

Grain Count Distributions in Labeled Cell Populations

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A quantitative mathematical formalism for the grain count distribution in radio-autographs of cells prepared at various times following exposure to radioactive thymidine is developed. The theory is formulated in the context of a particular model of cell proliferation proposed by Lajtha. The connection between the distribution of tritium atoms among the cells calculated from the theory and observable quantities such as the labeling index, median grain count, etc., are worked out in detail. Various possible extensions of the model are discussed and these appear to be sufficiently flexible so that they would correctly represent observations such as those of Clarkson and others on leukemic myeloblast cell populations. The time evolution of this cell population following an arbitrary initial state is also presented.

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1. Introduction

The introduction of tritiated thymidine as a radioactive label in proliferating cells and subsequent radioautographic study of them has greatly stimulated the study of mammalian cell kinetics in recent years (Cleaver, 1967).[‡] In particular, a great deal of information has been collected about differentiating cell systems such as blood cells (Cleaver, 1967, section 7.12).

The analysis of these experiments has in general been based only on the gross features of the labeling data such as the labeling index and the fraction of labeled mitotic cells. For this purpose the theoretical age-time (Scherbaum & Rasch, 1957; von Foerster, 1959) and more recently maturity-time (Rubinow, 1968) descriptions of cell populations is sufficient. However, there exists in addition detailed information concerning the distribution of

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‡ This book is an excellent and thorough account of the labeling process and its theoretical implications. Discussions of most of the aspects alluded to in this paper may be found there together with numerous references to the current literature.

grain counts in cells following exposure to radioactive thymidine (Cleaver, 1967, section 1.10; Clarkson, Ohkita, Ota & Fried, 1967). These observations, which will hopefully increase in precision and reliability, contain much quantitative information about cell kinetics. A theoretical analysis of such data for obtaining median generation times has been given recently by Fried (1968).

It is our purpose in this note to formulate a mathematical representation of cell populations in which the amount of radioactive label contained in a cell is included as one of the independent variables. Thus, equations for cell density functions are introduced which depend on the variables age, maturity, time and radioactive label. These equations are related to similar equations that have been introduced by various authors (von Foerster, 1959; Bell & Anderson, 1967; Bell, 1968; Rubinow, 1968). They are here used for quantitative mathematical treatment of experiments on cell populations which are subject to pulse labeling but can also be used for continuous infusion experiments.

Our equations are presented in the context of a physical model of cell proliferation proposed by Lajtha, Gilbert, Porteous & Alexanian (1964) to help explain how stem cells in the bone marrow may recover from radioactive damage. It utilizes the concept of a non-proliferative or resting state which has been suggested (Lajtha, Oliver & Gurney, 1962; Mendelsohn, 1962) to help understand kinetic features of cell renewal systems. In this view mitosis is normally followed by a G_0 or resting phase (Lajtha *et al.*, 1964) from which the normal cell cycle consisting of the phases G_1 , S, G_2 and M (Howard & Pelc, 1953) is triggered by an appropriate mechanism. This concept helps explain the very characteristic feature of leukemic marrow cells following a pulse injection of tritiated thymidine, namely that only an abnormally small fraction of them are initially labeled (Gavosto, Maraini & Pileri, 1960; Mauer & Fisher, 1963; Clarkson, Ota, Ohkita & O'Connor, 1964; Killman, 1965). A very simplistic study (Rubinow, unpublished) of the proliferative cycle for such cells suggests that a large proportion of the cells are in a resting state.

Our mathematical model hopefully provides, by the appropriate choice of the parameters involved, an adequate representation of the essential features of the *in vivo* and *in vitro* myeloblast proliferative system. It supposes two compartments of cells, an active compartment in which all cells undergo proliferation and a resting compartment in which no cells divide. Both compartments may lose cells at characteristic fractional rates per unit time β_1 and β_0 , respectively. A cell entering the active compartment is characterized by zero maturity. After such a cell has spent the time necessary for it to reach a certain level of maturity, assumed to require a definite time

interval T_A , it will divide into two cells. Of these newborn cells a fraction δ goes into the resting state and the remainder go back to the beginning of the active compartment. In addition the resting state contributes at a fractional rate α per unit time to the beginning of the active compartment (see Fig. 1).

There are five characteristic parameters of the system introduced thus far: α , δ , β_0 , β_1 and T_A . In addition the durations of the G_1 , S, G_2 and M phases T_1 , T_S , T_2 and T_M , with $T_1 + T_S + T_2 + T_M = T_A$, will enter into the description of labeling experiments. Further complexity can be introduced into the model, for example, by permitting variations in the times different cells spend in G_1 , S, G_2 and M. However, we have not done this here because initially it seems desirable to keep the model as simple as possible while still retaining consistency with the essential features of the myeloblast proliferative system.

The differential equations describing the proliferative system require for the uniqueness of their solution a specification of the initial state of all the cells, and knowledge of the manner in which newborn labeled cells make their appearance. It appears proper to assume that only those cells which are in the DNA synthesizing phase S will incorporate radioactive thymidine which is present in the environment, although there is some suspicion that cells in G_1 phase can also incorporate small amounts of thymidine (Pelc, 1963). The amount of radioactive material present in the nucleus of a cell at the time when the cells are killed and prepared for radioautography is measured in units x equal to the average number of observable grains on the photographic plate expected from this cell. In Appendix 1 it is shown that under normal conditions of exposure, x is directly proportional to the number of tritium atoms contained in a cell. The proportionality factor depends on the half-life of tritium, the duration of the exposure, and the efficiency of the photographic process. We assume further that when a cell divides each of the daughter cells receives half of its radioactive material. This is only true on the average after the first division because the chromosomes, which eventually incorporate the tritium from thymidine, may divide very unevenly; we shall consider a detailed theory of this in Appendix 2. Because it is rather awkward to express equal division in terms of a discrete variable, we treat x as a continuous variable. This appears justifiable when x is small compared to the number of tritium atoms present in the nucleus. This condition is easily satisfied for the experiments we wish to describe (see Appendix 1).

From an operational point of view, cells in different parts of G_1 and G_0 are at the present time indistinguishable from each other. Therefore, a different but equivalent interpretation of our model may be given as follows.

The cell population consists of a single proliferative compartment in which all members have the same time intervals assigned to the S, G₂ and M phases. However, the time interval assigned to the G₁ phase is variable from cell to cell, although always greater than or equal to a minimum time interval T₁. A certain number of G₁ cells enter the S phase per unit time, and cells are lost to the outside at any stage of maturation. The fractional loss rate may be different for cells in G₁ phase from the fractional loss rate for cells in the other phases.

The outline of this paper is as follows. In section 2 we derive and formally solve the kinetic equations for the total cell densities irrespective of labeling. We also discuss there the steady state of this system. In section 3 these equations are generalized to include the amount of tritium in the cell as an independent variable. Their solution is presented for an arbitrary initial state of the system. Section 4 contains expressions for the expected grain count distribution and some other directly observable quantities which are obtainable from the solution. Section 5 is a discussion of some of the uses and shortcomings of our model. The mathematical analysis of the expected grain count distribution is given in Appendix 1. In Appendix 2 we obtain in quantitative form the grain count distribution when the localization of DNA in the chromosomes is taken into account. The asymptotic behavior of the cell densities for long times is examined in Appendix 3.

2. Kinetics of the Total Population

We start by considering the kinetics of our cell population independent of labeling. Let $n(\mu, t) d\mu$ be the number of cells at time t in the active compartment with "maturity" parameter μ lying between μ and $d\mu$. The maturity variable μ will be taken to vary between zero and some maximum value m when cell division takes place. Also let $Q(a, t) da$ be the number of cells in the resting compartment G₀ between a and $a+da$, where a is the chronological age of a cell measured from time of birth. The governing equations of our model are assumed to be the following for all positive times:

$$\frac{\partial n(\mu, t)}{\partial t} + \frac{\partial(\dot{\mu}n(\mu, t))}{\partial \mu} = -\beta_1 n(\mu, t), \quad 0 \leq \mu \leq m, \quad (1)$$

$$\frac{\partial Q(a, t)}{\partial t} + \frac{\partial Q(a, t)}{\partial a} = -(\alpha + \beta_0)Q(a, t), \quad 0 \leq a \leq \infty, \quad (2)$$

together with the boundary conditions

$$Q(0, t) = 2\delta n(m, t)\dot{\mu}(m, t), \quad (3)$$

$$\dot{\mu}(0, t)n(0, t) = 2(1-\delta)n(m, t)\dot{\mu}(m, t) + \alpha \int_0^\infty Q(a, t) da. \quad (4)$$

Here $\dot{\mu}$ is the rate of maturation of cells in the active phase which can in principle depend on μ and t . It has to be given to make these equations complete. In addition, $\dot{\mu}$ may be expected to vary from cell to cell. This

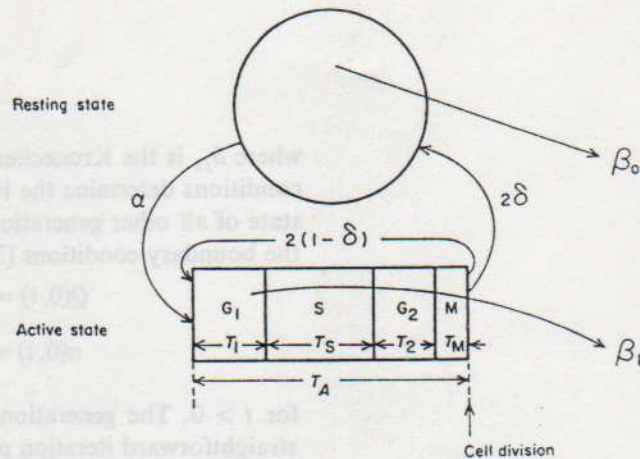


FIG. 1. Schematic representation of the mathematical model represented herein. The system consists of an active state in which cells mature and divide, and a resting state in which cells merely age without changing their maturity state. All the parameters of the system are indicated: the time intervals associated with the phases of the cell cycle, T_1 , T_S , T_2 and M ; the environmental fractional loss rates per unit time, β_0 and β_1 ; the fractional rate per unit time α at which resting cells enter the beginning of the active state; the fraction δ of newborn cells which enter the resting state.

would correspond to different cells spending different amounts of time in the active phase before undergoing division. We shall, however, assume herein that all cells mature at the same rate and that this rate is independent of t . We can therefore choose to measure μ in the same units as we measure time and set $\dot{\mu} = 1$ and $m = T_A$, the time each cell spends in the active compartment. We are also assuming here that α , β_0 and β_1 are constant, although more generally they may be expected to depend on μ or a and perhaps also on t . It is to be noted that according to the model, cells in the resting state do not mature with time, although they do become older.

In order to solve equations (1) to (4) it is necessary to give the values of $n(\mu, t)$ and $Q(a, t)$ at some initial time $t = 0$: $n(\mu, 0)$ and $Q(a, 0)$. The solution is then conveniently expressed in terms of the generation density functions (Rubinow, 1968) $n_j(\mu, t)$ and $Q_j(a, t)$, which are assigned to the j th generation, $j = 1, 2, \dots$. The cells present at $t = 0$ are naturally counted as belonging to the first generation for which $j = 1$. This will turn

out to be very useful later when we consider the evolution of labeled cell populations. Thus, we set

$$n(\mu, t) = \sum_{j=1}^{\infty} n_j(\mu, t), \quad (5)$$

$$Q(a, t) = \sum_{j=1}^{\infty} Q_j(a, t), \quad (6)$$

$$n_j(\mu, 0) = \delta_{j1} n(\mu, 0), \quad (7)$$

$$Q_j(a, 0) = \delta_{j1} Q(a, 0), \quad (8)$$

where δ_{j1} is the Kronecker delta. Equations (7) and (8) state that the initial conditions determine the initial state of the first generation only, the initial state of all other generations being null. In view of the discussion following the boundary conditions (3) and (4), the latter simplify to

$$Q(0, t) = 2\delta n(T_A, t), \quad (9)$$

$$n(0, t) = 2(1-\delta)n(T_A, t) + \alpha \int_0^{\infty} Q(a, t) da, \quad (10)$$

for $t > 0$. The generation functions at any time t are now obtained by a straightforward iteration procedure, e.g.

$$n_1(\mu, t) = \left[n_1(\mu-t, 0) + \alpha \int_0^{\infty} Q_1(a', t-\mu) da' \right] e^{-\beta_1 t}, \quad 0 \leq \mu \leq T_A, \quad (11)$$

$$Q_1(a, t) = Q_1(a-t, 0) e^{-(\alpha+\beta_0)t}, \quad 0 \leq a \leq \infty, \quad (12)$$

$$n_i(\mu, t) = [2(1-\delta)n_{i-1}(T_A, t-\mu) + \alpha Q_i(t-\mu)] e^{-\beta_1 t}, \quad i \geq 2, \quad (13)$$

$$Q_i(a, t) = 2\delta n_{i-1}(T_A, t-a) e^{-(\alpha+\beta_0)a}, \quad i \geq 2, \quad (14)$$

with the understanding that these functions are to be set equal to zero whenever any of their arguments are negative. In equation (13) we have defined

$$Q_i(t) = \int_0^{\infty} Q_i(a, t) da, \quad i \geq 1, \quad (15)$$

which is the total number of cells in G_0 belonging to the i th generation.

We shall now consider the simple case where the total cell population, labeled plus unlabeled, is in a steady state. Denoting the steady state values of $n(\mu, t)$ and $Q(a, t)$ by $\bar{n}(\mu)$, $\bar{Q}(a)$, and setting the time derivatives in (1) and (2) equal to zero, we easily find the solutions to these equations as

$$\bar{n}(\mu) = N_A \beta_1 e^{-\beta_1 \mu} / (1 - e^{-\beta_1 T_A}), \quad 0 \leq \mu \leq T_A, \quad (16)$$

$$\bar{Q}(a) = N_0 (\alpha + \beta_0) e^{-(\alpha+\beta_0)a}, \quad 0 \leq a \leq \infty. \quad (17)$$

N_0 and N_A represent the total number of cells in the resting and active compartments, respectively, when the cell population is in a steady state.

Substituting equations (16) and (17) into (9) and (10), there result two simultaneous linear homogeneous equations for N_0 and N_A . Their ratio is determined provided the determinant of the coefficient matrix is zero. This results in the steady-state condition

$$e^{-\beta_1 T_A} = \frac{\alpha + \beta_0}{2[\alpha + \beta_0(1 - \delta)]}. \quad (18)$$

Note that since $e^{-\beta_1 T_A}$ is ≤ 1 , it follows that $\delta \leq \frac{1}{2}(1 + \alpha/\beta_0)$. Provided equation (18) is satisfied, which is to say that a steady state is possible, the relative number of cells in each compartment in the steady state is given as

$$N_0/N_A = \frac{2\delta\beta_1}{[\alpha + \beta_0(1 - 2\delta)]}. \quad (19)$$

It is also useful to derive the average time T_0 spent by a cell in the resting state, or the mean age of one of its members,

$$T_0 \equiv \frac{1}{N_0} \int_0^{\infty} a \bar{Q}(a) da = \frac{1}{\alpha + \beta_0}. \quad (20)$$

Let T be the average time between the time when a cell undergoes division and the time it was born. It is obtained as the proportion of cells undergoing division, $2(1 - \delta)e^{-\beta_1 T_A}$, which went directly after their birth into the active state multiplied by the time T_A spent by cells in the active state, plus the remaining proportion of cells $[1 - 2(1 - \delta)e^{-\beta_1 T_A}]$ which went first into the resting state and thence to the active state, multiplied by the average time spent by cells that go through both the resting and active states, $T_0 + T_A$. Thus

$$T = T_A + \frac{\alpha \delta T_0}{\alpha + \beta_0(1 - \delta)}. \quad (21)$$

Designate the total number of cells in the steady state by N ,

$$N = N_0 + N_A. \quad (22)$$

The fractions of cells in the resting and active states are then obtained from (19) and (22) as respectively

$$\frac{N_0}{N} = \frac{2\delta\beta_1}{\alpha + \beta_0 - 2\delta(\beta_0 - \beta_1)}, \quad (23)$$

$$\frac{N_A}{N} = \frac{\alpha + \beta_0(1 - 2\delta)}{\alpha + \beta_0 - 2\delta(\beta_0 - \beta_1)}. \quad (24)$$

From their definitions, the fractions of cells in G_1 , S , G_2 and M in the steady

state are given, respectively, as follows.

$$\frac{N_1}{N} \equiv \frac{1}{N} \int_0^{T_1} \bar{n}(\mu) d\mu = \frac{N_A (1 - e^{-\beta_1 T_1})}{N (1 - e^{-\beta_1 T_A})}, \quad (25)$$

$$\frac{N_s}{N} = \frac{N_A}{N} e^{-\beta_1 T_1} \frac{(1 - e^{-\beta_1 T_s})}{(1 - e^{-\beta_1 T_A})}, \quad (26)$$

$$\frac{N_2}{N} = \frac{N_A}{N} e^{-\beta_1 (T_1 + T_s)} \frac{(1 - e^{-\beta_1 T_2})}{(1 - e^{-\beta_1 T_A})}, \quad (27)$$

$$\frac{N_M}{N} = \frac{N_A}{N} \frac{[e^{-\beta_1 (T_1 + T_s + T_2)} - e^{-\beta_1 T_A}]}{(1 - e^{-\beta_1 T_A})}. \quad (28)$$

When equation (18) is not satisfied, a steady state is not possible and the population will either continue to grow or decline depending on whether the left side is greater or smaller than the right side in that equation. This is shown in Appendix 3 where the long time asymptotic behavior of the cell densities is investigated. It is shown there that the approach to a steady (or bounded oscillatory) state from an arbitrary initial state is possible only when, in addition to (18), the parameters of the problem satisfy the following inequality,

$$e^{-\beta_1 T_A} < \frac{1 + T_A(\alpha + \beta_0)}{2(1 - \delta)}. \quad (29)$$

Also, the actual number of cells in the final steady state (when it exists) will depend on the initial distribution.

3. Kinetics of the Labeled Population

Let $n(x, \mu, t) dx$ and $Q(x, a, t) dx$ represent the cell densities with amount of radioactive thymidine between x and $x + dx$ in the active and resting phases, respectively. As mentioned in section 1 we shall treat x as a continuous variable. We assume that at division each daughter cell receives half of the amount of label of the parent cell (see also Appendix 2). This means that if a group of cells undergoing mitosis have an amount of label between x and $x + dx$, then their daughter cells will have an amount of label between $x/2$ and $(x + dx)/2$. These density functions therefore satisfy equations similar to (1) to (4), namely, with $t \geq 0$,

$$\frac{\partial n(x, \mu, t)}{\partial t} + \frac{\partial n(x, \mu, t)}{\partial \mu} + \dot{x}(\mu, t) \frac{\partial n(x, \mu, t)}{\partial x} = -\beta_1 n(x, \mu, t), \quad 0 \leq \mu \leq T_A, \quad 0 \leq x \leq \infty, \quad (30)$$

$$\frac{\partial Q(x, \mu, t)}{\partial t} + \frac{\partial Q(x, a, t)}{\partial a} = -(\alpha + \beta_0) Q(x, a, t), \quad 0 \leq a \leq \infty, \quad (31)$$

$$Q(x, 0, t) = 4\delta n(2x, T_A, t), \quad (32)$$

$$n(x, 0, t) = 4(1-\delta)n(2x, T_A, t) + \alpha \int_0^\infty Q(x, a, t) da. \quad (33)$$

In the interests of simplicity we have assumed in these equations that $\dot{\mu} = 1$ as set forth in the discussion following equation (4). In equation (30) \dot{x} is the rate of uptake of labeled thymidine by a cell of maturity μ at time t . This will depend on the amount of labeled thymidine present in its compartment at time t , the maturity of the cell at time t , and the individual characteristics of the cell (Cleaver, 1967). However, it is assumed to be independent of x , the amount of labeled thymidine already taken up by the cell. It follows from experimental observation (cf. Appendix 1) that there is great variability among the cells in their utilization of external thymidine (Cleaver, 1967, section 1.10; Clarkson *et al.*, 1967). Hence within the context of our model, $\dot{x}(\mu, t)$ should be thought of as a random variable which has some specified probability distribution function associated with it which depends on μ .

It is generally believed that there is only a short period of the order of one-half to one hour after an injection during which radioactive thymidine can be incorporated into the nucleus of a mammalian cell (Cleaver, 1967, section 2.7). After this time interval, it becomes degraded and unavailable for incorporation. This implies that $\dot{x}(\mu, t)$ is zero for t later than an hour after injection. Therefore, if we know the values of $n(x, \mu, t)$ and $Q(x, a, t)$ at any time t_0 after this period we can use equations (30) and (31) with $\dot{x}(\mu, t)$ set equal to zero to find their values at later times $t > t_0$. If we choose our time origin $t_0 = 0$ these known values then determine $n(x, \mu, 0)$ and $Q(x, a, 0)$. We find the solution for all subsequent times in a manner analogous to that utilized in section 2 as

$$n(x, \mu, t) = \sum_{j=1}^{\infty} n_j(x, \mu, t), \quad (34)$$

$$Q(x, a, t) = \sum_{j=1}^{\infty} Q_j(x, a, t), \quad (35)$$

$$n_j(x, \mu, 0) = \delta_{j1} n(x, \mu, 0), \quad (36)$$

$$Q_j(x, a, 0) = \delta_{j1} Q(x, a, 0), \quad (37)$$

$$n_1(x, \mu, t) = \left[n_1(x, \mu - t, 0) + \alpha \int_0^\infty Q_1(x, a', t - \mu) da' \right] e^{-\beta_1 t}, \quad 0 \leq \mu \leq T_A, \quad (38)$$

$$Q_1(x, a, t) = Q_1(x, a - t, 0) e^{-(\alpha + \beta_0)t}, \quad 0 \leq a \leq \infty, \quad (39)$$

$$n_i(x, \mu, t) = \left[4(1-\delta)n_{i-1}(2x, T_A, t - \mu) + \alpha \int_0^\infty Q_i(x, a', t - \mu) da' \right] e^{-\beta_i t}, \quad i \geq 2, \quad (40)$$

$$Q_i(x, a, t) = 4\delta n_{i-1}(2x, T_A, t-a)e^{-(\alpha+\beta_0)a}, \quad i \geq 2. \quad (41)$$

Having obtained the general solution of our equations for an arbitrary initial distribution we shall now consider more explicitly the case when cells in S phase only can incorporate appreciable amounts of radioactive thymidine into the nucleus. However, there is a very short time delay t_1 from the time of injection to the time of incorporation of radioactive thymidine in the nucleus. This occurs because of the time elapsed for transport, diffusion, and the various intermediate biochemical steps which are required for the incorporation to take place. Therefore, cells of maturity greater than $T_1 + T_S - t_1$ at $t = 0$ will be incapable of becoming labeled. In addition, cells with values of μ between $T_1 - t_0$ and T_1 can at the time of injection also become labeled, where t_0 is the time following injection when labeled thymidine is no longer available for incorporation. Consequently, the apparent S phase interval is $T_S + t_0 - t_1$, which is approximately the same as T_S and thus amounts to a slight redefinition of the S phase.

With this type of initial condition the total cell population divides naturally into two parts: those cells which are initially labeled with thymidine, and those cells which are initially unlabeled. Thus, set

$$n(x, \mu, t) = n'(x, \mu, t) + n''(\mu, t)\delta(x), \quad (42)$$

$$Q(x, \mu, t) = Q'(x, a, t) + Q''(a, t)\delta(x), \quad (43)$$

where the single primed functions represent the initially labeled cells, the double primed functions represent the initially unlabeled cells, and $\delta(x)$ is the Dirac delta function. Since all our equations are linear, this separation carries through for all times and generations. We shall first consider the solution for the initially labeled cells. According to our model

$$Q'(x, a, 0) = 0. \quad (44)$$

We shall now further assume that $n'(x, \mu, 0)$ can be written as a product of a function of μ and a function of x ,

$$n'(x, \mu, 0) = f(\mu)\varphi(x), \quad (45)$$

where

$$\int_0^\infty \varphi(x) dx = 1, \quad (46)$$

and $f(\mu)$ is zero when μ is not in the S phase. This means that the distribution of labeled thymidine $\varphi(x)$ among those cells which get labeled is independent of their maturity μ at the time of injection. This is clearly an approximation and is certainly not true for cells at the beginning and end of the S phase. Using this initial condition, the functional dependence of $n'(x, \mu, t)$ can be

expressed in a partially separable form,

$$n'_j(x, \mu, t) = 2^{j-1} \phi(2^{j-1}x) n'_j(\mu, t), \quad j \geq 1, \quad (47)$$

$$Q'_j(x, a, t) = 2^{j-1} \phi(2^{j-1}x) Q'_j(a, t), \quad j \geq 1. \quad (48)$$

By direct substitution it follows that (36) to (41) are satisfied when $n'_j(\mu, t)$ and $Q'_j(a, t)$ are given by (11) to (14) with $n'_1(\mu, 0) = f(\mu)$ and $Q'_1(a, 0) = 0$. Here $f(\mu)$ will be chosen, again for mathematical simplicity, equal to the initial value of the total cell density function $n(\mu, 0)$ for values of μ which define the S phase. The solution for the labeled cell population is now completed by assuming that the total cell population is in the steady state. Therefore

$$f(\mu) = \begin{cases} \bar{n}(\mu), & T_1 \leq \mu \leq T_1 + T_s, \\ 0, & \text{otherwise.} \end{cases} \quad (49)$$

It should be noted from equations (40) to (48) that $n'(\mu, t)$ and $Q'(a, t)$ represent the maturity density and age density functions respectively of all labeled cells, irrespective of amount of label x .

With regard to the solution for the initially unlabeled cells, we assume as initial conditions

$$n''(\mu, 0) = \bar{n}(\mu) - f(\mu), \quad (50)$$

$$Q''(a, 0) = \bar{Q}(a), \quad (51)$$

with $\bar{n}(\mu)$ and $\bar{Q}(a)$ given by (16) and (17). It follows directly from (32) and (33) that the boundary conditions for $Q''(0, t)$ and $n''(0, t)$ are the same as (9) and (10). It is intuitively clear that the description of the initially unlabeled cell population is given by the equations

$$n''(\mu, t) = \bar{n}(\mu) - n'(\mu, t) = \bar{n}(\mu) - \int_0^\infty n'(x, \mu, t) dx, \quad (52)$$

$$Q''(a, t) = \bar{Q}(a) - Q'(a, t) = \bar{Q}(a) - \int_0^\infty Q'(x, a, t) dx. \quad (53)$$

It is worth remarking that it is possible to treat a general initial labeled distribution $n'(x, \mu, 0)$ by writing it as a sum of terms

$$n'(x, \mu, 0) = \sum_i f^i(\mu) \phi^i(x). \quad (54)$$

Each term in this sum gives rise to later distributions which are representable by equations of the form (47) and (48).

4. Relation to Observable Quantities

We are now ready to compute various observable quantities on the basis of our model. For example, we shall now show how measurements of the

distribution of grain counts of cells in either interphase or in mitosis may be related to theory. Let $N'(t)$ be the number of cells containing tritium at time t . Then

$$N'(t) = \int_0^\infty F(x, t) dx, \quad (55)$$

where

$$F(x, t) = \int_0^{T_A} n'(x, \mu, t) d\mu + \int_0^\infty Q'(x, a, t) da. \quad (56)$$

Thus, $F(x, t) dx$ is the number of cells at time t whose radioactive material content is between x and $x+dx$. The number of unlabeled cells at any time is

$$N''(t) = N - N'(t), \quad (57)$$

where N is constant. Of course, if the system were not in study state, N would likewise depend on t .

In a similar way we define for labeled cells in mitosis

$$F_M(x, t) = \int_{T_A - TM}^{T_A} n'(x, \mu, t) d\mu. \quad (58)$$

Then the total numbers of labeled and unlabeled cells in mitosis are, respectively,

$$N'_M(t) = \int_0^\infty F_M(x, t) dx, \quad (59)$$

$$N''_M(t) = N_M - N'_M(t), \quad (60)$$

with N_M given by equation (28).

The quantities $F(x, t)$ and $F_M(x, t)$ may be related to the actual number of grain counts that are expected to be observed. This is done in Appendix 1 where it is shown that the fraction of cells with an observed count of exactly k grains is expected to be

$$p(k, t) = \frac{N''(t)}{N} \delta_{k,0} + \frac{1}{N} \int_0^\infty \frac{x^k}{k!} e^{-x} F(x, t) dx, \quad k = 0, 1, 2, \dots \quad (61)$$

For the mitotic cells, the expected fraction of cells with a count of k grains is

$$p_M(k, t) = \frac{N''_M(t)}{N_M} \delta_{k,0} + \frac{1}{N_M} \int_0^\infty \frac{x^k}{k!} e^{-x} F_M(x, t) dx, \quad k = 0, 1, 2, \dots \quad (62)$$

It is clear that even aside from extrinsic but probably unavoidable errors introduced by observations, it is intrinsically impossible to determine a precise $F(x, t)$ from observations of $p(k, t)$. Furthermore, even if $F(x, 0)$ were known, it would not determine $n(x, \mu, 0)$ and $Q(x, a, 0)$, the quantities required by the theory in order to make predictions at later times t . A reasonable approach for obtaining maximum information from our equations is to start with some initial condition such as that given in section 3 assuming some simple analytic form for $\varphi(x)$. This may be chosen to fit the actual measured values of $p(k, 0)$. Having done this, the equations are utilized to predict values of $p(k, t)$ and other quantities derivable from the solution for various choices of the parameters entering our model. These can then be compared with actual measurements to see which set of parameters, if any, fit the observations. As a practical problem of observation in the radioautographic process, there is background "noise" caused by cells with a small amount of contaminated label. It is therefore useful to introduce a threshold value of grain counts, below which a cell is not considered to be labeled. This number is usually taken to be five. Because of this, it is necessary to define the fraction of cells whose grain count k at time t is greater than a threshold value j as (cf. Appendix 1),

$$L(t; j) = \sum_{k=j}^{\infty} p(k, t) = \frac{1}{N} \int_0^{\infty} \left[1 - e^{-x} \left(\sum_{k=0}^{j-1} \frac{x^k}{k!} \right) \right] F(x, t) dx. \quad (63)$$

The same quantity defined for cells in mitosis is

$$L_M(t; j) = \frac{1}{N_M} \int_0^{\infty} \left[1 - e^{-x} \left(\sum_{k=0}^{j-1} \frac{x^k}{k!} \right) \right] F_M(x, t) dx. \quad (64)$$

The above quantities, with an appropriate choice of j , are referred to as the labeling index and the labeled mitotic index, respectively.

As an illustration of this procedure, suppose it is assumed that the normalized function $\varphi(x)$ introduced in equation (45) is a γ -distribution,

$$\varphi(x) = \frac{c^{\gamma+1} x^{\gamma} e^{-cx}}{\Gamma(\gamma+1)}. \quad (65)$$

The distribution of grain counts among labeled cells in fact has this general shape (Cleaver, 1967, section 1.10; Clarkson *et al.*, 1967). The values of the two parameters γ and c must however be assigned. We may do so by making the average grain count $\bar{k}(t)$ [see equation (A10)] and the labeled mitosis curve $L_M(t; j)$ agree with observation at some time t . It is clearly best to choose a time t which is greater than $T_2 + T_M$ and smaller than $T_2 + T_S$. According to our model,

$$F_M(x, t) = N_M \varphi(x) \quad (66)$$

for t in this interval. Substitution into equation (64) therefore yields

$$L_M(t; j) = 1 - \sum_{k=0}^{j-1} \frac{c^{\gamma+1} \Gamma(\gamma+1+k)}{(1+c)^{\gamma+1+k} \Gamma(\gamma+1)}. \quad (67)$$

From equation (A10) it follows directly that, with N in that equation interpreted as N_M ,

$$\bar{k}(t) = (\gamma+1)/c. \quad (68)$$

From the observed values of $\bar{k}(t)$ and $L_M(t; j)$, the parameters γ and c are obtainable by the method of least squares.

Another quantity that is readily observable is the fraction v of all those cells which are considered to be labeled whose grain count is above a specified number ξ ,

$$L(t; \xi) = vL(t; j), \quad \xi \geq j, \quad 0 \leq v \leq 1. \quad (69)$$

As a practical matter of observation, Clarkson *et al.* (1967) kept v constant in time and measured ξ as a function of the time. Therefore, we may view (69) as defining $\xi = \xi(t; v)$. For $v = \frac{1}{2}$, ξ is the median grain count of the "labeled" cells. In the "grain-count halving" method (Killman, Cronkite, Flidner & Bond, 1962), the time T_g that it takes ξ to reduce to one-half of its starting value is interpreted as the mean generation time of the population. Thus, T_g is defined by

$$\xi(T_g; v) = \frac{1}{2} \xi(0; v). \quad (70)$$

It is clear from this expression, however, that T_g in general depends on v , $T_g = T_g(v)$. It is also clear that T_g depends implicitly on the initial labeled thymidine distribution function $\varphi(x)$, and more generally on $n'(x, \mu, 0)$. The value $T_g(\frac{1}{2})$ is called the median generation time [8]. We can define a similar quantity ξ_M for those cells which are in mitosis, with $\xi_M = \xi_M(t; v)$ given by

$$L_M(t; \xi_M) = vL_M(t; j). \quad (71)$$

5. Discussion

We have developed a simple quantitative theory for obtaining the distribution of grain counts in cells as a function of the time after pulse labeling. It is hoped that a quantitative theory of this type will prove useful in extracting maximum information from labeling experiments such as those of Clarkson *et al.* (1967) on leukemic myeloblast cell populations. The success of such an attempt will depend primarily on the precision and reliability of the experiments. With the availability of such reliable information one can "guess" at a correct model of the kinetics of the cell system being studied and then refine and verify such a model through a comparison between observations and the predictions of a quantitative mathematical theory.

The theory is presented in this paper in the context of a specific model which is certainly an oversimplified one. Thus, by assuming a fixed T_S and T_2 for all cells and no initial labeling for cells outside S phase, it predicts that the labeled mitotic index goes to zero for times t between $T_S + T_2 + T_M$ and $T_S + T_2 + T_M + (T_1 + T_2)$. This is never observed as some small but finite value of the index is seen in this interval. There are various possible simple modifications of our model which would explain this. One is to permit some cells in G_1 to be initially labeled. Another possibility which mitigates the problem considerably is to set T_1 equal to zero. A careful examination of the grain count distribution of cells in this interval would help explain this phenomenon.

Another oversimplification in our model, which also has bearing on the point just discussed, is the assumption of fixed T_S and T_2 , and also of T_A . It is well established that even under ideal similar environments, cells of a particular type exhibit variable generation times (Prescott, 1959). An additional modification of the model which could take this into account is to introduce a distribution of subpopulations each with different generation times (Rubinow, 1968). This could be expected to represent the real population over a time span of several generations at least.

Finally, the assumption that all cells in the S phase get labeled with the same distribution is not true in general. It is well known that the rate of DNA replication varies within the S phase, generally increasing towards the end of the phase (Cleaver, 1967, section 5.6). Such behavior could be represented by an appropriate initial condition, as for example with an initial distribution of the form given in (54).

Even within the confines of our simple model, it is possible to reinterpret some of the quantities involved for use in different situations. For example, by setting $\alpha = 0$, G_0 could be interpreted to correspond to a functional compartment distinguishable from the active compartment. An example of this would be the non-proliferative metamyelocyte class of red blood cells which follows the proliferative myelocyte class. One could then study separately the labeling of cells in G_0 and in the active compartment.

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Appendix 1

CALCULATION OF THE GRAIN COUNT DISTRIBUTION

Consider a sample containing N cells which have been exposed to a pulse radioactive marker at time zero and radioautographed at time t . These cells are exposed on film for a period of time τ_0 and the fraction of cells with grain count k , $p(k, t)$ is then determined, $k = 0, 1, 2, \dots$. Let $W(A, t)$ be the number of cells in the sample of time t with A radioactive atoms so that

$$N = \sum_{A=0}^{\infty} W(A, t). \quad (A1)$$

Although each W varies with time, the sample size N of course does not. Let ϵ , the efficiency, be the fraction of disintegrations which get recorded on the film and τ be the mean life of the radioactive atom. The average number of grains observed during the time τ_0 from cells containing A radioactive atoms is then

$$x = \epsilon[1 - e^{-\tau_0/\tau}]A \equiv \rho A. \quad (A2)$$

If the radioactive marker is tritiated thymidine, then the radioactive atom is tritium for which $\tau = 17.1$ years. The values of magnitude of τ_0 and ε are $\varepsilon \sim 0.1$ and $\tau_0 \sim 1$ week. For these values, $\tau_0/\tau \ll 1$ and the proportionality constant ρ is likewise quite small,

$$\rho \approx \frac{\varepsilon \tau_0}{\tau} \sim 10^{-4}. \quad (\text{A3})$$

The probability of observing k grains for a cell contained in such a sample is $x^k e^{-x}/k!$. This expression is valid for all $k \ll x/\rho$ which is always satisfied in our case with a probability close to unity since ρ is such a small number. The percentage of cells having k grains will then be, assuming a sufficiently large sample,

$$p(k, t) = N^{-1} W(0, t) \delta_{k,0} + N^{-1} \sum_{x \neq 0} \frac{x^k}{k!} e^{-x} W(x/\rho, t), \quad (\text{A4})$$

where we have separated out the cells which have no external radioactive thymidine in them. The sum in the second term in (A4) goes over the values $x = \rho, 2\rho, \dots$. Note that $\sum_{k=0}^{\infty} p(k, t) = 1$, as it should be. At the same time equation (A1) may be rewritten as

$$1 = N^{-1} W(0, t) \delta_{k,0} + N^{-1} \sum_{x \neq 0} W(x/\rho, t). \quad (\text{A5})$$

We shall now assume that this sum over x can be converted to an integral over x with a weight function, i.e. $W(x/\rho, t) \rightarrow F(x, t) dx$. Then (A4) becomes

$$p(k, t) = N^{-1} W(0, t) \delta_{k,0} + N^{-1} \int_0^{\infty} \frac{x^k}{k!} e^{-x} F(x, t) dx. \quad (\text{A6})$$

$F(x, t) dx$ is the number of cells in the sample having between x/ρ and $(x+dx)/\rho$ tritium atoms. The normalization constant for $F(x, t)$ is the total number of labeled cells:

$$\int_0^{\infty} F(x, t) dx = N - W(0, t). \quad (\text{A7})$$

What is actually assumed here is that the distribution of tritium atoms in the different cells, i.e. the uptake of external thymidine, is sufficiently regular for $F(x, t)$ to be reasonably smooth. For example, it does not consist of a sum of delta functions. It is because of this requirement that we separated out the term $W(0, t)$ representing the cells without any tritium. This group of cells can be thought of as including also cells which contain a small number ($\ll 1/\rho$) of tritium atoms since the probability of these producing

even one grain is very small. It is seen from (A6) that for k large, $x^k e^{-x}/k!$ will be sharply peaked at $x = k$, so that $p(k, t) \approx F(k, t)$, $k \gg 1$. The function $F(x, t)$ is given in terms of our notation in the text by

$$F(x, t) = \int_0^{T_A} n'(x, \mu, t) d\mu + \int_0^\infty Q'(x, a, t) da \quad (\text{A8})$$

when we consider the labeling of all cells, and by

$$F_M(x, t) = \int_{T_A - TM}^{T_A} n'(x, \mu, t) d\mu \quad (\text{A9})$$

when we consider the labeling of cells in mitosis only.

It follows with the use of (A6) and (A7) that the average number of grain counts at time t is

$$\bar{k}(t) = \sum_{k=0}^{\infty} k p(k, t) = N^{-1} \int_0^\infty x F(x, t) dx = \left(1 - \frac{W(0, t)}{N}\right) \langle x \rangle, \quad (\text{A10})$$

where

$$\langle x \rangle = \frac{\int_0^\infty x F(x, t) dx}{\int_0^\infty F(x, t) dx} \quad (\text{A11})$$

is the average of x over those cells which have taken up some external thymidine, $N - W(0, t)$. Because, in order to avoid background problems, only cells with a minimum threshold number of grains, say j , are usually counted as labeled, it is useful to define averages over this labeled subset instead of over the entire population as in equation (A10). Denoting the threshold number j as a subscript, the average number of grain counts at time t with this definition is $\bar{k}_j(t)$ given by the expression

$$\bar{k}_j(t) = \frac{\sum_{k=j}^{\infty} k p'(k, t)}{\sum_{k=j}^{\infty} p'(k, t)} = \frac{\int_0^\infty \left[1 - e^{-x} \sum_{m=0}^{j-2} \frac{x^m}{m!}\right] x F(x, t) dx}{\int_0^\infty \left[1 - e^{-x} \sum_{m=0}^{j-1} \frac{x^m}{m!}\right] F(x, t) dx}, \quad (\text{A12})$$

where

$$p'(k, t) = p(k, t) - N^{-1} W(0, t) \delta_{k,0}.$$

Obviously, $\bar{k}_0(t) = \langle x \rangle$. Similarly, the mean square number of grain counts

defined over the labeled population is

$$\overline{k_j^2(t)} = \frac{\sum_{k=j}^{\infty} k^2 p'(k, t)}{\sum_{k=j}^{\infty} p'(k, t)} = \overline{k_j(t)} + \frac{\int_0^{\infty} \left[1 - e^{-x} \sum_{m=0}^{j-3} \frac{x^m}{m!} \right] x^2 F(x, t) dx}{\int_0^{\infty} \left[1 - e^{-x} \sum_{m=0}^{j-1} \frac{x^m}{m!} \right] F(x, t) dx}. \quad (\text{A13})$$

From this it follows that the mean square fluctuation in k when the threshold value j is zero is

$$\overline{k_0^2(t)} - \overline{k_0(t)}^2 = \langle x \rangle \left(1 + \frac{\langle (x - \langle x \rangle)^2 \rangle}{\langle x \rangle} \right), \quad (\text{A14})$$

where $\langle x^2 \rangle$ is defined in a manner analogous to equation (A1). The first term in the bracket on the right side of (A14) is just the fluctuation due to the randomness of the decay process while the second term represents the fluctuation in the distribution of tritium atoms in the labeled cells. Now if the incorporation of external thymidine was an independent random process, i.e. each thymidine molecule was incorporated independently and with equal probability by all cells which are in S phase during the pulse labeling, then the second term in (A14) should be approximately $2\rho \sim 2 \times 10^{-4}$. In practice however the dispersion is very much larger with the term in the bracket varying between five and ten for mammalian cells (Cleaver, 1967, section 1.10; Clarkson *et al.*, 1967). This appears to be true even when one singles out cells which were at approximately the same maturity level in the S phase during the injection of the tritiated thymidine. Such cells can be singled out either by looking at first generation cells in mitosis (Clarkson *et al.*, 1967) or by looking at cells with the same amount of DNA (Cleaver, 1967, section 5.6). This large dispersion does not appear to apply to labeled bacteria (Painter, Drew & Giauque, 1960) or to labeled phage in bacteria (Caro & Schnös, 1965).

Experiments with cells *in vitro* would seem to rule out variation due to different amount of thymidine reaching the cells. One explanation for this large variation, assuming it doesn't arise simply from classifying intrinsically different cell groups as the same population, involves the assumption of some randomness in the replication schedule of the different chromosomes (Alpen & Johnston, 1967). Alternatively, if the rate of synthesis of DNA varies rapidly with the position of the cell in the S phase, then the duration of the pulse and the duration of the mitotic phase could give rise to large variations in the grain count of the labeled mitotic cells and of cells with approximately the same DNA content. In any case this question deserves further investigation.

Appendix 2

EFFECT OF CHROMOSOMES

Because of the existence of chromosomes, a radioactive DNA label such as tritium is not divided equally among the granddaughters and subsequent generations of an initially labeled cell (Cronkite, Greenhouse, Brecher & Bond, 1961). We shall present here the essential aspects of an extended theory of labeled cell populations which considers the labeling of each chromosome individually.

Let J be the total number of chromosomes in the cell. Every chromosome consists of two halves (chromatids) each of which replicates itself. After pulse labeling at $t = 0$ the two new strands of each labeled chromosome will be labeled. We can assume that the two strands have equal label. On division, every chromosome of the daughter cells will consist of one labeled and one unlabeled chromatid (semi-conservative replication). During all subsequent mitoses, labeled chromosomes become chromosomal pairs consisting of one labeled and one unlabeled chromosome (neglecting cross-over). We shall assume that on division the labeled one is equally likely to go to either one of the daughters.

In order to treat this situation we introduce the vector $\mathbf{x} = (x_1, \dots, x_J)$, where x_i specifies the amount of tritium in the i th chromosome. If the i th chromosome is unlabeled, then $x_i = 0$. Denoting by

$$n(x_1, \dots, x_J, \mu, t) dx_1 \dots dx_J \equiv n(\mathbf{x}, \mu, t) d\mathbf{x}$$

and

$$Q(x_1, \dots, x_J, a, t) dx_1 \dots dx_J \equiv Q(\mathbf{x}, a, t) d\mathbf{x}$$

the cell densities containing between x_i and $x_i + dx_i$ amount of tritium in the i th chromosome $i = 1, 2, \dots, J$, it follows that

$$n(\mathbf{x}, \mu, t) = \int_0^\infty n(\mathbf{x}, \mu, t) \delta\left(x - \sum_{i=1}^J x_i\right) d\mathbf{x}, \quad (\text{A15})$$

$$Q(\mathbf{x}, a, t) = \int_0^\infty Q(\mathbf{x}, a, t) \delta\left(x - \sum_{i=1}^J x_i\right) d\mathbf{x}, \quad (\text{A16})$$

where $n(\mathbf{x}, \mu, t)$ and $Q(\mathbf{x}, a, t)$ have the same meanings as before. These equations show how the previously defined cell density functions may be obtained from the chromosomal density functions which are defined here. These latter functions satisfy equations which are similar to (30) and (31), with the vector $\mathbf{x} = (x_1, \dots, x_J)$ replacing x , and the velocity term involving \dot{x} in equation (30) replaced by its generalization

$$\dot{\mathbf{x}} \cdot \nabla n(\mathbf{x}, \mu, t) = \sum_{i=1}^J \dot{x}_i \frac{\partial n}{\partial x_i}(\mathbf{x}, \mu, t).$$

For consideration of pulse labeling experiments, this term may be taken to be zero as discussed in section 3. To solve these generalized equations, we shall again divide the cells according to generations, viz.,

$$n(x, \mu, t) = \sum_{i=1}^{\infty} n_i(x, \mu, t), \quad (\text{A17})$$

$$Q(x, a, t) = \sum_{i=1}^{\infty} Q_i(x, a, t). \quad (\text{A18})$$

At $t = 0$ we have the initial conditions

$$n_i(x, \mu, 0) = \delta_{i1} n(x, \mu, 0), \quad (\text{A19})$$

$$Q_i(x, a, 0) = \delta_{i1} Q(x, a, 0), \quad (\text{A20})$$

where $n(x, \mu, 0)$ and $Q(x, a, 0)$ are assumed to be known functions. These equations are similar to equations (34) to (37) with x replaced by the vector x . The same change in equations (38) to (39) will give the time evolution of the first generation. The same change in equations (40) and (41) with $i = 2$ will give the correct result for the second generation. For subsequent generations the results are somewhat different, as follows:

$$n_i(x, \mu, t) = n_i(x, 0, t - \mu) e^{-\beta_1 \mu}, \quad i \geq 3, \quad (\text{A21})$$

$$Q_i(x, a, t) = Q_i(x, 0, t - a) e^{-(\alpha + \beta_0)a}, \quad i \geq 3, \quad (\text{A22})$$

with

$$n_i(x, 0, t) = 2(1 - \delta)(\frac{1}{2})^J \sum_{(\sigma_j=0,1)}^{\infty} \int_0^{\infty} \prod_{j=1}^J \delta(x_j - \sigma_j x'_j) n_{i-1}(x', T_A, t) dx' + \alpha \int_0^{\infty} Q_i(x, a', t) da', \quad i \geq 3, \quad (\text{A23})$$

$$Q_i(x, 0, t) = 2\delta(\frac{1}{2})^J \sum_{(\sigma_j=0,1)}^{\infty} \int_0^{\infty} \prod_{j=1}^J \delta(x_j - \sigma_j x'_j) n_{i-1}(x', T_A, t) dx', \quad i \geq 3. \quad (\text{A24})$$

In equations (A23) and (A24), the summation symbol means that every σ_j is summed independently over the two values 0 and 1. Note that we have not assumed that the different chromosomes were equally labeled initially. In fact, we can easily choose $n(x, \mu, 0)$ to represent situations in which some chromosomes have no label at all. Considerable simplification may, however, be achieved by assuming that all chromosomes which are labeled have the same amount of label and then let K , the number labeled, vary among cells. It is then not necessary to introduce the separate variables (x_1, \dots, x_J) but treat each group of cells with a given K separately and average over K . We outline below such a modified treatment.

Let x_0 be the amount of tritium measured in grain counts in one labeled chromatid at $t = 0$. Then the first generation cells with K labeled chromo-

some will have $2Kx_0$ units of tritium. Subsequent generations with K labeled chromosomes will have only Kx_0 units of tritium. Let $n_i(K', \mu, t; K)$ and $Q_i(K', a, t; K)$ be i th generation cell densities with $K'x_0$ tritium units which are all descendents of first generation cells with K labeled chromosomes. The first argument of n_i and Q_i denotes the number of labeled chromosomes at time t , while the last argument denotes the number of labeled chromosomes present at $t = 0$ in the original labeled cell of which these are i th generation descendents. It then follows that

$$n_1(K', \mu, t; K) = \left[n_1(K', \mu - t, 0; K) + \alpha \int_0^\infty Q_1(K', a', t - \mu; K) da' \right] \times \delta_{K', 2K} e^{-\beta_1 t}, \quad (\text{A25})$$

$$Q_1(K', a, t; K) = Q_1(K', a - t, 0; K) \delta_{K', 2K} e^{-(\alpha + \beta_0)t}, \quad (\text{A26})$$

$$n_2(K', \mu, t; K) = \left[2\delta n_1(2K', T_A, t - \mu; K) + \alpha \int_0^\infty Q_2(K', a', t - \mu; K) da' \right] e^{-\beta_1 \mu}, \quad (\text{A27})$$

$$Q_2(K', a, t; K) = 2(1 - \delta) n_1(2K', T_A, t - a; K) e^{-(\alpha + \beta_0)a}. \quad (\text{A28})$$

For later generations it follows on the assumption of random division of the labeled chromosome between the daughter cells that

$$n_i(K', \mu, t; K) = n_i(K', 0, t - \mu; K) e^{-\beta_1 \mu}, \quad i \geq 3, \quad (\text{A29})$$

$$Q_i(K', a, t; K) = Q_i(K', 0, t - a; K) e^{-(\alpha + \beta_0)a}, \quad i \geq 3, \quad (\text{A30})$$

with

$$n_i(K', 0, t; K) = 2\delta \sum_{K''=K'}^K \binom{K''}{K'} n_{i-1}(K'', T_A, t; K) - \alpha \int_0^\infty Q_i(K', a', t; K) da', \quad (\text{A31})$$

$$Q_i(K', 0, t; K) = 2(1 - \delta) \sum_{K''=K'}^K \binom{K''}{K'} n_{i-1}(K'', T_A, t; K), \quad (\text{A32})$$

and $\binom{K''}{K'}$ denotes the binomial coefficient. If we let $P(K)$ be the fraction of cells with K labeled chromosomes at $t = 0$, i.e. at the time of the pulse labeling, then the density of cells in the i th generation with $K'x_0$ tritium units at time t is

$$n_i(K', \mu, t) = \sum_{K=0}^J P(K) n_i(K', \mu, t; K), \quad i \geq 1, \quad (\text{A33})$$

and

$$Q_i(K, a, t) = \sum_{K=0}^J P(K) Q_i(K', a, t; K), \quad i \geq 1, \quad (\text{A34})$$

where $\sum_{K=0}^J P(K) = 1$ and J is the total number of chromosomes in the cell.

Integrating over μ and summing over i then yields for the number of cells Ψ with Kx_0 units of tritium time t the expression

$$\Psi(K, t) = \sum_{i=1}^{\infty} \left[\int_0^{T_A} n_i(K, \mu, t) d\mu + \int_0^{\infty} Q_i(K, a, t) da \right]. \quad (\text{A35})$$

The grain count distribution is now given, as in (A6), by

$$p(k, t) = N^{-1} \sum_{K=0}^{2J} \frac{(Kx_0)^k}{k!} e^{-Kx_0} \Psi(K, t). \quad (\text{A36})$$

Appendix 3

LONG TIME BEHAVIOR OF THE CELL DENSITIES

The long time behavior of $n(\mu, t)$ and $Q(a, t)$ for specified $n(\mu, 0)$, $Q(a, 0)$ may be readily obtained through the use of the Laplace transform. Let

$$\tilde{n}(\mu, s) = \int_0^{\infty} e^{-st} n(\mu, t) dt, \quad (\text{A37})$$

$$\tilde{Q}(a, s) = \int_0^{\infty} e^{-st} Q(a, t) dt. \quad (\text{A38})$$

Equations (1) to (4) with $\dot{\mu} = 1$, $m = T_A$, now become

$$s\tilde{n}(\mu, s) + \frac{\partial}{\partial \mu} \tilde{n}(\mu, s) = n(\mu, 0) - \beta_1 \tilde{n}(\mu, s), \quad (\text{A39})$$

$$s\tilde{Q}(a, s) + \frac{\partial}{\partial a} \tilde{Q}(a, s) = Q(a, 0) - (\alpha + \beta_0) \tilde{Q}(a, s), \quad (\text{A40})$$

$$\tilde{Q}(0, s) = 2\delta \tilde{n}(T_A, s), \quad (\text{A41})$$

$$\tilde{n}(0, s) = 2(1 - \delta) \tilde{n}(T_A, s) + \alpha \int_0^{\infty} \tilde{Q}(a, s) da. \quad (\text{A42})$$

These equations have the solution

$$\begin{aligned} \tilde{n}(\mu, s) = & \left\{ 2 \left[\frac{s + \alpha + \beta_0 - \delta(s + \beta_0)}{s + \alpha + \beta_0} \right] \tilde{n}(T_A, s) + \alpha \int_0^a \int_0^a e^{-(s + \alpha + \beta_0)(a - a')} \times \right. \\ & \left. \times Q(a', 0) da' da + \int_0^{\mu} e^{(s + \beta_1)\mu'} n(\mu', 0) d\mu' \right\} e^{-(s + \beta_1)\mu}, \quad (\text{A43}) \end{aligned}$$

$$\tilde{Q}(a, s) = \left[2\delta\tilde{n}(T_A, s) + \int_0^a e^{(s+\alpha+\beta_0)a'} Q(a', 0) da' \right] e^{-(s+\alpha+\beta_0)a}, \quad (A44)$$

with

$$\tilde{n}(T_A, s) = \left\{ \alpha \int_0^\infty \int_0^a e^{-(s+\alpha+\beta_0)(a-a')} Q(a', 0) da' da + \int_0^{T_A} e^{(s+\beta_1)\mu} n(\mu, 0) d\mu \right\} (s+\alpha+\beta_0) / D(s), \quad (A45)$$

$$D(s) = (s+\alpha+\beta_0)e^{(s+\beta_1)T_A} - 2[s+\alpha+\beta_0-\delta(s+\beta_0)]. \quad (A46)$$

The long time behavior of the cell densities is determined by the zeros of $D(s)$. For $n(T_A, t)$ to approach a steady or oscillatory state and thus maintain a bounded non-vanishing cell population, it is necessary and sufficient that $D(s)$ not vanish for $\text{Re } s > 0$ and be $o(s)$ as $s \rightarrow 0$. The latter requirement leads to equation (18). The first requirement implies that $D(s) > 0$ for all $s \geq 0$. Now

$$D'(s) = e^{(\beta_1+s)T_A} [1 + T_A(s+\alpha+\beta_0)] - 2(1-\delta), \quad (A47)$$

$$D''(s) = T_A e^{(\beta_1+s)T_A} [2 + T_A(s+\alpha+\beta_0)] > 0 \quad \text{for } s \geq 0, \quad (A48)$$

where the prime denotes differentiation with respect to argument. Hence the existence of a bounded non-vanishing cell population as $t \rightarrow \infty$ requires that $D'(0) > 0$, or

$$e^{\beta_1 T_A} [1 + T_A(\alpha + \beta_0)] - 2(1 - \delta) > 0. \quad (A49)$$

When (18) is not satisfied and $D(0) < 0$, the cell population will grow exponentially, while $D(0) = 0$ and $D'(0) = 0$ imply a growth which is linear in time.

While we have not proven rigorously the sufficiency of the above conditions for the asymptotic approach to a bounded population they do appear to be sufficient. It is clear that when the final state is stationary in time the cell densities will be given by

$$\lim_{t \rightarrow \infty} n(\mu, t) = \lim_{s \rightarrow 0} s\tilde{n}(\mu, s) = \tilde{n}(\mu), \quad (A50)$$

$$\lim_{t \rightarrow \infty} Q(a, t) = \lim_{s \rightarrow 0} s\tilde{Q}(a, s) = \tilde{Q}(a), \quad (A51)$$

with $\tilde{n}(\mu)$ and $\tilde{Q}(a)$ given in section 2. The final total cell population N will be related to the initial values $n(\mu, 0)$ and $Q(a, 0)$ by means of the relation expressing the equality in the steady state of the birth rate $\tilde{n}(T_A)$ and the loss rate $(\beta_0 N_0 + \beta_1 N_A)$, or

$$N = \tilde{n}(T_A) \left/ \left(\beta_0 \frac{N_0}{N} + \beta_1 \frac{N_A}{N} \right) \right., \quad (A52)$$

where N_0/N and N_A/N are given by equations (23) to (24) and

$$\bar{n}(T_A) = \lim_{s \rightarrow 0} s \bar{n}(T_A, s). \quad (\text{A53})$$

Thus

$$N = (\alpha + \beta_0)[\alpha + \beta_0 - 2\delta(\beta_0 - \beta_1)] \left\{ \alpha \int_0^\infty \int_0^a e^{-(\alpha + \beta_0)(a-a')} Q(a', 0) da' da + \right. \\ \left. + \int_0^{T_A} e^{\beta_1 \mu} n(\mu, 0) d\mu \right\} / 2\beta_1 [\delta\alpha + T_A(\alpha + \beta_0)(\alpha + \beta_0 - \beta_0\delta)]. \quad (\text{A54})$$

Using (A54) it is easy to compute the ultimate fraction of cells which are the descendants of cells in some particular phase, say the S phase, at $t = 0$ when the cell population is in a steady state.