AGE DISTRIBUTION OF HUMAN DIPLOID FIBROBLASTS

A Stochastic Model for In Vitro Aging

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ABSTRACT Variation in the lifespan of mass cultures and clones of human diploid fibroblasts can be explained on the basis of variation in the length of the mitotic cycle. This variation is of biological significance; the intrinsic standard deviation of culture lifespan is equal to about 10% of the mean. We constructed a two-parameter stochastic model based on the following assumptions: the time between successive divisions of a given cell is of random duration; cells divide or lose the ability to divide independently of one another; the probability that a cell can undergo further division is constant up to some maximum number of divisions and zero thereafter. We determined numerically the proportion of nondividing cells and the distribution of cell generations. Samples taken by Monte Carlo means from a hypothetical in vitro population were compared with clonal survival data obtained experimentally. The fit between experimental and theoretical findings was within the range of sampling variation. If we accept our model as being applicable to human diploid cell culture, we can draw the following conclusions: the proportion of dividing cells is an inadequate index of a population's age; even in populations in which almost all cells are still capable of division, a majority of the cells have less than eight generations remaining to them. At each subcultivation the ultimate fate of a culture is determined by the disposition of a relatively small number of "young" cells.

INTRODUCTION

Human diploid fibroblasts have only a finite lifespan in culture. Many investigators use cultures of human diploid fibroblasts for the study of aging (1). Our objective is to provide a quantitative framework for use in the experimental design and analysis of such studies (2).

Human diploid fibroblasts can be serially propagated in culture for no more than 40–60 population doublings (3) or, correcting for cell loss and inability to divide, an estimated 120–160 cell generations (2). The lifespan, expressed in terms of population doublings, varies widely from culture to culture (3). The extent of variation in doubling potential among individual cells in a single culture became evident during a series of cloning experiments (4). 30 or more cells were individually cloned. Regardless of the age of the culture of origin, many of the cells ceased division immediately, while others could be propagated for more than 30 additional population doublings. The

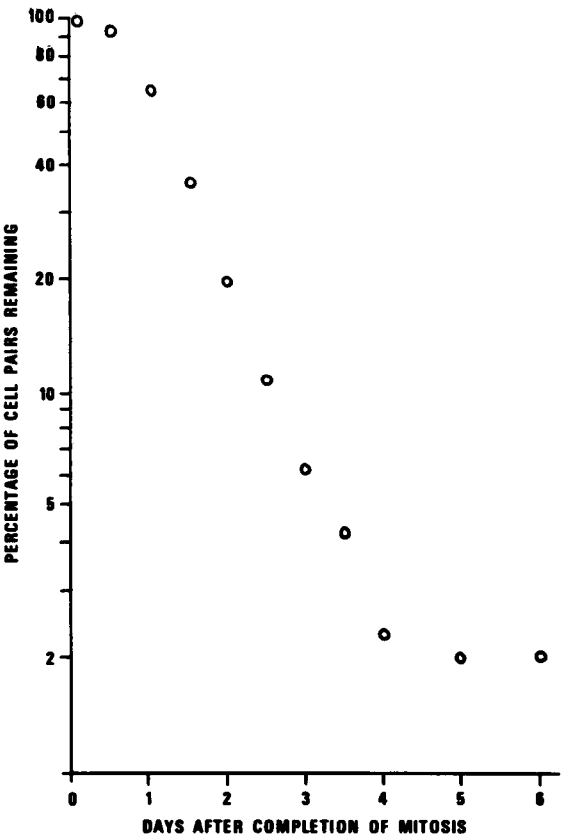

FIGURE 1 The percentage of original mitotic pairs remaining as a function of days after completion of mitosis. (Data provided by George Merz, Institute for the Study of Mental Retardation, Staten Island, N.Y.) Abscissa, days after completion of mitosis; ordinate, percentage of cell pairs remaining.

shape of the clonal survival curve, that is, the proportion of old and young cells, was related to the age of the culture of origin. Similar variation was noted in chick heart muscle cells (5) and *Paramecium* (6).

Cristofalo and Sharf (7) found that subcultures derived from three sublines of WI-38 cells and maintained in closely matched environments could vary in lifespan from 56 to 73 population doublings. Their observation suggests that a random mechanism underlies population development. We wanted to learn whether random variation in the length of the cell cycle could account for variation in the lifespan of cultures and of clones of human diploid fibroblasts.

George Merz observed individual WI-38 human diploid fibroblasts that had just completed mitosis; he found that the length of the mitotic cycle varied widely from cell to cell, from 15 h to 5 days (Fig. 1). Thus, some cells may go through two or three divisions while others go through none (8). Numerous studies have been made of the effect

on population growth of cell-to-cell variation in the interdivision interval (9–12). Our study took into consideration the declining ratio of dividing to nondividing cells with successive population doublings in culture (2, 13, 14).

A mathematical model is constructed in the next section. A computer program based on the model is used to develop a set of hypothetical results—the expected number of dividing and nondividing cells after various periods of time, the generation age distribution, and the cloning characteristics of samples taken from such hypothetical distributions. These theoretical results are compared with experimental findings for mass cultures and clones of WI-38 in the Results section.

MODEL

Assume that cells divide or lose the ability to divide independently of one another subject to the restriction that the offspring of a single mitosis will either both divide or both fail to divide (15). Assume that in any small interval of time $(t,t + \Delta t)$ each nth generation dividing cell can undergo the following transitions:

(*a*) It is preserved without change with probability $1 - a\Delta t - o(\Delta t)$, where $\lim \Delta t \downarrow 0\, o(\Delta t)/\Delta t = 0$;

(*b*) it divides and is replaced by two $(n + 1)$th generation dividing cells with probability $a\Delta t p_n + o(\Delta t)$, where $0 \leq p_n < 1$;

(*c*) It divides and is replaced by two nondividing cells with probability $a\Delta t(1 - p_n) + o(\Delta t)$.

For simplicity, let time be measured in mean cell generations; that is, $a = 1$.

As is the case with WI-38 (16), the interval between successive divisions of a single cell will vary from a few hours to a few days. Some cells will have many ancestors while others will have only one or two; the population will be a mixture of cells of various generations.

Let $c_n(t + t_o \mid j,t_o)$ denote the expected number of nth generation dividing cells at time $t + t_o$ arising from a single jth generation cell at time t_o. Our definitions do not depend on the starting time t_o and we may write

$$c_n(t + t_o \mid j,t_o) = c_n(t \mid j,0) = c_n(t \mid j).$$

Kharlamov (17) has shown that

$$c_n(t \mid 0) = \left(\prod_{i=0}^{n-1} p_i\right)(2t)^n \exp(-t)/n! \qquad (1)$$

where p_i is the probability that an ith generation dividing cell will produce two $i +$ first generation dividing cells.

Daniel and Young (18) observed that the loss of ability to divide in vivo is associated with the number of ancestors of a given cell. Smith and Rubenstein (19) found a similar phenomenon in *Podospora*. We incorporate their observation in our model as

follows:

Let p_i be a non-zero constant PDIV up to the MAXAGE generation and zero thereafter. Each MAXAGE generation cell will give rise to two nondividing cells; each dividing cell of an earlier generation may give rise with probability 1 − PDIV to two nondividing cells. Let $h(t \mid j)$ denote the expected number of nondividing cells at time t arising from a single jth generation cell at time 0. $h(t \mid 0)$ is the solution of the differential equation

$$dh/dt = 2(1 - \text{PDIV}) \sum_{n=0}^{\text{MAXAGE}} c_n(t \mid 0) + 2c_{\text{MAXAGE}}(t \mid 0). \tag{2}$$

With the passage of time, the population will consist exclusively of nondividing cells. The expected number of cells in the limit is shown in the appendix to be approximately

$$(2\text{PDIV})^{\text{MAXAGE}}/(2\text{PDIV} - 1). \tag{3}$$

One may interpret this latter expression as the reproductive potential of a single 0th generation cell. We generalize to the reproductive potential of a culture as follows:

Let $a_0, \ldots, a_{\text{MAXAGE}}$ denote the number of 0th, ..., MAXAGE generation dividing cells and a_h the number of nondividing cells in the culture. Let $X_j^i(t)$ denote the number of cells at some later time t that are descended from the ith jth generation cell. Let $X_j^i = \lim X_j^i(t)$; one may approximate this limit in practice, as in the Smith-Hayflick cloning experiments (4), by choosing a suitably long interval t. We define the reproductive potential of the culture as the random variable:

$$R(a_0, \ldots, a_k, \ldots; a_h) = \sum_{k=0}^{\text{MAXAGE}} \sum_{i=1}^{a_k} X_j^i + a_h. \tag{4}$$

When individual cells are cloned, some may fail to propagate while others divide repeatedly (4). According to our assumptions, a cell prior to MAXAGE may divide with probability 1 − PDIV to form two nondividing cells. A cell of the (MAXAGE-j)th generation will be unable to form a clone of size greater than 2^{j+1}. For $2k < 2^{j+1}$, neglecting the effects of age, the probability of a clone of exactly $2k$ nondividing cells is given by the following expression (20):

$$\binom{2k-1}{k} \text{PDIV}^{k-1}(1 - \text{PDIV})^k/(2k - 1). \tag{5}$$

In the next section the distribution of clone size is plotted along with the expected number of nondividing and dividing cells, for several values of the parameters PDIV and MAXAGE.

RESULTS

The expected number of cells and the proportions of dividing and nondividing cells at various times after the initiation of a hypothetical population are given in Table I for PDIV = 0.95 and MAXAGE = 215. The proportion of dividing cells decreases in a few generations from 1.0 to 0.9, a normal value for early passage

TABLE I

EXPECTED PROPORTIONS OF DIVIDING AND NONDIVIDING CELLS, CELL NUMBER, AND DOUBLING TIME FOR A HYPOTHETICAL POPULATION WITH PDIV = 0.95, MAXAGE = 215

Generations	Proportion of dividing cells	Proportion of nondividers	Total number of cells	Generations per population doubling
1	0.920	0.080	2.67E + 00	1.136
5	0.901	0.099	1.00E + 02	1.152
9	0.900	0.100	3.66E + 03	1.152
13	0.900	0.100	1.34E + 05	1.152
17	0.900	0.100	4.90E + 06	1.152
21	0.900	0.100	1.79E + 08	1.152
25	0.900	0.100	6.57E + 09	1.152
29	0.900	0.100	2.40E + 11	1.152
33	0.900	0.100	8.80E + 12	1.152
37	0.900	0.100	3.22E + 14	1.152
41	0.900	0.100	1.18E + 16	1.152
45	0.900	0.100	4.31E + 17	1.152
49	0.900	0.100	1.58E + 19	1.152
53	0.900	0.100	5.78E + 20	1.152
57	0.900	0.100	2.11E + 22	1.152
61	0.900	0.100	7.74E + 23	1.152
65	0.900	0.100	2.83E + 25	1.152
69	0.900	0.100	1.04E + 27	1.152
73	0.900	0.100	3.79E + 28	1.152
77	0.900	0.100	1.39E + 30	1.152
81	0.900	0.100	5.08E + 31	1.152
85	0.900	0.100	1.86E + 33	1.152
89	0.900	0.100	6.80E + 34	1.152
93	0.899	0.101	2.49E + 36	1.152
97	0.897	0.103	9.03E + 37	1.155
101	0.889	0.111	3.22E + 39	1.161
105	0.873	0.127	1.10E + 41	1.174
109	0.848	0.152	3.43E + 42	1.196
113	0.814	0.186	9.52E + 43	1.227
117	0.774	0.226	2.28E + 45	1.265
121	0.731	0.269	4.62E + 46	1.310
125	0.687	0.313	7.87E + 47	1.360
129	0.644	0.356	1.13E + 49	1.414
133	0.601	0.399	1.35E + 50	1.473
137	0.559	0.441	1.37E + 51	1.536
141	0.519	0.481	1.18E + 52	1.603
145	0.481	0.519	8.74E + 52	1.674
149	0.444	0.556	5.55E + 53	1.749
153	0.409	0.591	3.05E + 54	1.830
157	0.376	0.624	1.47E + 55	1.915
161	0.344	0.656	6.19E + 55	2.006
165	0.314	0.686	2.31E + 56	2.102
169	0.285	0.715	7.64E + 56	2.206
173	0.258	0.742	2.26E + 57	2.317
177	0.232	0.768	6.03E + 57	2.437
181	0.208	0.792	1.45E + 58	2.566
185	0.185	0.815	3.18E + 58	2.707
189	0.163	0.837	6.37E + 58	2.860
193	0.142	0.858	1.17E + 59	3.027
197	0.123	0.877	1.99E + 59	3.211
201	0.105	0.895	3.13E + 59	3.414
205	0.088	0.912	4.60E + 59	3.639
209	0.073	0.927	6.35E + 59	3.890
Limiting no. of nondividers			1.81E + 60	

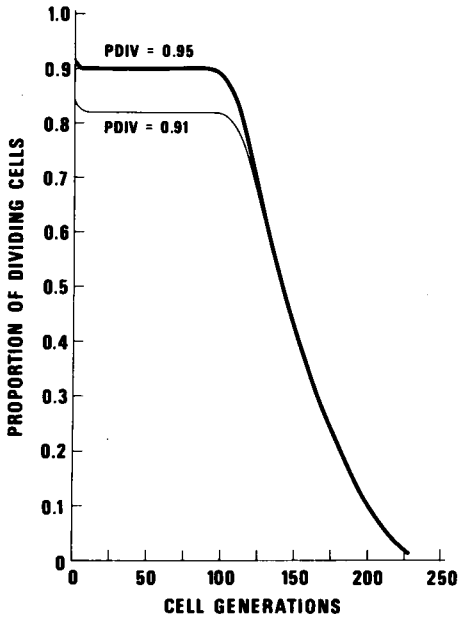

FIGURE 2

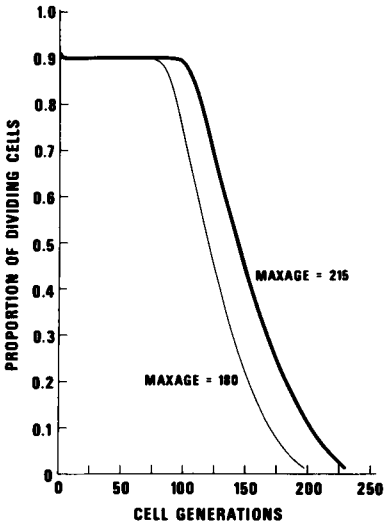

FIGURE 3

FIGURE 2 Decline in proportion of dividing cells with successive generations. For a hypothetical population with PDIV = 0.95 or 0.91, MAXAGE = 215. Abscissa, cell generations; ordinate, proportion of dividing cells. PDIV = 0.95 (———), PDIV = 0.91 (———).
FIGURE 3 Decline in proportion of dividing cells with successive generations. For a hypothetical population with PDIV = 0.95, MAXAGE = 215 or 180. Abscissa, cell generations; ordinate, proportion of dividing cells. MAXAGE = 215 (———), MAXAGE = 180 (———).

WI-38 (7), and remains at this level from the 4th through the 93rd generation. Finally, cells near the maximum age predominate and the proportion of dividing cells declines to zero.

When PDIV is increased (Fig. 2), the level of the initial plateau and the rate of descent from the plateau increase. The limiting number of cells in the population increases also (Eq. 3). When MAXAGE is increased (Fig. 3), the initial plateau is

TABLE II
COMPARISON OF AGING MEASURES FOR A HYPOTHETICAL POPULATION OF HUMAN DIPLOID FIBROBLASTS

Population doublings in vitro	Cell generations (theoretical)	Percent of dividing cells	Percent of cells with less than 8 divisions remaining to them
0	84	90	8 (hypothetical)
9	94	90	11
22	109	85	36
30	119	75	63

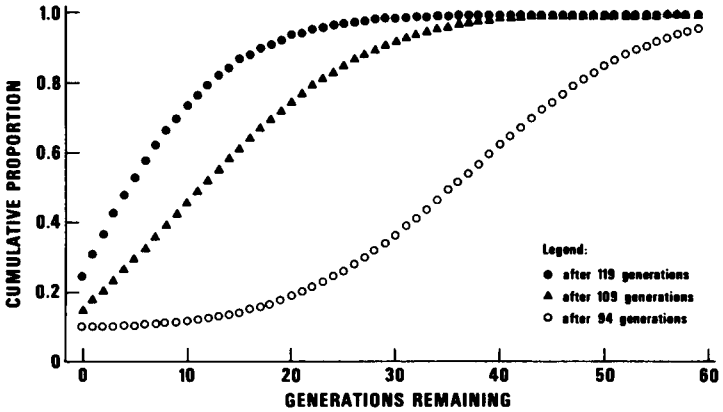

FIGURE 4 Cumulative age distribution of a hypothetical population with PDIV = 0.95 and MAXAGE = 215. Abscissa, generations remaining; ordinate, cumulative proportion. After 94 generations (ooo), after 109 generations (▲▲▲), after 119 generations (●●●).

extended at the same level and the rate of descent from the plateau increases. The limiting cell number also increases.

Included in Table I is the ratio of cell generations to population doublings. As the proportion of dividing cells decreases, this ratio increases (2). At the 192nd generation the proportion of dividing cells drops to less than 15%, and three or more cell generations are required for each subsequent doubling. These changes parallel those observed in cultures of human diploid fibroblasts (3, 7, 14).

The percentage of dividing cells or the doubling time are not always accurate gauges of a population's age. In a hypothetical population with PDIV = 0.95 and MAXAGE = 215, the proportion of nondividers does not increase appreciably between the 94th and 109th cell generations (Tables I and II). The generation age distribution does change (Fig. 4). 11% of the cells at the 94th generation and 36% at the 109th have eight or less generations remaining to them.

A shift in the age distribution can be observed experimentally when cells are cloned (4). Figs. 5 *a*, *b*, and *c* depict the results of a real cloning experiment and a hypothetical one in which we assumed that the age distribution of the sample was that of the population as a whole. Both sets of curves share the following three attributes: an initial drop when the cells in each clone are few in number and there is a significant probability that all will be nondividers (Table III); a uniform decline as the cells at or near the modal age reach the end of their dividing lifespans; and an asymptotic approach to zero as the young cells in the tail of the age distribution proliferate (Fig. 4).

In Fig. 5 *a* the survival curve expected at the 109th generation is compared with the experimental findings of Smith and Hayflick (4) for a sample of 40 cells at the 22nd population doubling. There are eight doublings between the 22nd and 30th population doubling or, using Table II, 10 cell generations. In Fig. 5 *b* the expected values at the 119th generation are compared with the experimental findings for the 30th population

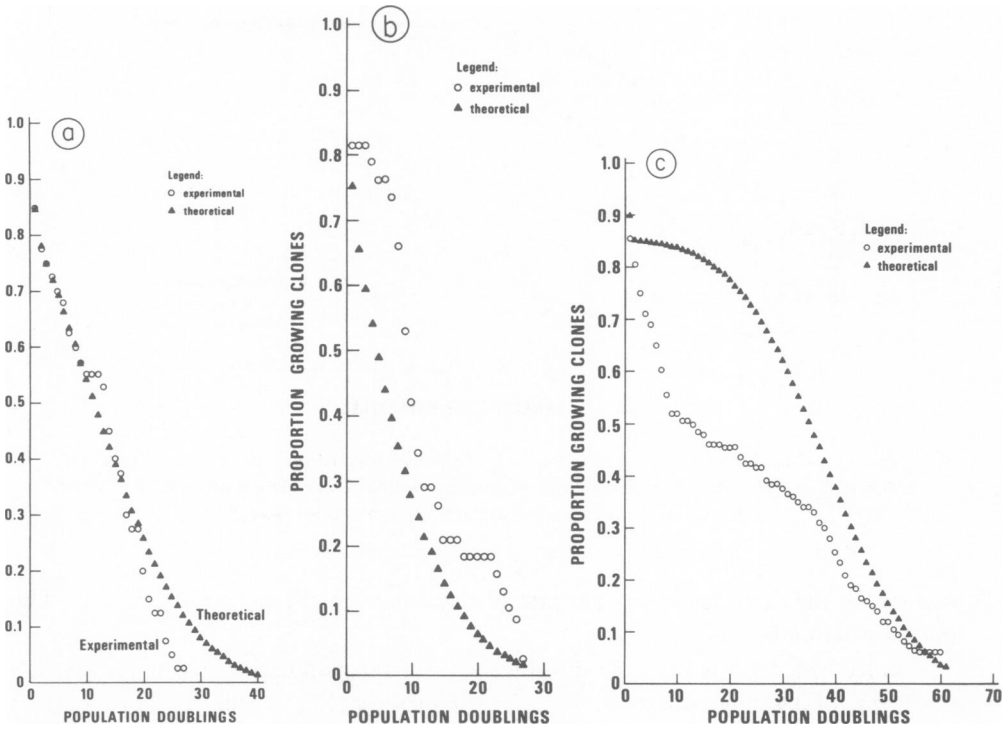

FIGURE 5 Growth potential of isolated cells. Percentage of cells able to undergo a given number of doublings. Comparison of theoretical and experimental results.

FIGURE 5 *a* Population doubling 22 or generation 109.

FIGURE 5 *b* Population doubling 30 or generation 119.

FIGURE 5 *c* Population doubling 9 or generation 94. Abscissa, population doublings; ordinate, proportion growing clones. Theoretical (▲▲▲), experimental (ooo).

doubling. While the theoretical curve drops uniformly, the experimental curve based on a sample of 38 cells has two plateaus.

Fig. 5 *c* depicts the Smith-Hayflick (4) results for a sample of 216 cells at the ninth population doubling and the clonal survival curve expected of a theoretical population at the 94th generation. The theoretical curve has a plateau between the 4th and 10th doublings, signifying the relative absence of very old cells. The experimental curve has an initial abrupt drop.

The Smith-Hayflick (4) experiments dealt with samples of 250 or fewer cells taken from a single culture. An addition to our computer program of a Monte Carlo sampling scheme allows us to assess the effects of sample-to-sample variation. Four such samples, each of 40 cells, taken from a 109th generation population, are depicted in Fig. 6. The deviations between observed and expected values at the 109th generation (Fig. 6) are within the range of sampling variation. The results of sampling from a 119th generation population (not shown) were similar and included the plateaus found experimentally (Fig. 5 *b*). None of over 100 samples taken by Monte Carlo means from

TABLE III

PROBABILITY OF CLONES OF VARIOUS SIZES, WHEN THE LOSS OF THE ABILITY TO DIVIDE IS A CONSTANT, 1 – PDIV, INDEPENDENT OF THE AGE OF THE CELL

Ultimate size of the clone	PDIV	
	0.91	0.95
Less than 2^1 cells	0.820009	0.100000
2^1 to 2^2 cells	0.089999	0.049999
2^2 to less than 2^3 cells	0.008578	0.002600
2^3 to less than 2^4 cells	0.000321	0.000031
2^4 to less than 2^5 cells	0.000001	0.000000

a 94th generation population had the sharp initial drop observed experimentally (Fig. 5 *c*).

It is easy to see that the number of cells arising from a single progenitor may vary widely from clone to clone. It is less easy to see, but no less true, that the number of cells arising from a culture of a million or more cells, and hence the ultimate lifespan of the culture, may vary widely. We defined this number in the model as R, the reproductive potential of the culture (expression 4). The mathematical expectation of R may be written as the sum of the mathematical expectations of the X_j^i, the reproductive potentials of the individual cells. The variance of R may be written as the sum of the covariances of the X_j^i taken in pairs. We assumed that cells divide or lose the ability to divide independently of one another; that is, that the X_j^i are independent of one another. The covariance of X_j^i and $X_{j'}^{i'}$ is zero unless $i = i'$ and $j = j'$. Thus, the variance of R may be written as the sum of the variances of the X_j^i.

In an appendix we show that the expected value of X_j^i is approximately $(2\text{PDIV})^{\text{MAXAGE}-j}/(2\text{PDIV-1})$ for small j, and the variance of X_j^i is approximately $K(2\text{PDIV})^{2(\text{MAXAGE}-j)}$ for small j. If the initial age distribution of the culture is $\{a_0, \ldots, a_{\text{MAXAGE}}; a_h\}$, then we may write the mean and variance of the reproductive potential as:

$$K \sum_{j=0}^{\text{MAXAGE}} a_j/(2 \times \text{PDIV})^j + a_H, \tag{6}$$

$$K' \sum_{j=0}^{\text{MAXAGE}} a_j/(2 \times \text{PDIV})^{2j}, \tag{7}$$

respectively, where K, K' are constants whose values depend on PDIV and MAXAGE.

The standard deviation of the reproductive potential is equal to approximately 10% of the mean in the parameter ranges we consider. This ratio agrees with that found experimentally for culture lifespan (3). Replicate serial subcultivations would reduce the standard error of the mean.

The reproductive potential is primarily a function of the youngest cells in the population. In expression 6 for the mean successive terms are divided by increasing powers of 2 × PDIV. In expression 7 for the variance successive terms are divided by in-

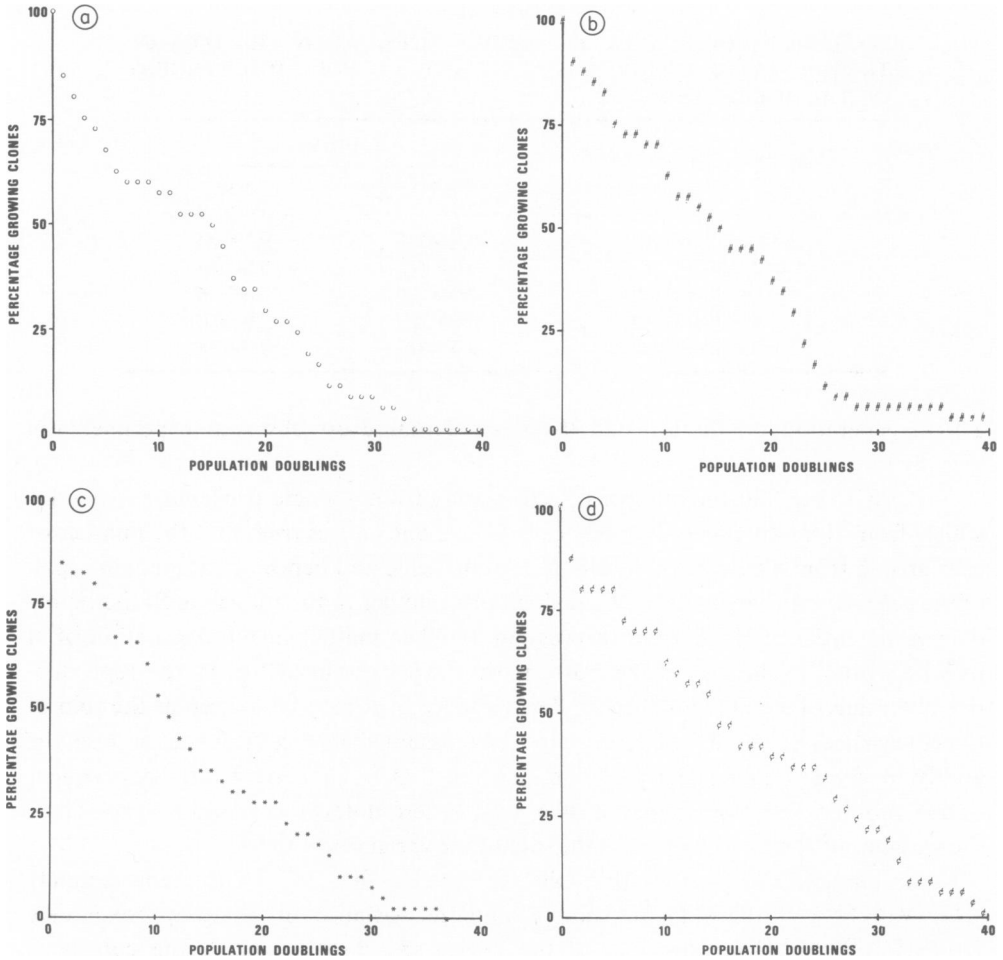

FIGURE 6 Growth potential of isolated cells in four samples taken at random from a hypothetical population with PDIV = 0.89, MAXAGE = 215. Abscissa, population doublings; ordinate, percentage growing clones. (*a*) Sample 1, 40 cells (○). (*b*) Sample 2, 40 cells (#). (*c*) Sample 3, 40 cells (*). (*d*) Sample 4, 40 cells (¢).

creasing powers of $(2 \times \text{PDIV})^2$. In the present example $2 \times \text{PDIV} = 1.9$ and $(2 \times \text{PDIV})^2 = 3.6$; the mean and, to a greater extent, the variance are dominated by the initial terms of expressions 6 and 7. These terms correspond to those cells in the population which have undergone the fewest initial divisions. For example, in a 109th generation population with MAXAGE = 215 and PDIV = 0.95, less than 0.001 of the cells have more than 60 generations remaining to them. It is these few cells which will determine the mean and variance of the resulting lifespan.

DISCUSSION

In the literature on in vitro aging little or no attention has been paid to the effect of variation on experimental design. Not infrequently, a comparison of the lifespan of

"treated" and "control" cultures has been made on the basis of a single series of subcultivations. A two-parameter stochastic model was presented in the hope of providing a quantitative framework for the design and analysis of such studies. Within the framework of this model, variation in the lifespan of cultures and of clones follows from variation in the interdivision times of individual cells.

The proportion of nondividing cells in an abstract, theoretical population remains constant for many cell generations (Table I). With successive divisions a greater and greater proportion of the cells approach their limiting lifespan (Fig. 4), and the proportion of dividing cells declines (Figs. 2 and 3). These changes mirror those observed in cultures of human diploid fibroblasts (3, 14).

With successive cell generations (and subcultivations) there is a decline in the reproductive potential of the model population (expression 6). This decline is highly variable. According to the model, the expected value of the standard deviation of the reproductive potential is equal to about 0.1 the expected value of the mean. This ratio agrees with that found experimentally (3). In each experiment the mean and standard deviation depend on the growth of a relatively small number of cells with a high initial reproductive potential (expressions 6 and 7). The Cristofalo-Sharf experiments (7) began with parallel subcultures of a million or more cells each. The differences these workers observed in culture lifespan can be explained by differences in the proportions of "young" cells in each of the starting populations. Studies of culture lifespan will require repeated parallel serial subcultivations to reduce this initial variation.

The model can account for the variation in clonal lifespan reported by Smith and Hayflick (4). The theoretical clonal survival curve (Fig. 5) has the following characteristics: an abrupt drop for the first one to four generations—the result of the accidental loss of dividing ability in small clones (Table III): a uniform drop for 10–20 generations, reflecting a corresponding uniformity in the age distribution (Fig. 4); a final asymptotic decline—the result of a reservoir of young cells in the tail of the age distribution. These same three phenomena are found in the experimental curves (Fig. 5), though masked in part by random fluctuations. The plateaus in the experimental curve for population doubling level 30 (Fig. 5 *b*) are to be found in results obtained by Monte Carlo sampling (Fig. 6).

We made the simplifying assumption that the probability of a cell dividing during a given time interval was independent of the time that had elapsed since the preceding division. There appears to be a minimum interval between successive divisions of a given WI-38 fibroblast (16, 21). The different generations arise one by one with a delay of n times the minimum interval before cells of the nth generation appear in the population. Even with this delay the generation age distribution will converge to the distribution given by the present model (22). The agreement will be incomplete during the period of interest. If there were agreement in the tail of young cells with many generations remaining to them, there would be disagreement in the hump of old cells, and vice versa. In particular, when the population nears the end of its lifespan, there will be fewer young cells in the tail of the age distribution than predicted by the

present model (22). This is precisely what was observed (Fig. 5). Part of the discrepancy between theoretical and experimental results can be explained by our use of this simplifying assumption. (See also references 11, 23.)

Albright and Makinodan (24) suggested that in vivo there was a progressive loss with age of progenitor or "stem" cells that are normally utilized to replace terminally differentiated, dying cells. Daniel and Young (18) found that the loss of ability to divide in mouse mammary epithelium was associated with the number of previous divisions. The present model rests on the assumption that each cell is capable of only a finite number of divisions. The model should be verifiable in the system of lymphocytes studied by Albright and Makinodan (24) and in mouse mammary epithelium (18). Variation in clonal lifespan was observed in chick heart muscle cells (5) and *Paramecium* (6) to which the model should also prove applicable.

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APPENDIX

Mean and Variance of the Limiting Population Size

Let X_j denote the ultimate size of the clone which arises from a given jth generation cell.

$$Pr\{X_j = 2\} = 1 - \text{PDIV}; \tag{8}$$

for $n > 2$

$$Pr\{X_j = n\} = \text{PDIV} \sum_{n_1+n_2=n} Pr\{X'_{j+1} = n_1\}\, Pr\{X''_{j+1} = n_2\}, \qquad (8\,A)$$

where X'_{j+1} and X'_{j+1} are the sizes of the clones which arise from the offspring of the initial division.

Define $g_j(s) = \Sigma\, s^n Pr\{X_j = n\}$: Using Eq. 1 to form a matrix of coefficients of (s^{n_1}, s^{n_2}) as in reference 25, p. 266,

$$g_j(s) = \text{PDIV}\, g^2_{j+1}(s) + (1 - \text{PDIV})s^2. \qquad (9)$$

It is well known that

$$EX_j = dg_j(s)/ds \mid_{s=1},$$
$$E[X_j(X_j - 1)] = d^2g_j(s)/ds^2 \mid_{s=1}.$$

From Eq. 9,

$$dg_j(s)/ds = 2\text{PDIV}\, g_{j+1}(s) \cdot dg_{j+1}(s)/ds + 2(1 - \text{PDIV})s,$$
$$d^2g_j(s)/ds^2 = 2\text{PDIV}[g_{j+1}(s) \cdot d^2g_{j+1}(s)/ds + (dg_{j+1}(s)/ds)^2] + 2(1 - \text{PDIV}).$$

$EX_j = 2\text{PDIV}\, EX_{j+1} + 2(1 - \text{PDIV})$; by definition $EX_{\text{MAXAGE}} = 2$, so that

$$EX_{\text{MAXAGE}-j} = [(2\text{PDIV})^{j+1} - 1]/(2\text{PDIV} - 1) + 1$$

for large j

$$EX_{\text{MAXAGE}-j} = (2\text{PDIV})^j/(2\text{PDIV} - 1). \qquad (10)$$

$$EX_j(X_j - 1) = 2\text{PDIV}(E[X_{j+1}(X_{j+1} - 1)] + (EX_{j+1})^2) + 2(1 - \text{PDIV}).$$

$$\text{Var}\, X_j = EX_j(X_{j-1}) + EX_j - (EX_j)^2;$$

for large j

$$\begin{aligned} \text{Var}\, X_{\text{MAXAGE}-j} &= 2\text{PDIV}[\text{Var}\, X_{\text{MAXAGE}-j+1} \\ &\quad + 2\text{PDIV}(1 - \text{PDIV})(EX_{\text{MAXAGE}-j+1})^2] \\ &= C(EX_{\text{MAXAGE}-j+1})^2 \\ &= \frac{(2\text{PDIV})^{2j+2}(1 - \text{PDIV})}{(2\text{PDIV} - 1)^3}. \end{aligned} \qquad (11)$$