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TISSUE GROWTH AND HOMEOSTASIS: CONSEQUENCES OF CONTROL IN SYNCHRONOUS CELL POPULATIONS

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A simple growth model for synchronous tissue cell populations is analyzed to assess the long term consequences of three growth control mechanisms.

The model classifies cells as either proliferating (population, P) or non-proliferating (population, N) and takes into account the probability of P cells moving into N (probability α), of N cells moving to P (probability β), and N cells dying (probability δ). The mechanisms of growth control examined are sensitive to the number of cells in the controlling population (P or N) and operate by alterations in the probability of cell movement along one of the three available pathways: P to N (mitotic inhibition), N to P (mitotic stimulation), or N to death. We have determined the criteria for the tissue to attain a stable steady-state size if control is operating on one cellular flux and the probability of cellular movement along the other two pathways is constant.

Insight into the kinetic and steady state characteristics of a tissue utilizing one of these growth control mechanisms is obtained by examining the tissue growth characteristics when no control is operating.

INDEX TERMS: Mathematical model, proliferation, growth control, cell number homeostasis, tissue growth, synchronous division

THE BASIC MODEL

In the hypothetical synchronous tissue denote by P and N the numbers of cells in the proliferating and non-proliferating pools respectively. P and N are generally functions of time, and in the discrete formulation of the model used here the generation time, T_p, will be assumed constant and used as the basic unit of time measure. Rather than deal with the time durations, $t_m = mT_p$ $(m = 1,2, \ldots)$, time is denoted by the dimensionless parameter $m = t_m/T_p$, an integer. To denote the temporal (m) dependence of the cell population size the notation P_m and N_m is used.

The tissue model is schematically illustrated in Figure 1. In the model during the m^{th} generation a fraction α_m of the cells in P may transfer

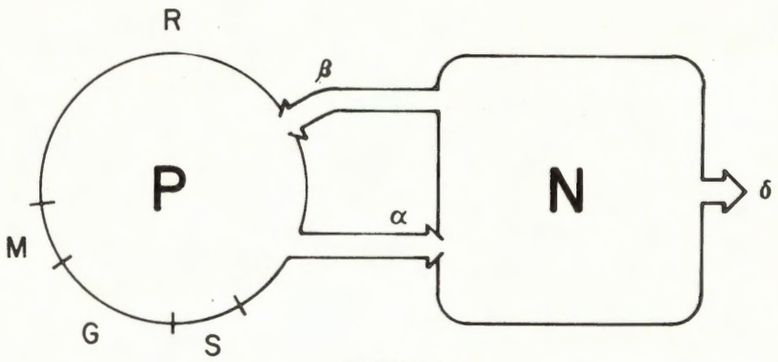

FIGURE 1

A schematic representation of the cell tissue kinetic model under investigation. Proliferating cells have a population P, the non-proliferating cells have a population N. The probability per generation time that a P cell will become an N cell is α, the probability that an N cell will re-enter P is β, and the probability that an N cell will die is δ. ($\beta + \delta \leqslant 1$). Any, or all, of the three probabilities α, β, and δ may change with time, as may P and N. M, R, S, and G denote the mitotic, post-mitotic, DNA synthesis, and pre-mitotic cell cycle phases respectively.

to N; a fraction β_m of the N cells may go to P; and a fraction δ_m of the N cells die and are removed from observational capacity. Any or all of the probability (or transfer fraction) parameters α_m, β_m, δ_m may depend on the populations P_{m-1} and N_{m-1} or on m, and it is through the dependence of transfer fractions on pool population sizes that the element of control is introduced. By definition the transfer fractions are pure numbers between 0 and 1; $\beta_m + \delta_m \leqslant 1$ for all m. In terms of current concepts related to cellular kinetics, a control mechanism operating on the probability that a cell will transfer from P to N would be related to a process of mitotic inhibition (Bullough and Deol, 1971). Conversely, triggers of mitotic activity would presumably operate via the probability of cell transfer from N to P (Baserga, 1965).

The model may be formally described by the following set of coupled difference equations:

$$P_m = 2(1 - \alpha_m)P_{m-1} + \beta_m N_{m-1} \tag{1}$$

and

$$N_m = (1 - \beta_m - \delta_m)N_{m-1} + \alpha_m P_{m-1} \tag{2}$$

These equations are written for $m \geqslant 1$. P_0 and N_0 respectively denote the numbers of cells in the proliferating and non-proliferating

pools between $t = 0$ and $t = T_p$. Equations 1 and 2 are 'conservation of cell' equations and do not explicitly contain any of the elements of control. Thus (1) is an expression of the fact that, during the m^{th} generation the number of cells in P_m is determined by the sum of the number of cells that entered P from N, $\beta_m N_{m-1}$, and the number of cells left in P from the $(m-1)^{th}$ generation time, $2(1-\alpha_m)P_{m-1}$, where the factor 2 accounts for mitosis. $\alpha_m P_{m-1}$ of the cells in P transferred to N. In equations (1) and (2) we have implicitly assumed that the transit time for a cell in N to return to P, as well as the lifespan of an N cell, are both equal to T_p. While probably unrealistic biologically, this assumption aids in obtaining a first quantitative characterization of the model tissue characteristics.

For the totally asynchronous tissue (cells passing through mitosis continuously) the discrete time difference equation formulation is inappropriate and recourse must be taken to a continuous time representation. The continuous time differential equation analogs to (1) and (2) are

$$\dot{P}(t) = -P(t) + 2(1-\alpha)P(t-T_p) + \beta N(t-T_p) \qquad (3)$$

and

$$\dot{N}(t) = \alpha P(t) - \beta N(t+T_N) - \delta N(t+T_D) \qquad (4)$$

where any or all of α, β, and δ may depend on P, N, t, T_p, T_N (the transit time between entry of a cell into N and its exit back into P), and T_D (the lifespan of a cell remaining in N), and $\dot{X}(t)$ denotes a time derivative.

Various authors have analyzed specific cases of the model presented here. Burns and Tannock (1970) utilized a continuous model with $\alpha \equiv 1$ (obligatory passage of cells through N before re-entry into P), constant β and δ, $T_N = T_D = 0$, and equal *a priori* probability of cellular death from P and N. We assume P cells do not die. Good (1972, 1973) has also analyzed a similar model with control over α; β and δ constant; and in the first instance $T_P = T_N = T_D = 0$ (Good, 1972), relaxing this later to $T_P = T_D = 0$ with $T_N \neq 0$ but small (Good, 1973). Woolley and DeRocco (1973) have considered in some detail the relaxation of a synchronous proliferating population to an asychronous one.

The Consequences of No Control

Before examining the effects of various control mechanisms on growth in the model of Figure 1 and equations 1 and 2, the behaviour of the model in the absence of any dependence of α_m, β_m, and/or δ_m on the cellular populations will be examined for constant T_p. In this case $\alpha_m = \alpha$, $\beta_m = \beta$, and $\delta_m = \delta$ are all constant, and the only constraint on equations 1 and 2 is $\beta + \delta \leqslant 1$, for $\beta + \delta > 1$ would imply that a fraction of cells greater than one was lost from the nonproliferating pool per generation time.

In Appendix 1 the large m properties of the analytical solutions to the coupled linear difference equations 1 and 2 for constant coefficients are discussed. The interested reader may refer to that section for the basis of the following discussion.

As shown in Appendix 1 there is a crucial relationship between α, β, and δ that determines the qualitative growth patterns of the model tissue described by equations 1 and 2. If a "critical" value of α is defined by (see equation 1A.8, Appendix 1)

$$\alpha_c = (\beta + \delta)/(\beta + 2\delta)$$

then for $\alpha < \alpha_c$, the total tissue cellular population will be an increasing function of the growth time. The tissue growth pattern is "exponential", and the tissue cell number doubling time is a function of α, β, and δ. A decrease in α and/or δ, and/or an increase in β, serves to increase the tissue growth rate. Qualitatively this is expected, for the result of all three of these changes is to increase the number of cells engaged in mitosis. Also, not unexpectedly, the effect of changing α is much more potent than changes in β or δ. Thus, in the no control model when $\alpha < \alpha_c$, tissue growth will be exponential and the total tissue cellular population will increase indefinitely.

When $\alpha_c < \alpha \leqslant 1$ the behaviour of the tissue growth pattern is the reverse of the behaviour of $\alpha < \alpha_c$; tissue evolution as a function of the number of generation times is properly termed regression. Changes in α, β, or δ have the effects one would qualitatively expect on the tissue cell number regression rate. Thus in the absence of control if tissue cell population transfer probabilities are such that $\alpha_c < \alpha \leqslant 1$, the tissue will eventually regress and disappear.

The growth or regression in the absence of control are separated by the kinetic characteristics of the tissue when $\alpha = \alpha_c$. As shown in Ap-

pendix 1 when $\alpha = \alpha_c$ the tissue always approaches a steady state size defined by α, β, δ and the initial conditions. The approach to this non-zero steady state may be via growth or regression; the path is dependent on the initial relationship between the number of proliferating and non-proliferating cells.

Thus the model in Figure 1 and equations 1 and 2, in the absence of any specific growth control mechanism, leads us to conclude that unless the kinetic determinants of the model tissue are so arranged that $\alpha \equiv \alpha_c$, the tissue will either regress and disappear, or grow indefinitely. The requirement that *a priori* $\alpha \equiv \alpha_c$ for tissue homeostasis to be maintained seems to be particularly strenuous, and does not allow for any correction on the part of the organism in response to external alterations of P and/or N (e.g., wounding reactions). However, it will be clear from our considerations of control mechanisms that the requirement $\alpha = \alpha_c$, is of special import for a steady state tissue size to be reached and maintained.

Growth and Its Control

Why Control?

If the pre- and post-uterine growth patterns of normal mammalian organisms is examined, the most constant feature is that overall volume as a function of time generally follows a sigmoid curve (Laird, 1969). Initially it grows slowly, then goes through a phase of rapid growth, and finally attains a constant size (negligible growth rate). At the level of the individual tissues growth becomes more complex as embryogenesis and organogenesis proceeds, involving different growth rates, relative sizes and time scales in different tissues. Certain tissues may also achieve maximum size and then regress at some time prior to maturity of the organism. These differential growth phenomena are well described by the law of allometry (Laird, Barton, and Tyler, 1968).

It has been a matter of some interest (Laird, 1969) that the sigmoidal pattern of growth observed in normal pre- and post-uterine tissue, and some tumor systems, is empirically described by the Gompertz equation:

$$V(t) = V_0 \exp\{A[1 - \exp(-Bt)]\}.$$

In this equation $V(t)$ and V_0 are measures of tissue volume at general times (t) and zero time respectively, and A and B are adjustable parameters. Although the Gompertz equation can empirically describe a

variety of growth data, its basic usefulness is limited because the parameters A and B have no discernible connection with experimentally measurable cell kinetic characteristics.

In spite of this limitation, the fitting of growth data to the Gompertz function has established some interesting and suggestive relationships. Laird (1966), using normalized weight and time, was able to fit the accumulated growth data from the embryos of 15 mammalian and avian species with a single set of parameters (A,B) for the Gompertz function. Similarly Laird (1965), using the same normalization procedure, was able to fit the combined growth data from 12 different tumor systems in three different species with a single set of parameters. The implications of this work are open to debate. We share with Laird (1969) the feeling that, at the very least, they imply the existence of inherent growth control mechanisms operating across species and tissue types for the lifetime of an organism. Whether the expression of this control is achieved in a similar fashion for normal and neoplastic tissue is unknown, but evidence summarized by Bullough and Deol (1971) would strongly support a common mechanism.

The foregoing, in conjunction with the results obtained from the simplified conceptual model when the transfer probabilities are all constant (no control), provides strong incentive for examining the consequences of control of cell tissue kinetics at biologically reasonable points within the model.

Possible Points of Control

Several hypotheses have been advanced to account for the regulation of growth. One is that the availability of diffusion limited nutrient supplies restricts growth (Burton, 1966; Summers, 1966). This hypothesis, formulated for solid tumours, is untenable for other tissues such as blood, whether normal or abnormal, and Laird (1969) has argued quite convincingly that nutrient deficiencies cannot generally be the primary source of tissue growth regulation. Thus, although such deficiencies may play a role in regulation, we assume here that they are not operating.

Other workers have suggested that growth control might be exercised via changes in the generation time (Laird, 1964). It is well known that in normal embryonic tissue the generation time decreases as growth increases. Further, there is abundant evidence from ascites tumor sys-

tems (Frindel *et al.*, 1969; Lala and Patt, 1966, 1968) that the generation time increases as ascites tumor growth progresses. Thus, a variable generation time may be a source of control in some normal and neoplastic tissues. However, based on the growth characteristics of the no control model, control of generation time cannot be the *sole* source of tissue volume maintenance unless T_P becomes much larger than the life span of the tissue. This does not, however, rule out its importance for tissue responses to an external stimulus; or its interplay with other control mechanisms. Again, we assume that in our model changes in T_P are not operating.

Other mechanisms proposed for the control of growth implicate the proliferating pool of cells as the control site (Baserga, 1968). On teleological grounds the proliferating pool seems a logical choice. If the goal of a cell is to minimize its energy expenditure, then it is perhaps reasonable to suppose that it will prefer to refrain from going through the metabolically expensive process of DNA synthesis in preparation for mitosis unless required to by overall tissue needs.

Mitotic Inhibition. Bullough (1965, 1967, 1969) and subsequent investigators (summarized in Bullough and Deol, 1971) have marshalled an impressive body of experimental evidence from different sources arguing for the existence of intrinsic tissue specific mitotic inhibitors. The hypothetical control scheme is envisioned as follows. A tissue specific protein (chalone) is produced by the cells of a tissue. Chalone, in the presence of adrenaline, inhibits mitotic activity. Thus, a decrease of chalone concentration (due, e.g., to a loss of cells in a tissue and the resultant decrease in absolute chalone production) increases activity in the proliferative pool of cells and the tissue mass is gradually built back up to its previous level. The cellular origin of the mitotic inhibitor (proliferating and/or non-proliferating cells) is unspecified, but its action seems to be confined to the late R and G phases of the cell cycle. Although we have included the possibility that cells in P are the source of the mitotic inhibitor (see below) it seems much more likely that it derives from cells in N. The differentiated cells that perform tissue specific functions are in N, and when they exist in sufficient numbers they would be the likely candidates to limit further production.

Riley (1969) has analyzed a simple one component tissue model incorporating the chalone concept and demonstrates that it leads to a

stable tissue size. Goods (1973) model included a form of mitotic inhibition control also suggested by the chalone concept. A more complex model has been proposed for the red blood cell-stem cell tissue system involving two basic control mechanisms (Kirk, Orr and Hope, 1968). In their model chalone is produced by the stem cell population which, in turn inhibits stem cell mitotic activity. In addition erythropoietin, produced by the juxtaglomerular cells of the kidney, acts to control eventual stem cell differentiation into red blood cells. They have proposed equations describing this four component system, and the results calculated from their model are in satisfactory agreement with available data. This model is specifically designed for the erythrocyte series, and has not been generalized.

In the context of our model the mitotic inhibitor hypothesis may be viewed as a control of the fraction (α_m) of cells leaving P. We postulate that increasing amounts of chalone will increase the transfer of cells from P to N, and thus decrease the number of cells going through mitosis. The origin of chalone could be cells in P and/or N so two possible control functions, given in Table 1, are examined. In writing these control functions it has been tacitly assumed that the concentration of chalone is proportional to the number of cells involved in producing it. We make no specific statements about the loss of chalone from the system through metabolism, but assume only that it is lost as chalone

TABLE 1
SUMMARY OF THE VARIOUS CONTROL FUNCTIONS EXAMINED FOR THEIR ABILITY TO GIVE STABLE TISSUE SIZES

Point of Control	Origin of Control	Control Function
α	$X_{m-1} = P_{m-1}$ or N_{m-1}	$\alpha_m = \dfrac{X_{m-1}}{A + X_{m-1}}$
β	$X_{m-1} = P_{m-1}$ or N_{m-1}	$\beta_m = \dfrac{(1-\delta)}{A + X_{m-1}}$
δ	$X_{m-1} = P_{m-1}$ or N_{m-1}	$\delta_m = \dfrac{(1-\beta)X_{m-1}}{A + X_{m-1}}$

The factor A that appears throughout has the dimension of cell number, and the factors $(1-\delta)$ and $(1-\beta)$ in the β_m and δ_m functions respectively ensure that $\beta + \delta \leqslant 1$ for all m. The value of A determines the size of the control population of cells at which the control function is at its half maximal value.

producing cells disappear, either through transfer to another pool or death.

Note that in the case of α-control small populations of mitotic inhibitor producing cells give $\alpha_m \to 0$ so most cells will tend to be left in P. On the other hand, as the number of cells producing the mitotic inhibitor becomes large the probability of cells being transferred from P each generation time approaches 1. The parameter A controls the behavior of α with respect to P or N. The potentiating role of adrenaline in the chalone hypothesis can be qualitatively mimiced by having A a monotonic decreasing function of adrenaline concentration. An increase in adrenaline levels will decrease A and increase the corresponding transfer fractions from P to N.

Mitotic Stimulation. It is also of interest to examine the consequences of control by mitotic stimulation, i.e. cells stimulated to re-enter P from N in response to an appropriate signal. With respect to the model being considered here, this reduces to a control operating on β_m. Baserga (1968) has reviewed the extensive biochemical evidence supporting this concept, and the review of Epifanova and Terskikh (1969) lends further support to the concept. As in the case of mitotic inhibitor, biochemical evidence would implicate late R and G as the cell cycle sites of mitotic stimulator action.

If the action of the mitotic stimulator is to control the fraction of cells re-entering P (and, thereby, the number going through mitosis) then P and/or N cells could be the stimulus source and two possible expressions for β_m are shown in Table 1. The qualitative behaviour of this type of control is different than the α control, for an increase in the controlling population of cells decreases the number of cells re-entering P. The factor $(1 - \delta)$ in the numerator of the control equations insures that $\beta_m + \delta \leqslant 1$. As in the mitotic inhibitor control, cells in N seem to be the logical source of a mitotic stimulator.

Bronk, Dienes, and Johnson (1970) have examined the early time growth patterns of a continuous model with mitotic stimulator control operating and find many of the early time behaviours exhibited by our model under β control. Most of their considerations were, however, for a tissue with no cellular death and their model does not apparently exhibit non zero stable steady state solutions. In light of the results obtained here (see below) this is expected.

Burton (1971) and Burton and Canham (1973) have analyzed an

intriguing model for contact inhibition where control is mediated via an oscillating concentration of mitotic stimulator and cell-cell communication is via tight junctions. Their work was inspired by the results of Loewenstein (see Loewenstein, 1968), and they are able to explain the heretofore paradoxical observation that small cell populations exhibit contact facilitation, and that contact inhibition is the observed state for larger cell aggregates. They do not consider the role of time lags (e.g., the generation time) in stability, but do give some quite interesting conclusions about the possible role of asychrony in determining cellul population stability.

Cellular Death. Laird (1969) suggests that the characteristic growth patterns described by the Gompertz equation are due to controlled cellular death as tissue mass increases. It is difficult to argue convincingly for or against this hypothesis on the basis of available experimental evidence, but it is known that there is significant cellular death in some tumor systems (Steel, 1968; Steel and Lamerton, 1969).

In an effort to examine the consequences of controlled cellular death we have included a form of control in which the fraction of cells in N that die per generation time is an increasing function of the number of cells in the controlling population (N or P). The control functions appropriate for this hypothesis are also given in Table 1. The term $(1 - \beta)$ plays the same role as does $(1 - \delta)$ in the β-control case, insuring that $\beta + \delta_m \leqslant 1$.

The Consequences of Control

We are interested in the long term behaviour of the model of Figure 1, described by equations 1 and 2, when "control" is operating according to one of the schemes considered above. Specifically, do these simple quantifications of hypotheses related to tissue growth control actually predict equilibrium cell populations P_∞ and N_∞? Under what conditions will these equilibria, if they exist, be stable with respect to changes in P and/or N? All of the mathematical baggage necessary to answer these questions is summarized in Appendix 2; we give here the conclusions.

For every type of control mechanism proposed, the determination of P_∞ and N_∞ and the requirements for their existence are easily obtained. The determination of the stability at P_∞ and N_∞ is rather more difficult. The results of the determinations for the existence of P_∞ and N_∞, their values and the requirements for their stability are presented

in Table 2. There, for each point of control discussed earlier (α, β, δ) and each possible controlling cellular population (P or N) are shown the stable steady state pool sizes, P_∞ and N_∞ and the conditions under which they are attained.

With respect to the criteria set forth in Table 2 for the existence of stable tissue size, some interesting comparisons can be made with the previous results of our model growth characteristics in the absence of control. The last column in Table 2 gives the relation that the uncontrolled probabilities must satisfy for the tissue to reach a stable steady state. What is even more illuminating is the behaviour of the *controlled* probabilities as a steady state is approached. In Table 3 the values of each of the six possible control functions at the initial time ($m = 0$) are shown, as well as their final value when a steady state has been reached. The first important point is that the controlling cellular population (P or N) has no effect on the eventual steady

TABLE 2

THE STEADY STATE PROLIFERATING AND NON-PROLIFERATION CELLULAR POOL SIZES PRODUCED BY EACH TYPE OF CONTROL, AND THE CRITERIA FOR THE TISSUE TO REACH THIS STEADY STATE

Point of Control	Origin of Control	Steady State Population	Criteria for Steady State
α	P	$P_\infty = A\left(\frac{\beta+\delta}{\delta}\right);\ N_\infty = \frac{P_\infty}{\beta+2\delta}$	$0<\delta\leqslant 1$
	N	$P_\infty = \frac{A(\beta+\delta)(\beta+2\delta)}{\delta};\ N_\infty = \frac{P_\infty}{\beta+2\delta}$	
β	P	$P_\infty = \frac{A(1-\alpha-\alpha\delta)}{\delta(2\alpha-1)};\ N_\infty = P_\infty\left(\frac{1-\alpha}{\delta}\right)$	$\frac{1}{2}<\alpha<\frac{1}{1+\delta}$
	N	$P_\infty = \frac{A(1-\alpha-\alpha\delta)}{(1-\alpha)(2\alpha-1)};\ N_\infty = P_\infty\left(\frac{1-\alpha}{\delta}\right)$	$0<\delta\leqslant 1$
δ	P	$P_\infty = \frac{A\beta(1-\alpha)}{2\alpha-1-\alpha\beta};\ N_\infty = P_\infty\left(\frac{2\alpha-1}{\beta}\right)$	$\frac{1}{2-\beta}<\alpha$
	N	$P_\infty = \frac{A\beta^2(1-\alpha)}{(2\alpha-1)(2\alpha-1-\alpha\beta)};\ N_\infty = P_\infty\left(\frac{2\alpha-1}{\beta}\right)$	$0<\beta\leqslant 1$

TABLE 3
THE CONTROL FUNCTIONS AT $m = 0$ (INITIAL POPULATION $X_0 = P_{i_0}$ OR N_0) AND WHEN THE TISSUE HAS REACHED A STEADY-STATE

Point of Control	Origin of Control	Control Function	
		Initial	At Stable Steady State
α	$X = P$ or N	$\alpha_0 = \frac{X_0}{A + X_0}$	$\alpha \to \frac{\beta + \delta}{\beta + 2\delta}$
β	$X = P$ or N	$\beta_0 = \frac{A(1 - \delta)}{A + X_0}$	$\beta \to \frac{\delta(2\alpha - 1)}{1 - \alpha}$
δ	$X = P$ or N	$\delta_0 = \frac{X_0(1 - \beta)}{A + X_0}$	$\delta \to \frac{\beta(1 - \alpha)}{2\alpha - 1}$

The criteria for the attainment of a steady state are given in Table 2.

state value of the controlled probability. Secondly, for control by mitotic inhibition the tissue settles down in a steady state such that $\alpha \to \alpha_c$. This is the requirement in the no control model for growth or regression to a stable non-zero tissue size ($\alpha = \alpha_c$). If the steady state values for the controlled β and δ probabilities are examined it can be shown quite simply that the limiting forms of these control functions are such that the tissue adjusts to make $\alpha \equiv \alpha_c$. Thus, the result of every control mechanism examined here, if it is to lead to a stable tissue size, is to force the tissue into a situation such that $\alpha = \alpha_c$ irregardless of the origin and point of control.

It is to be noted that only with mitotic inhibition (α) control by P or N do we always achieve a stable tissue mass. If either the probabilities β (mitotic stimulation) or δ (cellular death) are the single controlled pathways, then stability is achieved only for a suitable combination of α and δ, or α and β, respectively. A further point of interest in Table 2 is the requirement for non-zero cellular death ($\delta \neq 0$) in order for a stable steady state to be achieved. Steel (1968) has maintained that cellular death is important for tissue homeostasis and his data, in conjunction with our results, support this contention.

The results presented in Table 2 also lead to another conclusion: if a tissue is to have a maximal safety factor with respect to maintaining a constant size, then it would seem imperative to include some form of control over the probability of cells leaving the proliferating

pool (α control). It is clear that with β or δ control alone, fluctuations in an uncontrolled probability could leave a tissue growing in an unrestrained fashion.

In reality it would be expected that a given tissue probably has potential control over α, β, and δ. The degree of control at each point may change with a number of parameters but a tissue would seem to retain the maximum in flexibility by having all three growth control mechanisms in its repertoire.

From the results of our analysis of the no control model, and a knowledge of the initial ($m = 0$) value of the control functions qualitative statements may be made about the early growth patterns of the model with control. Thus by examining the initial control functions given in Table 3 to see if they correspond to the $\alpha = \alpha_c$, $\alpha > \alpha_c$, or $\alpha < \alpha_c$ cases of the no control model we are able to characterize early growth patterns. For example with α control by P, if $(A/P_0) >> 1$ so $\alpha_0 << 1$ and $\alpha_0 < \alpha_c$, then the tissue cell population will initially be an increasing function of m. As control becomes more influential tissue growth will decelerate and finally a constant size is approached. Conversely, if $(A/P_0) << 1$ so $\alpha_0 \simeq 1$ and $\alpha_0 > \alpha_c$ then the cell population will decrease with time until control is felt strongly enough to lead it to level off at a non-zero steady state size. Exactly the same considerations apply to all of the control mechanisms examined here for the determination of the qualitative nature of the model early growth characteristics.

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APPENDIX 1

To determine the way in which P_m and N_m, from (1) and (2) with constant coefficients, behave with increasing m proceed as follows. Equation 1 may be rearranged to give

$$\beta N_m = P_{m+1} - 2(1 - \alpha)P_m \qquad (1A.1)$$

and this, when substituted into (2), yields immediately

$$P_{m+2} - [2(1 - \alpha) + (1 - \beta - \delta)]P_{m+1}$$

$$+ [2(1-\alpha)(1-\beta-\delta) - \alpha\beta]P_m = 0 \quad (1A.2)$$

From (1A.2), the characteristic equation for the system, (1) and (2), is given by

$$\lambda^2 - [2(1-\alpha) + (1-\beta-\delta)]\lambda + [2(1-\alpha)(1-\beta-\delta) - \alpha\beta] = 0 \quad (1A.3)$$

The solutions of the characteristic equation,

$$\lambda_\pm = \tfrac{1}{2}[2(1-\alpha) + (1-\beta-\delta) \pm \sqrt{D}] \quad (1A.4)$$

wherein the discriminant of (1A.3), D, is given by

$$D = [2(1-\alpha) + (1-\beta-\delta)]^2 - 8(1-\alpha)(1-\beta-\delta) + 4\alpha\beta \quad (1A.5)$$

will give all the information needed to determine the behaviour of P_m and N_m for large m since the general solution of (1A.2) is (Jordan, 1960)

$$P_m = \Gamma_1\lambda_+^m + \Gamma_2\lambda_-^m \quad (1A.6)$$

when $\lambda_+ \neq \lambda_-$, or

$$P_m = (\Gamma_1' + m\Gamma_2')\lambda^m \quad (1A.7)$$

when $\lambda_+ \equiv \lambda_- = \lambda$. In (1A.6) and (1A.7), the constants Γ_1, Γ_2 and Γ_1', Γ_2' are determined from known values of P_m and N_m at a given m.

Note that D as defined by (1A.5) may be written in the alternate form

$$D = [2(1-\alpha) - (1-\beta-\delta)]^2 + 4\alpha\beta$$

thus illustrating that $D \geqslant 0$ for all values of α, β, and δ. Hence, λ_+ and λ_- will always be real. Further, inspection of (1A.4) shows $\lambda_+ \geqslant \lambda_-$ for all α, β, and δ and from the fact that the coefficient of λ in (1A.3) is non-positive, λ_+ must be non-negative.

Now define λ^* as

$$\lambda^* = \text{maximum of } (1\lambda_+1, 1\lambda_-1)$$

so the long term (large m) behaviour of P_m and N_m will be determined by λ^*. There are 2 distinct cases:

Case 1: $\lambda_+ \equiv \lambda_- = \lambda$, thus $\lambda^* = 1\lambda1$. In this case, with increasing m, if: (i) $\lambda^* > 1$, P_m and N_m increase exponentially; (ii) $\lambda^* = 1$,

P_m and N_m increase linearly; or (iii) $\lambda^* < 1$, P_m and N_m decrease exponentially to zero.

Case 2: $\lambda_+ \neq \lambda_-$. For this situation, with increasing m, if: (i) $\lambda^* > 1$, P_m and N_m exponentially increase; (ii) $\lambda^* = 1$, P_m and N_m approach a steady state through either exponential growth or regression; or (iii) $\lambda^* < 1$, P_m and N_m decrease exponentially to zero.

With the above considerations in mind, the determination of necessary and sufficient conditions for $\lambda^* \leqslant 1$ will give the constraints between α, β, and δ necessary for finite steady state tissue size in the absence of control. Manipulation of (1A.4) shows that the necessary *and* sufficient condition for $\lambda^* \leqslant 1$ is $\alpha \geqslant \alpha_c$, where α_c is defined by

$$\alpha_c = (\beta + \delta)/(\beta + 2\delta) \tag{1A.8}$$

It is of interest to know how α, β, and δ affect the tissue growth rate. A decrease in α at constant β, δ has the effect of increasing λ^* (and thus the growth rate) for $\lambda^* > 1$. If the rate of change of α_c with respect to β and δ is calculated,

$$\frac{\partial \alpha}{\partial \beta} = \frac{\delta}{(\beta + 2\delta)^2} \geqslant 0 \tag{1A.9}$$

and

$$\frac{\partial \alpha_c}{\partial \delta} = -\frac{\beta}{(\beta + 2\delta)^2} \leqslant 0 \tag{1A.10}$$

Thus, an increase in $(\alpha_c - \alpha)$ corresponds to an increase in growth rate. From equation 1A.9 an increase in β increases α_c, and thus the growth rate. Further from (1A.10) an increase in δ decreases α_c and therefore the growth rate also decreases. It is to be noted that the effect of a change in α on the growth rate is much more pronounced than the effect of a change in β or δ. Thus, since growth comes about by cells going through mitosis, a decrease in α and/or an increase in β will serve to increase the number of cells in the proliferating pool. In addition a decrease in the rate of cell death from the non-proliferating pool will make the contribution of the proliferating pool to growth felt even more.

The above comments about the effects of α, β, and δ on the tissue growth rate for $\lambda^* > 1$ can be extended to the $\lambda^* < 1$ case. A decrease in α will bring λ^* closer to one and this results in a slower decline to

zero tissue volume. An increase in β and/or a decrease in δ has the same effect.

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APPENDIX 2

The stability theorem presented here is due to Bellman (1947) and Perron (1929).

Consider the system of two first order difference equations

$$\begin{aligned} \Delta X_1(m) &= F_1(X_1, X_2) \\ \Delta X_2(m) &= F_2(X_1, X_2) \end{aligned} \tag{2A.1}$$

where the difference operator, Δ, is defined by

$$\Delta f(m) = f(m+1) - f(m)$$

and the F_i $(i = 1,2)$ are real valued functions.

DEFINITION: The set of singular points P for the system (1) is defined by the solutions of

$$F_1(X_1, X_2) = 0, \quad F_2(X_1, X_2) = 0.$$

Assume there is at least one P, $P_0 = (p_0, q_0)$. With the transformations

$$\begin{aligned} X_1 &= p_0 + Z_1(m) \\ X_2 &= q_0 + Z_2(m) \end{aligned}$$

the system of equations 2A.1 become

$$\begin{aligned} \Delta Z_1(m) &= F_1(p_0 + Z_1, q_0 + Z_2) \\ \Delta Z_2(m) &= F_2(p_0 + Z_1, q_0 + Z_2) \end{aligned} \tag{2A.2}$$

DEFINITION: A set of solutions (X_1, X_2) to the system of difference equations 2A.1 are absolutely stable (unstable) at P_0 if and only if (Z_1, Z_2) approach (diverge from) (0, 0) for large m.

DEFINITION: A set of solutions (X_1, X_2) to the system of difference equations 2A.1 show limit cycle behaviour at P_0 if and only if (Z_1, Z_2) oscillate uniformally about (0, 0) for large m.

If, in equations 2A.2, F_1 and F_2 are expanded about (p_0, q_0) we have immediately that

$$\Delta Z_1(m) = \alpha_{11}Z_1(m) + \alpha_{12}Z_2(m) + \psi_1(Z_1, Z_2)$$

and (2A.3)

$$\Delta Z_2(m) = \alpha_{21}Z_1(m) + \alpha_{22}Z_2(m) + \psi_2(Z_1, Z_2)$$

wherein

$$\alpha_{ij} = \left(\frac{\partial F_i}{\partial Z_j}\right)_{(p0,q0)} \qquad (i, j = 1, 2)$$

$$\psi_1(Z_1, Z_2) = \sum_{n=2}^{\infty}\sum_{j=0}^{n} \frac{Z_1^{n-j} Z_2^{j}}{(n-j)!\,j!}\left(\frac{\partial^n F_1}{\partial Z_1^{n-j}\,\partial Z_2^{j}}\right)_{(p0,q0)}$$

and

$$\psi_2(Z_1, Z_2) = \sum_{n=2}^{\infty}\sum_{j=0}^{n} \frac{Z_1^{n-j} Z_2^{j}}{(n-j)!\,j!}\left(\frac{\partial^n F_2}{\partial Z_1^{n-j}\,\partial Z_2^{j}}\right)_{(p0,q0)}$$

With the definition of Δ, equations 2A.3 may be rewritten as

$$Z_1(m+1) = (1 + \alpha_{11})Z_1 + \alpha_{12}Z_2 + \psi_1(Z_1, Z_2)$$

and

$$Z_2(m+1) = \alpha_{21}Z_1 + (1 + \alpha_{22})Z_2 + \psi_2(Z_1, Z_2)$$

DEFINITION: The characteristic equation, $f(\lambda)$, of the system of difference equations 2A.1 at the singular point P_0 is

$$f(\lambda) = \begin{vmatrix} 1 + \alpha_{11} - \lambda & \alpha_{12} \\ \alpha_{21} & 1 + \alpha_{22} - \lambda \end{vmatrix} = 0$$

and the solutions (λ_1, λ_2) of $f(\lambda) = 0$ are the (generally complex) characteristic roots of the system (2A.1) at P_0.

DEFINITION: Denoting by $|\lambda_i|$, $i = 1,2$, the modulus of λ_i (absolute value if real) define

$$\lambda^* = \max(|\lambda_1|, |\lambda_2|)$$

THEOREM: for a system of difference equations 2A.1 the stability characteristics of the solutions X_1, X_2 near the singular point P_0 are determined by the characteristic roots of the system at P_0 such that:

1. For $\lambda^* < 1$ the solutions are absolutely stable.
2. For $\lambda^* > 1$ the solutions are absolutely unstable.
3. For $\lambda^* = 1$ the solutions show limit cycle behaviour.

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