

Mathematics of Cellular Control Processes

I. Negative Feedback to One Gene

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Repression of mRNA synthesis is discussed for situations in which the repressor is either the protein encoded by the mRNA or a metabolite formed under the catalytic control of that protein. Following Goodwin (1965), plausible physicochemical equations are set up. They contain a non-linear element. Standard methods of the theory of non-linear equations are used to determine the properties of these equations for general values of the parameters contained therein. Undamped oscillations in the concentrations of the components can never occur for any values of the parameters when the repression is accomplished by the protein. Such oscillations are possible when repression is due to the metabolite, but only when there is a co-operative repression of such a high order as to be unlikely in practice.

1. Introduction

Considerable interest exists in the control equations involving protein synthesis and the regulation of mRNA production. Two questions particularly have arisen. One asks in what circumstances stable oscillations in the concentrations of the components can occur? (Goodwin, 1963, 1965). The other asks when a particular arrangement of control interactions can lead to more than one possible stable, i.e. enduring, set of concentrations. The second question is especially important in relation to the mechanism of differentiation, proposed by Monod & Jacob (1961), and subsequently used as a basis for explanatory hypotheses about various biological problems (Bonner, 1965; Roberts & Flexner, 1966; Griffith, 1967*a,b*).

One theoretical method of tackling these questions is by means of computer simulations. However, simulations can only be run for certain chosen values of the parameters in the control equations and, although these may range over many choices, they obviously cannot work through all of them. Hence it is desirable, as far as this is possible, to complement these simulations with general mathematical proofs of the existence or non-existence of oscillations

or other features for wide classes of equations. The equations concerned are non-linear and this fact increases the mathematical difficulties enormously. Nevertheless we shall find that useful information can be obtained by applying standard methods of the theory of non-linear equations. We shall consider several simple and representative examples and start, in this paper, with the simplest kind of self-repressing, or negative feedback, system. In it, a single gene G produces a mRNA, M , which codes for a protein E . Either this protein, or a metabolite, P , formed in a reaction catalysed by E , acts as a repressor for the same gene G . This situation has been discussed previously by Goodwin (1963, 1965) and Maynard Smith (1965), and very similar equations, in more variables, to the ones we shall use have appeared in the work of Walter (1968) on enzyme-catalysed sequences of reactions.

2. The Equations

Following Goodwin (1963, 1965) we shall suppose the repressor, R , combines with the gene, G , according to the equation



and that G , but not GR_m , is active for the production of mRNA. R may be either E or P . Current experimental evidence on repression suggests that the case $m = 1$ will prove the most important (Koch, 1967), but we shall make our analysis for other values of m also. It follows from equation (1) that the proportion of time G is active is given by

$$p = \frac{1}{1 + Kx^m} \quad (2)$$

where x is the concentration of R , and K is the equilibrium constant of equation (1). Equation (2) becomes inaccurate near $x = 0$ (see Koch, 1967), but we shall be interested primarily in the behaviour of the control equations away from $x = 0$ and so shall neglect that fact here.

If we assume we can write ordinary macroscopic equations for the concentrations of mRNA and protein then, in the case that E is the repressor, we shall take them as

$$\begin{aligned} \dot{M} &= \frac{dM}{dt} = \frac{a}{1 + KE^m} - bM \\ \dot{E} &= \frac{dE}{dt} = cM - dE, \end{aligned} \quad (3)$$

where a , b , c , d are positive constants. Equations (3) are of the type discussed by Goodwin (1965) and assume that the rate of messenger production is proportional to the fraction of time G is active, and that the protein is synthesized at a rate proportional to the amount of messenger present.

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M and E decay in proportion to their concentrations, which must surely often happen, even if it is not always so. It follows at once from equations (3) that if, initially, $M \geq 0$ and $E \geq 0$, then they both remain non-negative subsequently. This is obviously necessary, because of the physical significance of M and E , and in the mathematical analysis which follows, we shall not concern ourselves with negative values for them.

Equations (3) contain a large number of constants and in a mathematical analysis it is desirable to simplify them as much as we can do without thereby reducing the generality of the problem. We do this by a change of units for each of M , E and t . After a little simple algebra, it is apparent that the units can be chosen so that

$$\begin{aligned} M &= \frac{1}{1+E^m} - \alpha M \\ \dot{E} &= M - \beta E \end{aligned} \quad (4)$$

without loss of generality. α and β are both positive constants. The analogous equations to (3), which arise when the metabolite P acts as the repressor, can similarly be shown to be reducible to the form

$$\begin{aligned} \dot{M} &= \frac{1}{1+P^m} - \alpha M \\ \dot{E} &= M - \beta E \\ \dot{P} &= E - \gamma P \end{aligned} \quad (5)$$

with α , β and γ positive.

Goodwin (1963) has remarked that a two-variable equation may be regarded as a limiting case of a three-variable one. Suppose γ is very large in equation (5). Then P will change much more rapidly than M or E and will usually be close to its "equilibrium" value for fixed E , i.e. $P = \gamma^{-1}E$. This enables us to simplify equations (5) by eliminating P and thus reducing them to the form (3) which can then, by a change of units, be transformed to the form (4). Evidently we can go further and, in (4), let β be large. This suggests considering, together with (4) and (5), the one-variable equation

$$\dot{M} = \frac{1}{1+M^m} - \alpha M. \quad (6)$$

In this equation, as M runs from 0 to ∞ so $(1+M^m)^{-1}$ runs monotonically from 1 to 0. Hence $\dot{M} = 0$ for a unique value $M = M_0$ satisfying

$$\alpha M_0(1+M_0^m) = 1. \quad (7)$$

When $M < M_0$, $\dot{M} > 0$ and when $M > M_0$, $\dot{M} < 0$. Hence $M \rightarrow M_0$, whatever the initial value of M .

3. The Two-variable Case

Here we use standard techniques of the theory of non-linear equations (see, e.g. Leimanis & Minorsky (1958); Andronov, Vitt & Khaikin (1966). First we set $M = E = 0$ in equations (4) to obtain the stationary points. They occur when M and E satisfy

$$\begin{aligned} M &= \beta E, \\ \alpha \beta E(1+E^m) &= 1. \end{aligned} \quad (8)$$

As with equation (7) there is just one solution and so just one stationary point, (M_0, E_0) say. We expand near this point by writing $M = M_0 + X$, $E = E_0 + Y$ and obtain

$$\begin{aligned} \dot{X} &= -m\alpha^2\beta^2E_0^{m+1}Y - \alpha X + O(Y^2), \\ \dot{Y} &= X - \beta Y. \end{aligned} \quad (9)$$

E_0 , α and β are all positive and therefore the point satisfies the conditions for stability for all values of the parameters (for these conditions see Leimanis & Minorsky (1958, p. 121).

We conclude the analysis by showing that there are no limit cycles and that the motion in the phase plane (the M , E plane) contains no trajectories which go off towards infinity. Both these are easy. The quantity

$$\frac{\partial}{\partial M}(M) + \frac{\partial}{\partial E}(E) = -\alpha - \beta,$$

has constant sign everywhere which allows us to apply the Bendixson criterion (Andronov *et al.*, 1966, p. 305) which says that it follows that no motion can describe a closed trajectory in the phase plane. So there are no limit cycles and hence no stable oscillations for any values of α , β and m . The behaviour towards infinity is treated by observing that the motion is entirely inwards across the boundaries of the rectangle having vertices $(0, 0)$, $(0, A)$, $(\beta A, 0)$, $(\beta A, A)$ for any A satisfying $\alpha\beta A > 1$.

It is interesting to refer here to the equations discussed by Goodwin (1963), which are of the form

$$\begin{aligned} \dot{M} &= \frac{1}{1+E^m} - \alpha, \\ \dot{E} &= M - \beta. \end{aligned} \quad (10)$$

He showed that these give oscillatory behaviour of arbitrary amplitude. These oscillations are of indifferent stability (Leimanis & Minorsky, 1958, p. 129) rather than limit cycles. However, equations (10) are not satisfactory when E and M are small because if we start the system with $M = E = 0$ we find $\dot{M} = 1 - \alpha$, $\dot{E} = -\beta$ and so E subsequently becomes negative, which

is physically unacceptable. Hence the best we can do is to replace β with some function $f(E)$ such that $f(E) \approx \beta$ for E large, but $\lim_{E \rightarrow 0} f(E) = 0$. If $f(E)$ is also a monotonic function of E with $f'(E) > 0$, for all E , we may apply Bendixson's criterion to show there can be no stable oscillations. However, there may still be damped oscillations with very slow decay of amplitude.

4. The Three-variable Case

Again, there is a unique stationary point (M_0, E_0, P_0) , where

$$\begin{aligned} M_0 &= \beta E_0, \\ E_0 &= \gamma P_0, \\ \alpha \beta \gamma P_0(1 + P_0^m) &= 1. \end{aligned} \quad (11)$$

The stability of this point is discussed by expanding about it by writing $M = M_0 + X$, $E = E_0 + Y$, $P = P_0 + Z$. Let us write R for the column vector (X, Y, Z) and retain only the first order terms in X, Y and Z . We readily find $\dot{R} = QR$, where Q is the matrix

$$Q = \begin{bmatrix} -\alpha & 0 & \phi m(\phi P_0 - 1) \\ 1 & -\beta & 0 \\ 0 & 1 & -\gamma \end{bmatrix}$$

and we have set $\phi = \alpha \beta \gamma$. The point is stable if and only if the real parts of all the eigenvalues of the matrix Q are negative. To test this, we use Hurwitz's criteria (Uspenski, 1948) applied to the characteristic equation, which is

$$(E + \alpha)(E + \beta)(E + \gamma) + \phi m(1 - \phi P_0) = 0. \quad (12)$$

Hurwitz's criteria applied to a cubic equation

$$E^3 + p_2 E^2 + p_1 E + p_0 = 0$$

are satisfied if and only if $p_1 > 0$ and $p_1 p_2 - p_0 > 0$. As $p_1 = \alpha \beta + \beta \gamma + \gamma \alpha$, it is evidently positive. The other condition reads

$$D \equiv \sum (\alpha)(\sum \alpha \beta) - \phi - \phi m(1 - \phi P_0) > 0 \quad (13)$$

where

$$\sum \alpha = \alpha + \beta + \gamma \quad \text{and} \quad \sum \alpha \beta = \alpha \beta + \beta \gamma + \gamma \alpha.$$

We now use the well-known result (Hardy, Littlewood & Pólya, 1952) that for all positive α, β, γ

$$\frac{1}{3} \sum \alpha \geq \left(\frac{1}{3} \sum \alpha \beta \right)^{\frac{1}{2}} \geq (\alpha \beta \gamma)^{\frac{1}{3}},$$

and equality holds only if $\alpha = \beta = \gamma$. Hence

$$\sum (\alpha)(\sum \alpha \beta) \geq \sqrt{3} (\sum \alpha \beta)^{3/2} \geq 9 \alpha \beta \gamma. \quad (14)$$

So we have

$$D \geq 8\phi - \phi m(1 - \phi P_0) = \phi(8 - m) + m\phi^2 P_0 \quad (15)$$

and therefore $D > 0$, and thus the point is stable, for $m \leq 8$ and any values of α, β, γ .

On the other hand, when $m > 8$, we can always find values of α, β and γ which make D negative and the stationary point unstable. For if we let $\alpha = \beta = \gamma$, equations (14) and (15) become equalities, and we then require

$$\phi P_0 < 1 - 8m^{-1}, \quad \phi P_0(1 + P_0^m) = 1. \quad (16)$$

Now as

$$\phi \rightarrow 0, \quad \text{so } P_0 \rightarrow \infty \quad \text{and } \phi P_0 = (1 + P_0^m)^{-1} \rightarrow 0,$$

so this can always be done by taking ϕ small enough.

No trajectories can go off to infinity. This is easily seen by using equations (5) to show that all motion is inwards across the faces of the rectangular box which has its sides parallel to the axes of co-ordinates and two opposite vertices at $(0, 0, 0)$ and $(\beta \gamma \lambda, \gamma \lambda, \lambda)$, where $\alpha \beta \gamma \lambda > 1$.

The foregoing results indicate strongly that there will be one limit cycle whenever $m > 8$ and $D < 0$, and none in any other circumstances. I have been unable to establish rigorously that there are none under the latter conditions. However, we have run a large number of simulations on a digital computer, with various choices for the parameters, in complete agreement with the predictions. In these simulations, whenever an oscillation has been found, it has been carefully checked that it is really a limit cycle and it has been observed to be unique. The present results are inconsistent with the report of Goodwin (1965), who claimed to have found a limit cycle for $m = 1$ and certain values of α, β and γ . He informs me, however, that he now considers his result to have arisen erroneously out of errors in the analogue simulation which he employed.

Our mathematical formulation has depended upon equations (1) and (2), which do not represent the only ways in which repression could occur. The inclusion of an aporepressor in the scheme, or replacing equation (2) with the more complicated one suggested in Koch's (1967) work would greatly complicate the analysis. However, the absence of limit cycles in the two-variable case should still hold, whilst in the three-variable case it would be surprising if limit cycles appeared for very low values of m . Thus the present work must be regarded as casting serious doubt on the possibility that negative feedback from a product of a single gene can ever give rise in practice to undamped oscillations in the concentrations of cellular constituents. It has not, however, discussed at all the question of whether such oscillations might arise in systems in which two or more genes are inductively or repressively coupled.

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Mathematics of Cellular Control Processes

II. Positive Feedback to One Gene

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Equations are set up to describe the induction of activity in a gene by the protein for which it codes, or by the metabolic product of that protein. The equations are analysed by methods closely paralleling those in paper I (Griffith, 1968). When the induction is due to the combination of one molecule of inducer with the genetic locus, it is found that there is only one stable set of concentrations (which may or may not be zero). When more than one molecule of inducer combines at the same locus, the state in which all concentrations are zero is always stable, and there is either no other or one other stable state, depending on the values of the parameters.

1. Introduction

In this paper we shall discuss equations which are precisely analogous to those in Part I (Griffith, 1968), except that the feedback is now of the positive, or inductive, type. Specifically, we shall suppose that the inducer I combines with the gene G according to the equation



G is inactive, and GI_m active, in the production of the mRNA, M , which codes for the protein E , which catalyzes the production of the metabolite P . I is either E or P . We shall now say that the fraction of time that G is active is

$$p = \frac{Kx^m}{1 + Kx^m} \quad (2)$$

where K is the equilibrium constant of equation (1) and x is the concentration of I . The question of the biochemical plausibility of these equations will be considered at the end of the paper.

Just as we found in Part I, the resulting equations may be reduced, by a change of units, to the form

$$\begin{aligned} \dot{M} &= \frac{E^m}{1 + E^m} - \alpha M, \\ \dot{E} &= M - \beta E, \end{aligned} \quad (3)$$

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in the two-variable case, and to

$$\begin{aligned} \dot{M} &= \frac{P^m}{1+P^m} - \alpha M, \\ \dot{E} &= M - \beta E, \\ \dot{P} &= E - \gamma P, \end{aligned} \quad (4)$$

in the three-variable case.

As we saw there, it is also of some interest to investigate the corresponding equation in one variable, which is

$$\dot{M} \equiv g(M) = \frac{M^m}{1+M^m} - \alpha M. \quad (5)$$

The stationary values of M are given by the equation

$$\alpha M(1+M^m) = M^m \quad (6)$$

whose properties are treated in the Appendix. When $m = 1$ and $\alpha \geq 1$, it has only the one solution $M = 0$ and, as

$$g'(M) = M(1-\alpha-\alpha M)/(1+M) < 0$$

whenever $M > 0$, all other values of M tend towards this. The point $M = 0$ is a stable stationary point. If $\alpha < 1$, there are two stationary points, namely $M = 0$ and $M = \alpha^{-1} - 1$. By considering the sign of $g'(M)$ it follows that the first is unstable and the second is stable.

When $m > 1$, the behaviour in the neighbourhood of $M = 0$ is given by

$$\dot{M} = -\alpha M + O(M^2)$$

and so $M = 0$ is a stable stationary point for all values of α . When (see the Appendix) $m^m \alpha^m > (m-1)^{m-1}$, this is the only stationary point and as $g'(M)$ is now continuous and non-vanishing for all $M > 0$, it follows that $M < 0$ for all positive M .

When $m^m \alpha^m < (m-1)^{m-1}$, there are two non-zero stationary points M_1 and M_2 (take $M_1 < M_2$). Now $g(M)$ is a continuous function of M and is negative for M small. It is zero only at $M = 0$, M_1 , or M_2 and it is easily seen that $g'(M_1) \neq 0$ and $g'(M_2) \neq 0$. Therefore, $g(M)$ changes sign at M_1 and M_2 and is negative for $M < M_1$ and $M > M_2$, but positive for $M_1 < M < M_2$. Hence $M = 0$ and $M = M_2$ are stable stationary points while $M = M_1$ is an unstable stationary point. Finally, when $m^m \alpha^m = (m-1)^{m-1}$ there is just one non-zero stationary point and it is unstable.

This is obvious from the continuity of $g(M)$, considered as a function of α . The mathematics we have used in this preliminary treatment of the one-variable case has been quite elementary. The general features we have found here are preserved in the two and three-variable cases, and in that respect

the present results differ from those found in the previous paper. Consequently it is possible for the reader to pass from this point immediately to the discussion, if he wishes.

2. The Two-variable Case

The stationary points are obtained by setting $\dot{M} = \dot{E} = 0$ in equations (3) and therefore satisfy

$$\begin{aligned} M &= \beta E \\ \alpha \beta E(1+E^m) &= E^m. \end{aligned} \quad (7)$$

We see that we have the same form of equation for E as we had for M in the one-variable case. As a consequence, there is always a stationary point $M = E = 0$ and none, one or two others according to the same conditions on $\alpha\beta$ as applied there in relation to α .

If there are no other stationary points, we can easily solve the whole problem by means of the Lyapunov function

$$L = M + \alpha E. \quad (8)$$

It follows from equations (3) that

$$\dot{L} = \frac{E^m}{1+E^m} - \alpha \beta E \quad (9)$$

The expression on the right of equation (9) has the same general form as $g(M)$ in equation (5). Using the properties found for $g(M)$ it follows that, when $M = E = 0$ is the only stationary point, $L < 0$ for all M, E except $E = 0$ (when $M < 0$ for all positive M). Therefore $L \rightarrow 0$ which, as neither M nor E can become negative, means $M \rightarrow 0$ and $E \rightarrow 0$ separately.

When $m = 1$ and $\alpha\beta < 1$, there are the two stationary points

$$M = E = 0 \quad \text{and} \quad M = M_0 = \alpha^{-1} - \beta, \quad E = E_0 = (\alpha\beta)^{-1} - 1.$$

Near $E = M = 0$, we have

$$\begin{aligned} \dot{M} &= E - \alpha M + O(E^2) \\ \dot{E} &= M - \beta E, \end{aligned}$$

which is unstable because $\alpha\beta < 1$ (see Leimannis & Minorsky (1958)). Near (M_0, E_0) we set

$$\begin{aligned} M &= M_0 + X, \quad E = E_0 + Y \\ \dot{X} &= \alpha^2 \beta^2 Y - \alpha X + O(Y^2) \\ \dot{Y} &= X - \beta Y \end{aligned}$$

which is stable because $\alpha\beta > \alpha^2 \beta^2$.

When $m > 1$, the behaviour in the neighbourhood of the origin is given by

$$\begin{aligned}\dot{M} &= -\alpha M + 0(E^m) \\ \dot{E} &= M - \beta E\end{aligned}$$

and so the origin is always stable. If there are two other stationary points, having $E = E_1$ and $E = E_2$ and $E_1 < E_2$, we expand near them to obtain

$$\begin{aligned}\dot{X} &= \alpha\beta m(1 - \alpha\beta E_i)Y - \alpha X + 0(Y^2) \\ \dot{Y} &= X - \beta Y,\end{aligned}\tag{10}$$

where $i = 1$ or 2 . Such a point is stable if

$$\alpha\beta m(1 - \alpha\beta E_i) < \alpha\beta$$

and unstable if the inequality is the other way round. Hence we can use equations (A3) and (A4) to deduce that the point with $E = E_1$ is unstable and that with $E = E_2$ is stable.

The analysis may be completed by noting that Bendixson's criterion applies and so there are no limit cycles. Finally, the motion towards infinity is entirely inwards as is seen by considering the sides of the rectangle having vertices at $(M, E) = (0, 0)$, $(\beta A, 0)$, $(0, A)$, $(\beta A, A)$, when $\alpha\beta A > 1$.

3. The Three-variable Case

The stationary points occur at

$$M_0 = \beta E_0 = \beta \gamma P_0, \quad \phi P_0(1 + P_0^m) = P_0^m,\tag{11}$$

where $\phi = \alpha\beta\gamma$. As in the two-variable case, whenever ϕ and m are such that $P_0 = 0$ gives the only non-negative solution of (11), there is a Lyapunov function. It is

$$L = M + \alpha E + \alpha\beta P.\tag{12}$$

In a precisely parallel way, this yields a rigorous proof that $M = E = P = 0$ is a stable stationary point towards which all trajectories tend.

For other values of ϕ and m we expand about the stationary points by setting $M = M_0 + X$, $E = E_0 + Y$, $P = P_0 + Z$, R for the column vector (X, Y, Z) and retain only the first-order terms. Then $\dot{R} = QR$, where Q is the matrix

$$\begin{bmatrix} -\alpha & 0 & B \\ 1 & -\beta & 0 \\ 0 & 1 & -\gamma \end{bmatrix}.\tag{13}$$

$B = \delta_{m1}$ for the point $M_0 = E_0 = P_0 = 0$, and $B = \phi m(1 - \phi P_0)$ for any other stationary point. We now apply Hurwitz's criteria to the characteristic equation, which is

$$(E + \alpha)(E + \beta)(E + \gamma) - B = 0.\tag{14}$$

These are readily seen to be

$$\begin{aligned}\phi - B &> 0, \\ (\sum \alpha)(\sum \alpha\beta) - \alpha\beta\gamma + B &> 0.\end{aligned}\tag{15}$$

When $m = 1$ and $\phi < 1$, $B = 1$ at the origin and $B = \phi^2$ at the other stationary point. Thus the first of the inequalities (15) is satisfied at the other point, but not at the origin. Using equation (14) of Part I, we have

$$(\sum \alpha)(\sum \alpha\beta) - \alpha\beta\gamma + B \geq 8\phi + \phi^2 > 0$$

so the second inequality is always satisfied. Therefore the origin is unstable and the other point stable.

When $m > 1$, $B = 0$ at the origin, which is therefore always stable. At any other stationary point we have

$$(\sum \alpha)(\sum \alpha\beta) - \alpha\beta\gamma + B \geq 8\phi + \phi m(1 - \phi P_0)$$

and clearly

$$\phi P_0 = P_0^m(P_0^m + 1)^{-1} < 1,$$

so the second condition is always satisfied. The first becomes

$$1 - m + \phi P_0 > 0$$

which, from equations (A3) and (A4), is satisfied by the larger of the two non-zero P_0 but not by the smaller. Hence the larger P_0 corresponds to a stable stationary point and the smaller to an unstable one.

Thus we have found the same general behaviour in all three cases. In the present case, we can show that no phase trajectories reach infinity by considering the rectangular box formed by the co-ordinate planes and the point $(\beta\gamma A, \gamma A, A)$, for $\phi A > 1$. However, I have not proved that there can be no limit cycles, except in the case discussed at the beginning of this section. The equations (4) have been run on a digital computer for a selection of values of α, β, γ, m and initial conditions. Although some of the phase trajectories show a spiralling tendency which is, of course, absent in the two-variable case, the simulations showed no sign of limit cycle behaviour.

4. Discussion

We have found that the qualitative behaviour of the equations is similar in the one, two and three-variable cases. Our treatment was rigorous for the first two cases but incompletely so for the third. When $m = 1$ in equation (1), there are two possibilities. Let $\phi = \alpha, \alpha\beta$ or $\alpha\beta\gamma$, in the three cases respectively. Then if $\phi \geq 1$, the state in which all concentrations are zero is the only stable one and all others tend towards this. If $0 < \phi < 1$ there is a state with non-zero concentrations which is stable. There is also a metastable state with concentrations zero, and all other states tend towards the state

with non-zero concentrations. This means that if we start with zero concentrations, that state will persist indefinitely. However, if we introduce even a single molecule of any component, it will be liable to start a progressive growth of all concentrations, leading the system into the other, stable, state.

When $m > 1$, the state with all concentrations zero is stable for every choice of positive α, β, γ and of m . If we write $x = (m-1)^{1/m}$, then if $x+x^{1-m} > \phi^{-1}$ there are no other stationary sets of values for the concentrations. If $x+x^{1-m} = \phi^{-1}$, there is just one other, but it is metastable. If $x+x^{1-m} < \phi^{-1}$ there is one non-zero set of concentrations which is metastable and one which is stable. This means that in this latter case there are two distinct stable sets of values for the concentrations. Each of them will persist if left alone and, for each, the concentrations can be altered by a small amount, whereupon they will return again to the stable values. The extent to which they can thus be altered depends upon the values of α, β, γ and m and is not a difficult matter to compute in any particular instance.

Our treatment has been based upon equation (1) which is probably the simplest way in which induction could occur. Experimentally, most evidence is in favour of induction taking place through the inducer I combining with a repressor R , and thus protecting G from repression. Positive control has been found in the l-arabinose system (Sheppard & Englesberg, 1967) and the l-thiamine system (Power, 1967), although the metabolite combines first with an "apoinductor" rather than directly with the gene. However, very few cases have been investigated in any detail and it may well be that induction according to equation (1) will be found to occur. In any event, equation (1) and its consequences are a natural starting point in a mathematical treatment because the resulting equations (3) and (4) are relatively simple, while other methods of induction lead to a vastly more complicated expression for the induction term in the equation for M . Note, however, that this term will still be a function of E alone in equations (3) and therefore we can still apply the Bendixson criterion to show there are no limit cycles. The same remark applies to the two-variable case with negative feedback. The one qualitative difference which is to be expected with more complicated induction mechanisms is that we no longer have the induction term exactly zero when there is no inducer and hence in the case ($m = 1, \phi < 1$) when the origin was found to be metastable, it should now cease to be a stationary point and become actually unstable.

Finally, we must consider the point raised by Koch (1967), that for very low values of x , equation (2) is no longer accurate. Using the correct equations, we readily find that

$$p = (1 + K)^{-1} K x \delta_{m-1} + O(x^2)$$

near $x = 0$. As a consequence, the stability for $m > 1$ is unaffected, while for $m = 1$ the same qualitative conclusions hold as before except that the stability and existence of both stationary points now depend on whether

$$\phi > \frac{1}{1+K}$$

rather than $\phi > 1$. We expect K to be small in interesting cases, otherwise G would be almost completely combined for minute concentrations of I .

Inasmuch as our results are directly applicable to the biological situation, they suggest that the simple self-inductive mechanism which has been invoked by Monod & Jacob (1961) in connection with differentiation, and Griffith (1967) in relation to the disease Scrapie is only likely to give sufficient stability in the two states required if the induction is by a co-operative mechanism with $m \geq 2$.

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Appendix

Here we obtain various properties of the equation

$$\phi x(1+x^m) = x^m \quad (\text{A1})$$

which are needed in the text. We are only interested in non-negative solutions, of which $x = 0$ is always one, and there are sometimes others.

The behaviour is rather different for $m = 1$ and we consider this first. Equation (A1) has the second solution $x = \phi^{-1} - 1$ which is allowable only when it is non-negative, i.e. when $\phi \leq 1$. Otherwise $x = 0$ is the only allowable one.

If $m > 1$, any positive solution of (A1) must satisfy

$$f(x) \equiv x + x^{1-m} = \phi^{-1}. \quad (\text{A2})$$

Now

$$f(0) = f(\infty) = +\infty$$

and $f'(x) = 0$ only for $x = x_0$, where

$$x_0 = (m-1)^{1/m}.$$

Hence equation (A2) has no real positive solutions if $f(x_0) > \phi^{-1}$. If $f(x_0) = \phi^{-1}$ it has just one, and if $f(x_0) < \phi^{-1}$ it has just two distinct ones, which we shall call x_1 and x_2 (with $x_1 < x_0 < x_2$). When $m = 2$, this means there are two solutions of (A2) when $\phi < \frac{1}{2}$. Note that, for general m , the condition may be rearranged to read that there are 0, 1 or 2 distinct solutions according as $m^m \phi^m >$, = or $< (m-1)^{m-1}$.

As $f'(x) < 0$ for $x < x_0$, we find that x_1 satisfies the two equations

$$f'(x_1) = 1 + (1-m)x_1^{-m} < 0,$$

$$f(x_1) = x_1(1+x_1^{-m}) = \phi^{-1},$$

from which we readily deduce that

$$m-1-m\phi x_1 > 0. \quad (\text{A3})$$

In a similar way we obtain

$$m-1-m\phi x_2 < 0. \quad (\text{A4})$$

The Life Span of the Cultured Normal Cell: Concepts Derived from Studies of Human Lymphoblasts

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There is substantial but not conclusive evidence that lymphoblastoid cell lines derived from normal individuals are made up of normal cells with an infinite life span. In contrast, similar cell lines that originated from the blood and tumor tissues of patients with malignant hematopoietic diseases had some characteristics of malignancy. These abnormal cell lines had one or more of these features: an abnormal karyotype, relatively high cloning efficiencies, and synthesis of an abnormal cell product. There was no difference in growth rates between normal and abnormal cell lines. There was no evidence of "aging" of the normal cell lines during continuous culture for more than 20 months. It seems reasonable to suggest that some criteria for normalcy for one kind of cultured human cells in a restricted environment may not necessarily apply to other kinds of such cells.

1. Introduction

As cell culture methodology expands and becomes more diversified and additional kinds of cells are grown, appellative terms like *normal* require redefinition. Furthermore, the criteria currently used in establishing the "normalcy" of cultured cells are restrictive in the sense that they are applicable to certain kinds of cells but not to others.

The complexity of these issues is illustrated in this paper by a brief review of the unique biological features of cultured human hematopoietic cells. This review is more than an academic exercise, since these seemingly normal lymphoblastoid cell lines have an infinite life, maintain the ability to produce important end products (i.e. immunoglobulins), and may have clinical usefulness as vital cells. Accordingly, criteria for normalcy and benignancy are extremely important. Lastly, these cells can be stored "indefinitely", perhaps retaining much of the genetic pattern of the donor, and hence may raise ethical and moral questions.