COOPERATIVE REGULATION OF CELLULAR PROLIFERATION BY INTERCELLULAR DIFFUSION

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ABSTRACT A theoretical model for the cooperative control of cellular kinetics is investigated. A critical substance A is produced by the cells whose concentration in a given cell determines whether that cell can divide. The substance A can leak out of the cells into the surrounding medium as well as be reabsorbed by the cells. This feature then implies communication between the cells since all concentrations will be functions of the population density. The substance A also has a lifetime, i.e. decays, for example, by denaturation. This system can be described by three coupled nonlinear differential equations which can be solved analytically in certain limiting cases and can, of course, be studied in detail by computer techniques. Our investigations have shown that (*a*) there is a critical initial cell population density below which cell proliferation will not occur, (*b*) cell proliferation can be stimulated by supplying substance A to the medium and there is a critical initial concentration in the medium for initiating proliferation when the cell population density is subcritical, and (*c*) a well-defined induction period prior to exponential growth may exist whose length depends on the system parameters and initial conditions.

INTRODUCTION

The intimate environment of an individual cell in a complex biological organism consists of neighboring cells and of chemical species including some produced by distant cells. The future state of an individual cell is partially determined by the present state of all the other cells of that organism. For example, during the course of development the individual cell must stop dividing when the tissue to which it belongs has reached its appropriate size. In a given organ it must produce less or more of a characteristic product when called on to do so. The information controlling a cell's function may arrive via a physical or chemical (e.g. hormonal) signal from distant parts of the organism, or it may consist of a subtle quantitative change in the local environment of the cell.[1]

[1] The well-known phenomenon of regeneration following partial hepatectomy is probably such a case. Tumanishvili and Salamatina (1) have given evidence that the mitotic activity of chicken liver cells is determined by the ratio of nuclear material to cytoplasmic material.

While the understanding of the organization of organisms is a long-range aim, in vivo experiments are difficult to interpret since too many effects occur simultaneously. One must first try to understand the systematics of *cell-cell population* interactions in vitro, where the environment can be controlled to a much greater extent. In this paper we explore mathematical aspects of one of the simpler types of such interactions. The specific interactions we have in mind are those in which the total number of cells present determine whether or not proliferation occurs.

Cooperative effects among cell populations were hypothesized by a number of people by the early part of the century (2, 3). They wished to explain such observations as the enhancement of growth rate as an optimal density is approached. The idea that growth may be enhanced by self-feeding among cells was named "allelocatalysis" by Robertson (3), who claimed to observe the effect with a species of ciliated protozoan. The status of the effect remained controversial for some time.[2] More modern experiments (5–8) give rather convincing evidence that such cooperative effects exist for cultured animal cells. One such example is the use of a feeder layer of irradiated cells (6) which often improves single-cell cloning efficiency. Another interesting example for which our approach will yield testable results, is given in the experiments of Eagle and Piez (7) in which critical population densities were found for sustained growth and for the production of various essential metabolites.

We direct our attention in this paper to the type of phenomenon in which the critical part of the environment[3] is produced by the cells themselves. We suppose that the cells produce a regulating substance, A, which diffuses to the environment and is reabsorbed by the cells. Its concentration *inside the cell* then determines the state with respect to proliferation depending on whether it is above or below a certain critical level. We study changes in the concentration of A which give rise to the following three conditions of the cells:

(a) proliferating;

(b) reversibly inhibited from proliferating;

(c) irreversibly inhibited (including cell death).

The cells are assumed to be in suspension with sufficient mixing so that at any instant each cell has essentially the same environment. It is assumed that, except for the concentration of A, the medium is kept constant. A loss rate, λ, is assumed for the substance A. This could represent the denaturation of A or removal of the substance from the system. For maximum simplicity we assume that the number of cells per unit volume is kept uniform.[4] It is consistent with both physical and bio-

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[2] In 1932 Buchsbaum (4) reviewed the experimental situation and lists a number of people disclaiming the phenomenon with a number of others supporting its existence.

[3] Some theoretical work on related aspects of the interaction of a cell and its environment may be found in references (9–13).

[4] Interesting effects which are probably due to spatial variation in the concentration of a regulatory substance have been observed in experiments where the distribution of cells is nonuniform (8).

logical experience to assume that the characteristic time of diffusion over intercellular distances is short with respect to the doubling time and hence the concentration of A is spatially uniform. It follows that one can treat an "average cell" and develop the theory by means of differential equations.[5]

THE BASIC MODEL

The central idea of the model is that the cell proliferation rate, α, is a function of X, the number of molecules of A inside a cell. Hence for ρ, the number of cells per cc of medium, we have

$$\frac{d\rho}{dt} = \alpha(X)\rho. \tag{1}$$

We will first consider a system in which we can neglect the possibility of irreversible inhibition. A cell with a sufficient supply of A is assumed to proceed through the cycle and divide. If the supply is below the critical level, the cell is reversibly inhibited from division. A realistic model would not necessarily require the same critical concentration, X_c, for each cell. Therefore, $\alpha(X)$ is taken to be a differentiable function which increases monotonically in value from zero below X_c to α_0, the log-phase growth rate constant, above X_c. The region in which $d\alpha/dX$ is appreciably different from zero is assumed to be limited to a finite band around X_c. We call this band *the σ region*, below the band, *the zero region*, and above the band, *the α region*. For the numerical examples in this paper we choose

$$\alpha(X) = (\alpha_0/\sqrt{2\pi\sigma^2}) \int_{-\infty}^{X} \exp\left(-[X' - X_c]^2/2\sigma^2\right) dX', \tag{2}$$

and its limiting step-function form for $\sigma = 0$, (see Appendix I),[6] where σ is a parameter which measures both variation in X_c and variation away from the average concentration, X, in individual cells.

In addition to ρ and X, the concentration of A (molecules per cc) exterior to the cells, Y, is a variable in the theory. Transport across a cell membrane is taken to be proportional to the concentration of A on the initiating side so that the total flux leaving a cell is $(LX - MY)$, where L and M are independently adjustable parameters (as they would be in the case of active transport [see Appendix II]). The critical factor, A, is produced inside the cell at an average rate, K (molecules/hr per cell). We also assume that A is denatured, or removed from the medium at a rate, λ.

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[5] Underlying this deterministic description we have a probabilistic ensemble in mind. Thus, a large total number of cells, entailing either a large volume or the statistical average of several experiments, must be considered.

[6] The finite σ case is simpler mathematically and the reader may, if he wishes to avoid the mathematical complications, simply interpret the $\sigma = 0$ examples as having "very small σ." The $\sigma = 0$ discussion in Appendix I does, however, give some insight into the effective value of α which occurs during the log phase.

TABLE I

STANDARD VALUES OF THE PARAMETERS

$X_c = 1.0 \times 10^6$	*molecules/cell*
$X_0 = 1.1 \times 10^6$	*molecules/cell*
$K = 10^6$	*molecules/(cell hr)*
$L = 1.0$	$(hr)^{-1}$
$M = 10^{-2}$	*cc/(hr cell)*
$\lambda = 10.0$	$(hr)^{-1}$
$\alpha_0 = 0.1$	$(hr)^{-1}$
$Y_0 = 0.0$	*molecules/cc*
$\rho_0 = 1.0 \times 10^8$	*cells/cc*

The dynamics of the system is then described by the differential equations

$$\frac{dX}{dt} = K - LX + MY - \alpha X \qquad (3\,a)$$

$$\frac{dY}{dt} = L\rho X - M\rho Y - \lambda Y. \qquad (3\,b)$$

The last term is in equation 3 *a* accounts for the reduction in the value of X during doubling. Since the above equations are nonlinear, a considerable part of the discussion will be based on numerical solutions obtained with a computer. A set of parameters chosen for convenience in computation is designated as "standard" for numerical illustrations, and is listed in Table I. Those parameters deviating from standard in a given computation are indicated in the text and in the figure captions. A discussion is given in Appendix II to indicate how one may estimate the order of magnitude of the parameters as more information about A becomes available, and of some of the relationships among the parameters.

GENERAL CHARACTERISTICS OF THE GROWTH CURVES

In this section we discuss the behavior predicted by the model. The general pattern of growth is that log phase is delayed when the level of A in a cell is reduced by leaking out to the medium. A period of slow growth with $\alpha(X) < \alpha_0$ takes place until the concentration in the medium is sufficiently high that leakage back into the cell together with production of A overcomes leakage out. A typical example similar to experimentally observed behavior is illustrated in Figs. 1–3. The growth curves of Fig. 1 are similar to experimental observations (5) in that progressively higher initial cell populations give decreasing lag-phase periods. The behavior of X giving rise to these growth curves is shown in Fig. 2. In the present case with $\alpha(X)$ given by equation 2, the σ region is roughly a distance σ on either side of X_c (between the light dashed lines of Fig. 2). It is for X in this region that intermediate values of α occur. The corresponding behavior of Y, which should be experimentally accessible once

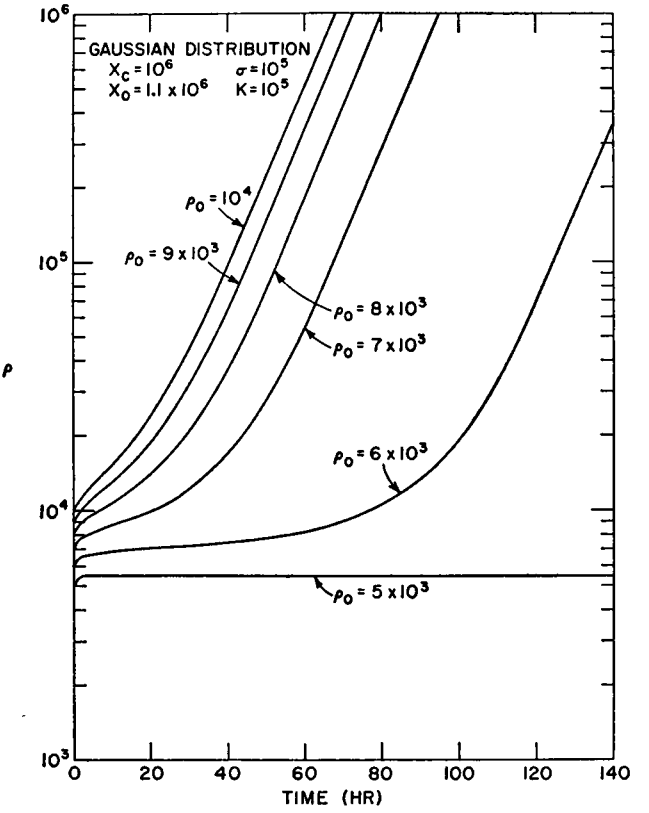

FIGURE 1 Population density, ρ, vs. time curves for a set of initial ρ_0 values ($\sigma = 10^5$).

the substance A is identified (7), is shown in Fig. 3. If the initial population density is small enough, as shown for $\rho_0 = 5 \times 10^3$, Y and hence X remain small and the population does not grow.[7] An additional prediction illustrated in Fig. 1 is that if the initial values of X are above X_c, then a very short period of normal growth may be observed prior to the lag phase.

For the above illustration, σ was chosen to be $X_c/10$. The limiting $\sigma = 0$ case is similar in behavior and the corresponding growth curves are shown in Fig. 4 with all other parameters the same as for Fig. 1.

As mentioned earlier, the parameters for the numerical examples are chosen primarily for convenience. In Fig. 5 a possibly more realistic set of parameters is seen to give quite similar results. The rationale for estimating the parameters of Fig.

[7] Since there is no finite cutoff for the particular form of α chosen, eventual growth is mathematically predicted after a very long time but we may say that there is effectively no growth since death would usually intervene. Actually a decline in population is usually seen for too low an initial population. This may be due to a critical lower level for X and may easily be included in the model as indicated in the section on cell death.

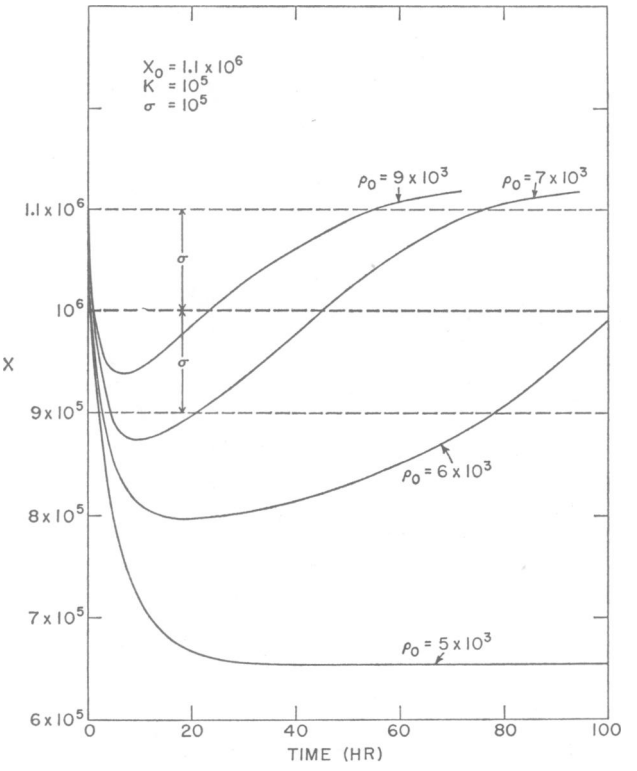

FIGURE 2 Concentration of critical substance inside the cell, X, vs. time curves for various initial population densities, $\rho_0(\sigma = 10^5)$. The middle dashed line is the value of X_c with σ indicated by the other dashed lines.

5, as well as the reasons why apparently quite different sets of parameters give rise to similar results, are discussed in Appendix II.

The family of growth curves shown in Fig. 6 depends on the concentration of A added to the medium at zero time, Y_0, in a way similar to its dependence on ρ_0 shown in Fig. 1. As an illustration of a similar dependence on one of the parameters of the system, the production rate of A, K, was varied for the growth curves of Fig. 7. The result was a similar family of curves.

An important feature of the model is the approach to steady state of X and Y. This is conveniently discussed by eliminating Y from the equations of motion. Differentiating equation 3 a and substituting expressions for Y and $\dot{Y}$ obtained from equations 3 a and 3 b gives

$$\ddot{X} + (L + \alpha + M\rho + \lambda)\dot{X} + \frac{d\alpha}{dX}\dot{X}X + ([M\rho + \lambda]\alpha + \lambda L)(X - X_s) = 0 \quad (4)$$

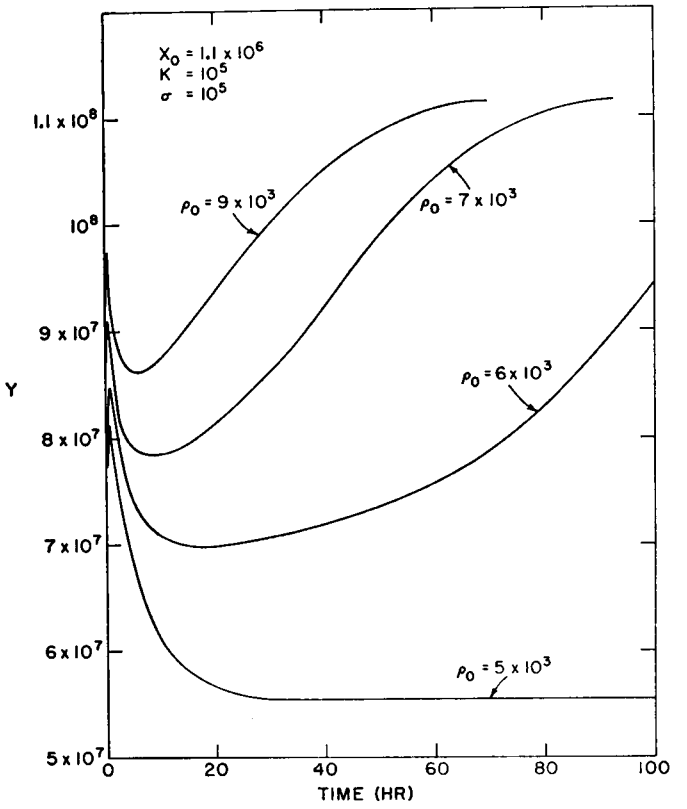

FIGURE 3 Concentration of critical substance in the medium, Y, vs. time curves for various initial population densities, $\rho_0(\sigma = 10^5)$.

with

$$X_s = \frac{K(M\rho + \lambda)}{(M\rho + \lambda)\alpha + \lambda L}. \tag{5}$$

From equations 4 and 5 we see that any two of the conditions

$$\ddot{X} = 0$$

$$\dot{X} = 0$$

$$X = X_s \tag{6}$$

imply the third. X_s, however, is not a true steady state since it increases monotonically with ρ.

We may also relate the state of equation 6 to the behavior of Y. It is easy to show from equations 3 *a* and 3 *b* that any two of the following conditions imply the other two.

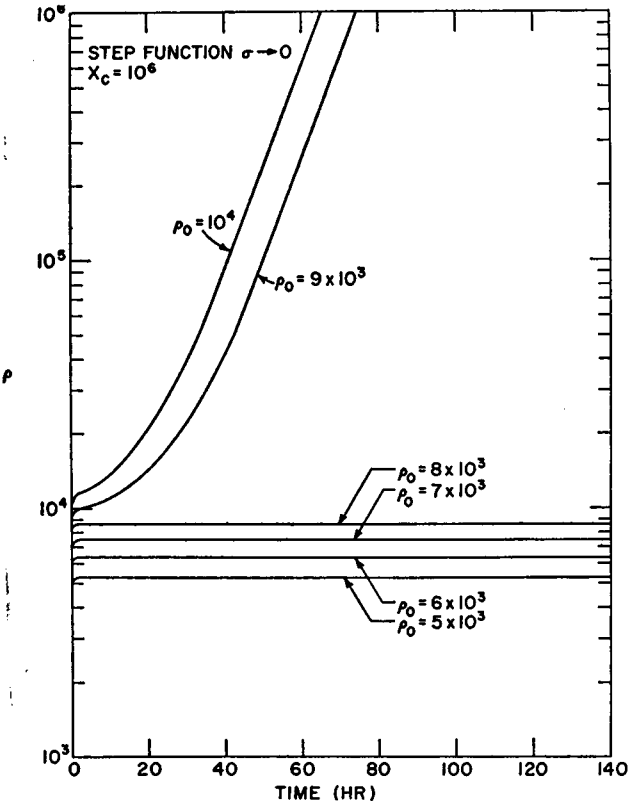

FIGURE 4 Population density, ρ, vs. time curves for a set of initial ρ_0 values ($\sigma = 0$).

$$\dot{X} = 0 \qquad X = X_s$$
$$\dot{Y} = 0 \qquad Y = Y_s \tag{7}$$

where

$$Y_s = \frac{L\rho K}{(M\rho + \lambda)\alpha + \lambda L}. \tag{8}$$

Taking the first derivative of equation 3 *b* reveals that $\ddot{Y} \neq 0$ when conditions of equation 7 hold. The equation which we obtain is

$$\ddot{Y} + (L + [M\rho + \lambda])\,\dot{Y} + (M\rho\alpha + L\lambda)(Y - Y_t) = 0 \tag{9}$$

with

$$Y_t = \frac{L\rho K}{M\rho\alpha + L\lambda}. \tag{10}$$

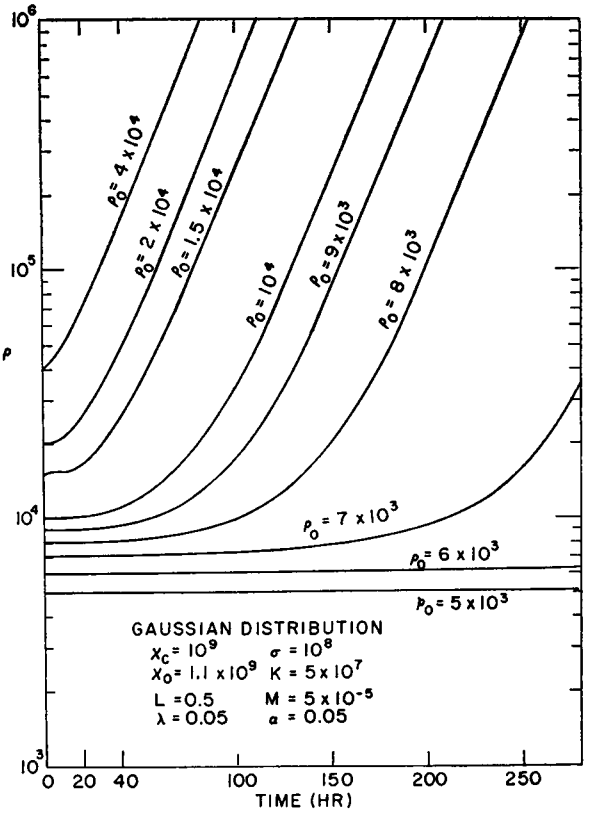

FIGURE 5 Population density, ρ, vs. time curves for a set of initial ρ_0 values ($\sigma = 10^8$) with a more "realistic" set of parameters differing from those in Fig. 1.

Y_t and Y_s are equal only when X is in a region where $\alpha = 0$, or when X is in a finite α region with $\lambda = 0$. We note that Y_t and Y_s both approach $(L/M)X_s$, X_s in turn approaches (K/α) for large ρ, and therefore both X and Y approach an asymptotic steady state as ρ increases.

Let us use equation 4 to study the behavior of X in the regions far enough away from X_c so that the term $(d\alpha/dX)\dot{X}X$ is negligible. For X in the zero region ($X < X_c$), the solution of equation 4 has the form

$$(X - X_s) = a_1 e^{m_1 t} + a_2 e^{m_2 t}, \tag{11}$$

where

$$m_{1,2} = (1/2)\,(-[L + \alpha + M\rho + \lambda] \pm \sqrt{([M\rho + \lambda] + [L + \alpha])^2 - 4([M\rho + \lambda]\alpha + \lambda L)}). \tag{12}$$

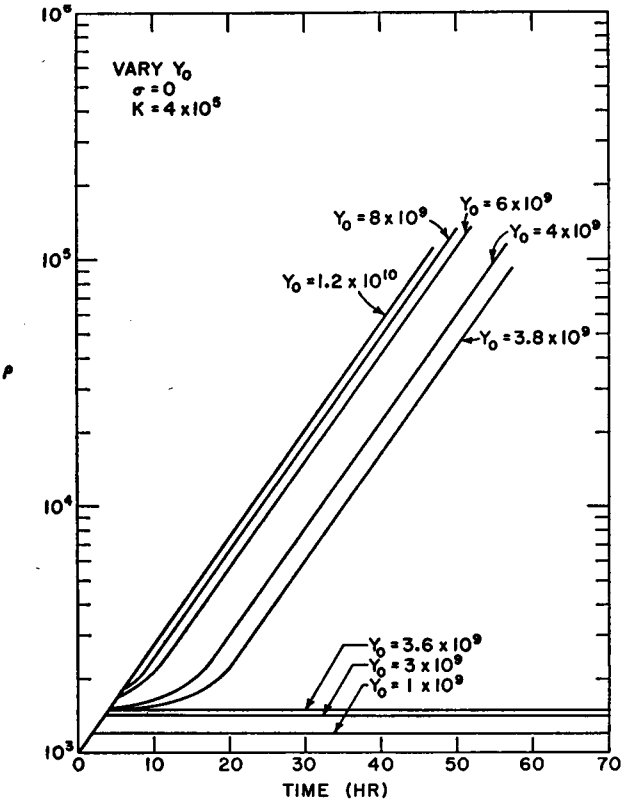

FIGURE 6 The influence of the initial concentration of the critical substance added to the medium, on the growth curves ($\sigma = 0$).

We may obtain a_1 and a_2 from the initial conditions on X and $\dot{X}$ giving

$$a_j = \left(\frac{m_i}{m_i - m_j}\right)\left\{(X_0 - X_s) - \frac{1}{m_i}(K - [L + \alpha]X_0 + MY_0)\right\} \quad (13)$$

with $i, j = 1, 2$, and $i \neq j$.

If R is the expression under the radical in equation 12, we see that

$$R > ([M\rho + \lambda] + [L + \alpha])^2 - 4([M\rho + \lambda][L + \alpha])$$

$$= ([M\rho + \lambda] - [L + \alpha])^2 > 0 \quad (14)$$

and, therefore, there are no oscillations in X for constant ρ. Since the square root of R is less in magnitude than the first term in the parentheses of equation 12, m_1 and m_2 are both negative so that X approaches X_s exponentially whenever equations 11 and 12 are applicable. The above solution also gives a useful approximation for times short with respect to the doubling time when ρ is not constant. Consequently,

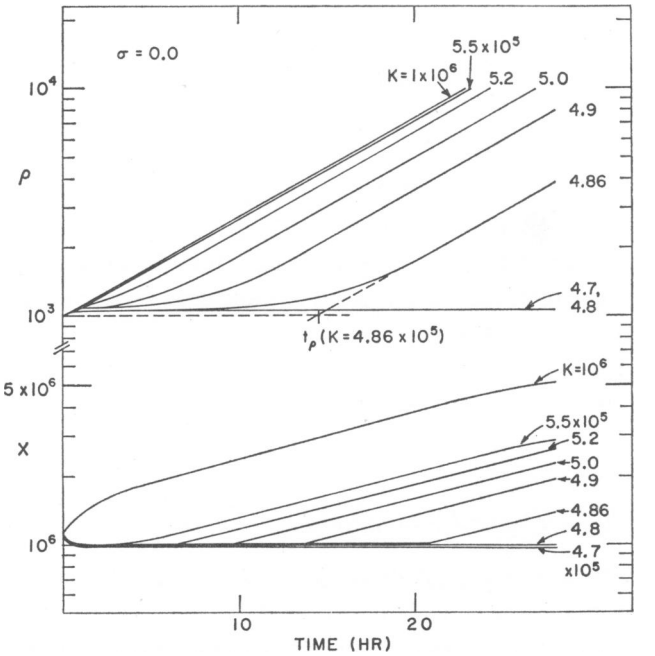

FIGURE 7 Population density, ρ, and concentration of critical substance inside the cell, X, vs. time curves for a set of production rates, $K(\sigma = 0, X_e = 10^6)$.

$X \rightarrow X_e$ exponentially and without oscillations over such periods in any region far away from $X = X_c$.

We next proceed to the case where ρ is increasing exponentially (the α region) and verify that X again approaches X_e exponentially and with no oscillations of long period. After a sufficiently long time, $M\rho$ becomes large compared to the other coefficients in equation 4, X_e is nearly constant, and we have for $g \equiv (X - X_e)$

$$\ddot{g} + M\rho\dot{g} + M\rho\alpha g = 0 \tag{15}$$

or

$$\frac{d}{dt}(\dot{g} + M\rho_a e^{\alpha t} g) = 0,$$

where ρ_a is the population density at the time when equation 15 is first appropriate. Using the integrating factor

$$p(t) = \exp([\rho_a/\alpha][e^{\alpha t} - 1]), \tag{16}$$

we find

$$g(t) = \frac{g_0}{p(t)} + \frac{C\int_0^t p(t')\,dt'}{p(t)}. \tag{17}$$

Using L'Hospital's rule to evaluate the second term we find for t large

$$g(t) \sim \frac{C}{\rho_a} e^{-\alpha t}, \tag{18}$$

where C is a constant.

One may discuss the behavior of Y in the same manner. For the region of steady growth one obtains an expression for $(Y - Y_t)$ exactly of the form shown in equation 18 with the constant C altered appropriately. For the region where the growth rate is negligible, or when the time interval is short compared to the doubling time, one has again, from equation 9

$$Y - Y_t = b_1 e^{n_1 t} + b_2 e^{n_2 t}, \tag{19}$$

where

$$n_{1,2} = (1/2)\{-([M\rho + \lambda] + L) \pm \sqrt{([M\rho + \lambda) + L)^2 - 4(M\rho\alpha + \lambda L)}\}. \tag{20}$$

Oscillations in $Y(t)$ are very unlikely to occur since α would have to be relatively very large to give imaginary roots in equation 20.

The above discussion indicates that an overdamped rather than a true oscillatory behavior is expected for X in the zero or α region. The situation is similar in the σ region, where a hovering behavior occurred in many of the cases studied (see the next section), but never oscillations. For a large value of σ (small $d\alpha/dX$) the discussion following equation 11 indicates that gradual changes rather than oscillations are to be expected. For small σ the situation is discussed in Appendix I.

THE INDUCTION PERIOD

One of the principal features reproduced by the model is an induction period during which X may linger at (for $\sigma = 0$) or near (for finite but small σ) X_c. During this period ρ grows at an effective rate α_{eff} until a critical population density is reached which is sufficiently high for X to break away from X_c and climb toward a steady-state value. This "lag time," since it is experimentally measurable, is worth some quantitative study although the analysis is necessarily crude. We develop a simple formula in this section to estimate this time and compare the estimates with values obtained in computer runs.

The existence of the induction period may be discussed in terms of X_s, the value toward which X tends (equation 5). We therefore define

$$X_s^{\alpha} \equiv \frac{K(M\rho + \lambda)}{(M\rho + \lambda)\alpha_0 + \lambda L} \ (X_s \text{ for the } \alpha \text{ region}) \tag{21 a}$$

$$X_s^{0} \equiv \frac{K(M\rho + \lambda)}{\lambda L} \ (X_s \text{ for the zero region}). \tag{21 b}$$

X_s^α is an increasing function of ρ and is less than X_s^0, hence if X_s^α becomes larger than $(X_c + \sigma)$, then it stays larger and $X \to X_s^\alpha \to (K/\alpha_0)$ as ρ increases. Similarly, if X_s^0 is well below $(X_c - \sigma)$ at the time X crosses into the zero region then, according to equation 11, X will tend to approach the now constant X_s^0. For both of these situations the approach is approximately exponential and without oscillations. The other important case to examine if for $X_s^\alpha < X_c < X_s^0$. In this situation if X is in the zero region, it will tend toward the α region, and if it is in the α region, it will tend to approach the zero region. One might expect an oscillatory behavior to result, however, in the examples X instead was found to hover in the σ region climbing slowly to its final steady-state value. The reasons for this hovering together with a method for estimating the effective value of α, $0 < \alpha_{\text{eff}} < \alpha_0$, occurring during this time is given (for small σ) in Appendix I.

For the above reasons the relation $X_s^0 > X_c > X_s^\alpha$ is normally found to hold for cases with significant induction periods. We immediately gain some insight into the critical effect of the initial population density, and initial concentration in the medium for if ρ_0 is so large that $X_s^\alpha(\rho_0) > X_c$, there is no induction period. The effect of a large initial value of Y is to delay the fall of X to X_c since X initially increases. Because of this, ρ increases to larger values before X_c is reached. Hence, smaller values of ρ_0 are needed to achieve a given value of $X_s^\alpha(\rho)$ when $X = X_c$.

Computer runs support the above discussion. The data are shown in Table II ($\sigma = 0$). (We limit the examples to [$\sigma = 0$] runs but very similar results were obtained for small but finite σ.) Most of the data of Table II are shown in Fig. 7. In the run with $K = 10^6$ (not in the Table) $X_s^\alpha > X_c$. In this case the minimum value of X was greater than X_c and no lag occurred. When X_s^0 is less than X_c at the time when X falls below X_c, then X tends to remain below X_c so that the induction time becomes infinite, ρ remaining forever at the value it had for $X = X_c$ as illustrated in runs 1 and 2.

If X is bracketed by X_s^0 and X_s^α at the time it initially approaches X_c, we expect a true induction. We may therefore use the time required for X_s^α to increase until it equals X_c as an estimate of the induction time. Although α_{eff} is increasing during this time, we may estimate the rate of increase of X_s^α by assuming that a constant value of $(\alpha_0/2)$ is sufficiently close to the average of α during the induction period. (See Appendix I.) We define t_X as the time X hovers at X_c and from the above we estimate, for $\sigma = 0$, that

$$
\begin{aligned}
t_X &\equiv \infty && X_s^0 < X_c \\
t_X &\equiv 0 && X_s^\alpha > X_c \\
t_X &\approx \frac{(X_c - X_s^\alpha)}{\left.\dfrac{dX_s^\alpha}{dt}\right|_{\substack{\alpha=\alpha_0/2 \\ \rho=\rho(X_c)}}} && X_s^0 > X_c > X_s^\alpha.
\end{aligned}
\tag{22}
$$

The values for X_s^0 and X_s^α are calculated when X first hits X_c using the values of $\rho \equiv$

TABLE II

DERIVED QUANTITIES FOR A SERIES OF COMPUTER RUNS (σ = 0)

Parameters changed from standard values	$\rho(X_c)$ ($\times 10^9$)	X_s^{α} ($\times 10^6$)	X_s^{0} ($\times 10^6$)	$(X_c - X_s^{\alpha})$ ($\times 10^6$)	$X_s^{\alpha/2}$ ($\times 10^6$)	t_x formula	t_x computer	$\rho_c(\alpha_0)$ ($\times 10^9$)	$t_{\rho c}(\alpha_0)$	$\rho_c(\alpha_0/2)$ ($\times 10^9$)	$t_{\rho c}(\alpha_0/2)$	t_ρ	Run
$K = 4.7 \times 10^5$	1.06	0.799	0.963	0.201	—	∞	∞	1.70	∞	1.38	∞	∞	1
$K = 4.8 \times 10^5$	1.06	0.820	0.989	0.180	—	∞	∞	1.63	∞	1.32	∞	∞	2
$K = 4.86 \times 10^5$	1.06	0.830	1.001	0.170	0.908	8.0	17.0 ± 1.0	1.59	19.0	1.29	16.1	14.5	3
$K = 4.9 \times 10^5$	1.06	0.837	1.009	0.163	0.915	7.6	11.0 ± 0.5	1.56	14.7	1.27	9.7	7.5	4
$K = 5.0 \times 10^5$	1.06	0.854	1.03	0.146	0.934	6.7	7.5 ± 0.5	1.50	8.0	1.22	4.9	4.0	5
$K = 5.1 \times 10^5$	1.08	0.878	1.06	0.122	0.961	5.4	6.0 ± 0.5	1.43	5.8	1.17	3.0	2.4	6
$K = 5.2 \times 10^5$	1.09	0.899	1.09	0.101	0.984	4.3	3.9 ± 0.3	1.38	4.6	1.13	1.7	1.7	7
$K = 5.3 \times 10^5$	1.09*	0.916	1.107	0.084	1.003	3.6	3.8 ± 0.3	1.32	3.5	1.08	0.8	0.9	8
$K = 5.4 \times 10^5$	1.11*	0.941	1.139	0.059	1.031	2.4	2.5 ± 0.3	1.27	2.9	1.04	0.4	0.4	9
$K = 5.5 \times 10^5$	1.21*	0.962	1.166	0.038	1.055	1.4	1.4 ± 0.3	1.10	0.95	1.00	0.0	0.0	10
$K = 5.0 \times 10^5$ $X_c = 1.01 \times 10^6$	1.06	0.854	1.03	0.156	0.934	7.2	10.0 ± 0.5	1.53	10.4	1.24	7.4	6.5	11
$K_c = 5.0 \times 10^5$ $X = 0.99 \times 10^6$	1.08	0.861	1.04	0.129	0.942	5.8	6.7 ± 0.3	1.25	4.43	1.20	3.7	3.0	12

* Value taken at the time X reaches minimum.

$\rho(X_c)$ obtained at that time. We evaluate the denominator for t_x using equation 21 as

$$\left.\frac{dX_s^{\alpha}}{dt}\right|_{\substack{\rho=\rho(X_c)\\ \alpha=(\alpha_0/2)}} = \frac{(X_s^{\alpha/2})^2(\alpha_0/2)M\rho}{X_s^0(M\rho+\lambda)}. \tag{23}$$

By $X_s^{\alpha/2}$ we mean simply that $(\alpha_0/2)$ is substituted for α in equation 21.

The value of t_x obtained from the formula is listed in Table II next to a value estimated graphically for t_x. For longer induction periods, $(\alpha_0/2)$ is too high an estimate for α and the values from the computer become substantially larger than the estimate.

An experimentally measurable quantity is t_ρ, which we define as the time at the intersection of the tangent to the log ρ vs. t curve with the horizontal line $\rho = \rho_0$. (For run 3, t_ρ is indicated in Fig. 7.) We also define ρ_c, a critical value for ρ, to be the solution of equation 21 a at $X_s^{\alpha} = X_c$. This is given by

$$\rho_c = (\lambda/M)\,([(X_cL)/(K-\alpha X_c)]-1).$$

At any $\rho > \rho_c$ no induction period is expected since $X_s^{\alpha} > X_c$. The value of the time at which ρ reaches ρ_c is taken from the computer readout for each run. We observe that $t_{\rho c}(\alpha_0)$ (the time when $\rho = \rho_c[\alpha_0]$) is close to t_x. We also note that $t_{\rho c}(\alpha_0/2)$ is close to the value of $t\rho$.

We now consider some effects of the initial concentration in the medium, Y_0, and the dynamics of Y during induction. The critical dependence on Y_0 (illustrated, in Fig. 6 for $K = 4 \times 10^5$ and $\sigma = 0$) indicates how an insufficient concentration of cells is compensated for by increasing Y_0. (This is similar to an effect noted experimentally in reference 7.) The system is subcritical for values of $Y_0 \leqq 3.6 \times 10^9$.

As indicated by equation 3 a, X initially increases extremely rapidly for large Y. X reaches a maximum and then falls to a steady-state value at or below X_c if the system is subcritical. If the system gets trapped at $X = X_c$, and Y has a minimum sufficiently large such that $\alpha_{eff} > 0$, then growth occurs. This critical minimum value is, from equation 35 of Appendix I, given by

$$Y_{\min} > \frac{LX_c - K}{M} \equiv Y_1. \tag{24}$$

During the induction period when $X = X_c$ and equation 24 is satisfied, we obtain from equation 3 b and $\rho = \rho(X_c)e^{\alpha_s t}$

$$\dot{Y} \approx L\rho(X_c)e^{\alpha_s t}X_c - M\rho(X_c)e^{\alpha_s t}Y - \lambda Y, \tag{25}$$

where $\rho(X_c)$ is the value of ρ at the beginning of the induction period. If growth is to occur, the minimum value of Y must be greater than Y_1. In this case Y increases un-

til $\alpha_e \geqq \alpha_0$ (or K/X_c if this is less than α_0)[8] or, equivalently, (see Appendix I, equation [35])

$$Y \geqq (X_c\alpha_0 - K + LX_c)/M \equiv Y_c\,, \tag{26}$$

at which time ρ begins log-phase growth. If $Y_{\min}$ is greater than Y_c, then we have no kink in the log ρ vs. t graph (i.e. ρ always increases at rate α_0) as illustrated in Fig. (6) for $Y_0 = 1.2 \times 10^{10}$.

It is also of interest to discuss briefly the behavior of the total concentration of A, namely $W \equiv (\rho X + Y)$ which is described by the equation

$$\frac{dW}{dt} = K\rho - \lambda Y. \tag{27}$$

When W increases without leveling off, the system will exhibit log-phase behavior, since if X becomes large we soon have $X > X_c$, and similarly if Y monotonically increases, then eventually α_e becomes α_0. Both cases imply log-phase growth. Similarly, if ρ becomes very large, X and Y approach asymptotic steady-state values. If in addition $(K/\alpha_0) > X_c$, then $X_s^\alpha > X_c$ and X becomes sufficiently large for log-phase growth. The importance of the decay parameter[9] λ is underscored by the fact that if λ were zero, according to equation 27, W would always increase. Hence, by the above arguments, there would be self-sustained proliferation for any initial conditions.

CELL DEATH

For simplicity we have assumed that all cells in the population are either proliferating or reversibly inhibited from proliferating so that the cell population density, ρ, never decreases. In some experiments, however, the population is found to decrease through cell death (5, 7) when the initial population density is too low. In this section we discuss briefly a simple modification to the equations by means of which the phenomenon of irreversible inhibition or cell death may be included.

Suppose that there is a critical concentration, X_L, for the regulating substance,[10]

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[8] If an induction period occurs with $K/X_c < \alpha_0$ then the system is trapped forever in the α_{eff} region. At long time (large ρ) $dY/dt \to 0$ and $Y \to (LX_c)/M$. Thus, $\alpha_{eff} \to K/X_c$ and an effective log-phase behavior is observed with that value for α_e.

[9] It is easy to generalize equations 3 to include decay inside the cell. In this case one has a term $(-\lambda_1 X)$ in equation 3 *a* and $(-\lambda_2 Y)$ in equation 3 *b*. The equations corresponding to equations 4 and 5 assume the exact same form except that λ_2 replaces λ, and $(\alpha + \lambda_1)$ replaces α everywhere so that all qualitative features of the model are unchanged.

[10] The formalism of this section can be modified to handle the situation where the substance A is toxic at a sufficiently high concentration inside the cell. To represent this case one simply replaces β everywhere in the equations of this section with the function $\beta' = -(\beta - \beta_0)$. (For $\sigma = 0$ this is a step function increasing from $\beta' = 0$ to $\beta' = \beta_0$ at $X = X_L$.) In this case the region of toxicity where cell death occurs is $X > X_L$. We will not study this case in detail but we merely indicate here how, for

such that when $X < X_L$ cells are removed at the rate $\beta(X)$, but no cells are removed for $X > X_L$. The rate constant β does not enter into the equation for dX/dt since removing a cell does not change the average amount of A in the remaining cells. The dynamical equations are now written

$$\frac{d\rho}{dt} = (\alpha[X] - \beta[X])\rho$$

$$\frac{dX}{dt} = K - LX + MY - \alpha(X)X$$

$$\frac{dY}{dt} = L\rho X - M\rho Y - \lambda Y + \beta(X)\gamma\rho X, \qquad (28)$$

where $0 \leqq \gamma \leqq 1$.

The parameter γ has been inserted to allow for possible variation in the utilization of A from the dead cells.[11] A set of equations may be derived that are similar in form to equations 4 and 5 except that an additional term $(-M\rho\beta\gamma)$ now occurs in the coefficient of $(X - X_s)$ and the denominator of X_s. Y_s is increased by a multiplying factor $(L + \beta\gamma)/L$. It is important to note that the various arguments indicating that X does not oscillate still hold in the present case.

Let us now consider a simple example, solved numerically, to show how a constant death rate affects criticality. A constant death rate independent of concentration is described within the above formalism simply by choosing $X_L = +\infty$. We choose $\beta = 0.05$ (one-half the proliferation rate α_0) and $\sigma_\alpha = 10^5$. A family of curves showing the critical effect of initial population density is drawn in Fig. 8 for $\gamma = 1.0$ and $K = 5.14 \times 10^5$. It was found that an almost identical set of graphs (not shown) resulted for $\gamma = 0.0$ when the value of K was increased by about 5 % to 5.38×10^5. This increased production rate makes up for the loss of A due to removal of the material from the dead cells. Quantitatively one finds that the change in K is just sufficient to give equal values for X_s in the critical region for the two cases.

$X_L > X_c$, one can in certain cases obtain an oscillating solution of equation 44. Consider, for example, the case of $(\beta_0 = 2\,\alpha_0)$, $(\gamma = 0)$, $(\sigma_\beta = \sigma_\alpha = 0)$, $([K/\alpha_0] > X_L > [K/(\alpha_0 + L)] \gg X_c)$, $(X_L > X_0 > X_c)$. Initially ρ would increase and X would approach (K/α_0). When X crosses X_L, ρ begins to decrease. The slope of X changes continuously as does the second derivative. (The second derivative of X but not the first would be discontinuous at X_L for $\gamma \neq 0$.) As ρ decreases $X_s \to K/(\alpha_0 + L)$. Consequently, X decreases below X_L, but again the derivative of X doesn't change sharply so that X continues to decrease while ρ again starts its increase and the process repeats.

[11] If dead cells with most of their internal supply of A are removed from the system, then $\gamma = 0$ is appropriate. If dying cells lyse in a time short with respect to doubling and release all their supply of A to the medium, $\gamma = 1$ is appropriate. A situation where the substance is released slowly could be represented roughly by an intermediate value of γ.

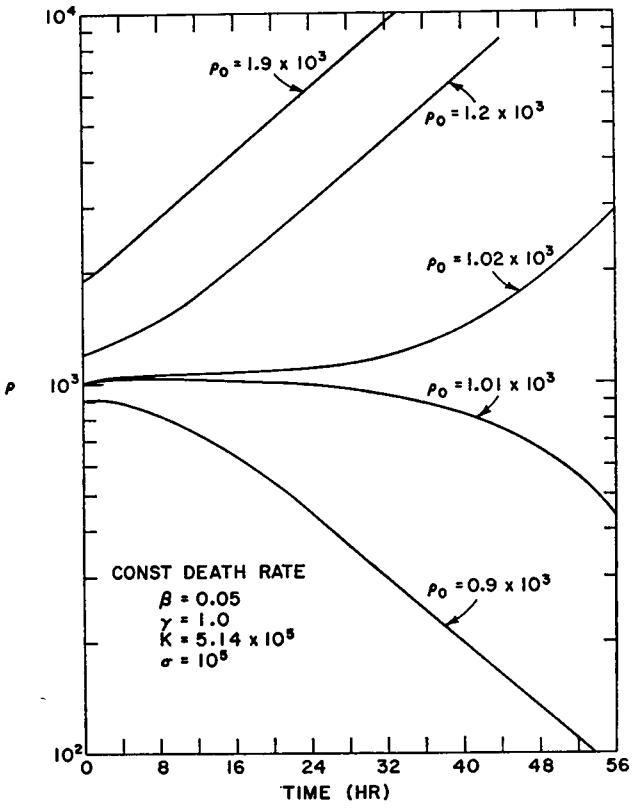

FIGURE 8 The influence of the death rate, β, on the growth curves for various initial population densities ($\sigma = 10^5$, $K = 5.14 \times 10^5$). Critical substance from dead cells released into medium ($\gamma = 1.0$).

A METHOD FOR THE DETERMINATION OF PARAMETERS

In this section we indicate that the parameters of the model can be estimated from experiments before attempting curve fitting. As an illustration, let us consider the following situation. Let ρ and Y be measured experimentally for various initial ρ_0 values as already illustrated in Fig. 4 for ρ. From log-phase growth we know α and Y_s^U, where Y_s^U is the value at which Y levels off for large ρ. Y_s^U is obtained from equation 8 for large ρ as

$$Y_s^U = \frac{LK}{M\alpha}. \tag{29}$$

For any subcritical experiment, (i.e. $\alpha = 0$), we can get the steady-state value of Y, Y_s^L, which again from equation 8 is given by

$$Y_s^L = \rho K/\lambda. \tag{30}$$

During the induction period the change in ρ is governed by an effective α, $\alpha_e(t)$, which can be determined as the slope of the ρ vs. t curve. During this same period $Y(t)$ may be measured. If we now eliminate t parametrically we have a relation between α_e and Y, so that we can plot α_e vs. Y at identical values of t. These two quantities are connected by equation 35 (see Appendix I) which can be written as

$$\alpha_e = a + bY,$$

where a and b are given by

$$a = \frac{K}{X_c} - L \tag{31}$$

$$b = \frac{M}{X_c}. \tag{32}$$

Hence, we may obtain b as the slope and a as the intercept of the α_e vs. Y plot. Since it was assumed that Y is measurable as a function of time, we can obtain λ from an independent experiment, namely the decay of Y in the nutrient solution with no cells present. Thus, there are four unknowns, K, L, M, and X_c in the four equations given above. We tested the use of the above method by using it to estimate the input parameters for a computer experiment. We found that the estimates gave L and M within about 10% and X_c within a few percent.

SUMMARY

The model described in this paper is a minimum model in the sense that all the parameters, K, L, M, X_c, and λ are necessary to obtain the effects discussed. If λ were zero the amount of substance A would always increase and the system would always exhibit growth after a sufficiently long time. If any of the other constants were zero the "communication" among the cells would not exist. As shown in the last section, the parameters of the model can be determined from suitable experiments.

The important features derived from the analysis of the model are: (*a*) the existence of a critical initial cell population density below which proliferation does not occur, (*b*) the stimulation of cell proliferation by supplying substance A to the medium, (*c*) the existence of a critical initial concentration of A in the medium for initiating proliferation when the initial population density is subcritical, and (*d*) the existence of a well-defined induction period prior to exponential growth whose length depends on the system parameters and initial conditions. Items (*a*) and (*b*) above are in good qualitative agreement with some experiments as pointed out in the introduction. Items (*c*) and (*d*) essentially are predictions from the model to be tested by future experiments.[12]

[12] Some further results concerning oscillatory effects when the model is extended to include progression through the cell cycle are given in Brookhaven National Laboratory Report 13872. Further details on such items as cell death and the induction period for small but finite σ are also given there.

APPENDIX I

In this appendix we discuss the behavior of the model in the limiting, $\sigma = 0$, case. For any finite σ, all time derivatives of X, Y, and ρ are well-defined by equations 1, 2, and 3 in the text. Hence, solutions exist for all finite σ. A series of computer experiments were run with decreasing values of σ and the other parameters equal to those of Fig. 1. The graphs (not shown) for various ρ_0 values appeared to continuously approach those shown in Fig. 4 for $\sigma \to 0$, indicating that $\rho(t)$ approaches a continuous limiting function as σ becomes small. The mathematics, however, becomes more complicated in the limiting case in which $\alpha(X)$ becomes a step function.

$$\lim_{\sigma \to 0} \alpha(X) \equiv \alpha_{\lim}(X) = \begin{cases} \alpha_0 & X > X_c \\ \alpha_0/2 & X = X_c \\ 0 & X < X_c. \end{cases} \tag{33}$$

Some additional definitions are needed since the kinetic equations do not, as they stand, always have solutions in the usual sense for $\sigma = 0$. To see this, suppose that $\sigma = 0$ and X approaches X_c with the magnitude of $\dot{X}$ less than $|\alpha_0 X_c|$. It follows from equations 3 *a* and 3 *b* that if X arrives at X_c at time t, there exists a positive constant ϵ such that for $(\delta < \epsilon)$, $\dot{X}(Y[t], X_c + \delta) < 0$ but $\dot{X}(Y[t], X_c - \delta) > 0$. Hence the dynamic behavior of X in the neighborhood of X_c becomes undefined. For a similar situation with small but finite σ, X remains near X_c, and in the next paragraph we see that X can be made to remain arbitrarily near X_c by reducing σ. It is reasonable therefore, for $\sigma = 0$ and X having arrived at X_c with $\dot{X}$ in the critical range, to define

$$\left.\begin{aligned} \dot{X} &= 0 \\ X &= X_c \end{aligned}\right\} \text{for } 0 < \alpha_{\text{eff}} < \alpha_0 \tag{34}$$

$$\alpha_{\text{eff}} \equiv \frac{K - LX_c + MY}{X_c}. \tag{35}$$

When α_{eff} is in the range given in equation 34 and σ is sufficiently small, X cannot cross from the α region to the zero region (see discussion prior to equation 2) or vice versa since $\dot{X}$ will go to zero in a continuous manner with X in the σ region. Solving equation 3 *a* for $\alpha(X)$ with $\dot{X} = 0$, one obtains again the right-hand side of equation 35. Hence, α_{eff} as given in equation 35 is the correct value to use for the growth rate constant when the conditions of equations 34 and 35 are satisfied. To see that the hovering behavior prescribed by equation 34 for $\sigma = 0$ is the correct counterpart of the behavior of X for small but finite σ, consider the situation when X approaches X_c with $0 < \alpha_{\text{eff}} < \alpha_0$ for very small σ. Then when $\dot{X}$ becomes zero, X is in the σ region so that $d\alpha/dX$ is very large. In this case the coefficient of $\dot{X}$ in equation 4 is very large, and of the same sign as $\ddot{X}$, so that while $\ddot{X}$ will have a sign which tends to make X approach X_s, $\dot{X}$ must remain very small until X leaves the σ region.

APPENDIX II

We exhibit here a set of numerical values for the parameters based on available experimental information in order to show how the orders of magnitude of these quantities may be initially estimated, to understand relationships among the parameters, and to see why sets of parameters which are apparently drastically different give qualitatively similar results. Since quantitative information is scanty the actual value suggested for a given parameter is only intended

as a plausible example. A family of curves was computed using these values, and is shown in Fig. 5. The qualitative appearance of the family of graphs is quite similar to that of Fig. 1, even though the magnitudes of the parameters are quite different. Some of the reasons for this are discussed below.

Two scaling laws exist among the parameters due to the form of equations 1, 2, and 3 which result in identical graphs for certain particular changes of scale. These are:

(*a*) Multiplying K, X_c, X_0, Y_0, and σ by a numberical factor, C, results in $X(t)$ and $Y(t)$ being multiplied by the same factor, and $\rho(t)$ being unaffected.

Proof: Define $\alpha'(X')$ as $\alpha(X)$ is defined in equation 2 except that $\sigma' \equiv C\sigma$, $X_c' \equiv CX_c$, and $X' \equiv CX$. Then $\alpha'(X') \equiv \alpha(X)$. Similarly if $Y' \equiv CY$ and $K' \equiv CK$, equations 3 for X and Y are transformed to similar equations for X' and Y' with K' replacing K. Hence $X'(t) = CX(t)$, $Y'(t) = CY(t)$, and $\rho'(t) = \rho(t)$ are the correct solutions of the kinetic equations with the new values of the parameters.

(*b*) Dividing α_0, K, L, M, and λ by a constant, C', to give new parameters yields the same equations as equations 1, 2, and 3 if t is replaced by $t' \equiv C't$. Hence if the solutions of the equations with the new parameters are called X', Y', and ρ', then $X'(t') = X(t)$, $Y'(t') = Y(t)$ and $\rho'(t') = \rho(t)$.

With the above in mind, we start with a plausible guess for X_c and see what this implies for the other parameters. X_c was chosen as equal to 10% of the average number of molecules of a given protein in a cell. Reference 14 indicates that this number is about 0.5×10^6 for bacterial cells, so 10^9 is a reasonable estimate for mammalian cells, taking into account the volume difference. For cells reinoculated from a growing population one expects X to be somewhat higher than X_c, so we standardize X_0, the initial value of X, at 1.1 X_c. The log-phase growth rate constant, α_0, is taken to be 0.05, corresponding to a generation time of about 14 hr. The average production rate, K, is expected to be sufficient to overcome dilution by doubling and is therefore taken as equal or greater than $\alpha_0 X_c$. Similarly, leakage out should overcome production if feedback from other cells is to be important so L is chosen as equal to 0.5 $(\text{hr})^{-1}$. Since active participation of the cell is to be expected, we assume that L and M are independent. (If there were no active participation of the cell, $[L/M]$ would be restricted to [1/cell volume] or $\sim 10^9$ [cm^3/hr cell].) One may calculate an approximate *upper bound* for M by assuming that the interaction takes place on the surface of the cell. Using a steady-state solution of the diffusion equation for an absorbing sphere (see, for example, reference 15), one finds $M \sim 4\pi RD$ where D is the diffusion constant for A, and R is a cellular radius. Taking $R \sim 10^{-3}$ cm and $D \sim 4 \times 10^{-2}$ cm^2/hr as appropriate for an amino acid (reference 16, p. 502) gives $M \lesssim 4 \times 10^{-4}$ (cm^3/hr cell) consistent with the above. We actually use a value of $M = 5 \times 10^{-5}$, an order of magnitude less than this upper bound giving us a steady-state value for Y of the order of LX_c/M or about 0.017 μmoles, which would be small, but not unheard of for a regulatory substance. Finally, a value of 0.05 $(\text{hr})^{-1}$ is chosen for λ in order to bring λY within range of the other terms in the equation for $\dot{Y}$, and the values of ρ_0 are taken in the range 10^3–10^4 cells/cc in accordance with experiments in which critical cell populations are found. The graphs obtained using these parameters are shown in Fig. 5 and are seen to have the same qualitative features as for a comparable set in Fig. 1 where the parameters of Table I are used.

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REFERENCES

1. TUMANISHVILI, G. D., and N. V. SALAMATINA. 1968. *J. Embryol. Exp. Morphol.* **20**:53.
2. MCKENDRICK, A. G., and M. K. PAI. 1910. *Proc. Roy. Soc. Edinburgh.* **31**:649.
3. ROBERTSON, T. B. 1921. *Biochem. J.* **15**:595; 1924. *Aust. J. Exp. Biol. Med. Sci.* **1**:151.
4. BUCHSBAUM, R. M. 1932. *J. Exp. Zool.* **63**:483.
5. EARLE, W. R., K. K. SANFORD, K. J. EVANS, H. K. WALZ, and J. E. SHANNON. 1951. *J. Nat. Cancer Inst.* **12**:133.
6. PUCK, T., and P. I. MARCUS. 1955. *Proc. Nat. Acad. Sci. U. S. A.* **41**:432.
7. EAGLE, H., and K. PIEZ. 1962. *J. Exp. Med.* **116**:29.
8. REIN, A., and H. RUBIN. 1968. *Exp. Cell Res.* **49**:666.
9. TSANEV, R., and B. SENDOV. 1966. *J. Theoret. Biol.* **12**:327.
10. DEAN, A. C. R., and C. HINSHELWOOD. 1966. Growth, Function and Regulation in Bacterial Cells. The Oxford University Press, London. Especially Chap. III.
11. MONTROLL, E. W. 1967. *J. Appl. Prob.* **4**:281; 1967. Lectures on Nonlinear Rate Equations. Summer Institute in Theoretical Physics, University of Colorado, Boulder.
12. FREDRICKSON, A. G., D. RAMKRISHNA, and H. M. TSUCHIYA. 1967. *Math. Biosci.* **1**:327.
13. SAIDEL, G. M. 1968. *J. Theoret. Biol.* **19**:287.
14. WATSON, J. 1965. Molecular Biology of the Gene. W. A. Benjamin, Inc., New York. 85.
15. DIENES, G. J., and G. H. VINEYARD. 1957. Radiation Effects in Solids. Interscience Publishers, Inc., New York. Equations 5–17.
16. SETLOW, R. B., and E. C. POLLARD. 1962. Molecular Biophysics. Addison-Wesley Co., Inc., Reading, Mass. Sects. 2–4.