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THE WASTE-PRODUCT THEORY OF AGING: CELL DIVISION RATE AS A FUNCTION OF WASTE VOLUME

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SUMMARY

The rate of cell division is calculated as a function of waste product volume in U-787CG human diploid glial cells grown *in vitro*. The calculation is based on two earlier mathematical models. One is a compartmental analysis in which cell division rate is obtained from data on the fraction of cells which become sterile as the passage level increases. A second model is used to calculate the amount of waste per cell from the observed rate of waste accumulation in a non-dividing population and from the division rate calculated with the use of the first model. Results from the two models are correlated to obtain the desired function relating cell division rate to waste volume. If cellular aging is taken to mean loss of the ability of cells to divide, and if, as in the waste-product theory, this loss is attributed to waste accumulation, the calculated results show that aging is evident at waste levels well below those at which non-dividing populations can survive. Thus the process of cell division may be much more sensitive to waste accumulation than other cellular processes needed for the maintenance of life.

Key words: Waste products; Age pigments; Lipofuscin; Cell division rate; Mitosis; Aging; Cellular aging

INTRODUCTION

The waste product theory of aging, in its simplest form, proposes that cellular aging is caused by the accumulation of intracellular waste which can be diluted by cell division but cannot otherwise be eliminated or destroyed [1]. The nature of the waste as well as the mechanism by which it is formed remains uncertain. Age pigments found in residual bodies or secondary lysosomes, such as lipofuscin, have been studied extensively [2], in part because it is believed that they may be the waste products in question. However, there is no conclusive evidence that age pigments are injurious to cellular function [3,4]. The question whether age pigments are responsible for cellular aging is not examined here. A related problem, to obtain a quantitative relation between cell division rate and age pigment accumulation, is the subject of this paper.

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The word "aging", in the context of a cellular theory of aging, may signify the loss of the ability of a cell to divide, i.e. the "phase III" phenomenon [5], or it may represent events leading to the metabolic death and final dissolution of a cell. This report is concerned with the former process.

It is clear that cell division and waste accumulation are intimately related. Deamer and Gonzales [6] established a clearcut correlation between mitotic potential and the level of fluorescent waste in human diploid WI-38 fibroblasts. Collins and Brunk [7] demonstrated the influence of cell division on waste dilution by experiments on U-787CG human diploid glial cells. However, quantitative data relating cell division rate to the volume or mass of intracellular waste have not, as far as can be determined, been published. Fortunately, if certain reasonable assumptions are made, it is possible to obtain the same information by mathematical treatment of other available data. The result, derived and presented here, quantifies the amount of waste which might be expected to affect cell division and is, therefore, of interest in its own right. Moreover, it promises to be useful in a simulation study of the waste-product theory of aging which is now in progress.

CALCULATION OF THE CELL DIVISION RATE AS A FUNCTION OF WASTE VOLUME

Calculation of the cell division rate as a function of waste volume is a three-part process. First the cell division rate as a function of time is obtained from data on the fraction of cells in culture which are unable to reproduce. This is accomplished with the help of the cell sterility model of culture growth described by Hirsch and Curtis [8]. Next the calculated cell division rate, together with data on waste accumulation in a non-dividing cell population, is used to obtain the volume of waste per cell as a second function of time. A waste dilution model presented several years ago [9] is employed for this purpose. Finally the cell division and waste dilution functions are compared at equal values of time; the time parameter is eliminated to obtain tabular and graphic representations of cell division rate as a function of waste volume per cell.

Cell division rate as a function of time

Data reported by Merz and Ross [10] inspired the creation of the cell sterility model (Hirsch and Curtis [8], hereafter designated HC) which is used here to calculate cell division rate as a function of time. Merz and Ross observed that the number of sterile, i.e. non-dividing, WI-38 cells increases approximately exponentially with passage level. Hirsch and Curtis then proposed a mathematical model containing two parameters which can be adjusted to give a good fit to the sterility data. The model also reproduced with reasonable accuracy the population dynamics and, in particular, the phase III behavior of a diploid cell culture.

The sterility model, illustrated in Fig. 1, consists of three pools of cells: reproducing, sterile, and dead. Transfers of cells between pools are described by differential equations.

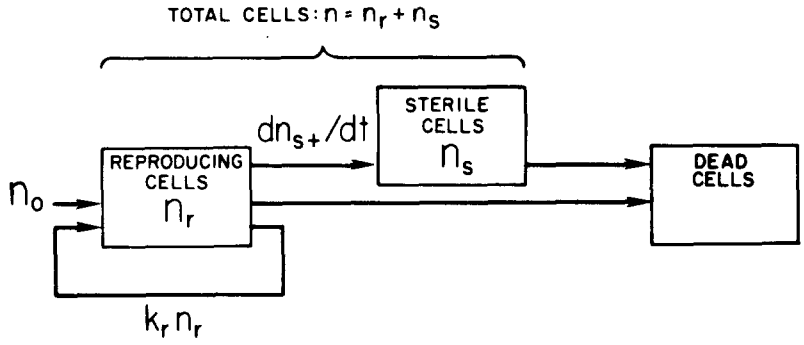

Fig. 1. A three-compartment model of a diploid cell culture. (Hirsch and Curtis [8].) Symbols are defined in the text.

It is assumed that the number of cells in each pool is sufficiently large to allow statistical fluctuations to be ignored. Thus, although the initial number of cells can be normalized to unity for the sake of convenience, it should not be inferred from this that the culture is cloned from a single cell.

Dead cells are presumed to decompose too rapidly to be counted. Consequently

$$n = n_r + n_s, \tag{1}$$

where n is the total number of cells in the culture, n_r is the number of reproducing cells, and n_s is the number of sterile cells. Results given in HC indicate that cell death has a relatively small effect on the sizes of the reproducing and sterile cell populations. Consequently, death-rate terms are omitted in the calculations which follow.

The differential equations which constitute the HC model and govern the population sizes n_r, n_s, and n yield solutions that are continuous functions of time. The step-like interruptions in culture growth patterns imposed by serial subcultivation are ignored. Values of time represent the age of the culture as a whole rather than the period elapsed since the start of a particular passage.

The cell division rate of the total culture population is, by definition,

$$k_b = \frac{1}{n}\frac{dn}{dt}. \tag{2}$$

With respect to the numbers of reproducing and sterile cells,

$$k_b = k_r \frac{n_r}{n_r + n_s}, \tag{3}$$

where k_r is the division rate of the reproducing cells. Time, t, is measured from an origin at the beginning of the first passage. Although eqn. (3) is, perhaps, intuitively obvious, a brief proof is given in Appendix 1.

To use eqn. (3) to arrive at the cell division rate, $k_b(t)$, it is necessary to obtain the time functions $n_r(t)$ and $n_s(t)$. These functions were calculated in HC by assuming a specific sterility-rate function

$$f(t) = \frac{1}{n_r} \frac{dn_{s+}}{dt} \tag{4}$$

that increases as an exponential or power function of time. The derivative dn_{s+}/dt represents the rate at which reproducing cells because sterile. Therefore $f(t)$ is the fraction of the reproducing cell pool which becomes sterile per unit time. The power-law specific sterility rate function is used in the present study because it corresponds more closely to available data than the exponential function. Thus

$$f(t) = ct^{N-1}, \tag{5}$$

where C and N are adjustable parameters. If N is restricted to integral values, it can be interpreted as the number of sequential steps or events which lead to sterility [11,12].

Values of the parameters C and N, as well as other numerical results based on WI-38 sterility data, which appear in HC are of little use here because appropriate data on waste accumulation in WI-38 cells are not available. However, data have been published which can be used to calculate both the cell division rate and the waste level in U-787CG human diploid glia. Two such publications [13,14] contain information on the fraction, n_s/n, of U787CG cells which fail to divide at various passage levels, P. Either provides data which can be used to calculate cell division rate as a function of passage level or time.

The more recent of these papers, by Collins, Willems, Alva-Willems, and Thaw [13], describes experiments in which tritiated thymidine is used to detect cell division. When n_s/n is plotted against P in logarithmic coordinates, the data can be fit with a straight line having a correlation coefficient of approximately 0.90. A somewhat earlier paper by Blomquist *et al.* [14] reports data on the fraction of sterile cells obtained by direct observation through the use of a minicloning technique. The correlation coefficient between $\log(n_s/n)$ and $\log P$ is in excess of 0.99.

The data provided by Blomquist *et al.* [14] differ significantly from those presented by Collins *et al.* [13]. Since the former were the product of direct observation, and since they fit a power function so exactly, they have been adopted for use in the present work. When the parameters in eqn. (5) are adjusted to fit the data of Blomquist *et al.* by the methods described in HC, and N is restricted to integral values, the result is $N = 2.0$ and $C = 9.0 \times 10^{-3}$ (generation times)$^{-2}$. The degree of agreement between the data and the calculations based on the HC model is illustrated in Fig. 2.

Since the waste production data to be used farther on are presented in units of volume per hour, it is necessary to express the value of C in units of h^{-2} rather than (generation time)$^{-2}$. The generation time of U-787CG cells does not appear in the literature, but Pontén *et al.* [15] assign a 24-h generation time to related glia-like cells in culture. With

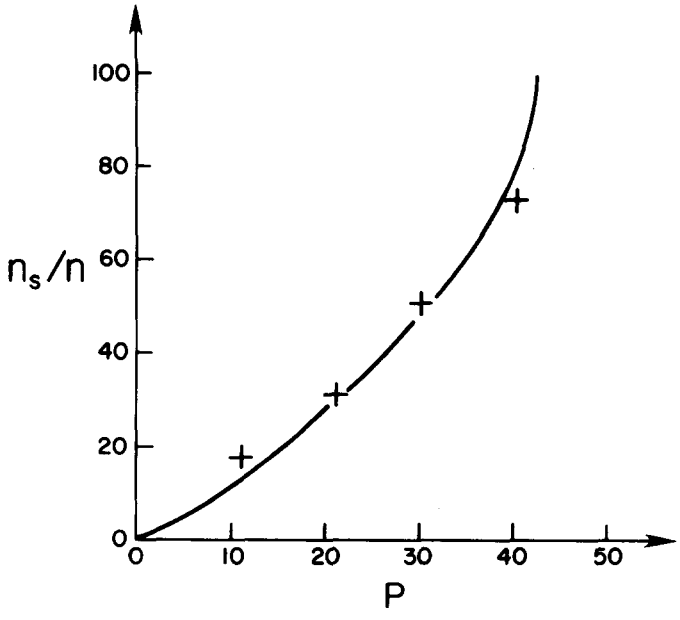

Fig. 2. Fraction of sterile cells, n_s/n, as a function of passage level, P. Crosses represent data on U-787CG human diploid glia taken from Blomquist *et al.* [14]. The solid line was fitted to the data as described in the text.

the use of this value, $k_r = \ln 2/24 = 2.888 \times 10^{-2}$ h^{-1}, and $C = 9.0 \times 10^{-3}/(24)^2 = 1.5625 \times 10^{-5}$ h^{-1}.

As shown in HC, eqn. (24), the number of reproducing cells is

$$n_r(t) = n_0 e^{k_r t - (c/N)t^N}, \tag{6}$$

where $n_0 = n(0)$ can be normalized to unity. The number of sterile cells (HC, eqn. (13) is obtained by integrating

$$\frac{dn_s}{dt} = n_r(t) f(t). \tag{7}$$

With the use of eqns. (5) and (6),

$$\frac{dn_s}{dt} = n_0 c t^{N-1} e^{k_r t - (c/N)t^N}. \tag{8}$$

Numerical values of the cell division rate as a function of time can easily be computed with the help of eqns. (3), (6), and (8) together with the parameter values given above.

TABLE I

CALCULATED VALUES OF THE CELL DIVISION RATE, k_b, WASTE VOLUME PER CELL, w', AND SPECIFIC STERILITY RATE, f, IN U-787CG HUMAN DIPLOID GLIAL CELLS

The cell division rate has been normalized such that its value at time $t = 0$ is 1.000. To obtain true values, multiply by $k_r = 2.888 \times 10^{-2}\ h^{-1}$.

$t(h)$	k_b	$w'(\mu^3/cell)$	$f(h^{-1})$
0	1.000	0.0	0.0
10	0.999	17.7	0.000156
20	0.997	30.8	0.000313
40	0.991	48.3	0.000625
60	0.983	58.3	0.000938
80	0.974	64.2	0.00125
100	0.964	67.8	0.00156
120	0.954	70.2	0.00188
160	0.933	73.2	0.00250
200	0.912	75.3	0.00313
300	0.859	80.0	0.00469
400	0.806	85.0	0.00625
500	0.753	90.7	0.00781
600	0.701	97.0	0.00938
800	0.597	112.7	0.0125
1000	0.495	133.7	0.0156
1200	0.394	163.1	0.0188
1400	0.297	206.1	0.0219
1600	0.207	272.0	0.0250
1800	0.126	376.6	0.0281
2000	0.063	544.5	0.0313
2200	0.023	800.0	0.0343
2400	0.005	1140.7	0.0375

These computations, as well as others reported here, were performed with the use of the IBM continuous systems modeling program (CSMP) on the University of Kentucky's IBM 3081 mainframe. The results are presented in Table I and illustrated in Fig. 3. Table I also contains values of the specific sterility-rate function, which increases linearly with time. The cell division rate falls nearly linearly with time for the first 1500 h. It drops to half its initial value after approximately 1000 h and approaches zero at 2500 h.

Waste volume as a function of time

The volume of waste per cell, w', can be obtained as the solution of a first-order differential equation (Hirsch [9], hereafter designated HW):

$$\frac{dw'}{dt} + k_b w' = r_w. \tag{9}$$

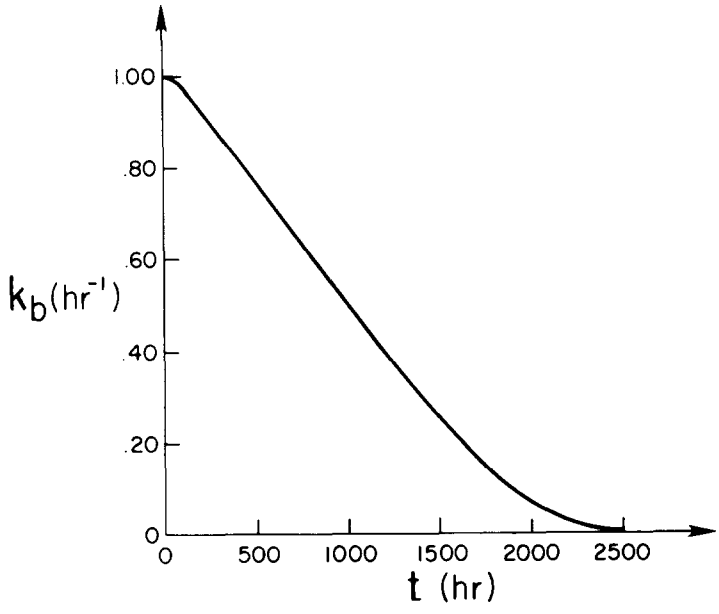

Fig. 3. Cell division rate, k_b, as a function of time, t. The cell division rate has been normalized such that its value at time $t = 0$ is 1.00. To obtain true values, multiply by $k_r = 2.888 \times 10^{-2}$ h^{-1}.

The rate of waste accumulation, r_w, as well as the cell division rate, k_b, may, in general, depend upon time, t, and upon w' itself. However data cited below indicate that r_w is, in fact, constant. In the HW model, as in the HC model, the effects of serial subcultivation are not considered.

Values of $k_b(t)$ are calculated as shown in the preceding section. Data provided by Collins and Brunk [7] on residual body volume in U-787CG cell cultures are used to obtain a value of r_w. Their Fig. 6 contains a graph of residual body volume as a function of the length of time during which a culture has been contact inhibited. A linear regression fits the data with a correlation coefficient of 0.97. The slope of the regression line, 2.033 μ^3/cell/h, is the value of r_w used in solving eqn. (9).

A numerical solution of eqn. (9), computed with the use of CSMP, is displayed in Table I and Fig. 4. The initial rise in waste volume is rapid, but it is soon limited by waste dilution attendant upon cell division. Once the cell division rate has declined sufficiently, the rapid increase in waste accumulation resumes.

Cell division rate as a function of waste volume

Table I lists values of the cell division rate, waste volume, and specific sterility rate at increasing values of time. The first three of these quantities were obtained with the use of differential equations in which time was the independent variable, so it is natural to think of them as functions of time. However there is no mathematical or logical requirement to do so. They are simply simultaneous variables. If the values in the table had

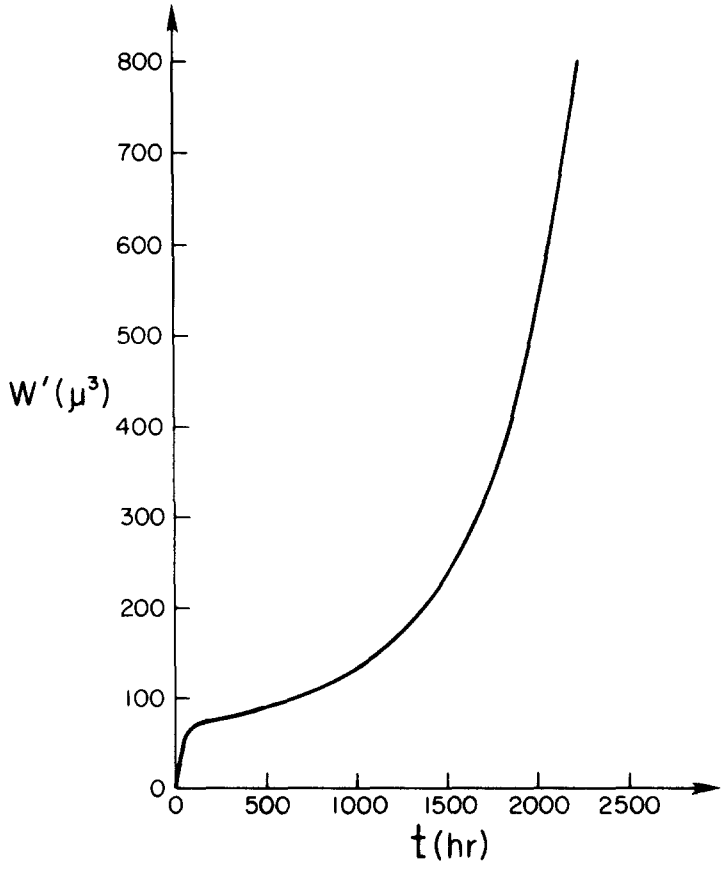

Fig. 4. Waste volume per cell, w', as a function of time, t.

been gathered in the course of an experiment, the date and hour of each measurement would have been noted, but no assumption would have been made *a priori* as to the importance of time in interpreting the data. The decision whether to choose time or some other quantity as the independent variable when reporting the data would depend upon the nature of the results as perceived by the experimenter. The same option to choose an independent variable is open to the mathematical investigator.

Consistent with the preceding remarks, the cell division rate and specific sterility rate may be regarded as functions of the waste volume, as shown in Figs. 5 and 6, respectively. The cell division rate falls quite rapidly with increasing waste volume, dropping to half its initial value when the waste volume is approximately 131 μ^3 or 2% of the average cell volume. A parallel rise in the specific sterility rate is evident.

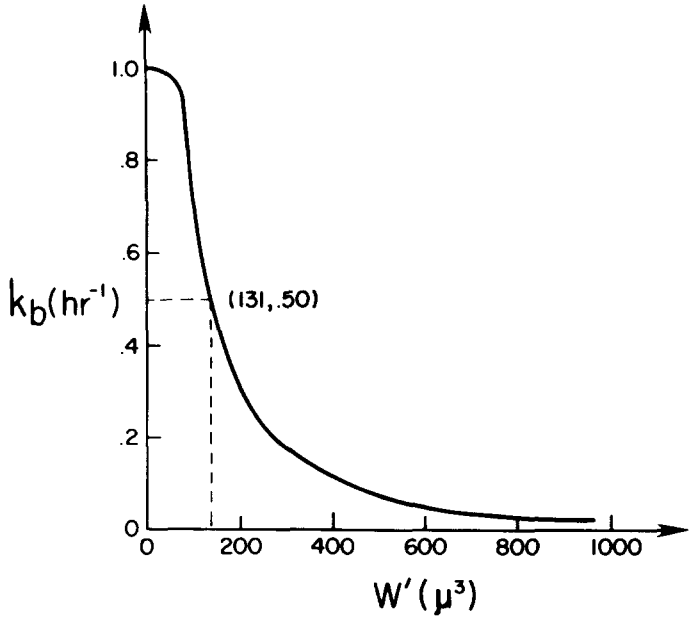

Fig. 5. Cell division rate, k_b, as a function of waste volume per cell, w'. The cell division rate is normalized as in Fig. 3.

DISCUSSION

Data relating cell division rate and waste accumulation are few, and, regrettably, it appears that none have been reported in quantitative form which would allow their comparisons with the results presented in Table I and Figs. 5 and 6. Deamer and Gonzales [6], for example, found an increase in the percentage of WI-38 cells in late passage displaying high levels of fluorescence, presumably reflecting a greater accumulation of age pigments. They also reported that the highly fluorescent cells were unable to incorporate thymidine, signifying a loss of the ability to divide. However, they expressed the fluorescence levels in five qualitative categories which cannot be translated into quantitative units. Other studies, e.g. those of Collins and Brunk [7], which concern cell division and waste accumulation, are even less susceptible to quantitative expression in a form which would allow their comparison to the mathematically derived results reported here. Consequently, these results must be evaluated without the benefit of direct experimental verification.

Relatively few biological assumptions enter into the calculations by which the mathematical results were obtained. The HC model is a simple compartmental analysis in which the choice of compartments is based on the observations of Merz and Ross [10]. Once the compartments have been defined, the only important assumption associated with the

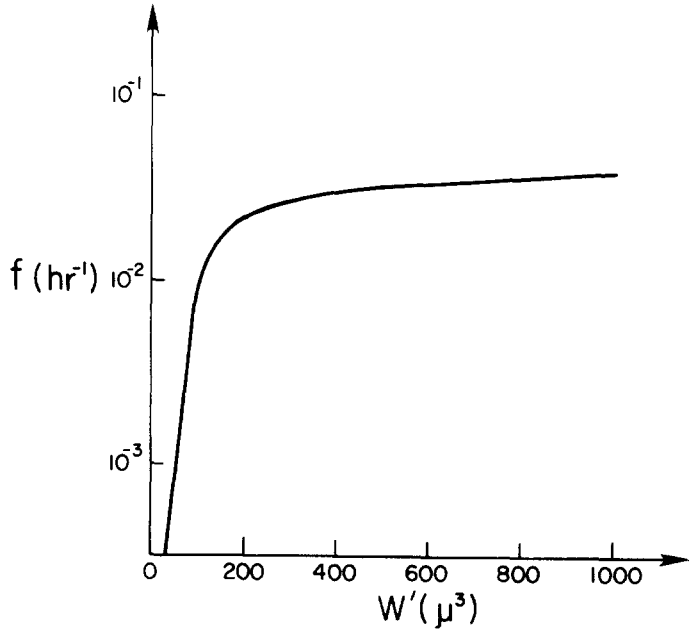

Fig. 6. Specific sterility rate, f, as a function of waste volume per cell, w'. The ordinate scale is logarithmic.

HC model is that of a progressive increase in the fraction of sterile cells. The HW model rests on just on major assumption, namely that waste is conserved during cell division.

Numerical values of the small number of parameters which appear in the models were taken directly from experimental data or were obtained by fitting equations based on the models to such data. Some reservation may be justified with respect to the value of the waste accumulation rate constant, r_w, which was evaluated with the use of data on a non-dividing population and applied to calculations involving a dividing population. However, neither this value nor the value of any other parameter was adjusted arbitrarily. Given the simplicity of the mathematical models and the close relation between the parameter values and the data, the results obtained here appear to be sufficiently trustworthy to merit further examination even in the absence of confirming experimental observations.

A divergence between the observations of Collins and Brunk [7] and the present work should be noted. Collins and Brunk report that U-787CG cells in log phase growth during passage 19 have 9.6% of the cytoplasmic volume taken up by residual bodies. This percentage corresponds to a residual body volume of approximately 600 μ^3. According to the cell-division-rate *vs.* waste-volume function (Fig. 5), the division rate in the presence of 600 μ^3 of waste would be only 5% as large as in the complete absence of waste. Such a division-rate value is clearly too low for cells in logarithmic growth in phase II. Two explanations may be offered for the large observed value of waste volume: 1. Collins and Thaw [4] state, "The quantitation of age pigment in secondary lysosomes may be an

overestimate, due to their content of other material which may be difficult to distinguish from age pigment morphologically." 2. In addition, there may be a buildup of waste due to growth inhibition resulting from serial subcultivation. The effects of subcultivation are omitted from both the HC and HW models.

The experiments performed by Collins and Brunk [7] shows that the effects on cell division of the relatively high waste levels which develop after contact inhibition of culture growth are reversible after serial subcultivation. It would be expected, if the waste product theory were to explain the phase III phenomenon, that the effect of waste accumulation on cell division would be irreversible. However, the observations of reversibility were made in the 19'th passage, at which level sufficient mitotic potential remains to "rejuvenate" the culture solely by waste dilution in the subpopulation of cells which retain the capacity to divide. After approximately 40 passages, the division rate declines, whether as the result of waste accumulation or for other reasons, to such a degree as to render the dilution mechanism inoperative. It would therefore be of interest to repeat the experiments of Collins and Brunk at a passage level approaching the end of phase II.

The results of the present study are relevant to the waste-product theory of cellular aging, but cannot be used to verify or invalidate it. As stated above, it is optional to assume that the cell division rate is a function of waste volume. Table I and Figs. 5 and 6 show some of the quantitative consequences of this assumption. *If* waste products can slow and, ultimately, completely halt cell division, i.e. if waste accumulation is responsible for the phase III phenomenon, a rather small amount of waste, of the order of magnitude of a few percent of the cell volume, can have a very significant effect on mitotic rate. This inference is surprising and unexpected. Observations on non-dividing cell populations *in vivo* [2] and *in vitro* [4,7] reveal functioning cells which, if the results presented here are valid, contain volumes of waste that would completely stop cell division. Thus large volumes of waste need not cause the metabolic death of a cell, but much smaller volumes may be of importance in influencing cell division.

CONCLUSIONS

The results of this study show a correlation between waste accumulation and a decrease in cell division rate. The correlation need not be causal and may, in fact, be principally a consequence of waste dilution or of some undiscovered mechanism underlying both the cell division and waste production processes. In any event, the calculations show that a large decline in cell division rate occurs at a remarkably low level of waste accumulation. Reproductive aging may be influenced by waste accumulation much more strongly than metabolic aging.

APPENDIX 1: CELL DIVISION RATE AS A FUNCTION OF THE REPRODUCING AND STERILE POPULATION SIZES

Calculation of the cell division rate, k_b, in terms of the number, n_r, of reproducing cells and the number, n_s, of sterile cells is most easily accomplished with the help of the

simple compartmental analysis illustrated in Fig. 1. The time derivative of eqn. (1) is divided by n, the total population size, and the definition of k_b, eqn. (2), is applied to obtain

$$k_b = \frac{1}{n}\left(\frac{dn_r}{dt} + \frac{dn_s}{dt}\right). \tag{A1}$$

If cell death can be ignored, as proposed in the comments following eqn. (1), the rate equations

$$\frac{dn_r}{dt} = k_r n_r - \frac{dn_{s+}}{dt} \tag{A2}$$

and

$$\frac{dn_s}{dt} = \frac{dn_{s+}}{dt} \tag{A3}$$

follow from inspection of Fig. 1. Substitution of eqns. (1), (A2), and (A3) in eqn. (A1) yields eqn. (3), which is the desired result.

If cell death is not negligible and if sterile and reproducing cells die at the same rate, k_d, a compartmental analysis similar to that presented above shows that the net growth rate is

$$k_b - k_d = k_r \frac{n_r}{n_r + n_s}. \tag{A4}$$

Thus the inclusion of the death rate makes no essential change in the result given in eqn. (3).

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