KINETICS OF LYSOSOMAL STORAGE OF INDIGESTIBLE MATTER

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ABSTRACT In lysosomal storage diseases and in the accumulation of lipofuscin in the lysosomes there is a gradual eroding of the lysosomal system due to overloading the lysosomes by molecules which cannot be digested or expelled. The kinetics of this accumulation is examined for tissue cultures in terms of the cell growth rate, lysosomal production rate, and rate of generation of the indigestible element.

INTRODUCTION

There are a number of instances in which lysosomes are unable to break down ingested molecules and as a consequence these molecules accumulate within the lysosomes. In some cases this is pathological and is due to a deficiency of some enzyme. This is a genetic defect and is the cause of many lysosomal storage diseases. There are other cases in which even the normal cell lacks a digestive mechanism for treating ingested matter or its metabolic residue. An example of the latter is the production of lipofuscin in lipid peroxidation. This residue is called "age pigment" because of the striking correlation between the amount of accumulating pigment and the age of the cell. Stehler et al. (1959), for example, has shown that the rate of accumulation of pigment in human heart cells is about 1% per cell volume per decade.

In both instances, lysosomal storage diseases and accumulation of age pigment, one finds that the lysosomes become increasingly swollen as they become saturated with the indigestible element and as a result become progressively less functional. This puts a strain on less bloated lysosomes which must therefore become saturated all the more rapidly.

In the hope of modeling some current experiments (Deamer, 1973) we will focus our attention on a culture of proliferating cells whose lysosomes are unable to digest some molecule. In what follows we shall refer to this mass of indigestible material as pigment. It could, however, be glycogen in someone suffering from glycogen storage disease or any element that cannot be broken down or discharged from the lysosomes. The cells are growing and dividing continuously. As part of the growth process any cell will be generating new lysosomes so that at mitosis it will have double the original number lysosomes. In this way each daughter cell will have approximately the same number of lysosomes. (There will of course be some distribution about this average,

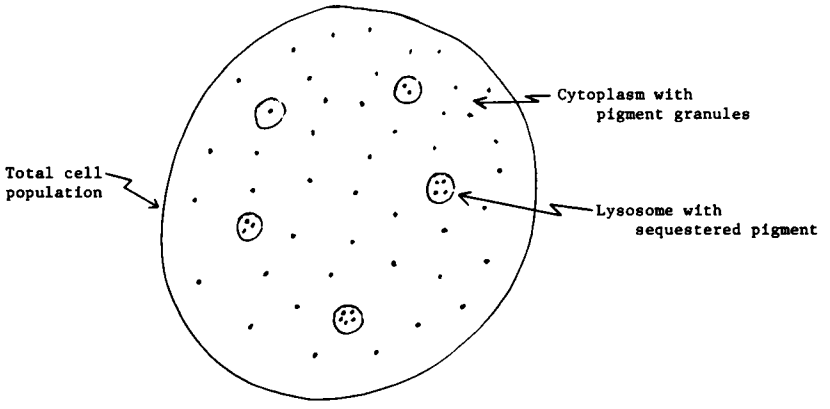

FIGURE 1 Equivalent representation of cell culture.

both in cell size at birth and in the number of lysosomes. We will not consider this problem here. When we speak of individual cells we are speaking of the average cell).

For conceptual purposes it is convenient to imagine a contiguous, homogeneous body having a volume equal to the total volume of the growing cell population (see Fig. 1). The system then possesses a generic "cytoplasm" containing a uniform distribution of lysosomes and pigment (or glycogen or other indigestable element). This does not imply that the individual cells are in any way interacting but is just a device which facilitates the mathematical description. The system is dynamic. The total cell volume doubles in a time equal to the average time between mitoses. The lysosomes are increasing at the same rate. Pigment is continuously being generated in the "cytoplasm" and absorbed by the lysosomes. Newly generated lysosomes will have little pigment and the older lysosomes much more. The rate at which a lysosome ingests pigment may depend on how much pigment there is about it and also on how much it has already taken in; indeed, the ingestion process stops altogether if the lysosome becomes saturated with pigment.

We would like to describe the kinetics of such a system; i.e. given the rate at which pigment is generated in the cytoplasm, the rate at which this pigment is being ingested by the lysosomes, the rate of growth of the cell population, and the rate of production of lysosomes, how does the system evolve in time? At any given moment we have some distribution of lysosomes—some newly born with little pigment and some old lysosome with much pigment. Pigment is being created, ingested by lysosomes and diluted due to growth of the cytoplasm. Questions we would like to ask are: What is the density of pigment in the cytoplasm and the distribution of pigment in the lysosomes as a function of time? Will this cytoplasm or the lysosomes ever become so saturated that normal cell processes, for instance, mitosis, are inhibited? Or will a steady state be reached in which a constant density of cytoplasmic pigment is attained and the cell population continues to grow indefinitely?

There has been some speculation that the observed finite lifespan (50 generations)

discovered in embryonic lung cells by Hayflick and Moorhead in 1961 might be due to an accumulation of damaging reactions like lipid peroxidation within the cell[1] (Tappel, 1965; Packer et al., 1967; Deamer, 1973). This lipid peroxidation results in the accumulation of lipofuscin in lysosomes and so the lipofuscin serves as a measure of the degenerative effects. If an indefinite growth in the cell population resulted in an *ever increasing* pigment density in lysosomes, then surely we have a contradiction. The contradiction is resolved if the cell population does not have an indefinitely long lifespan. We will examine the conditions under which cell death would occur.

ANALYSIS

The kinematics of our model system is governed by four equations. Two of these are continuity equations for the lysosomes and pigment and the other two, rate equations for cell division and lysosome generation.

Accumulation of Pigment: A Continuity Equation

If $M(t)$ is the total mass of pigment in the cytoplasm at time t then the rate of increase of M is given by

$$\mathrm{d}M/\mathrm{d}t = (\text{rate of production in the cytoplasm}) - (\text{rate of absorption by the lysosomes}).$$

Let k_p be the rate at which pigment is produced per unit volume. Since we expect some interference of cellular activity due to the presence of cytoplasmic pigment, we allow k_p to be some function of the density (D) of pigment in the cytoplasm. Thus, $k_p = k_p(D)$ and we can now write:

$$(\text{rate of production}) = k_p(D)\, V(t),$$

where $V(t)$ is the volume of the total cell population.

We have already suggested that ingestion rate of a given lysosome will be affected not only by the amount of pigment in its environment, but also by how much pigment it has already ingested: we let $I(p, D)$ be the rate at which a single lysosome containing pigment p ingests cytoplasmic pigment when the density of pigment in the cytoplasm is D.

If we let $N(t,p)\,\mathrm{d}p$ be the total number of lysosomes at time t which contain an amount of pigment between p and $p + \mathrm{d}p$ then the total rate of ingestion by all the lysosomes is given by the integral below:

$$(\text{rate of absorption by all the lysosomes}) = \int_0^\infty I(p,D)N(t,p)\,\mathrm{d}p.$$

[1] Packer, L., and J. Smith. Extension of the life span of cultured normal human diploid cells by vitamin E. Submitted to *Proc. Natl. Acad. Sci.*

We have finally an expression for the time evolution of $M(t)$:

$$(\mathrm{d}M(t)/\mathrm{d}t) = k_p(D)V(t) - \int_0^\infty I(p,D)N(t,p)\,\mathrm{d}p. \tag{1}$$

Distribution of Lysosomes: A Second Continuity Equation

Lysosomes are created when the requisite enzymes, manufactured at the ribosomal sites, are wrapped in a membrane. It is not unreasonable to suppose that these newly born lysosomes contain no pigment granules. If we further suppose that all lysosomes survive as long as the integrity of the culture is maintained, then the following partial differential equation expresses the fact of conservation of lysosomes:

$$(\partial/\partial t)N(t,p) + (\partial/\partial p)I(p,D)N(t,p) = 0. \tag{2}$$

The equation above is very much analogous to the equation of continuity familiar in the study of fluid flow without source or sink, or the conservation of electrical charge in electrodynamics (Feynman, Eqs. 13.4 and 13.8). Our equation expresses the simple idea that the rate of change of the number of lysosomes in any given interval $\mathrm{d}p$ is just equal to the number that enter the interval per unit time minus the number that leave.

Volume Growth Rate

A proliferating cell population with a constant doubling time is described by the equation $\mathrm{d}V/\mathrm{d}t = k_v V(t)$, where k_v is a constant representing the rate of volume increase per unit volume. The solution of this equation is given by $V(t) = V(0)\exp(k_v t)$. We may identify k_v^{-1} with the "e-folding time", i.e., k_v^{-1} is the time required for the population to increase its number by the factor e. We do not, however, expect our culture to have a constant growth rate. We instead expect the culture to reflect the inhibitive effects of accumulated cytoplasmic pigment. We allow for this possibility by letting k_v be a function of the pigment density: $k_v = k_v(D)$. Thus, our growth rate equation is

$$\mathrm{d}V(t)/\mathrm{d}t = k_v(D)V(t). \tag{3}$$

Production of Lysosomes

The rate of increase in the total number of lysosomes will be the rate per unit volume (k_L) times the total volume V:

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_0^\infty N(t,p)\,\mathrm{d}p = k_L(D)V(t). \tag{4}$$

We may make a considerable simplification of the above equation. If the derivative is brought inside the integral sign we have

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_0^\infty N(t,p)\,\mathrm{d}p = \int_0^\infty \frac{\partial N}{\partial t}\mathrm{d}p = -\int_0^\infty \frac{\partial \mathrm{IN}}{\partial p}\mathrm{d}p = [\mathrm{IN}]_{p=0},$$

where we have employed Eq. 1 and assumed that $[\mathrm{I\,N}]_{p=0} = 0$. Eq. 4 now becomes

$$I(0, D)N(t, 0) = k_L(D)V(t). \tag{5}$$

Summary

The system of integro-differential equation derived above, together with initial data forms a well-posed problem, i.e., has a unique solution. The notation is summarized below.

LIST OF SYMBOLS

$V(t)$	Total volume of the population mass at time t
$M(t)$	Total mass of pigment in the cytoplasm of the population
p	Mass of pigment in any given lysosome
$D(t)$	Density of pigment in the cytoplasm ($= M/V$)
$N(t,p)\,\mathrm{d}p$	Number of lysosomes at time t with pigment between p and $p + \mathrm{d}p$
$n(t,p)\,\mathrm{d}p$	Number of lysosomes *per unit volume* with pigment between p and $p + \mathrm{d}p$: $n = N/V$
$k_v(D)$	Rate of volume increase per unit volume
$k_L(D)$	Rate of production of lysosomes per unit volume
$k_p(D)$	Rate of production of pigment per unit volume

APPLICATIONS

It is convenient to write our main equations above in terms of the densities $n(t,p)$ and $D(t)$ for two reasons. First, since the k's and I are functions of the density D, it would be helpful to have an equation which determines D *directly*, instead of solving Eqs. 1 and 3 for $M(t)$ and $V(t)$, respectively, to obtain $D(t) = M(t)/V(t)$. Secondly, and more importantly, we have assumed a homogeneous system and so a knowledge of what is happening in any unit volume should determine the behavior of the entire culture. Since we are free to choose the scale length it will often be convenient to choose the unit volume to be the volume of a single cell. Thus all densities become amounts of pigment or number of lysosomes per cell.

Dividing Eq. 1 by V, setting $M = VD$ and employing Eq. 3, we obtain

$$\frac{\mathrm{d}D}{\mathrm{d}t}(t) = k_p(D) - \int_0^\infty I(p, D)n(t,p)\,\mathrm{d}p - k_v(D)D. \tag{6}$$

In a similar way we may combine Eqs. 2 and 3 to give

$$(\partial/\partial t)\mathrm{n}(t,p) + (\partial/\partial p)I(p, D)n(t,p) + k_v(D)n(t,p) = 0. \tag{7}$$

Finally, dividing Eq. 5 by V we have

$$I(0, D)n(t, 0) = k_L(D). \tag{8}$$

We wish to solve Eqs. 6 and 7 subject to the constraint expressed by Eq. 8. To do so, of course, we must first be given the functions $k_v(d)$, $k_p(D)$, $k_L(D)$, and $I(p, D)$. Presumably, the growth rate function $k_v(D)$ could be experimentally determined; the rate of production of pigment could also be observed since the pigment is fluorescent. A determination of the production rate of lysosomes will be aided by the fact (proved shortly) that $k_L(D)$ must be directly proportional to $k_v(D)$ or else the population very quickly develops an imbalance of lysosomes per cell. Finally, the function $I(p, D)$ may be obtained by choosing some reasonable function with parameters adjusted to fit experimental data.

First we shall show how these equations can be put in a form which permits a trivial numerical solution.

Solution of the Time-Dependent Problem: $I = I(D)$

As a special case we assume first that I is a function of D alone. This is certainly not a realistic assumption when we note that pigment-bloated lysosomes should suffer some loss of ingestion efficiency as their pigment content increases. Nevertheless, we gain much insight into the more general problem by solving first this restricted case.

If I is independent of p we may write Eq. 6 in the form

$$\mathrm{d}D/\mathrm{d}t = k_p(D) - I(D)L - k_v(D)D, \tag{9}$$

where $L = \int_0^\infty n(t,p)\,\mathrm{d}p$ is the total number of lysosomes per unit volume.

If we now integrate Eq. 7 over p from zero to infinity and use Eq. 8, we obtain

$$\mathrm{d}L/\mathrm{d}t = k_L(D) - k_v(D)L. \tag{10}$$

The pair of equations above are first order, nonlinear differential equations comprising an autonomous system whose numerical solution for any choice of the functions k_p, k_v, and k_L is straightforward.

Of particular interest are the circumstances under which a steady state is reached (the Liapounov problem). If we write Eqs. 9 and 10 in the more general form

$$\mathrm{d}D/\mathrm{d}t = f(D,L),$$
$$\mathrm{d}L/\mathrm{d}t = g(D,L),$$

and the lines

$$f(D,L) = 0$$
$$g(D,L) = 0$$

intersect, then a steady state exists ($\mathrm{d}D/\mathrm{d}t = \mathrm{d}L/\mathrm{d}t = 0$). If they do not intersect, there is no steady state. The stability of this steady state depends on the behavior of f and g in the neighborhood of the point of intersection. A stable steady state is illustrated in Fig. 2.

The general conditions then for the existence of a steady state is that the equations

$$k_p - IL - k_v D = 0 \tag{11}$$

and

$$k_L - k_v L = 0 \tag{12}$$

have a solution for D and L.

In most cell cultures the number of lysosomes per unit volume is constant. Thus we expect that L should approach a steady-state value independent of D. This can be achieved in the present model by simply assuming k_L is proportional to k_v, i.e.

$$k_L = L_o k_v,$$

when L_o is the constant of proportionality. This equation says that the rate of production of lysosomes is proportional to the rate of growth of the cells cytoplasm. This should describe a culture with constant lysosomal density. That this is indeed the case is easily seen from Eq. 10

$$dL/dt = k_L - k_v L = k_v(L_o - L).$$

Clearly as L approaches L_o, dL/dt approaches zero i.e. as L gets close to L_o it stops changing. Thus L_o is the steady-state lysosomal density.

Eq. 12 becomes now

$$k_v(L_o - L) = 0, \tag{13}$$

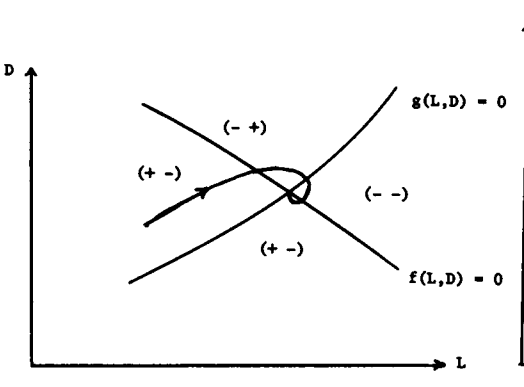

FIGURE 2

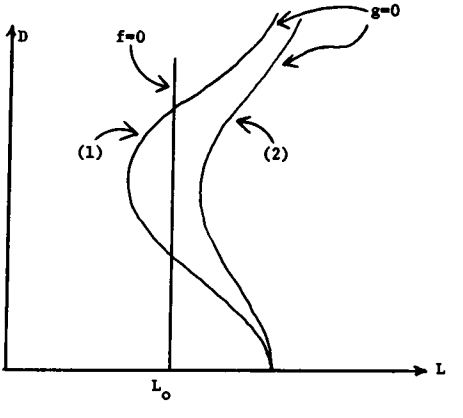

FIGURE 3

FIGURE 2 Example of a stable steady state. The notation (+ −) denotes that $f > 0$ and $g < 0$ in the indicated quadrant.

FIGURE 3 Graph of the curves $f(L,D) = 0$ and $g(L,D) = 0$ when $k_L = L_o k_v$. Curve 1 corresponds to a system with a larger volume growth rate than that of system 2.

whose solution is $L = L_o$. If this value of L is inserted into Eq. 11 we obtain

$$k_p - IL_o - k_v D = 0. \tag{14}$$

Our condition for a steady state then becomes that Eq. 14 have a real positive solution for D.

In Fig. 3 we have plotted the curves $f(L,D) = 0$ and $g(L,D) = 0$ when $k_L = L_o k_v$. Curve 1 corresponds to a system with a larger volume growth rate than that of system 2. The latter system does not intersect the line $L = L_o$ and therefore does not reach steady state. The density of pigment within this population increases without bound, whereas a stable density of pigment is reached for system 1. The physical reason for this is simple: the faster cells are growing, the greater is the dilution of the pigment.

If a steady state does exist and if $k_v(D)$ is not zero for this steady state then the population will continue to grow indefinitely. $k_v(D)$ of course determines the growth rate of the population (see Eq. 3). Only when $k_v(D) = 0$ does the population stop growing. We may determine the size of the population in the following way. We have from Eq. 3 that

$$V(t) = V(0) \exp \int_0^\infty k_v(D)\, dt. \tag{15}$$

We may make a change of variable to write this equation as

$$V(D) = V(0) \exp \int_o^D k_v(D) \left(\frac{dD}{dt}\right)^{-1} dD. \tag{16}$$

For the special case we considered earlier we have (if $L(0) = L_o$)

$$\frac{V(D)}{V(0)} = \exp \int_o^D \frac{k_v(D)\, dD}{k_p(D) - I(D)L_o - k_v(D)D}. \tag{17}$$

This expression will give us the number of doublings of the population as a function of the density of pigment D.

General Case: $I = I(p,D)$

Let us now turn to the more realistic case where the ingestion rate is a function of both p and D. It is no longer possible to simplify Eqs. 6 and 7 as before to obtain the autonomous system (Eqs. 9 and 10). However, it is still possible to find the steady-state solution if one exists.

To derive the necessary condition for the attainment of steady state for this general case we return to Eqs. 6–8 and set the time derivatives equal to zero.

$$(d/dp)I(p,D)n(p) + k_v(D)n(p) = 0, \tag{18}$$

$$k_p(D) - \int_o^\infty I(p,D)n(p)\,dp - k_v(D)D = 0, \tag{19}$$

$$I(0,D)n(0) = k_L(D). \tag{20}$$

The general solution of Eq. 18 is

$$n(p) = \frac{k_L(D)}{I(p,D)} \exp - k_v(D) \int_o^p \frac{dp}{I(D,p)}, \tag{21}$$

where we have made use of Eq. 20. If we insert this expression into Eq. 19, we obtain

$$k_p(D) - k_L(D) \int_o^\infty \exp\left[-k_v(D) \int_o^p \frac{dp}{I(p,D)} dp\right] - k_v(D)D = 0. \tag{22}$$

The steady-state value of L can be easily obtained by integrating Eq. 21. The result is

$$L = k_L(D)/k_v(D), \tag{23}$$

where D is the solution of Eq. 22.

If there exists a real, positive solution of Eq. 22, then the cell population will reach a steady state. If there is no solution the density D will increase without bound.

Illustration

We demonstrate how these results might be utilized by making an educated guess for the rate functions.

For purposes of numerical evaluation it is convenient to introduce dimensionless variables.

Let

$$D' = \frac{D}{k_p(0)k_v^{-1}(0)} = \frac{\text{(density of pigment in cytoplasm)}}{\text{(mass of pigment generated per unit volume in one } e\text{-folding time)}},$$

since as we have seen $k_v^{-1}(0)$ is the e-folding time for a cell with no pigment.

Let

$$p' = \frac{L_o p}{k_p(0)k_v^{-1}(0)} = \frac{\text{(mass of pigment in a lysosome)}}{\text{(mass of pigment generated per lysosome per } e\text{-folding time)}}$$

$$I' = \frac{L_o I}{k_p(0)} = \frac{\text{(mass of pigment ingested by lysosome in any volume per unit time)}}{\text{(mass of pigment generated in the same volume per unit time)}}$$

Let

$$k_p'(D) = k_p(\mathrm{D})/k_p(0),$$

and

$$k_v'(D) = k_v(D)/k_v(0).$$

In terms of these dimensionless variables and letting $k_L = L_o k_v$, Eq. 19 becomes

$$k_p' - k_v' \int_o^\infty \exp\left[-k_v' \int_o^{p'} \frac{dp'}{I'}\right] dp' - k_v' D' = 0. \tag{24}$$

To see how the existence of a steady state depends on the various factors we will illustrate the application of Eq. 24 by making a reasonable guess for the rate and ingestion functions.
Let

$$k_p' = 1,$$
$$k_v' = e^{-\gamma D'},$$

and

$$I' = \frac{\alpha D'}{1 + \beta D'} \eta(p_m' - p'),$$

where $\eta(x)$ is the unit step function ($\eta(x) = 1$ if $x > 0, \eta(x) = 0$ if $x < 0$).

With this choice we have a culture in which pigment is being generated throughout the cytoplasm at a constant rate ($k_p' = 1$), the culture grows steadily until $\gamma D'$ becomes comparable to one, (for much larger values of $\gamma D'$, $k_v' \cong 0$) and the lysosomes

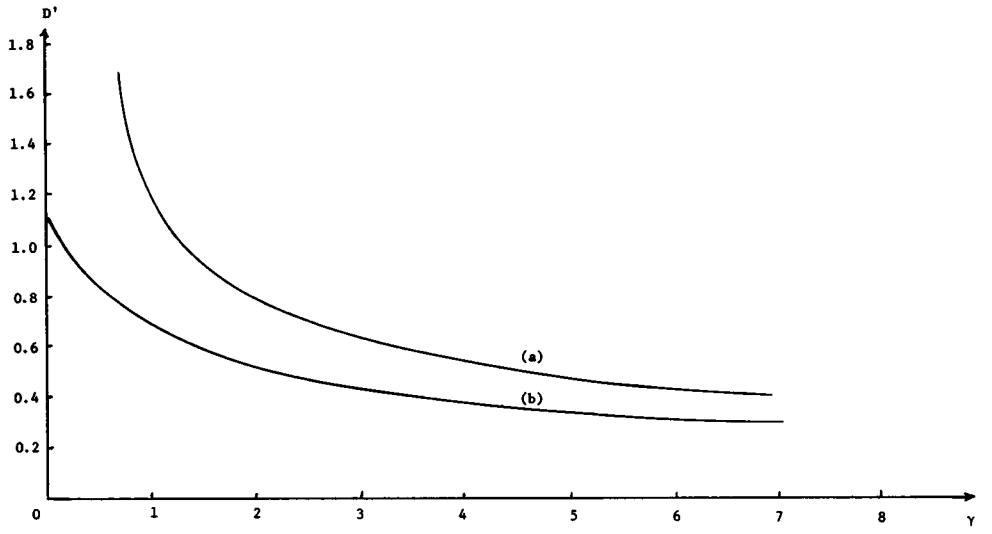

FIGURE 4 Steady-state pigment density as a function of γ when (*a*) $\beta = 1, \gamma = 0.5, p_m' = 1$ and (*b*) $\beta = 1, \gamma = 0.1, p_m = 1$.

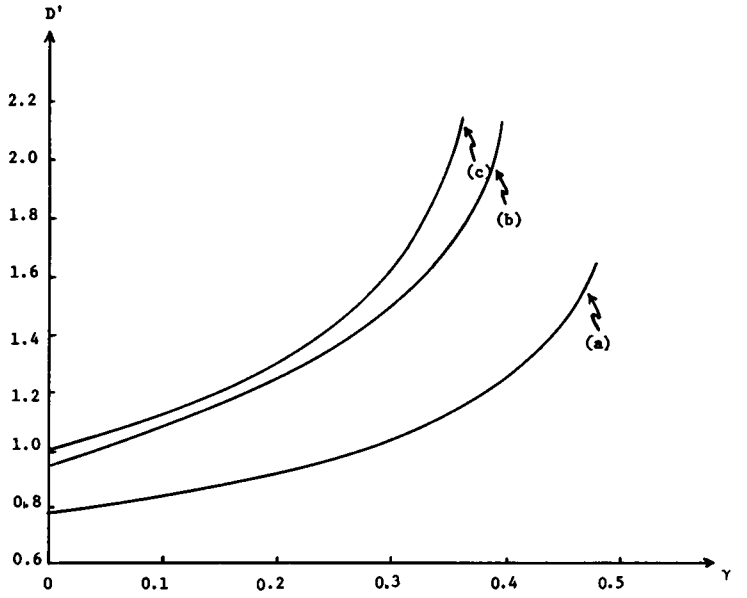

FIGURE 5 Steady-state pigment density as a function of γ when (*a*) $\alpha = 0.5, \beta = 1, p'_m = 1$. (*b*) $\alpha = 0.1, \beta = 1, p'_m = 1$, (*c*) $\alpha = 0.1, \beta = 1, p'_m = 0$.

ingest pigment until $p' > p'_m$ (where p'_m is some constant) and then cease to function. They ingest pigment in direct proportion to the density of pigment in the cytoplasm until $\beta D'$ becomes comparable to 1 at which point this ingestion rate begins to saturate.

In Figs. 4 and 5 we have plotted the steady-state density as functions γ and α. We see that as γ increases there comes a point where the steady-state density becomes infinite, i.e. for such cultures the pigment density continues to increase indefinitely.

It is of course unrealistic to have infinite pigment density. This arises from the fact that we chose $k'_p = 1$. In fact k'_p must decrease as D' increases. If k'_p had been chosen so that it goes to zero as D' goes to infinity then there would be no singular solutions. In such a case there would always exist a steady state with finite D'. And since $k'_v = e^{-\gamma D'}$ it follows that the volume growth rate remains finite and the population would grow indefinitely. However, if D' became large, the growth rate would slow down to the point that it would be almost unobservable. Cell death (i.e. cessation of reproduction in the culture) can be incorporated by choosing k'_v to be a function that becomes zero for finite values of D'. If, for example, we choose $k'_v = e^{-\gamma D'} \eta(D'_m - D')$ so that for all $D' > D'_m$, $k'_v = 0$ then if D' grew so large as to reach D'_m the culture would stop growing. If D'_m were, say 1.4, then if $\alpha = 0.5, \beta = 1$ and $a'_m = 1$, all cultures will die if $\gamma > 0.45$ (see curve *c* Fig. 5). If $\gamma < 0.45$, the culture will grow indefinitely.

SUMMARY

We have developed a mathematical framework which enables one to make predictions regarding the behavior of a cell population undergoing deteriorative processes and

leading to the production of lipofuscin (pigment). We have developed equations whose solution permit one to follow the evolution of a culture in time (when $I = I(D)$) and in general ($I = I(D,p)$) to determine the asymptotic behavior of the culture, i.e., under what conditions a steady state exists and what this steady state is. These results may be inverted. Given the fact that a certain culture reaches a certain steady state, we may draw some conclusion regarding the nature of the various rate functions.

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