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The Effects of Cell Density and Metabolite Flux on Cellular Dynamics

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Summary. Density-dependent regulation of cell growth in tissue culture is a well-known phenomenon but the mechanism of regulation remains obscure. Here we explore the effects of cell density and metabolite flux on the collective dynamics of a cell population. The intracellular dynamics are modelled by positive feedback kinetic mechanisms of the kind known to apply to yeast cells. Several experimental observations related to glycolytic oscillations are predicted and it is suggested that the general conclusions may be applicable in a broader context.

Key Words: Glycolytic oscillations—Density-dependent growth control

1. Introduction

Some form of intercellular communication is necessary in many multicellular systems, whether it be to ensure global coordination of proliferation, spatial pattern formation and differentiation in a developing embryo, or merely for the purpose of controlling the replenishment of lost cells in a fully-developed tissue or organ. At least three generic modes of short-range communication between cells have been identified. Firstly, there can be direct exchange of low molecular weight cellular constituents via the tight gap junctions that form in many systems (Loewenstein and Kanno, 1966; Furshpan and Potter, 1968). Such exchange is often invoked in mathematical models for pattern formation in developing tissue (Othmer and Scriven, 1971, 1974) and the role it may play in the control of growth has been discussed by Loewenstein (1968). Secondly, there can be surface interactions that result from mechanical stresses or that occur via receptor molecules embedded in the membrane and such interactions can lead to changes in permeability and the rate of transport of nutrients into the cell (Hoffman et al., 1973; Sefton and Rubin, 1971). One fascinating example of the intracellular changes that

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can be triggered in response to deformation of the cell membrane occurs when a paramecium encounters an obstacle (Machemer and Eckert, 1973). Thirdly, cells can interact indirectly by uptake of nutrients available in limited amounts or by the release into their environment of waste materials or other substances that activate or inhibit cellular functions. This third mode of interaction has been called 'physiological competition' by Spiegelman (1945).

One objective of theoretical analyses is to characterize and classify the kinds of collective behavior possible in a population of cells that interact via one or more of the foregoing modes. For example, substantial progress has been made in the theoretical analysis of pattern formation in aggregates of cells coupled by diffusion through tight junctions (for a review see Othmer, 1977). The effects of changing kinetic and transport parameters in systems near steady states have been analyzed and the kinds of spatio-temporal patterns that can develop have been characterized in simple systems. Although there is still a large gap between theory and experiment, simple models of some specific systems appear promising (Wilcox et al., 1973). Much less has been done toward modelling cellular interactions of the surface type, primarily because such interactions are poorly understood and their effects on other cell functions have not been adequately characterized. In this paper we focus on indirect interactions, even though these are not always clearly separable from surface interactions.

There are numerous instances in which cell density in a population influences cellular dynamics. For example, it has been shown that myoblasts will only fuse upon reaching a critical cell density (Konigsberg, 1971), and many cell types show 'density-dependent regulation' of growth in cultures (Holley, 1975). A prototype system for theoretical studies of density effects and indirect communication between cells is a suspension of the yeast *S. carlsbergensis*. The effect on glycolytic metabolism of changes in the glucose supply rate have been characterized and a qualitatively correct description of the important reactions is available (Pye, 1971; Boiteux et al., 1975; Aldridge, 1976). The effects of changes in the cell density have also been reported (Aldridge and Pye, 1976). We shall use this system as a concrete example to illustrate some of our general conclusions.

The following section deals with the dynamics of a class of positive feedback mechanisms that are applicable to the phosphofructokinase reaction in the glycolytic pathway. It is shown that these mechanisms give rise to at most one positive steady state and that when this steady state is unstable there exists at least one time-periodic solution. While much of the analysis can be done for an arbitrary mechanism of this type, the direction and stability of bifurcating periodic solutions depend on the particular mechanism. In the third section we give a complete analysis of a mechanism that involves activation of PFK by a single molecule of AMP.

The fourth section is concerned with the effect of cell density on cell dynamics. We treat the general case of arbitrary n-species kinetics and illustrate the results using the mechanism studied in the third section. We show that communication with a surrounding pool can quench oscillations that exist in the absence of communica-

tion and that such communication can give rise to oscillations when none exist in its absence. The relevance of these results to general models of growth control is discussed in the concluding section.

2. Qualitative Analysis

The general kinetic scheme studied here is one in which the throughput is controlled at one reaction in a sequence via feedback of a product of that reaction. The overall reactions are irreversible and there are only two species whose concentrations vary on the time scale of interest. The overall reactions are of the form

$$\text{substrate} \xrightarrow{1} A_1 \xrightarrow{2} A_2 \xrightarrow{3} \text{products} \tag{1}$$

but the mechanism may be more complex. The substrate may itself be the product of previous reactions or it may be supplied directly from the environment. Let x and y denote the dimensionless concentration of A_1 and A_2, respectively, and let $\mathscr{R}_i$ be the rate of the ith reaction. The input flux $\mathscr{R}_1$ is assumed to be a constant, $\mathscr{R}_2$ is a function of x and y, and the output $\mathscr{R}_3$ depends only on y. Therefore, the governing differential equations for x and y have the form

$$\begin{aligned} \frac{dx}{dt} &= \delta - F(x, y) \\ \frac{dy}{dt} &= F(x, y) - G(y) \end{aligned} \tag{2}$$

where F and G are smooth functions of their arguments.

By virtue of the irreversibility of reactions 2 and 3, $F(x, y) \geqslant 0$ for $(x, y) \geqslant (0, 0)$ and $G(y) \geqslant 0$ for $y \geqslant 0$. Because neither A_1 nor A_2 can disappear when none is present, $F(0, y) = 0$ for $y \geqslant 0$ and $G(0) = 0$. To further restrict the mechanisms, we impose the following conditions on F and G.

$$\begin{aligned} &\text{(i)} \quad \partial F/\partial x > 0, \quad \partial^2 F/\partial x^2 \leqslant 0 \quad \text{for } (x, y) > (0, 0) \\ &\text{(ii)} \quad \partial F/\partial y > 0, \quad \text{for all } (x, y) > (0, 0) \\ &\text{(iii)} \quad dG/dy > 0, \quad d^2G/dy^2 \leqslant 0 \quad \text{for } y > 0, \quad dG/dy < \infty \quad \text{at } y = 0 \end{aligned} \tag{3}$$

The first condition implies that there is no autocatalysis or inhibition by reactant in the reactions $A_1 \rightarrow A_2$, and the third condition puts similar restrictions on the third reaction. The second condition requires that the feedback effect of y on the rate of the second reaction be positive at all concentrations.

It is readily shown that under the preceding conditions any solution of (2) that begins in the non-negative quadrant always remains there. If $F(x, 0) \equiv 0$ the x axis is invariant under the flow of (2) and there is a critical point at $(\infty, 0)$. If $F(x, 0) > 0$ for $x > 0$, then any solution that intersects the x axis at $t = t_0$ lies in the interior of the first quadrant for $t > t_0$. The topological character of the phase portrait is determined by the number of critical points, closed orbits and separatrices of saddle points and once their number is determined, the qualitative behavior of solutions can be established.

The steady state solutions of (2) satisfy the pair of equations

$$\delta = F(x, y) = G(y) \tag{4}$$

and these have at most one positive solution, as shown by the following proposition.

Proposition 1. *If*

$$\lim_{y \to \infty} G(y) > \delta \tag{5}$$

then there exists a unique positive solution y^* *of* $G(y) = \delta$. *If such a* y^* *exists and*

$$\lim_{x \to \infty} F(x, y^*) > \delta \tag{6}$$

then there exists a unique positive solution x^* *of* $F(x, y^*) = \delta$. *If either* (5) *or* (6) *is violated, then there is no positive steady state.*

Proof. The proof follows immediately from (5) and (6) and the monotonicity of G and F.

The condition (5) means that the maximum possible output from the third reaction must exceed the input or else there can be no positive steady state; x and/or y simply increase. This is a necessary but not sufficient condition and if there exists a y^* at which the input and output can balance, it is necessary that the maximum rate of the second reaction balance the input at that y^*. Evidently a mechanism may fail to have a positive steady state on either of these accounts, but hereafter we assume that one does exist for at least some values of δ. Note that there are no steady states on the finite portions of the positive x and y axes.

It is helpful, in proving the existence or non-existence of periodic solutions, to know the qualitative features of the isoclines $\dot{x} = 0$ and $\dot{y} = 0$. It follows from (3)(ii) that the equation $F(x, y) = \delta$ has a unique solution $y(x)$ that satisfies

$$\begin{aligned} &F(x, y(x)) = \delta \\ &\frac{dy}{dx} = \frac{-F_x}{F_y} \leqslant 0 \end{aligned} \tag{7}$$

where $F_x \equiv \partial F/\partial x$ and $F_y \equiv \partial F/\partial y$. Thus y is non-increasing along $\dot{x} = 0$ and either y approaches a horizontal asymptote $y_\infty \in [0, y^*)$ as $x \to \infty$, or there exists an x_0 such that $F(x_0, 0) = \delta$. It will be clear from the following that the latter case is trivial to deal with and so we consider only the former case. Since $F(0, y) \equiv 0$, $\dot{x} = 0$ can never cross the y axis and therefore it has a vertical asymptote at some $x \geqslant 0$.

Let Q_1 denote the interior of the first quadrant and let Q_1^+ denote Q_1 plus the x and y axes. By hypothesis (3)(i), the equation $\dot{y} = 0$ ($F(x, y) = G(y)$) has a unique solution $x(y)$, whenever (x, y) lies in Q_1. If $F(x, 0) \not\equiv 0$, this branch must pass through (0, 0). When $F(x, 0) \equiv 0$, the x axis is another branch. The latter case occurs when every pathway from A_1 to A_2 is catalyzed by A_2.

Along any branch of $\dot{y} = 0$

$$\frac{dy}{dx} = \frac{F_x}{G_y - F_y} \tag{8}$$

and therefore $\operatorname{sgn}(dy/dx) = \operatorname{sgn}(G_y - F_y)$. Even simple mechanisms can give rise to very complicated curves for the loci $G_y = F_y$ (see, e.g. Higgins (1967)), but if we restrict attention to the locus, $\dot{y} = 0$, there are only three possibilities for dy/dx: (a) non-negative everywhere, (b) one or more sign changes, (c) non-positive everywhere. Representatives of the three generic loci for $\dot{y} = 0$ are shown in Figure 1. In (a) and (b) we show cases where $F(x, 0) \not\equiv 0$, while in (c) we assume $F(x, 0) \equiv 0$. It is clear from the figures that (a) and (c) are opposite extremes of (b). In general, there may be more than two points along $\dot{y} = 0$ at which the tangent is vertical. If we allow $F(x, 0) \equiv 0$ in (a) or (b) the intersection of $\dot{y} = 0$ with the x-axis can occur at some positive x.

Asymptotic stability of the steady state (x^*, y^*) is governed by the eigenvalues of the matrix

$$K \equiv \begin{bmatrix} -F_x & -F_y \\ F_x & F_y - G_y \end{bmatrix}, \tag{9}$$

wherein all partial derivatives are evaluated at (x^*, y^*). The eigenvalues are

$$\lambda_{\pm} = \frac{\operatorname{trace} K \pm \sqrt{(\operatorname{trace} K)^2 - 4 \det K}}{2} \tag{10}$$

where

$$\operatorname{trace} K \equiv F_y - F_x - G_y$$
$$\det K = F_x G_y.$$

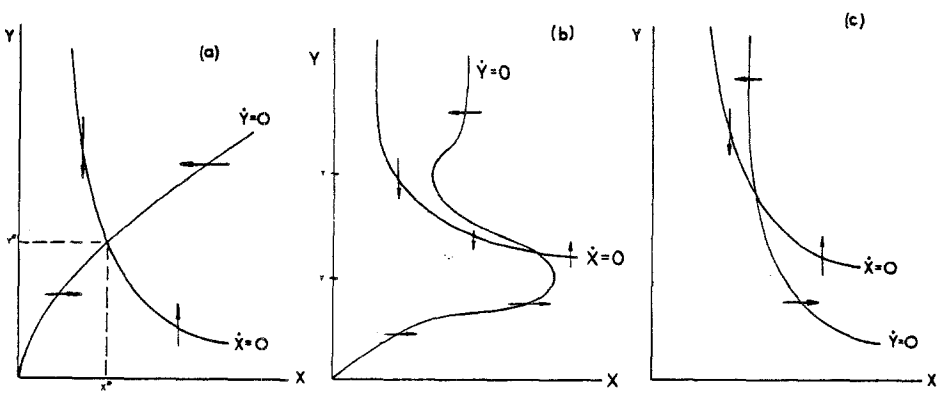


Fig. 1. The three generic $\dot{y} = 0$ loci and the $\dot{x} = 0$ locus

Because F_x and G_y are positive throughout Q_1, $\det K > 0$ and the steady state is always a node or focus, stable or unstable according as trace K is negative or positive. By utilizing (8) and (10) we can write

$$\text{trace } K = -F_x\left[1 + \left(\frac{dy}{dx}\right)^{-1}_{\dot{y}=0}\right] \tag{11}$$

and from this we obtain a geometric criterion for stability: *the steady state* (x^*, y^*) *is stable or unstable according as the slope at the steady state of* $\dot{y} = 0$ *lies outside or inside the interval* $(-1, 0)$. It follows that for the cases shown in Figure 1, the steady state is always stable in (a), can be unstable in (b) only when $y^* \in (y_1, y_2)$, and is unstable in (c) when y^* is sufficiently small.

Now consider what happens as the input flux δ is varied. If $(\hat{x}, \hat{y})$ denotes the point of intersection of the locus $\dot{x} = 0$ with the vertical line $x = \hat{x}$, then it follows from (2) that $d\hat{y}/d\delta = 1/F_y > 0$. If $F(\hat{x}, 0) \neq 0$, then there exists a $\delta^* > 0$ such that $\hat{y} \to 0$ as $\delta \to \delta^*$ from above. If $F(\hat{x}, 0) = 0$, then $\delta^* = 0$. In either case $\hat{y}$ increases monotonically from zero as δ increases from δ^*. Therefore, when $\dot{y} = 0$ is as shown in Figure 1b, there is at most one interval (δ_1, δ_2), $\delta_1 > 0$ in which the steady state can be unstable. At low and high fluxes it is necessarily stable. If $\dot{y} = 0$ passes through (0, 0) and has more than two points where the tangent is vertical, there may be several disjoint δ intervals, none of which contains 0, in which the steady state is unstable. In contrast to this, when $\dot{y} = 0$ is as shown in Figure 1c, its slope is monotone increasing in x. Now the steady state is unstable for all $\delta \in (0, \bar{\delta})$, where $\bar{\delta}$ is the δ value for which $(dy/dx)_{\dot{y}=0} = -1$ at (x^*, y^*). Here all low input fluxes lead to unstable steady states. We shall return to this point in the following section.

At those values of δ for which $(dy/dx)_{\dot{y}=0} = -1$ at the steady state, trace $K = 0$ and the eigenvalues of K are pure imaginary. Unless it happens that $d(\text{trace } K)/d\delta = 0$ at these values, the Hopf bifurcation theorem (see Appendix) guarantees the existence of a periodic solution in a one-sided neighborhood of these values. The direction and stability of these bifurcating solutions are governed by the non-linear terms in (2) and therefore no general statements can be made. An example is treated in detail in the following section. To complete the qualitative picture we have to analyze the global behavior of solutions, both when the steady state is stable and when it is unstable. The first step is to determine when solutions remain bounded for all $t > 0$.

The sum of x and y satisfies

$$\frac{d}{dt}(x + y) = \delta - G(y) \tag{12}$$

and the right-hand side is positive for $y < y^*$, negative for $y > y^*$. Along $y = y^*$ the vector field is tangent to a line $x + y = \text{constant}$. If $x(0) + y(0) > y^*$, then $x(t) + y(t) > y^*$ for all $t > 0$. Since $\dot{x} > 0$ and $\dot{y} < 0$ along the y axis, the trajectory through any point above the line $y = y^*$ ultimately crosses this line at a point whose x coordinate lies between 0 and x^*. Moreover, it follows from (12) that any solution which begins in the region bounded above by the line $y = y^*$ and below by the curve $\dot{x} = 0$ eventually crosses into the region $y > y^*$.

As a result, solutions can only escape to ∞ along a trajectory that remains beneath the curve $\dot{x} = 0$ for large x and small y. This happens when the substrate enters too fast to be consumed by the second reaction. An extreme case occurs when $F(x, 0) \equiv 0$, for then any solution that begins on the x axis remains there and $x \to \infty$ as $t \to \infty$. A necessary but not sufficient condition for unbounded solutions is that the locus $\dot{x} = 0$ have a non-negative horizontal asymptote, for if δ is so small that $\dot{x} = 0$ crosses the x axis at some $x_0 < \infty$, $\dot{x} < 0$ for $x > x_0$ and all solutions are certainly bounded. That the condition is not sufficient is shown by the following result.

Proposition 2. *Suppose that $F(x, 0) > 0$ for $x > 0$ and that the solution $y(x)$ of $\dot{x} = 0$ has a positive horizontal asymptote. Further, suppose that there exists an $x^{**} \geqslant x^*$ such that $F > G$ in the region R that lies to the right of x^{**}, above the x axis, and below $\dot{x} = 0$. Then all solutions of (2) that originate in Q_1^+ remain bounded for all $t > 0$.*

Proof. In light of the earlier remarks on the properties of solutions, it suffices to show that any solution that begins at a point $x_0 > x^{**}$ on the x axis crosses the curve $\dot{x} = 0$ for the first time at $\hat{t} < \infty$. From the hypotheses it follows that $dy/dx > 0$ along that part of any trajectory that lies in R, and if d^2y/dx^2 is always positive as well, the trajectory crosses $\dot{x} = 0$ at some finite t and the proposition is established. Next, suppose that $d^2y/dx^2 < 0$ at $(x_0, 0)$. Along a trajectory

$$\frac{d^2y}{dx^2} = \frac{[F_x + F_y(dy/dx)](\delta - G) - (\delta - F)G_y(dy/dx)}{(\delta - F)^2} \tag{13}$$

and this is positive near the curve $\dot{x} = 0$ in R. Therefore, either there exist an odd number of points on the trajectory in R at which d^2y/dx^2 changes sign, or there exists a horizontal asymptote $y = y^{**}$, $y^{**} < y^*$, of the trajectory. The latter requires that $(dy/dx)_{\text{trajectory}} \to 0$ as $x \to \infty$, but this is impossible because $F > G \geqslant 0$ in R. Therefore the trajectory crosses $\dot{x} = 0$ and the solution is bounded for all $t > 0$. The case where $d^2y/dx^2 > 0$ at $(x_0, 0)$ but not sign-definite, is handled in a similar fashion. This proves the proposition.

The physical interpretation of this result is as follows. If the input of A_1 exceeds the rate at which A_2 is degraded (i.e. when $y < y^*$) and the concentration of A_1 is large, the solution remains bounded if the rate at which A_1 is converted to A_2 also exceeds the rate at which A_2 is used. The result ensures boundedness whenever the $\dot{y} = 0$ locus is as shown in Figure 1a or 1b, but not in case $\dot{y} = 0$ is non-increasing, as in Figure 1c. We have not succeeded in proving a general result applicable to the latter case, but some particular forms of F and G are easy to treat. For instance, if $F(x, y)$ and $G(y)$ are given by

$$\begin{aligned} F(x, y) &= k_1 x y^\gamma \quad \gamma \geqslant 1 \\ G(y) &= k_2 y, \end{aligned} \tag{14}$$

then one can show that all trajectories of (2) for which $y(0)$ is sufficiently small tend to $(\infty, 0)$ as $t \to \infty$ (Selkov, 1968).

When F and G are such that all solutions of (2) remain bounded, it is sometimes possible to completely determine the nature of solutions.

Proposition 3. *Suppose that F and G are such that $F(x, 0) > 0$ and all solutions of (2) remain bounded. Then if $F_x + G_y > F_y$ throughout Q_1^+,*

$$\lim_{t\to\infty} (x(t), y(t)) = (x^*, y^*)$$

for all $(x(0), y(0)) \in Q_1^+$.

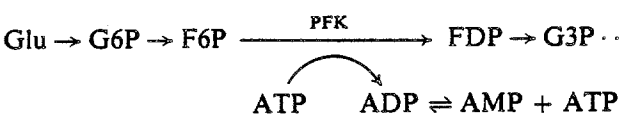
Proof. By hypothesis, (x^*, y^*) is asymptotically stable, and since $F_x + G_y > F_y$ throughout Q_1^+, Bendixson's criterion implies that there are no periodic solutions that lie entirely in Q_1^+. Since Q_1^+ is invariant under the flow of (2), the result follows.

A sufficient condition that ensures $F_x + G_y > F_y$ in Q_1^+ is $G_y > F_y$. Therefore, if the sensitivity of the output reaction is always greater than the sensitivity with respect to y of the intermediate reaction, the steady state is globally stable. A system that meets this condition is given by $F(x, y) = xy/(1 + x)$, $G(x, y) = ky$, $k > 1$.

When the steady state is unstable and all solutions remain bounded, the Poincare–Bendixson theorem (Hartman, 1973) implies the existence of at least one orbitally stable periodic solution. However, one cannot conclude *a priori* that this solution is unique and therefore, one cannot assert that a stable periodic solution that bifurcates when trace $K = 0$ is unique. Uniqueness must be decided on a case by case basis.

3. A Mechanism for the PFK Reaction

A wide variety of two-variable models that lead to equations like (2) have been proposed to explain the observed oscillations in glycolytic intermediates (see Gibbs and Murray (1976) for a review). The portion of the pathway of interest here is



The major control point resides in the phosphofructokinase reaction, in which fructose-6-phosphate (F6P) is phosphorylated to give fructose diphosphate (FDP). Phosphofructokinase (PFK) is activated by AMP and FDP and inhibited by ATP, but under conditions that lead to oscillations, PFK is fully activated with respect to FDP, and ATP has a negligible effect on activity (Betz, 1973). Therefore, if one assumes that a constant source of F6P exists, the major reaction is F6P → ADP. There is a net consumption of ADP in the entire glycolytic sequence and this can be accounted for with an ADP sink. Consequently, the overall reactions involving F6P and ADP can be modelled by (1), in which $A_1 \sim$ F6P and $A_2 \sim$ ADP.

The differences between existing two-variable models for glycolytic oscillations stem from the differences in the mechanism assumed for the PFK reaction. Here we assume that PFK is activated by a single molecule of AMP, that PFK exists in

either a low activity state or in an activated state, and that AMP is in equilibrium with ADP. Our mechanism is a variation of a general type of back activation mechanism proposed by Higgins (1967) and is similar to one studied by Gibbs and Murray (1976). The complete set of reactions is as follows.

$$
\begin{aligned}
&\xrightarrow{k} A_1 \\
&A_1 + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1A_1 \xrightarrow{k_2} E_1 + A_2 \\
&A_1 + E_1^* \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E_1^*A_1 \xrightarrow{k_4} E_1^* + A_2 \\
&A_2 + E_2 \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E_2A_2 \xrightarrow{k_6} E_2 + \text{Product} \\
&\hat{E}_1 + A_3 \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} \hat{E}_1^* \, 2A_2 \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} A_3 + A_4
\end{aligned}
\tag{15}
$$

Here E_1 and E_1^* represent the low activity and activated forms of free PFK, respectively; E_2 is the enzyme for the ADP sink reaction; E_1A_1, $E_1^*A_1$ and E_2A_2 are enzyme-substrate complexes; $\hat{E}_1^*$ and $\hat{E}_1$ are the total amounts of activated and low-activity enzyme, both in free and bound form; and A_3 and A_4 represent AMP and ATP, respectively. We assume that AMP binds to both the free and complexed form of low-activity PFK and that the interconversion between low-activity and activated form is always at equilibrium.

Let X and Y denote the dimensional concentrations of A_1 and A_2. The preceding assumptions, coupled with the pseudo-steady-state hypothesis, leads to the following differential equations for X and Y:

$$
\begin{aligned}
\frac{dX}{dt} &= K - \bar{F}(X, Y) \\
\frac{dY}{dt} &= \bar{F}(X, Y) - \bar{G}(Y).
\end{aligned}
\tag{16}
$$

Here

$$
\begin{aligned}
&\bar{F}(X, Y) \equiv \frac{1}{K_1 + Y^2}\left[\frac{V_{m_1}K_1X}{K_{m_1} + X} + \frac{V_{m_1}^*XY^2}{K_{m_1}^* + X}\right] \\
&\bar{G}(Y) \equiv \frac{V_{m_2}Y}{K_{m_2} + Y} \\
&K_1 \equiv \frac{k_{-7}k_{-8}A_4}{k_7k_8} \qquad V_{m_1} \equiv k_2E_1^T \\
&K_{m_1} \equiv \frac{k_{-1} + k_2}{k_1} \qquad V_{m_1}^* \equiv k_4E_1^T \\
&K_{m_1}^* \equiv \frac{k_{-3} + k_4}{k_3} \qquad V_{m_2} \equiv k_6E_2^T \\
&K_{m_2} \equiv \frac{k_{-5} + k_6}{k_5} \qquad E_1^T \equiv E_1 + E_1A_1 + E_1^* + E_1^*A_1 \\
&\qquad\qquad\qquad\quad E_2^T \equiv E_2 + E_2A_2.
\end{aligned}
\tag{17}
$$

The equations can be cast into the dimensionless form

$$\frac{dx}{d\tau} = \delta - F(x, y)$$
$$\frac{dy}{d\tau} = \alpha[F(x, y) - G(y)] \tag{18}$$

by defining

$$x = \frac{X}{K_{m_1}^*} \qquad y = \frac{Y}{K_{m_2}} \qquad k_1 = \frac{K_1}{K_{m_2}} \qquad k_2 = \frac{K_{m_1}}{K_{m_1}^*} \qquad \alpha = \frac{K_{m_1}^*}{K_{m_2}}$$
$$\beta = \frac{V_{m_2}}{V_{m_1}^*} \qquad \gamma = \frac{V_{m_1} K_1}{V_{m_1}^* K_{m_2}^2} \qquad \delta = \frac{K}{V_{m_1}^*} \qquad \tau = \frac{V_{m_1}^* t}{K_{m_1}^*} \tag{19}$$

and

$$F(x, y) = \frac{1}{k_1 + y^2}\left[\frac{\gamma x}{k_2 + x} + \frac{xy^2}{1 + x}\right]$$
$$G(y) = \frac{\beta y}{1 + y}.$$

The equations differ from those at (2) by the α factor in the y equation. It could be removed by setting $x = \bar{x}/\alpha$, $y = \bar{y}$, and redefining the other constants, but it proves more convenient to use the above form. The major parameters, and the only ones that we shall vary, are the dimensionless input δ and the dimensionless maximum output rate β. The others will be chosen to satisfy the conditions on F and G given at (3). From (19) it follows that

$$F_x = \frac{1}{k_1 + y^2}\left[\frac{\gamma k_2}{(k_2 + x)^2} + \frac{y^2}{(1 + x)^2}\right]$$
$$F_{xx} = \frac{-2}{k_1 + y^2}\left[\frac{\gamma k_2}{(k_2 + x)^3} + \frac{y^2}{(1 + x)^3}\right]$$
$$F_y = \frac{2xy[k_1 k_2 - \gamma + (k_1 - \gamma)x]}{(k_1 + y^2)^2(k_2 + x)(1 + x)} \tag{20}$$
$$F_{yy} = \frac{2x(k_1 - 3y^2)}{(k_1 + y^2)^3}\left[\frac{k_1 k_2 - \gamma + (k_1 - \gamma)x}{(k_2 + x)(1 + x)}\right]$$
$$G_y = \frac{\beta}{(1 + y)^2} \qquad G_{yy} = \frac{-2\beta}{(1 + y)^3}.$$

Therefore

$$\left.\begin{aligned} F_x &> 0 \\ F_{xx} &< 0 \\ G_y &> 0 \\ G_{yy} &< 0 \end{aligned}\right\} \quad \text{for all } (x, y) \in Q_1. \tag{21}$$

Furthermore, $F_y > 0$ for $(x, y) \in Q_1$ provided $k_1 > \gamma$ and $k_2 > \gamma/k_1$, while F_{yy} changes sign only once, at $y = \sqrt{k_1/3}$. All the conditions at (3) are fulfilled if k_1, k_2 and γ satisfy the foregoing restrictions.

The locus $\dot{x} = 0$ is given by

$$\delta = \frac{1}{k_1 + y^2}\left[\frac{\gamma x}{k_2 + x} + \frac{xy^2}{1 + x}\right] \tag{22}$$

and this yields the vertical asymptote

$$x = \frac{\delta}{1 - \delta}. \tag{23}$$

The solution of (22) is

$$y^2 = \left[\frac{(\gamma - \delta k_1)x - \delta k_1 k_2}{\delta + (\delta - 1)x}\right]\left[\frac{1 + x}{k_2 + x}\right], \tag{24}$$

which intersects the x axis at

$$x = \frac{k_1 k_2 \delta}{\gamma - k_1 \delta} \tag{25}$$

provided $\delta < \gamma/k_1$, and otherwise has the horizontal asymptote

$$y = \sqrt{\frac{\gamma - \delta k_1}{\delta - 1}}. \tag{26}$$

The latter requires that $\delta < 1$.

Along $\dot{y} = 0$, y is a solution of the equation

$$a(x)y^3 + b(x)y^2 + c(x)y + d(x) = 0 \tag{27}$$

where

$$\begin{aligned} a(x) &\equiv \frac{x}{1 + x} - \beta \\ b(x) &\equiv \frac{x}{1 + x} \\ c(x) &\equiv \frac{\gamma x}{k_2 + x} - k_1\beta \\ d(x) &\equiv \frac{\gamma x}{k_2 + x}. \end{aligned} \tag{28}$$

Clearly $b(x) > 0$ and $d(x) > 0$ for $x > 0$, $a(x) > 0$ for $x > \beta/(1 - \beta)$, and $c(x) > 0$ for $x > \beta k_2/(\gamma/k_1 - \beta)$. Generally $k_2 > 1$ and so $a(x) \geqslant c(x)$. The number of positive roots of (27) gives the number of intersections of $\dot{y} = 0$ with any vertical line $x =$ constant; this number varies with x as follows.

$$\begin{aligned} &x \in (0, \beta/(1 - \beta)): \text{ one or three positive real roots} \\ &x \in (\beta/(1 - \beta), \beta k_2/(\gamma/k_1 - \beta)): \text{ zero or two positive real roots} \\ &x \in (\beta k_2/(\gamma/k_1 - \beta), \infty): \text{ no positive real roots.} \end{aligned} \tag{29}$$

It is easily seen that the solution $y(x)$ of $\dot{y} = 0$ can be monotone increasing or monotone decreasing for all $x > 0$ only if $\beta > 1$. From (20) one sees that $G_y > F_y$

for y sufficiently small and therefore, $y(x)$ can never be monotone decreasing for all $x > 0$. If y is always monotone increasing along the isocline, the results of the previous section preclude the possibility of periodic solutions. Accordingly, we shall only consider the case $\beta < 1$, which gives the generic locus shown in Figure 1b.

The vertical asymptote of $\dot{y} = 0$ is at

$$x = \frac{\beta}{1 - \beta} \tag{30}$$

and the horizontal asymptotes, if any exist, are positive solutions of

$$ay^3 + by^2 + cy + d = 0. \tag{31}$$

Here $a \equiv (1 - \beta)$, $b \equiv 1$, $c \equiv \gamma - k_1\beta$ and $d = \gamma$. Equation (31) has either two or zero real positive roots, according as the discriminant

$$\Delta(\beta, \gamma, k_1) \equiv 18abcd - 4b^3d - 4ac^3 + b^2c^2 - 27ad^2, \tag{32}$$

is positive or negative. This discriminant is a quartic polynomial in β and is difficult to analyze in general. However, if there is no low-activity form of the enzyme, $\gamma \equiv 0$ and $y = 0$ is one branch of $\dot{y} = 0$. In this case one solution of (31) is $y = 0$ and the other positive solution is

$$y = \frac{-1 + \sqrt{1 + 4k_1\beta(1 - \beta)}}{2(1 - \beta)}. \tag{33}$$

If γ is small and positive the x axis is no longer invariant, but by continuity, (31) has two positive roots and the $\dot{y} = 0$ isocline is as shown in Figure 2. Clearly if the steady state lies on the upper branch of $\dot{y} = 0$, as shown, solutions that begin sufficiently close to the x axis approach the lower branch of $\dot{y} = 0$ asymptotically and $x \to \infty$ as $t \to \infty$. In general, whenever (31) has two positive roots and δ is sufficiently large, unbounded solutions will exist for some initial conditions. Moreover, their existence is independent of whether or not the steady state is stable.

The steady state (x^*, y^*) is the solution of the equations $\delta = F(x, y) = G(y)$. From these one finds that

$$y^* = \frac{\delta}{\beta - \delta} \tag{34}$$

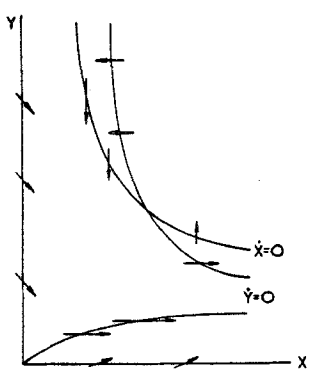


Fig. 2. The phase plane for a case in which unbounded solutions exist

and that x^* satisfies

$$\delta = \frac{1}{k_1 + y^2}\left[\frac{\gamma x}{k_2 + x} + \frac{xy^2}{1 + x}\right]. \tag{35}$$

Certainly y^* is positive if and only if $\beta > \delta$ and x^* is positive and finite provided that

$$\delta < \frac{1}{k_1 + (y^*)^2}[\gamma + (y^*)^2]. \tag{36}$$

Replacing the inequality with an equality yields a relation between δ and β that defines the locus along which $x^* = \infty$. Solving for β gives

$$\beta_\infty = \delta\left(1 + \sqrt{\frac{1 - \delta}{k_1\delta - \gamma}}\right). \tag{37}$$

Since $\gamma < k_1$, there is no positive steady state if $\delta > 1$ or if $\beta > \beta_\infty$. The absence of a steady state arises because the $\dot{y} = 0$ isocline has two horizontal asymptotes and the $\dot{x} = 0$ isocline passes between them. Thus the region whose boundary is given by (37) necessarily lies within the region where $\Delta > 0$. If γ and k_1 are fixed at 0.1 and 1.0, respectively, the region $\Delta > 0$ is found to be $\{(\delta, \beta) \mid 0 < \delta < 1;\ 0.74 \leqslant \beta < 1\}$. The region in which there is no positive steady state is shown in Figure 3 for the same choice of parameters. As δ increases across the left-hand boundary of the hatched region, the steady state leaves the lower branch of $\dot{y} = 0$ at $x = \infty$ and at the right-hand boundary it reappears on the upper branch. If $\beta > 0.74$ and δ lies to the right of the hatched region, any solution that begins beneath the lower branch of $\dot{y} = 0$ is unbounded as $t \to \infty$. Thus $F(x, 0) > 0$ is not sufficient to preclude unbounded solutions.

For the same values of γ and k_1, there are no horizontal asymptotes of $\dot{y} = 0$ when $\beta < 0.74$, and all the hypotheses of Proposition 2 are satisfied. Consequently, all solutions that originate in Q_1^+ remain bounded for all $t > 0$, and it only remains to determine the stability of the steady state as a function of δ and β. From results of the preceding section we know that the steady state is stable at low and high fluxes and perhaps unstable at intermediate values. The boundary of the region of instability is given by the solution of the simultaneous equations

$$\begin{aligned} &\delta = F(x, y) \\ &\delta = G(y) \\ &\text{trace } K = \alpha(F_y - G_y) - F_x = 0. \end{aligned} \tag{38}$$

There is no alternative to solving these numerically and this has been done for $\gamma = 0.1$, $k_1 = 1.0$, $\alpha = 0.1$ and $k_2 = 10.0$. The resulting locus of marginal (oscillatory) stability is the solid curve shown in Figure 3. At fixed β, trace $K > 0$ between the left and right branches of this locus.

Suppose that β is fixed at some value larger than the minimum on this locus. As we remarked in the preceding section, if $d(\text{trace } K)/d\delta \neq 0$ on the locus trace $K = 0$, the Hopf theorem guarantees the existence of a periodic solution for δ near this

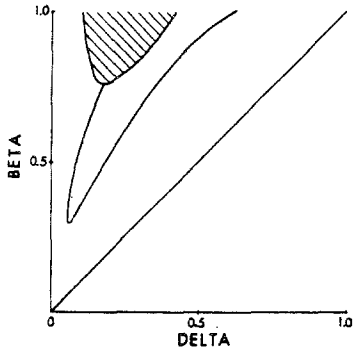


Fig. 3. The δ–β plane for $k_1 = 1.0$, $k_2 = 10.0$, $\gamma = 0.1$. No positive steady state exists for $\delta > \beta$ or for (δ, β) in the hatched region. The solid curve shows the locus trace $K = 0$

locus. However, the direction and stability of the bifurcating periodic solution must be established separately. In the Appendix we outline the procedure for doing this; the results are as follows. The bifurcating solution is stable along the entire locus of marginal stability. Along the left branch the bifurcating solution exists only to the right of the bifurcation point while on the right branch the solution exists only to the left of the bifurcation point. Thus a stable periodic solution appears along the left branch and disappears along the right branch. Throughout the region trace $K > 0$ the steady state is unstable, and the Poincare–Bendixson theorem implies the existence of at least one stable periodic solution. In principle, there could also be unstable solutions and other stable solutions, all of whose orbits are concentric, but numerical computations indicate that there is only one periodic solution.

The differential equations were integrated for $\beta = 0.6$ at various values of δ in the unstable region. Both the root-mean-square amplitude and the period were computed; the results are shown in Figure 4. Near the left boundary of the unstable region the amplitude first rises sharply, grows more slowly as δ increases, and then shrinks to zero at the right boundary. There is no evidence that periodic solutions exist when δ lies outside the unstable region. The period first increases sharply and then begins a slow decrease, until, near the right boundary, it drops sharply to the period of the linear system.

The periodic solutions are shown in Figure 5 for $\beta = 0.6$ and several δ values. Separate plots of the x component versus time show that near the left and right bifurcation points the waveform is nearly sinusoidal, even though the amplitude is large, while near the center of the region of instability the solution is more like a relaxation oscillation. It is apparent in Figure 5 that as the flux increases, x^* decreases and y^* increases. One can see that the net effect of increasing the flux from some value below the lower bifurcation point to a value above the upper bifurcation point is a transition from a steady state of large x and small y to one of small x and large y. The transition between these states, as δ increases across the unstable region, occurs via a sequence of periodic states of varying amplitude and period.

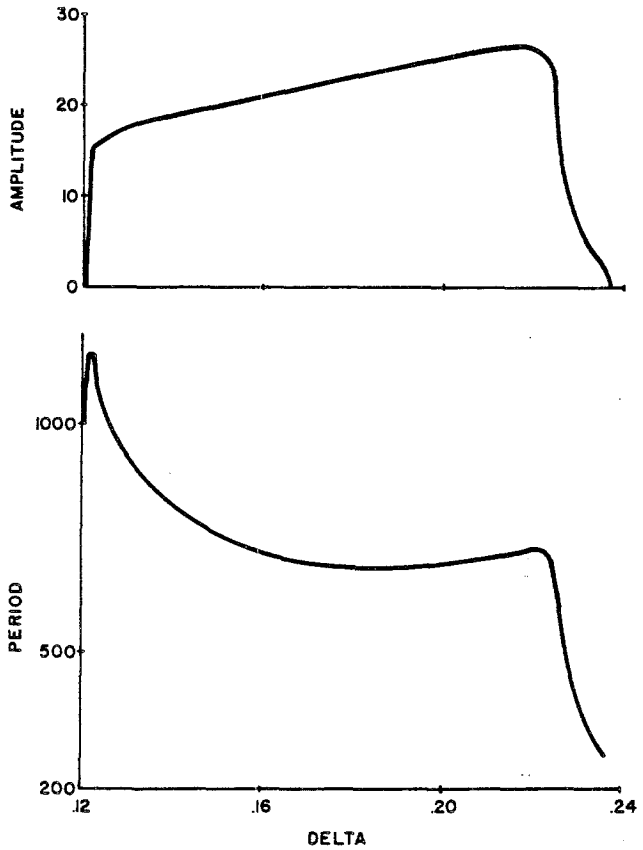


Fig. 4. The dependence of amplitude and period on the flux δ. $\beta = 0.6$ and other parameters are as in Figure 3. The bifurcation points are $\delta = 0.1205$ and $\delta = 0.237$

Qualitatively the computational results agree with observations on glycolytic oscillations. It is known that oscillations only exist over a range of input fluxes in yeast cell extract (Hess and Boiteux, 1973) and it has been shown that the oscillations vanish if glucose uptake is inhibited in intact cells (Aldridge, 1976). The period dependence on flux shown in Figure 4 also agrees qualitatively with results for yeast extract, at least in the intermediate regime where period decreases as the input flux increases (Hess and Boiteux, 1973). It would be difficult to obtain sufficiently accurate data to determine whether the initial sharp increase in period near the left bifurcation point occurs in reality. However, such qualitative agreement could be achieved with a wide variety of models of the kind studied in the preceding section and hence it cannot be regarded as confirmation of the particular mechanism we have analyzed. Since we are more interested in the generic properties of models like this, we shall not pursue a quantitative comparison of theory and experiment, but instead, we turn to an analysis of the role of cell density.

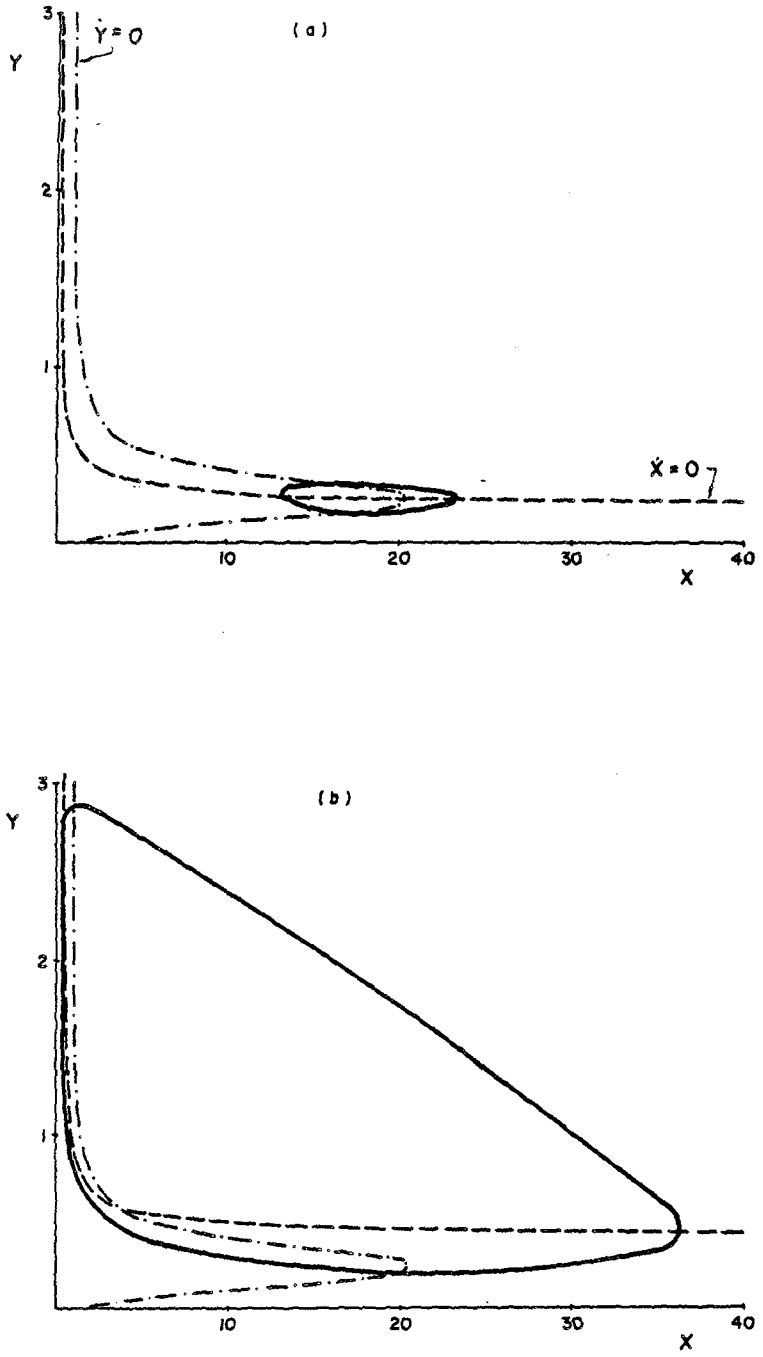


Fig. 5. The periodic solutions in the x–y plane. (a) $\delta = 0.121$, (b) $\delta = 0.22$, (c) $\delta = 0.23$. Other parameters are as in Figure 4; $\alpha = 0.1$ throughout

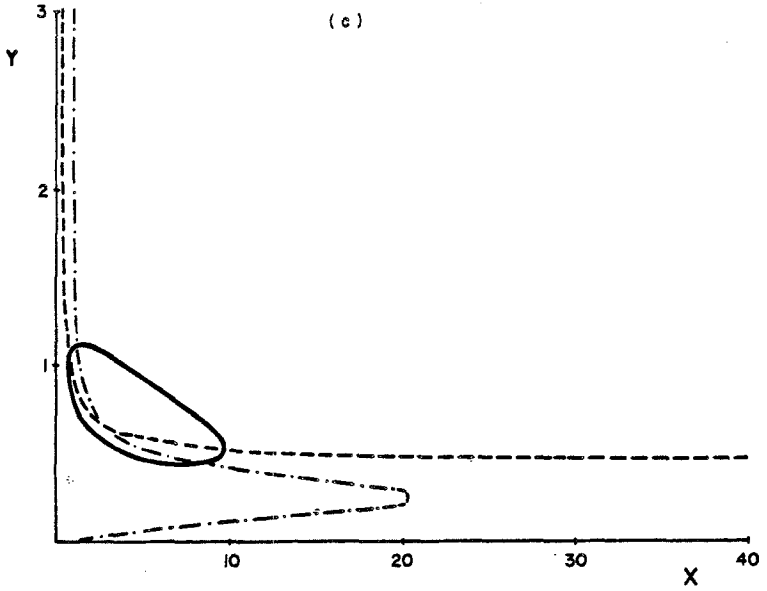


Fig. 5. (*continued*)

4. The Role of Cell Density

A. General Analysis

The conclusions of the preceding sections are valid under the assumption that the reacting mixture is homogeneous, as for instance, in experiments using yeast cell extract. However, as we have already noted, sustained oscillations of intracellular components are observed even in intact cell suspensions (Aldridge, 1976). Even if the cells are virtually identical, still, some mode of intercellular communication is needed to damp out inevitable concentration disturbances that would otherwise destroy the synchrony of the suspension. Since there is no apparent contact between cells, the communication must occur indirectly via the extracellular medium. Unfortunately, no messenger substances have been identified to date. For this reason, and because such indirect communication is likely to be important in many other systems, we carry the analysis here as far as possible using an arbitrary kinetic mechanism. Only later will we restrict ourselves to a two-variable, positive-feedback mechanism.

Consider a suspension of N identical cells, each of volume V_1, in a well-mixed medium of volume V_0. Suppose that there are n species involved in the intracellular reactions, that there is no reaction in the extracellular medium, and that one or more of the reacting species can diffuse across the cell membrane. Let $\mathscr{R}_i$ be the dimensionless net rate of production of the ith species, P_i the dimensionless permeability, x_i^j the dimensionless concentration of species i in the jth cell, and x_i^0 its

concentration in the medium. The equations governing the dynamics of the suspension are

$$\frac{dx^j}{d\tau} = \mathscr{R}(x^j) + P(x^0 - x^j) \qquad j = 1, \ldots, N \tag{39}$$

$$\frac{dx^0}{d\tau} = \varepsilon P(\bar{x} - x^0) \tag{40}$$

where

$$x^j \equiv \begin{pmatrix} x_1^j \\ \vdots \\ x_n^j \end{pmatrix} \qquad \mathscr{R}(x^j) \equiv \begin{pmatrix} \mathscr{R}_1(x^j) \\ \vdots \\ \mathscr{R}_n(x^j) \end{pmatrix}$$

$$P = \begin{bmatrix} P_1 & 0 & \cdots & 0 \\ & P_2 & & \\ \vdots & & \ddots & \\ 0 & & \cdots & P_n \end{bmatrix} \qquad \bar{x} \equiv \frac{1}{N} \sum_{j=1}^{N} x^j. \tag{41}$$

$$\varepsilon \equiv V_1 N / V_0.$$

The volumetric ratio ε provides a suitable measure of the relative cell density in the suspension. The dimensionless permeabilities P_i are related to their dimensional version by $P_i = A\mathscr{P}_i/V_1$, where A is the area of a cell. We do not consider the possibility of coupling between species in the rate of transport, nor do we consider more complicated modes of transport. Each of these possibilities may be important in some contexts.

If the cell density is small ($\varepsilon \ll 1$), it follows from (40) that x^0 varies slowly compared to x^j and the medium functions as a constant-concentration bath. At the other extreme, when $\varepsilon \gg 1$, (40) shows that x^0 varies rapidly compared to x^j and in the limit $\varepsilon \to \infty$, $x^0 \to \bar{x}$. We shall analyze the dynamical behavior between these extremes.

First consider the stability of a steady state in which the concentrations in all cells are identical and equal to those in the medium. Let X be the solution of $\mathscr{R}(X) = 0$, set $x^j = X + \xi^j$, and linearize (39); the result is the linear system

$$\frac{d\xi^j}{d\tau} = K\xi^j + P(\xi^0 - \xi^j) \qquad j = 1, \ldots, N \tag{42}$$

$$\frac{d\xi^0}{d\tau} = \varepsilon P(\bar{\xi} - \xi^0), \tag{43}$$

where $K_{ij} \equiv \partial R_i / \partial x_j|_{x_j = X_j}$.

By adding the N equations at (42) one gets

$$\frac{d\bar{\xi}}{d\tau} = K\bar{\xi} + P(\xi^0 - \bar{\xi}). \tag{44}$$

If this is subtracted from each of the N equations in (42), the result is

$$\frac{du^j}{d\tau} = (K - P)u^j \qquad j = 1, \ldots, N \tag{45}$$

where

$$u^j \equiv \xi^j - \bar{\xi}. \tag{46}$$

and $\sum_{j=1}^{N} u^j = 0$.

Consequently, (45) contains $n(N-1)$ independent equations. The remaining $2n$ equations needed are given by (43) and (44). Notice that the latter equations contain only $\bar{\xi}$ and ξ^0.

Evidently the evolution of disturbances of the steady state is governed by the eigenvalues of the matrix $K - P$ and those of

$$L \equiv \left[\begin{array}{c|c} K-P & P \\ \hline \varepsilon P & -\varepsilon P \end{array}\right]. \tag{47}$$

A distinction must be made here between the case of one cell in a medium and the multicellular case. In the former, only the eigenvalues of (47) come into consideration, because $N - 1 = 0$ and (45) is trivial. Later we shall see the consequences of this difference, but for the present we assume $N \geqslant 2$.

Changes in the cell density only affect the eigenvalues of L. If we write L in the product form

$$L = \left[\begin{array}{c|c} I & 0 \\ \hline 0 & \varepsilon I \end{array}\right]\left[\begin{array}{c|c} K-P & P \\ \hline P & -P \end{array}\right] \tag{48}$$

then

$$\begin{aligned} \det L &= \varepsilon^n \det \left[\begin{array}{c|c} K-P & P \\ \hline P & -P \end{array}\right] \\ &= (-\varepsilon)^n \det P \det K \end{aligned} \tag{49}$$

where $\det L$ denotes the determinant of L. Therefore, L has a zero eigenvalue if and only if one of the three factors in (49) vanishes.

The eigenvalues of L are the solutions of

$$\det [\lambda^2 I + \lambda(P - K + \varepsilon P) - \varepsilon PK] = 0. \tag{50}$$

At $\varepsilon = 0$ ($V_0 = \infty$), this reduces to

$$\det [\lambda^2 I + \lambda(P - K)] = 0 \tag{51}$$

and therefore, to zero order in ε,

$$\begin{aligned} \lambda_j &= \lambda_j^{K-P} \qquad j = 1, \ldots, n \\ \lambda_j &= 0 \qquad j = n+1, \ldots, 2n. \end{aligned} \tag{52}$$

(Here and hereafter λ_j^A denotes the jth eigenvalue of A.) To find the first-order terms for the zero eigenvalues, assume that the solutions of (50) are distinct for

small ε; the general case is only slightly more difficult. In this case (50) defines an algebraic function with $2n$ distinct branches for ε small. Of these, n are given above:

$$\lambda_j \sim \lambda_j^{K-P} + \mathcal{O}(\varepsilon) \tag{53}$$

where $f(\varepsilon) \sim \mathcal{O}(\varepsilon)$ means that

$$\lim_{\varepsilon \to 0} \frac{f(\varepsilon)}{\varepsilon} \tag{54}$$

is bounded.

To find the remaining n, set $\lambda = \varepsilon\bar{\lambda}$ in (50); then $\bar{\lambda}$ satisfies

$$\det [\bar{\lambda}(P - K) - PK] = 0 \tag{55}$$

and if $P - K$ is non-singular, $\bar{\lambda}_j = \lambda_j^{(P-K)^{-1}PK}$. Therefore

$$\begin{aligned} \lambda_j &\sim \varepsilon\bar{\lambda}_j + \mathcal{O}(\varepsilon^2) \\ &\sim \varepsilon\lambda_j^{(P-K)^{-1}PK} + \mathcal{O}(\varepsilon^2). \end{aligned} \tag{56}$$

If $P - K$ is singular, (50) must first be rescaled. A similar analysis can be done to find asymptotic expansions for the eigenvalues of L when ε is large. The results for both cases are summarized in the following table, along with the ε-independent eigenvalues.

Consider first the limit $\varepsilon \to \infty$, in which the cells are closely packed. All the permeabilities P_j are non-negative and so n modes decay rapidly, unless some $P_j \equiv 0$. For such a mode the next term has to be checked. The remaining modes have eigenvalues λ_j^K or λ_j^{K-P} and if the steady state is unstable in the absence of exchange with the medium, it remains unstable in its presence. On the other hand, even if K has only eigenvalues in the left-hand plane, the steady state will be unstable if $K - P$ has any eigenvalues with a positive real part. Such an instability is the first example of how indirect coupling between cells can affect cellular dynamics; if only one cell is present this instability can arise only in the limit $\varepsilon \to 0$, whereas it exists for all ε in the multicellular case. An example done later in this section will illustrate this.

The table shows that in the limit $\varepsilon \to 0$ there is no difference between the single cell and multicellular cases. Because the eigenvalues of K alone do not appear in this limit, the possibility seems to exist of choosing ε and P so as to stabilize a steady state that would be unstable in the absence of transport to the extracellular medium.

Table 1. Asymptotic expansions for the eigenvalues

$\varepsilon \to 0$	$\varepsilon \to \infty$
$\lambda_j = \lambda_j^{K-P}$ $j = 1, \ldots, n$ ($N - 1$ times)	
$\lambda_j \sim \lambda_j^{K-P} + \mathcal{O}(\varepsilon)$	$\lambda_j \sim \lambda_j^K + \mathcal{O}(\varepsilon^{-1})$
$\lambda_j \sim \varepsilon\lambda_j^{(P-K)^{-1}PK} + \mathcal{O}(\varepsilon^2)$	$\lambda_j = -\varepsilon P_j + \mathcal{O}(1)$

That is, the presence of the extracellular medium may serve to quench instabilities that exist in the kinetic mechanism. Such quenching will occur whenever P and ε can be chosen such that all the eigenvalues of $K - P$ and of L have negative real parts, even though one or more eigenvalues of K has a positive real part. A P always exists for which the eigenvalues of $K - P$ have negative real parts; simply choose $P = pI$, p a scalar, and make p sufficiently large. However, this choice of P may not ensure that all the eigenvalues of L have negative real parts, and in some cases, no choice of P will suffice for this.

Proposition 4. *Suppose that K is non-singular and that it has an odd number of real positive eigenvalues. Then L has an odd number of real positive eigenvalues for all $\varepsilon > 0$.*

Proof. Suppose first that P is non-singular. Since $\det A = \prod \lambda_i^A$, (49) can be rewritten

$$\prod_{i=1}^{2n} \lambda_i^L = (-\varepsilon)^n \left(\prod_{j=1}^{n} P_j \right) \left(\prod_{j=1}^{n} \lambda_j^K \right) \tag{57}$$

Since every P_j is positive, then for $\varepsilon > 0$

$$\operatorname{sgn} \left(\prod_{i=1}^{2n} \lambda_i^L \right) = (-1)^n \operatorname{sgn} \left(\prod_{j=1}^{n} \lambda_j^K \right).$$

If n is even K must have an odd number of real negative eigenvalues while if n is odd it has an even number; in either case

$$\operatorname{sgn} \left(\prod_{i=1}^{2n} \lambda_j^L \right) = -1 \tag{58}$$

and so L has an odd number of real negative eigenvalues. Since L has $2n$ eigenvalues, it must also have an odd number of real positive eigenvalues.

Next, suppose that $m < n$ permeabilities P_j are zero; without loss of generality it can be assumed that they are the last m. By deleting the last m rows and columns of L, one obtains the reduced matrix

$$L^* = \begin{bmatrix} K_{11} - P^* & K_{12} & P^* \\ K_{21} & K_{22} & 0 \\ \varepsilon P^* & 0 & -\varepsilon P^* \end{bmatrix}. \tag{59}$$

Here K has been partitioned to conform with P^*: K_{11} is $(n - m) \times (n - m)$, K_{22} is $m \times m$, etc. One finds by expanding (59) that

$$\det L^* = (-\varepsilon)^{n-m} \det P^* \det K. \tag{60}$$

Therefore,

$$\prod_{i=1}^{2n-m} \lambda_i^{L^*} = (-\varepsilon)^{n-m} \left(\prod_{j=1}^{n-m} P_j \right) \left(\prod_{j=1}^{n} \lambda_j^K \right). \tag{61}$$

Since all P_j in P^* are positive,

$$\operatorname{sgn} \left(\prod_{i=1}^{2n-m} \lambda_i^{L^*} \right) = (-1)^{n-m} \operatorname{sgn} \left(\prod_{j=1}^{n} \lambda_j^K \right). \tag{62}$$

Now the argument applied when P is non-singular can be used, except that here there are four cases since either n or m can be even or odd. This proves the proposition.

The proposition implies that eigenvalues of L can only cross the imaginary axis in pairs as ε is varied. When $\varepsilon > 0$ and $P_j > 0$ ($P_j \geqslant 0$), det L (det L^*) could vanish only if det $K = 0$. But det $K > 0$ and therefore, only complex conjugate pairs of eigenvalues can cross as ε varies. Consequently, changes in cell density can affect the collective dynamics qualitatively only by suppressing sustained oscillations that exist in the kinetic mechanism, or by generating sustained oscillations even though none are present in an isolated cell. Both possibilities are illustrated in the following example. For similar conclusions concerning the effect of capacitance terms on stability in different contexts, see Luss (1974); Othmer (1976) and Perelson (1976).

B. Generation and Suppression of Sustained Oscillations

Consider first the case of a single cell in the medium. Suppose that the intracellular reactions are described by (18) and that only x diffuses across the cell membrane. The equations that describe this situation are

$$\begin{aligned} \frac{dx}{d\tau} &= \delta - F(x, y) + P(x^0 - x) \\ \frac{dy}{d\tau} &= \alpha[F(x, y) - G(y)] \\ \frac{dx^0}{d\tau} &= \varepsilon P(x^0 - x) \end{aligned} \tag{63}$$

where F and G are given by (19) and x, y, x^0 and P are scalars.

The steady state (x^*, y^*, x^0) is the solution of the system

$$\begin{aligned} x^0 &= x^* \\ \delta &= F(x^*, y^*) = G(y^*) \end{aligned} \tag{64}$$

and the matrix for the linearization of (63) around this steady state is

$$L^* = \begin{bmatrix} k_{11} - P & k_{12} & P \\ k_{21} & k_{22} & 0 \\ \varepsilon P & 0 & -\varepsilon P \end{bmatrix}. \tag{65}$$

Here

$$\begin{aligned} k_{11} &= -F_x \\ k_{21} &= \alpha F_x \\ k_{12} &= -F_y \\ k_{22} &= \alpha(F_y - G_y). \end{aligned} \tag{66}$$

The characteristic equation for L^* is

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0 \tag{67}$$

where

$$\begin{aligned} a_1 &= (1+\varepsilon)P - T^K \\ a_2 &= D^K - \varepsilon P T^K - P k_{22} \\ a_3 &= \varepsilon P D^K \end{aligned} \tag{68}$$

and

$$\begin{aligned} T^K &\equiv \text{trace } K = k_{11} + k_{22} = \alpha(F_y - G_y) - F_x \\ D^K &\equiv \det K = k_{11}k_{22} - k_{12}k_{21} = \alpha F_x G_y. \end{aligned}$$

According to the Routh–Hurwitz criterion, the roots of (67) all have negative real parts if and only if

$$\begin{aligned} &a_1 > 0 \\ &a_3 > 0 \\ &H_2 \equiv a_1 a_2 - a_3 > 0. \end{aligned} \tag{69}$$

Since $P > 0$ and $D^K > 0$, $a_3 > 0$ for $\varepsilon > 0$, and we only have to check the first and third conditions. First suppose that the kinetic mechanism is stable at (x^*, y^*). Then $T^K < 0$ and $a_1 > 0$, which leaves

$$H_2(\varepsilon, P) = -\varepsilon^2 P^2 T^K - \varepsilon P[PT^K + Pk_{22} - (T^K)^2] - (D^K - Pk_{22})(T^K - P) \tag{70}$$

as the critical parameter. The locus in parameter space along which $H_2 = 0$ is the locus of marginal oscillatory instability and the Hopf theorem (see the Appendix) predicts that upon crossing this, a periodic solution usually appears or disappears. According to (66), $k_{11} < 0$ but k_{22} can have either sign. If $k_{22} < 0$, then since $D^K > 0$ and $T^K < 0$, $H_2 > 0$ for all $(\varepsilon, P) > (0, 0)$. Therefore *a necessary condition for oscillatory instability when the kinetics are stable is* $k_{22} > 0$. Furthermore, one can see from (70), by interchanging the roles of 1 and 2, that if the self-activating species (the one with $k_{ii} > 0$) diffuses into the medium rather than the self-inhibiting species, then $H_2 > 0$ for all $(\varepsilon, P) > (0, 0)$. If both species diffuse then (69) and (70) no longer apply, because the system is four-dimensional, but the general conclusion is similar: *if the self-inhibiting species diffuses fast enough relative to the self-activating species, an oscillatory instability may exist, even though the kinetics are stable.*

Accordingly, suppose that the parameters γ, k_1, k_2, δ and β are so chosen that $T_1^K < 0$ and $k_{22} > 0$. For $\gamma = 0.1$, $k_1 = 1.0$ and $k_2 = 10.0$, this region is shown in Figure 6. Let $\Delta(P)$ be the discriminant of (70), regarded as a quadratic in ε, and let $\hat{P}$ be the larger of the largest root of $\Delta(P) = 0$ and zero. Further, let

$$\bar{P} \equiv D^K / k_{22}$$

and when $k_{11} + 2k_{22} > 0$, let

$$\tilde{P} \equiv \frac{(T_1^K)^2}{k_{11} + 2k_{22}}. \tag{71}$$

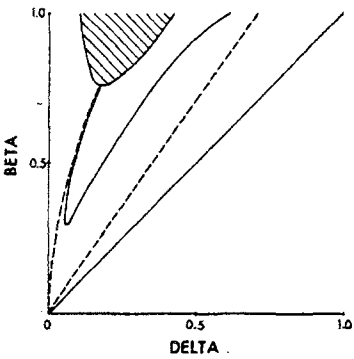


Fig. 6. The δ–β plane shown in Figure 4, with the addition of the locus $k_{22} = 0$ (broken curve). $k_{22} > 0$ between the left and right branches

Then $\Delta(P) > 0$ for $P > \hat{P}$, the coefficient of the linear term in (70) is negative for $P > \tilde{P}$, and the constant term in (70) is negative for $P > \bar{P}$. From this one concludes that

(i) if $P > \bar{P}$, then $H_2 < 0$ for $\varepsilon \in (0, \varepsilon_1)$, where ε_1 is the unique positive root of $H_2 = 0$,
(ii) if $\max\{\tilde{P}, \hat{P}\} < P < \bar{P}$, then $H_2 < 0$ for $\varepsilon \in (\varepsilon_1, \varepsilon_2)$, where ε_1 and ε_2 are the positive roots of $H_2 = 0$, (72)
(iii) if $P < \min[\max\{\tilde{P}, \hat{P}\}, \bar{P}]$, $H_2 > 0$ for all $\varepsilon > 0$.

As $T^K \to 0$, $\hat{P} \to 0$ and $\tilde{P} \to 0$, and $\varepsilon_2 \to \infty$ in (ii). For any fixed (β, δ) in the region $T^K \leqslant 0$ and $k_{22} > 0$, the locus $H_2 = 0$ is one of those shown in Figure 7a, b and c.

A similar analysis can be done when the kinetics are unstable by virtue of having $T^K > 0$. From (68), $a_1 > 0$ if $P > T^K/(1 + \varepsilon)$ and if we choose $T^K < \bar{P}$, the locus $H_2 = 0$ is as shown in Figure 7d. All the Routh–Hurwitz conditions are satisfied inside the horseshoe-shaped region. The transition from (a) → (b) → (c) → (d) in Figure 7 is accomplished by increasing k_{22}.

Consider what happens as the volume of the extracellular medium is increased (ε is decreased) at some fixed P. For the case shown in Figure 7a, the steady state is stable for all $\varepsilon > 0$ as long as $P < \bar{P}$. For $P = P_1 > \bar{P}$, a periodic solution bifurcates as ε crosses ε_1. In every case like this for which we computed the direction of bifurcation, we found that the bifurcating solution exists for $\varepsilon > \varepsilon_1$ and is *unstable*. It may be that there is an $\varepsilon_2 > \varepsilon_1$ at which the unstable solution merges with a stable solution so that there are no periodic solutions for $\varepsilon > \varepsilon_2$. We have not pursued this aspect because, as we shall see, these periodic solutions are of interest only in a single-cell system.

When $\max\{\tilde{P}, \hat{P}\} < \bar{P}$, as in Figure 7b, the computations show that a stable periodic solution appears as ε crosses ε_2 from above, and a stable periodic solution disappears as ε crosses ε_1 from above. Therefore, there is only a finite range of ε within which communication with the external medium leads to oscillatory instabilities. On the other hand, if the kinetics are unstable and ε and P are chosen

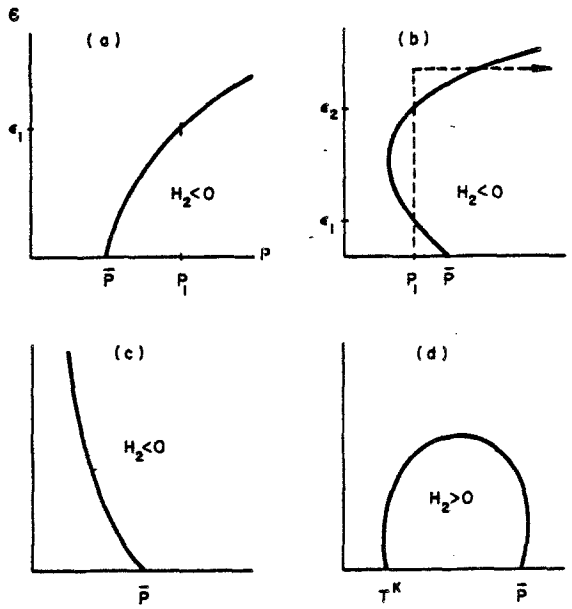


Fig. 7. Schematics of the generic $H_2 = 0$ loci. (a) $k_{11} + 2k_{22} < 0$, (b) $k_{11} + k_{22} < 0 < k_{11} + 2k_{22}$, (c) $k_{11} + k_{22} = 0$, (d) $k_{11} + k_{22} > 0$

to lie within the region $H_2 > 0$ in Figure 7d, the steady state is stable and disturbances of sufficiently small amplitude all decay. However we cannot say that *all* oscillations are suppressed for such (ε, P) pairs, since we have been unable to prove that the steady state is globally stable under these conditions.

If there is more than one cell present we have to examine the eigenvalues of $K - P$ as well. These are independent of ε and the analysis is straightforward. One finds that

(i) if $T_1^K < 0$, both eigenvalues of $K - P$ have negative real parts if $P < \bar{P}$, and one is real and positive if $P > \bar{P}$,
(ii) if $T_1^K > 0$, both eigenvalues have a positive real part if $P < T^K$, both have a negative real part if $T^K < P < \bar{P}$, and one is real and positive if $P > \bar{P}$. (73)

This information can be superimposed on that given in Figure 7 when dealing with a multicellular system. For example, the periodic solutions that arise in the case shown in Figure 7a are uninteresting in a multicellular system because the steady state is unstable for all $P > \bar{P}$ and *any* $\varepsilon > 0$. However, the conclusion on suppression of small amplitude oscillations, reached in connection with Figure 7d, applies equally well to a multicellular system when $P \in (T^K, \bar{P})$.

The fact that changes in the cell density can give rise to sustained oscillations over a finite range of densities, as in Figure 7b, may be relevant to observations on intact yeast cell suspensions. In Figure 8, experimental results on the amplitude and

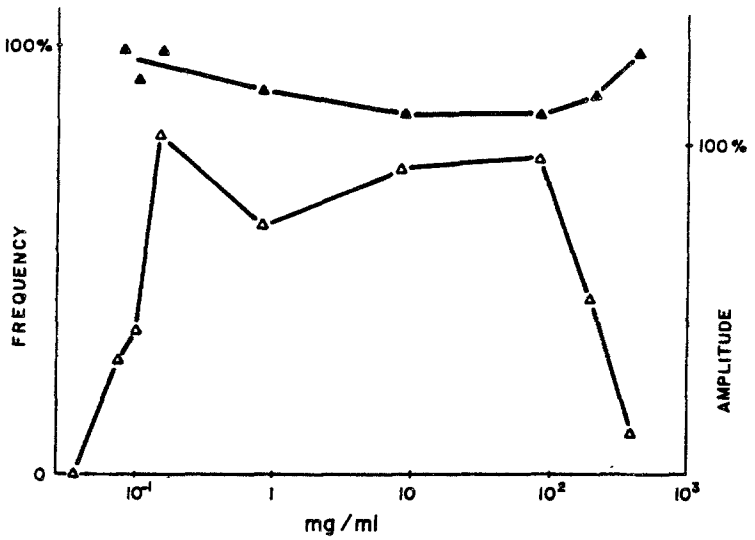


Fig. 8. The amplitude (△) and frequency (▲) dependence on cell density for intact yeast cell suspensions. Density is in mg wet weight of cells/ml solution. (After Aldridge and Pye (1976))

frequency of glycolytic oscillations are shown as a function of the cell density. Evidently the oscillations exist only over a limited range in the density. For comparison, we computed the periodic solution for a single cell with $\beta = 0.6$, $\delta = 0.239$, $P = 0.023$ and the remaining parameters as given in Figure 6[1]. [This point lies very close to the locus trace $K = 0$ in the $\delta - \beta$ plane.] The results for the period and amplitude are shown in Figure 9. Qualitatively the amplitude dependence on ε is quite similar to the experimental results shown in Figure 8. By adjusting the parameters somewhat one could extend the range over which the computed periodic solutions exist to better match the experimental results. However, there is little point in doing this at present, because there is as yet no concrete information on the identity of the molecule(s) used for intercellular communication. It is undoubtedly not F6P, but it has been suggested that a peptide may play the role in some yeasts (Kraepelin and Franck, 1973).

5. Discussion

The results of our analysis can be viewed from two perspectives. On the one hand, they establish the qualitative dynamical behavior of a whole class of models that includes many of those used to model the control steps in the glycolytic pathway. One can see, for example, what general properties the rate functions F and G must have in order for the model to predict that periodic solutions exist only over a limited range of the input flux. Furthermore, we have shown that the experimental observations on the density dependence of the oscillations can be qualitatively

[1] The effect of additional cells, when all are identical and begin in phase, is simply to rescale the density.

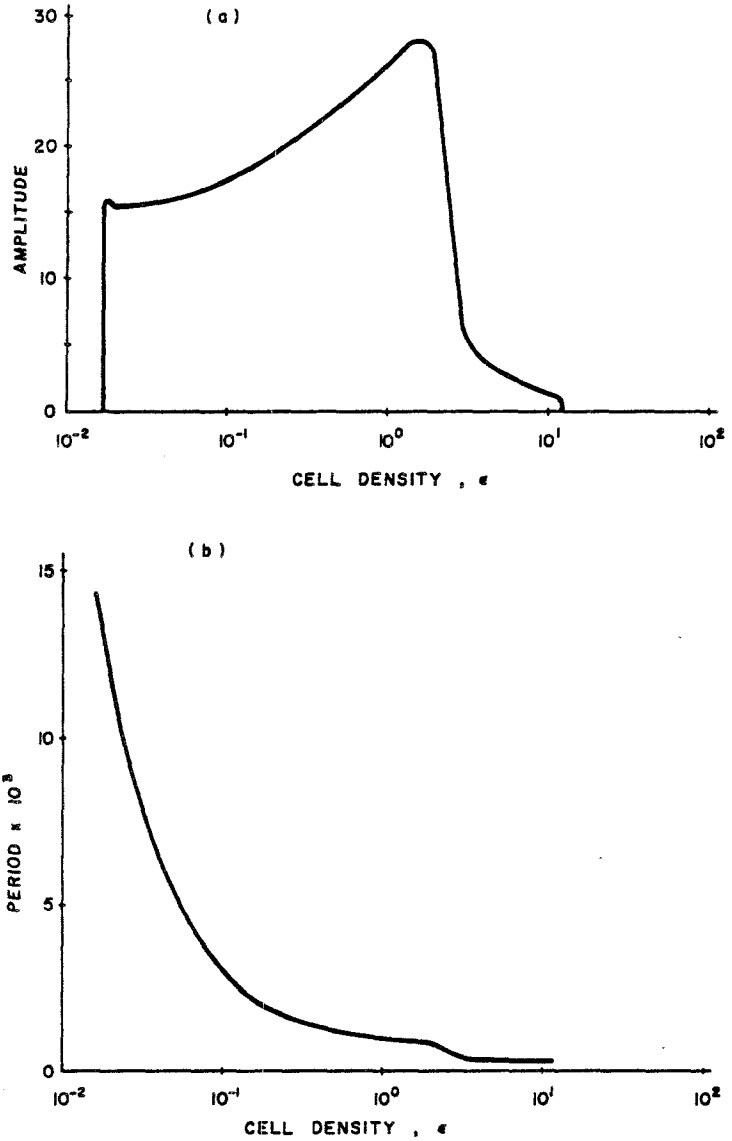


Fig. 9. The computed amplitude and period dependence on cell density for $\alpha = 0.1$, $\beta = 0.6$, $\delta = 0.239$, $P = 0.023$ and other parameters as in Figure 4

reproduced with simple models that include exchange between the cell and the medium. The only generic requirements of a two-variable kinetic model that are needed to reproduce the density effect are that one of the species be self-activating at the steady state and that this species diffuses slowly enough compared with the self-inhibiting species. Even these requirements can be relaxed if three-variable kinetic models are admitted. One can show that periodic solutions that arise

because of coupling between the cells and the medium are possible even if all three species are self-inhibiting (Othmer, 1977b).

On the other hand, the qualitative conclusions may be applicable in the broader context of growth and differentiation control in cell and tissue cultures. Cell proliferation in normal tissues must be controlled to maintain a homeostatic state and, as Folkman and Greenspan (1975) have emphasized, growth can be regulated at many levels. For example, the concentration of available glucose has been found to be important in initiation of DNA synthesis in 3T3 cells (Holley and Kiernan, 1974). Another factor whose effect may be similar is the presence of a diffusion boundary layer surrounding the cell. Stoker (1973) has demonstrated this effect very elegantly and conclusively by showing that division can be stimulated by exposing cells to a moving stream of the nutrient medium. Apparently this moving stream ensures a sufficient flux of some metabolite important in the mitotic cycle.

In other systems geometric factors appear to be the important variables. For instance, some cells fail to divide when freely suspended in a nutrient medium, but proliferate once a sufficiently large object is present which they can attach to and spread over. Conceivably the surface/volume ratio controls division via the supply rate of essential nutrients. A similar effect is observed for certain tumor cells: two-dimensional cultures can proliferate indefinitely but in three dimensions the tumor size is limited by the rate at which nutrients can be supplied. A mathematical model that predicts this effect and suggests new experiments to test the relative importance of various factors in growth control is given in Shymko and Glass (1976).

Our results demonstrate that even simple models lead to the prediction that nutrient supply rate and cell density can serve as control variables for cellular activity. As one can see from Figures 4 and 9, small changes in either of these variables can switch cells from a quiescent, time-independent state to an oscillatory state, or vice-versa. If cells are mitotically-active in one of these states, say the oscillatory state, but not the other, one can see how proliferation could be turned on or off by manipulating the control variables. Moreover, because cell density can function in this way, a proliferating population growing in a finite volume has a built-in mechanism for size regulation.

Suppose, for instance, that the kinetic parameters in a system are specified so that the P vs. ε diagram is as shown in Figure 7b. If a culture begins at too low a density, proliferation can never occur and eventually the cells die out; this is as observed experimentally (Sanford et al., 1948). If the cells are initially at or above a critical density ε_1, proliferation occurs and can continue until the density reaches a second, higher critical value ε_2, where it ceases. Thus there is density-dependent regulation of proliferation. Now if the density increases further, perhaps due to further growth of the non-proliferating cells, the point is reached where the cells will be close enough to form junctions of the kind mentioned in the Introduction. At this point one would expect the permeability between cells to be quite high, the change being as shown by the dotted line in Figure 7b. From the preceding section we know that if $P > \bar{P}$, the uniform, quiescent steady state is *unstable* and a nonuniform state develops. In this state the cells are differentiated into two subpopulations that

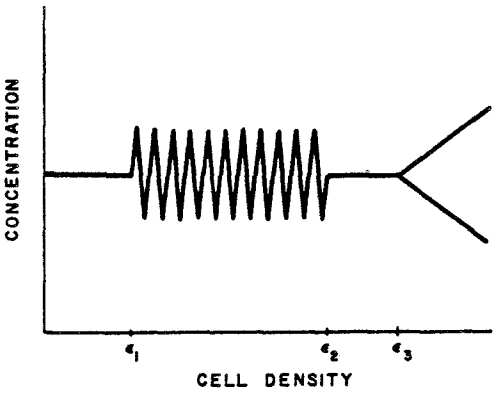


Fig. 10. A schematic showing how 'cell state', as measured by the concentration of some critical species, may depend upon cell density in a population of cells; $\varepsilon \in (0, \varepsilon_1)$; non-proliferating, $\varepsilon \in (\varepsilon_1, \varepsilon_2)$; proliferating, $\varepsilon \in (\varepsilon_2, \varepsilon_3)$; non-proliferating, $\varepsilon > \varepsilon_3$; terminally differentiated

differ from each other in the concentration of one or more species. In this terminally-differentiated state the cells may synthesize some product specific to their function in the organism, as is the case, for instance, with postmitotic myoblasts (Cohen et al., 1977). Density-dependent differentiation like that predicted by our model has been found experimentally. Nicolas et al. (1976) observed that differentiation of teratocarcinoma cells in vitro begins only after the culture reaches a critical cell mass, and that the differentiated cells have lost their tumorogenicity. Whether or not cell-to-cell junctions are involved is not known.

The sequence of changes that individual cells undergo as density varies from zero to confluence is shown schematically in Figure 10. As we have indicated, each of the individual steps is known to occur in various systems. It would provide strong support for the kind of regulative mechanism we have suggested if the entire sequence of changes were found to occur in a single system. To date we know of no such system.

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Appendix

The fundamental result on existence of periodic solutions is given by the following theorem.

Theorem [*Hopf*]. *Let* $x' = F(x, \mu)$ *be a real analytic autonomous system of differential equations with* x, $F \in R^n$ *and* $\mu \in R$. *Suppose that* $F(0, \mu) = 0$ *for* $\mu \in [-c, c]$, $c > 0$ *and let* $A(\mu)$ *be the linearization of* $F(x, \mu)$ *around* $(0, \mu)$. *Suppose that* $A(\mu)$ *has one pair of complex conjugate eigenvalues* $\lambda_{1,2}(\mu) = \alpha(\mu) \pm i\omega(\xi)$ *for which* $\alpha(0) = 0$, $\alpha'(0) \neq 0$, *and all other eigenvalues* $\lambda_j, j = 3, \ldots, n$, *have negative real parts for* $\mu \in [-c, c]$.

Under these conditions there exists an $\varepsilon_0 > 0$ and a functional relation $\mu = \mu(\varepsilon)$ such that for each $\varepsilon \in (-\varepsilon_0, \varepsilon_0)$ there exists a periodic solution $\tilde{x}(t, \varepsilon)$ with period $T(\varepsilon)$ of $x' = F(x, \mu)$. At $\varepsilon = 0$ we have $\mu(0) = 0$, $\tilde{x}(t, 0) = 0$ and $T(0) = 2\pi/\omega_0$,

and $\tilde{x}(t, \varepsilon) \neq 0$ for all sufficiently small $\varepsilon \neq 0$. Moreover $\mu(\varepsilon)$, $\tilde{x}(t, \varepsilon)$ and $T(\varepsilon)$ are analytic at $\varepsilon = 0$. These periodic solutions exist either only for $\mu > 0$, or only for $\mu < 0$, or only for $\mu = 0$. Furthermore, for each $L > T(0)$ there exist $a > 0, b > 0$ such that if $|\mu| < b$ then, except for the bifurcating periodic solutions $\tilde{x}(t, \varepsilon)$ with $\varepsilon > 0$, there is no non-constant periodic solution with period less than L which lies entirely in $\{x: \|x\| < a\}$.

For the proof of this, see Hopf (1942) or Ruelle and Takens (1971).

The theorem establishes existence and uniqueness of the bifurcating solutions, but gives no information on the direction of bifurcation (i.e. whether the periodic solution exists for $\mu < 0$, for $\mu = 0$, or for $\mu > 0$) or the stability of the bifurcating solution.

Consider the expansion of $\mu(\varepsilon)$:

$$\mu(\varepsilon) = \mu_1\varepsilon + \mu_2\varepsilon^2 + \mu_3\varepsilon^3 + \cdots. \tag{A1}$$

Hopf proved that the coefficient of the leading term is zero and so if $\mu_2 \neq 0$, the direction of bifurcation is determined by sgn μ_2. The characteristic exponents of the periodic solution can be written

$$\begin{aligned} &\beta^1(\varepsilon) \equiv 0 \\ &\beta^2(\varepsilon) = \beta_1\varepsilon + \beta_2\varepsilon^2 + \cdots \\ &\beta^j(\varepsilon) = \lambda_j(0)T + 0(\epsilon) \quad j = 3, \ldots, n. \end{aligned} \tag{A2}$$

Hopf established that $\beta_1 = 0$ and that

$$\beta_2 = -2\mu_2\alpha'(0). \tag{A3}$$

Therefore, the bifurcating period solution is stable if $\mu_2\alpha'(0) > 0$ and unstable if $\mu_2\alpha'(0) < 0$. If β_2 vanishes β_4 must be computed. Thus in general the direction and stability of the bifurcating solution is determined by sgn μ_2 and sgn $\alpha'(0)$.

Poore (1976) has derived an expression for $\mu_2\alpha'(0)$ that is particularly convenient for numerical computation. Let u and v denote any two left and right eigenvectors, respectively, of $A(0)$ corresponding to $\lambda_1(0) = i\omega(0)$, normalized so that $\sum u_i v_i = 1$. Then Poore's result is that

$$\begin{aligned} \text{sgn}\,(\mu_2\alpha'(0)) = \text{sgn Re}\{&-uF_{xxx}vv\bar{v} + 2uF_{xx}v(A(0))^{-1}F_{xx}v\bar{v} \\ &+ uF_{xx}\bar{v}(A(0) - 2i\omega(0)I)^{-1}F_{xx}vv\} \end{aligned} \tag{A4}$$

where $(F_x)_{ij} \equiv \partial F_i/\partial x_j$, $(F_{xx})_{ijk} \equiv \partial^2 F_i/\partial x_j\,\partial x_k$ and $(F_{xxx})_{ijkl} \equiv \partial^3 F_i/\partial x_j\,\partial x_k\,\partial x_l$. Furthermore,

$$uF_{xxx}vv\bar{v} \equiv \sum_{i,j,k,l=1}^{n} u_i \frac{\partial^3 F_i}{\partial x_j\,\partial x_k\,\partial x_l} v_j v_k \bar{v}_k, \tag{A4}$$

where $\bar{v}_i$ is the complex conjugate of v_i. Finally, it is elementary to show that

$$\alpha'(0) = \text{Re}\left\{u\left(\frac{dA}{d\mu}\right)_{\mu=0} v\right\}. \tag{A5}$$

The desired information about direction and stability of the bifurcating periodic solutions can thus be determined from (A5) and (A4). These quantities were computed numerically along the locus $\alpha(0) = 0$ in parameter space to arrive at the conclusions given in the text.

References

Aldridge, J.: Short-range intercellular communication, biochemical oscillations, and circadian rhythms. In: Handbook of Engineering in Medicine and Biology (Fleming, ed.), Cleveland: Chemical Rubber Co. (1976)

Aldridge, J., Pye, E. K.: Cell density dependence of oscillatory metabolism. Nature **259**, 670 (1976)

Betz, A.: Kinetics of yeast phosphofructokinase and the glycolytic oscillator. In: Biological and Biochemical Oscillators (Chance, B., Pye, E. K., Ghosh, A., Hess, B., eds.), New York: Academic Press (1973)

Boiteux, A., Goldbeter, A., Hess, B.: Control of oscillating glycolysis of yeast by stochastic, periodic, and steady source of substrate: A model and experimental study. Proc. Nat. Acad. Sci. (USA) **72**, 3829–3833 (1975)

Eagle, H., Piez, K.: The population-dependent requirement by cultured mammalian cells for metabolites which they can synthesize. J. Exp. Med. **116**, 29–43 (1962)

Folkman, J., Greenspan, H. P.: Influence of geometry on control of cell growth. Biochim. et Biophys. Acta **417**, 211–236 (1975)

Furshpan, E. J., Potter, D. D.: Low resistance junctions between cells in embryos and tissue culture. Curr. Top. Devel. Biol. **3**, 95–127 (1968)

Gibbs, R. G., Murray, J. D.: On models of oscillations in the glycolytic pathway. Preprint (1976)

Hartman, P.: Ordinary Differential Equations. Baltimore: P. Hartman (1973)

Hess, B., Boiteux, A.: Substrate control of glycolytic oscillations, in Biological and Biochemical Oscillators (Chance, B. et. al., eds.) New York: Academic Press, (1973)

Higgins, J.: Oscillating reactions. Ind. and Eng. Chem. Fund. **59**, 19–62 (1967)

Hoffman, R., Ristow, H. J., Veser, J., Frank, W.: Properties of two growth-stimulating proteins isolated from fetal calf serum. Exp. Cell. Res. **80**, 275–280 (1973)

Holley, R. W.: Control of growth of mammalian cells in cell culture. Nature **258**, 487–490 (1975)

Holley, R. W., Kiernan, J.: Control of the initiation of DNA synthesis in 3T3 cells: low molecular-weight nutrients. Proc. Nat. Acad. Sci. (USA) **71**, 2942–2945 (1974)

Hopf, E.: Abzweigung einer periodischen losung von einer stationaren losung eines differential systems. Ber. Math.-Phys. Klasse Sachs. Akad. Wiss. Leipzig **94**, 3–22 (1942)

Konigsberg, I. R.: Diffusion-mediated control of myoblast fusion. Dev. Biol. **26**, 133–152 (1971)

Loewenstein, W. R., Kanno, Y.: Intercellular communication and the control of tissue growth. Lack of communication between cancer cells. Nature **209**, 1248–1249 (1966)

Loewenstein, W. R.: Emergence of order in tissues and organs. Dev. Biol. Supp. **2**, 151–183 (1968)

Luss, D.: The influence of capacitance terms on the stability of lumped and distributed parameter systems. Chem. Eng. Sci. **29**, 1832–1836 (1974)

Machemer, H., Eckert, R.: Electrophysiological control of reversed ciliary beating in Paramecium. J. Gen. Physiol. **61**, 572–587 (1973)

Othmer, H. G., Scriven, L. E.: Instability and dynamic pattern in cellular networks. J. Theor. Biol. **32**, 507–537 (1971)

Othmer, H. G., Scriven, L. E.: Nonlinear aspects of dynamic pattern in cellular networks. J. Theor. Biol. **43**, 87–112 (1974)

Othmer, H. G.: Current problems in pattern formation. In Lects. on Math. in Life Sci. **9**, (S. Levin, ed.) Providence: American Mathematical Society (1977)

Othmer, H. G.: On the significance of finite propagation speeds in multicomponent reacting systems. J. Chem. Phys. **64**, 469–470 (1976)

Othmer, H. G.: The structure of kinetic mechanisms that can lead to oscillations in coupled cells, in preparation (1977b)

Perelson, A.: A note on the qualitative theory of lumped parameter systems. Chem. Eng. Sci. **31**, 170–173 (1976)

Poore, A.: On the theory and application of Hopf–Friedrichs bifurcation theory. Arch. Rat. Mech. Anal. **60**, 371–393 (1976)

Pye, E. K.: Periodicities in intermediary metabolism. In: Symp. on Biochronometry, Washington: Nat. Acad. Sci. Press (1971)

Ruelle, D., Takens, F.: On the nature of turbulence. Comm. Math. Phys. **20**, 167–192 (1971)

Sanford, K. K., Earle, W. R., Likely, G. D.: The growth in vitro of single isolated tissue cells. J. Nat. Cancer Inst., **9**, 229–246 (1948)

Sefton, B., Rubin, H.: Stimulation of glucose transport in cultures of density-inhibited chick-embryo cells. Proc. Nat. Acad. Sci. (USA) **68**, 3154–3157 (1971)

Spiegelman, S.: Physiological competition as a regulatory mechanism in morphogenesis. Quart. Rev. Biol. **20**, 121–146 (1945)

Stoker, M. G. P.: Role of diffusion boundary layer in contact inhibition of growth. Nature **246**, 200–203 (1973)

Wilcox, M., Mitchison, G., Smith, R.: Pattern formation in the blue-green alga Anabena. J. Cell Sci. **12**, 707–723 (1973)

Cohen, R., Pacifici, M., Rubinstein, N., Biehl, J., Holtzer, H.: Effect of a tumor promotor on myogenesis. Nature **266**, 538–540 (1977)

Shymko, R. M., Glass, L.: Cellular and geometric control of tissue growth and mitotic instability. Jour. Theor. Biol. **63**, 355–374 (1976)

Nicolas, J. F., Dubois, P., Jakob, H., Gaillard, J., Jacob, F.: Tératocarcinome de la souris: différenciation en culture d'une lignée de cellules primitives a potentialités multiples. Ann. de Microbiologie **126A**, 3–22 (1976)

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