vdots & \vdots & \ddots & \vdots \\ a_{s-1,2} & a_{s-1,2} & \ldots & a_{s-1,s-1} - z \\ a_{s1} & a_{s2} & \ldots & a_{s,s-1} \end{vmatrix} = (-1)^{s}a_{s1}(-z)^{s-2}[1 + O(1/z)]; \quad (A4)$$

Introducing equations (A3) and (A4) into equation (A2), we obtain

$$z^s - \lambda a_{s1}z^{s-2} + \ldots = 0. \quad (A5)$$

Expansion of $z$ as in equation (16) shows that $\alpha = 1/2$ and that $z_0$ is given by equation (17).
For the case $a_{s1} = 0$, i.e. $\Gamma_{s1} = 0$, the evaluation of $A_{1s}$ is also straightforward if more cumbersome. A similar procedure as given for the case $a_{s1} \neq 0$ shows that

$$A_{1s} = \Phi_{s1}(-z)^{3-3}[1 + O(1/z)],$$

yielding $\alpha = 1/3$ and equation (19).

**APPENDIX B**

**Equation for Separatrix**

If the system has multiple steady states, but no periodic solutions, then the concentration phase space may be divided into domains surrounding the stable steady states such that a system initially within a given domain will eventually approach the stable steady state of that domain. We now derive the equation for the hypersurface, the separatrix, which defines these phase space domains.

Division of the rate equations (4) by one rate, say $d n_1 / dt$, generates equations determining the trajectories in phase space, $(dn_i/dn_1) = F_i(n)$, for all species $i \neq 1$. We take the separatrix to be given by the general hypersurface $\phi(n) = \text{constant}$. The vanishing of the total differential $d\phi$ on this surface yields

$$\frac{\partial \phi}{\partial n_1} + \sum_{i=2}^{s} F_i(n) \frac{\partial \phi}{\partial n_i} = 0. \quad (B1)$$

The separatrix $\phi$ is then obtained as those solutions to (B1) which pass through the unstable steady states.