BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 32, 1970

# ON THE AVERAGE CELLULAR VOLUME IN SYNCHRONIZED CELL POPULATIONS*

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As was done by Sinclair and Ross (1969), we consider a cellular population that consists initially (at time zero) of $N_0$ newborn cells, all with the same volume, $v_0$. It is assumed that the occurrence of cell division is determined only by a cell's age, and not by its volume. The frequency function of interdivision times, $\tau$, is denoted by $f(\tau)$. If cell death is negligible, the expected number of cells, $N(t)$, will increase according to the laws of a simple age-dependent branching process. The expression for $N(t)$ is obtained as a sum over all generations; the $\nu$th term of this sum, in turn, is a multiple convolution integral, reflecting the life history of $\nu$th generation cells (i.e., the lengths of the $\nu$ successive interdivision periods plus the age of the cell at time $t$). Assuming that cell volume is a given function of cell age, e.g., linear or exponential, and that cellular volume is exactly halved at each division, it is possible to calculate the volume of a cell with a given life history, and thus the average cellular volume of the whole population as a function of time. If at time zero the volumes differ from cell to cell, the final equation must be modified by averaging over initial volumes. In the case of linear volume increase with age, a very simple asymptotic expression is found for the average cellular volume as $t \to \infty$. The case of exponential volume increase with age also leads to a simple asymptotic formula, but the resulting volume distribution is unstable.

The mean cellular volume at birth and the second moment of the volume distribution can be calculated in a similar manner.

*1. Introduction.* Recently there has been a considerable amount of work on the volume spectrum of growing cell populations. One question of interest in these investigations was whether the volume increase of individual cells with age

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* Work supported by the U.S. Atomic Energy Commission.

conforms more closely to the exponential or to the linear mode. Earlier studies along these lines include the remarkable paper by Scherbaum and Rasch (1957), but it was only the subsequent improvement in Coulter counter techniques that made it possible to obtain accurate volume spectra.

The present investigation arose in connection with the work of Sinclair and Ross (1969), who were interested in the mean cell volume as a function of time for a population that initially (at time zero) consists of $N_0$ *newborn* cells, all with the same volume, $v_0$. It is assumed that cell division is determined only by the cell's age and not by its volume. The probability density function for the distribution of interdivision times, $\tau$, is denoted by $f(\tau)$. It is further supposed that cell death is negligible, and so the number of cells in the population will increase according to the laws of a simple age-dependent branching process (Harris, 1959).

Two additional assumptions are made regarding cellular volume:

(a) The volume of each cell, $v$, is a given function of its age, $a$, and of its volume at birth, $v_b$:

$$v = \psi(a, v_b); \tag{1}$$

(b) When a cell divides, each of the two daughters has the same birth volume, equal to one-half that of the mother cell.

Special cases of equation (1), which Sinclair and Ross had in mind, are those of linear and exponential volume increase with age:

$$v = v_b + ra \quad (r = \text{constant} > 0), \tag{2}$$

and

$$v = v_b e^{ka} \quad (k = \text{constant} > 0). \tag{3}$$

Let $N(t)$ be the expected number of cells in the population at time $t$ and put

$$M(t) = N(t)/N_0. \tag{4}$$

The function $M(t)$ satisfies the renewal equation [Harris, 1959, eq. (2)]

$$M(t) = \phi(t) + 2\{M * f\}(t), \tag{5}$$

where

$$\phi(t) = 1 - \int_{\tau=0}^{t} f(\tau)\, d\tau = \int_{\tau=t}^{\infty} f(\tau)\, d\tau, \tag{6}$$

and $\{M * f\}(t)$, the convolution of the two functions $M$ and $f$, is defined by

$$\{M * f\}(t) = \int_{\tau=0}^{t} f(\tau) M(t - \tau)\, d\tau. \tag{7}$$

The solution of equation (5) can be written as an infinite series which converges for every finite value of $t$ (Harris, 1963, p. 161). It is as follows:

$$M(t) = \phi(t) + \sum_{\nu=1}^{\infty} 2^{\nu}\{\phi * f_{\nu}\}(t). \tag{8}$$

Here $f_\nu$ stands for the $\nu$ fold convolution of the function $f$, i.e.,

$$f_1(t) = f(t)$$

and

$$f_{\nu+1}(t) = \{f * f_\nu\}(t) \quad \text{for } \nu = 1, 2, 3, \ldots$$

Putting

$$F_\nu(t) = \int_{x=0}^{t} f_\nu(x)\, dx = \{1 * f_\nu\}(t), \tag{9}$$

we see that

$$\{f * F_\nu\} = \{f * 1 * f_\nu\} = F_{\nu+1} \tag{10}$$

and

$$\{\phi * f_\nu\} = F_\nu - F_{\nu+1}. \tag{11}$$

Hence, equation (8) can be written in the form

$$M(t) = 1 + \sum_{\nu=1}^{\infty} 2^{\nu-1} F_\nu(t), \tag{12}$$

from which it follows that

$$\dot{M}(t) = \sum_{\nu=1}^{\infty} 2^{\nu-1} f_\nu(t), \tag{13}$$

where

$$\dot{M}(t) = dM(t)/dt.$$

Also notice that in this model every cell disappearing from the population at mitosis is immediately replaced by two new cells of age zero and, therefore, the birth rate, $\alpha(t)$, must be equal to twice the net increase in number of cells:

$$\alpha(t) = 2N_0\dot{M}(t). \tag{14}$$

Sinclair and Ross (1969) used a special form of equation (12). Let $\bar{\tau}$ be the average generation time (assumed to be finite):

$$\bar{\tau} = \int_{\tau=0}^{\infty} \tau f(\tau)\, d\tau. \tag{15}$$

Suppose that the probability density $f(\tau)$ has a relatively small coefficient of variation and that the time, $t$, is not too large, so that no appreciable overlapping of generations occurs. Consider values of the variable $t$ restricted to a sufficiently small neighborhood of $s\bar{\tau}$, where $s$ is a positive integer. Then, in equation (12),

$$F_\nu(t) \approx 1 \quad \text{for } \nu < s, \qquad F_\nu(t) \approx 0 \quad \text{for } \nu > s,$$

and

$$M(t) \approx 1 + \sum_{\nu=1}^{s-1} 2^{\nu-1} + 2^{s-1}F_s(t) = 2^{s-1}[1 + F_s(t)],$$

or using the relation $1 = F_s(t) + \int_{x=t}^{\infty} f_s(x)\,dx$,

$$N(t) \approx N_0 \cdot 2^{s-1}\left[2\int_{x=0}^{t} f_s(x)\,dx + \int_{x=t}^{\infty} f_s(x)\,dx\right], \tag{16}$$

if $t \approx s\bar{\tau}$. This is Sinclair and Ross' equation (2).

*2. The Mean Cellular Volume.* Obviously, in equation (8) the expected number of cells appears as a sum over generations, the $\nu$th generation consisting of all the cells that have undergone exactly $\nu$ divisions since time $t = 0$. The term $\phi(t)$ represents the fraction of the original cells ("zeroth generation") that have not yet divided at time $t$. The quantity

$$N_0 \cdot 2^\nu \phi(a) f_\nu(t - a)\,da \tag{17}$$

is the expected number of cells present at time $t$, belonging to the $\nu$th generation ($\nu = 1, 2, 3, \ldots$), which have ages between $a$ and $a + da$.

The *life history* of a $\nu$th generation cell is specified by its $\nu$ interdivision times, $\tau_1, \tau_2, \ldots, \tau_\nu$ and by its age, $a$. Alternatively, one may use $a$ and the time periods

$$T_\lambda = \sum_{\mu=1}^{\lambda} \tau_\mu, \quad \lambda = 1, 2, \ldots, \nu,$$

so that $T_\lambda$ is the time at which the $\lambda$th division has occurred. These variables must satisfy the conditions

$$0 < T_\lambda < T_{\lambda+1} \quad \text{for } \lambda = 1, 2, \ldots, (\nu - 1),$$

and $T_\nu = t - a$ (see Fig. 1 for $\nu = 4$).

Writing the complete expression for the convolution integral, $f_\nu(t - a)$, in (17) it becomes evident that the expected number of $\nu$th generation cells present at

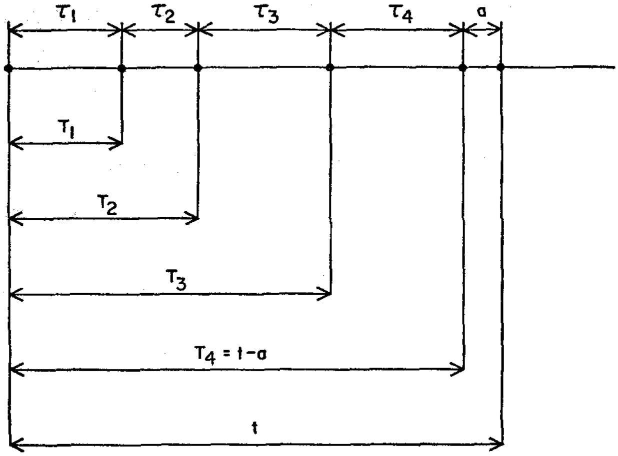

Figure 1.

time $t$, which have age $a$ (within $da$) and which, for $\lambda = 1, 2, \ldots, (\nu - 1)$, have given values of the $T_\lambda$'s (within $dT_\lambda$), is equal to

$$N_0 2^\nu \phi(a) f(T_1) f(T_2 - T_1) f(T_3 - T_2) \ldots$$
$$\ldots f(T_{\nu-1} - T_{\nu-2}) f(T_\nu - T_{\nu-1})\, dT_1\, dT_2 \ldots dT_{\nu-1}\, da \qquad (18)$$
$$[\nu = 2, 3, 4, \ldots],$$

where $T_\nu = t - a$. If $\nu = 1$ this expression reduces to $2N_0\phi(a)f(t - a)\, da$. Now, using equation (1) and halving the volume at each division, the volume of a $\nu$th generation cell with a given life history can be expressed as a function of the quantities $v_0, T_1, T_2, \ldots, T_{\nu-1}, t$, and $a$, say $\eta_\nu(v_0, t, a; T_1, T_2, \ldots, T_{\nu-1})$. Therefore the mean volume as a function of time, $\langle v(t)\rangle$, is obtained by multiplying the expression (18) with $\eta_\nu$, integrating over $a, T_1, T_2, \ldots, T_{\nu-1}$, summing over all generations, and finally dividing by $N(t)$. It is convenient to introduce $T_\nu = t - a$ as a new variable of integration instead of $a$. The resulting equation for $\langle v(t)\rangle$ is

$$\langle v(t)\rangle = \frac{1}{M(t)}\Bigg[\phi(t)\psi(t, v_0) + 2\int_{T_1=0}^{t} \phi(t - T_1) h_1(v_0, t, T_1)\, dT_1 +$$
$$\sum_{\nu=2}^{\infty} 2^\nu \int_{T_\nu=0}^{t} \phi(t - T_\nu) \int_{T_{\nu-1}=0}^{T_\nu} f(T_\nu - T_{\nu-1}) \int_{T_{\nu-2}=0}^{T_{\nu-1}} f(T_{\nu-1} - T_{\nu-2}) \ldots \qquad (19)$$
$$\ldots \int_{T_3=0}^{T_4} f(T_4 - T_3) \int_{T_2=0}^{T_3} f(T_3 - T_2) \int_{T_1=0}^{T_2} f(T_2 - T_1)$$
$$\times f(T_1) h_\nu(v_0, t; T_1, \ldots, T_\nu)\, dT_1\, dT_2 \ldots dT_\nu\Bigg],$$

in which $h_\nu$ is the same as $\eta_\nu$ with $t - T_\nu$ substituted for $a$.

To obtain the functions $h_\nu$, define the equations $v_j$ recursively by the equations

$$\begin{aligned} v_1 &= (1/2)\psi(\tau_1, v_0), \\ v_j &= (1/2)\psi(\tau_j, v_{j-1}) \quad \text{for } j \geqslant 2, \end{aligned} \tag{20}$$

so that $v_j$ is the cellular volume immediately after the $j$th division. For a $\nu$th generation cell present in the population at time $t = a + \sum_{\lambda=1}^{\nu} \tau_\lambda$, the volume will be $\psi(a, v_\nu)$. In this expression replace each $\tau_j$ by $T_j - T_{j-1}$ (with $T_0 = 0$) and $a$ by $t - T_\nu$; this gives

$$h_\nu(v_0, t; T_1, T_2, \ldots, T_\nu).$$

In particular, for $\nu = 0$, 1 and 2:

$$h_0 = \psi(a, v_0) = \psi(t, v_0)$$

(time and age being the same for cells of the zeroth generation),

$$h_1 = \psi[a, \tfrac{1}{2}\psi(\tau_1, v_0)] = \psi[t - T_1, \tfrac{1}{2}\psi(T_1, v_0)], \quad 0 < T_1 < t,$$

$$h_2 = \psi\{t - T_2, \tfrac{1}{2}\psi[T_2 - T_1, \tfrac{1}{2}\psi(T_1, v_0)]\}, \quad 0 < T_2 < t, \; 0 < T_1 < T_2.$$

For example, in the case of linear volume increase with age (eq. 2) one finds

$$v_\nu = \frac{v_0}{2^\nu} + \frac{r}{2^\nu} \sum_{\lambda=1}^{\nu} 2^{\lambda-1}\tau_\lambda \quad (\nu = 1, 2, 3, \ldots),$$

and hence

$$\begin{aligned} h_0 &= v_0 + rt, \\ h_\nu &= \frac{v_0}{2^\nu} + rt - \frac{r}{2^\nu} \sum_{\lambda=1}^{\nu} 2^{\lambda-1} T_\lambda \quad (\nu = 1, 2, 3, \ldots). \end{aligned} \tag{21}$$

It is also possible to derive equation (19) starting from Bell and Anderson's (1967) theory for cell growth and division. However, since the relevant calculations are rather lengthy, though not difficult, they will not be presented here.

*3. "Dispersionless" and Exponential Growth of Single Cells.* Equation (19) can be written in a more compact form if the function $\psi(a, v_b)$ is of the "dispersionless" type, as defined by Anderson *et al.* (1969, p. 261). This means that

$$\psi(a, v_b) = v_b \cdot g(a), \tag{22}$$

$g$ being a function of age only with $g(0) = 1$.

In that case, $h_0 = v_0 g(t)$,

$$h_1 = \frac{v_0}{2} g(T_1) g(t - T_1),$$

and

$$h_\nu = \frac{v_0}{2^\nu} g(t - T_\nu) g(T_1) g(T_2 - T_1) \ldots g(T_\nu - T_{\nu-1}) \quad \text{for } \nu \geqslant 2.$$

Thus equation (19) becomes

$$\langle v(t) \rangle = \frac{v_0}{M(t)} \left[ \rho(t) + \sum_{\nu=1}^{\infty} \{\rho * \zeta_\nu\}(t) \right], \tag{23}$$

where

$$\rho(t) = g(t)\phi(t), \tag{24}$$

$$\zeta(t) = g(t) f(t), \tag{25}$$

and $\zeta_\nu$ is the $\nu$fold convolution of the function $\zeta$.

The case of exponential volume increase with age (eq. (3)) is obtained by taking

$$g(a) = e^{ka}$$

in equation (22). This gives

$$h_\nu = \frac{v_0}{2^\nu} e^{kt}, \tag{26}$$

so that the volume of a $\nu$th generation cell is completely independent of its life history. From equations (19) or (23) one then finds

$$\langle v(t) \rangle = \frac{v_0 e^{kt}}{M(t)} \left[ \phi(t) + \sum_{\nu=1}^{\infty} \{\phi * f_\nu\}(t) \right] \cdot$$

Using equation (11), however, it is seen that the square bracket in this expression has the value 1, and therefore

$$\langle v(t) \rangle = \frac{v_0 e^{kt}}{M(t)}, \tag{27}$$

if $\psi(a, v_b)$ is given by equation (3).

Now it is known that for large values of $t$ the function $M(t)$ becomes approximately equal to $M_0 e^{ct}$, where the specific growth rate, $c$, is uniquely determined by the relation

$$2 \int_{x=0}^{\infty} e^{-cx} f(x)\, dx = 1, \tag{28}$$

and

$$M_0 = \frac{1}{2c\theta}, \tag{29}$$

with

$$\theta = 2 \int_{x=0}^{\infty} xe^{-cx} f(x)\, dx, \tag{30}$$

(eqs. (5) and (6) of Harris, 1959). These results follow from Feller's theorems on the renewal equation (Feller, 1941; see also Harris, 1963, pp. 161–163). Therefore, if $t \to \infty$, the right-hand side of equation (27) tends to a finite limit, $\langle v(\infty)\rangle > 0$, only if

$$k = c. \tag{31}$$

In fact, Bell and Anderson (1967) have shown that $k$ must be equal to $c$ ($\alpha = f_1$ in their notation) if there is no cell death and if the rate of volume increase with age is proportional to volume itself.

However, even with $k = c$ the volume distribution is asymptotically unstable, because it can be shown that its second moment becomes infinite as $t \to \infty$. More generally, a similar result holds for the case of "dispersionless" growth: the first and second moments of the volume distribution cannot both tend to a finite, nonzero limit for $t \to \infty$. This will be discussed at greater length in a subsequent paper (Trucco and Bell, 1970).

*4. Asymptotic Behavior of* $\langle \mathrm{v}(t)\rangle$ *for Linear Volume Increase with Age.* Consider instead the case of linear volume increase (eq. (2)). From equation (19) one can obtain a simple expression for the limiting value of $\langle v(t)\rangle$ as $t \to \infty$. It will be assumed that the function $f(t)$ is sufficiently well behaved, so that Feller's theorems (1941) are applicable, which is a very mild restriction. In particular, $f(t)$ must be regular at the origin ($F_1(0) = 0$).

It can easily be shown that

$$\sum_{\lambda=1}^{\nu} 2^{\lambda-1} T_\lambda = (2^\nu - 1)T_1 + \sum_{\lambda=2}^{\nu} (2^\nu - 2^{\lambda-1})(T_\lambda - T_{\lambda-1}) \quad (\nu = 2, 3, 4, \ldots). \tag{32}$$

Define the function $q(t)$ as follows:

$$q(t) = t \cdot f(t); \tag{33}$$

also notice that

$$\sum_{\lambda=1}^{\nu} (2^\nu - 2^{\lambda-1}) = 1 + (\nu - 1)2^\nu. \tag{34}$$

Inserting the expression given by equation (21) for $h_\nu$ into equation (19), and using the results (32) and (34), one finds

$$\langle v(t)\rangle = \frac{1}{M(t)}\left\{v_0\left[\phi(t) + \sum_{\nu=1}^{\infty}\{\phi * f_\nu\}(t)\right] + rtM(t)\right.$$
$$\left. -r\{\phi * q\}(t) - r\sum_{\nu=2}^{\infty}\left[(1 + (\nu - 1)2^\nu)\{\phi * f_{\nu-1} * q\}(t)\right]\right\}.$$

With the help of equation (11) this becomes

$$\langle v(t)\rangle = \frac{1}{M(t)}\left[v_0 + rtM(t) - r\sum_{\nu=1}^{\infty}\nu\cdot 2^{\nu-1}\{F_{\nu-1} * q\}(t)\right], \tag{35}$$

it being understood that

$$F_0(t) = 1. \tag{36}$$

Using equation (12) we can write

$$\langle v(t)\rangle = \frac{1}{M(t)}[v_0 + rt + rS(t)], \tag{37}$$

where

$$S(t) = \sum_{\nu=1}^{\infty} 2^{\nu-1}[tF_\nu(t) - \nu\{F_{\nu-1} * q\}(t)]. \tag{38}$$

Now, putting

$$tF_\nu(t) = \gamma_\nu(t),$$

one has the simple identity

$$\int_{u=0}^{t} f(u)(t-u)F_\nu(t-u)\,du = t\int_{u=0}^{t} f(u)F_\nu(t-u)\,du$$
$$- \int_{u=0}^{t} uf(u)F_\nu(t-u)\,du,$$

or

$$\{f * \gamma_\nu\}(t) = tF_{\nu+1}(t) - \{q * F_\nu\}(t), \tag{39}$$

and from this it follows easily that the function $S(t)$ satisfies the renewal equation

$$S(t) = B(t) + 2\{f * S\}(t), \tag{40}$$

with

$$B(t) = tF_1(t) - \int_{x=0}^{t} xf(x)\,dx = \int_{x=0}^{t} F_1(x)\,dx. \tag{41}$$

Feller's theorem then tells us that for large value of $t$

$$S(t) \approx S_0 e^{ct}. \tag{42}$$

Here $c$ is again determined by equation (28) and

$$S_0 = (1/\theta) \int_{t=0}^{\infty} e^{-ct} B(t)\,dt = 1/(2c^2\theta). \tag{43}$$

Consequently,

$$\lim_{t\to\infty} [S(t)/M(t)] = 1/c,$$

and so

$$\lim_{t\to\infty} \langle v(t)\rangle \equiv \langle v(\infty)\rangle = r/c. \tag{44}$$

This result is not unexpected because it can be shown (Bell and Anderson, 1967) that for a population in steady state of exponential growth the mean cellular volume is indeed equal to $r/c$ if equation (2) holds and if cell death is negligible. Intuitively, assuming that equation (2) is valid and that $N(t) \approx N_0 e^{ct}$, we can write the total volume increase in the population during the time interval $dt$ either as $N \cdot r\,dt$ (number of cells times volume increase of a single cell) or as $\langle v\rangle\,dN = \langle v\rangle cN\,dt$ (mean volume times increase in cell number), from which it follows that $r = c\langle v\rangle$.

*5. The Mean Cell Volume at Birth.* The mean cellular volume at birth, $\langle v_b(t)\rangle$, can be calculated in much the same way as $\langle v(t)\rangle$. The total expected number of newborn cells (cells with age between 0 and $dt$) present in the population at time $t$ is $\alpha(t)\,dt$, and the expected number of such cells belonging to the $\nu$th generation is obtained from (17) by setting $a = 0$, $\phi(0) = 1$. Cells of the zeroth generation have an age $> 0$ if $t > 0$, and the function $\alpha(t)$ is given by equation (14). Proceeding as in the derivation of equation (19) one finds, for $t > 0$:

$$\begin{aligned}
\langle v_b(t)\rangle = \frac{1}{M(t)} \Bigg[ & f(t) w_1(v_0, t) + \sum_{\nu=2}^{\infty} 2^{\nu-1} \int_{T_{\nu-1}=0}^{t} f(t - T_{\nu-1}) \\
& \times \int_{T_{\nu-2}=0}^{T_{\nu-1}} f(T_{\nu-1} - T_{\nu-2}) \int_{T_{\nu-3}=0}^{T_{\nu-2}} f(T_{\nu-2} - T_{\nu-3}) \cdots \int_{T_3=0}^{T_4} f(T_4 - T_3) \\
& \times \int_{T_2=0}^{T_3} f(T_3 - T_2) \int_{T_1=0}^{T_2} f(T_2 - T_1) f(T_1)\, w_\nu(v_0, t;\, T_1, T_2, \ldots T_{\nu-1}) \\
& \times dT_1\, dT_2 \ldots dT_{\nu-1} \Bigg],
\end{aligned} \tag{45}$$

where $w_\nu$ is equal to $h_\nu$ with $T_\nu$ replaced by $t$.

For the exponential case (eq. (3)) this gives

$$\langle v_b(t)\rangle = \frac{v_0 e^{kt}}{2\dot{M}(t)}\, G(t), \tag{46}$$

with

$$G(t) = \sum_{\nu=1}^{\infty} f_\nu(t). \tag{47}$$

The function $G(t)$ is a solution of the renewal equation

$$G(t) = f(t) + \{f * G\}(t),$$

and therefore tends to the finite limit $1/\bar{\tau}$ for $t \to \infty$. Differentiating both sides of equation (5) it is found that

$$\dot{M}(t) = f(t) + 2\{f * \dot{M}\}(t),$$

and thus, for large $t$,

$$\dot{M}(t) \approx \frac{e^{ct}}{2\theta} = cM_0 e^{ct}.$$

In the linear case (eq. (2)) one has

$$w_1 = \frac{v_0}{2} + \frac{rt}{2},$$

$$w_\nu = \frac{v_0}{2^\nu} + \frac{rt}{2} - \frac{r}{2^\nu}\sum_{\lambda=1}^{\nu-1} 2^{\lambda-1}T_\nu \quad \text{for } \nu = 2, 3, 4, \ldots,$$

and it is easy to see that $\langle v_b(t)\rangle$ tends to a finite value, $\langle v_b(\infty)\rangle$, as $t \to \infty$. The calculations are very similar to those used in deriving equation (44); the result is

$$\langle v_b(\infty)\rangle = 2r\int_{x=0}^{\infty} xe^{-cx}f(x)\,dx = r\theta. \tag{48}$$

Once again, equation (48) can be obtained formally from Bell's theory for a cellular population in steady state of exponential growth (Bell, 1968). In the following assume that (a) cellular volume increases according to equation (2), (b) Bell's function $D$ is equal to zero (no cell death), and (c) his function $P$ depends only on age but not on volume. Then Bell's quantity $\tilde{P}$ is the same as $f$. Let $m(v)$ be the distribution of birth volumes when the population has reached a steady state of balanced exponential growth. The integral equation for $m(v)$, equation (24) of Bell, 1968, now becomes, in our notation

$$m(v) = 4 \int_{a=0}^{2v/r} m(2v - ra)f(a)e^{-ca}\, da. \tag{49}$$

Multiplying both sides of this equation with $v$, integrating over $v$, from $v = 0$ to $v = \infty$, and interchanging the order of the two integrations, one finds

$$\begin{aligned}\langle v_b(\infty)\rangle &= \int_{v=0}^{\infty} vm(v)\, dv = r \int_{a=0}^{\infty} e^{-ca}f(a) \int_{v=ra/2}^{\infty} vm(2v - ra)\, dv\, da \\ &= \int_{a=0}^{\infty} e^{-ca}f(a) \int_{u=0}^{\infty} (u + ra)m(u)\, du\, da = (1/2)\langle v_b(\infty)\rangle + \frac{r\theta}{2},\end{aligned}$$

or

$$\langle v_b(\infty)\rangle = r\theta.$$

Here the relations

$$\int_{a=0}^{\infty} e^{-ca}f(a)\, da = \tfrac{1}{2} \quad \text{and} \quad \int_{u=0}^{\infty} m(u)\, du = 1$$

have been used.

The quantity $\theta$ is the first moment of the "carrier density" $2e^{-c\tau}f(\tau)$. It can be shown that, in steady state of exponential growth without cell death, $\theta$ is the average age at which the cells divide. Also, $\theta$ is slightly smaller than the doubling time, $T$, of the population [$T = (1/c) \ln 2$], and $T$ itself is smaller than the average generation time, $\bar{\tau}$. Thus: $\theta < T < \bar{\tau}$ (Trucco, 1965, page 469†; Brockwell and Trucco, 1970, Appendix D).

Equation (48) says that, as $t \to \infty$, the mean volume at birth becomes equal to the volume increment during the time interval $\theta$, a result which is intuitively very plausible. Notice that according to equation (2) the average cell volume of the zeroth generation will be increased by approximately $r\bar{\tau}$, which could be much more or much less than $v_0$ (of course, in real populations there may not be any cells with extremely large or extremely small volumes, but this is irrelevant for a discussion of the mathematical model). Subsequently, however, the

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† In Trucco, 1965, the probability density of interdivision times is denoted by $\delta$ and the "carrier density" by $f$. Also, the quantities $\bar{l}$ and $D$ of that paper correspond, respectively, to $\theta$ and $\bar{\tau}$. In Brockwell and Trucco, 1970, we write $Z$ instead of $\theta$ and $D$ for $\bar{\tau}$.

mean volumes, $\langle v(t)\rangle$ and $\langle v_b(t)\rangle$, will gradually reach their asymptotic values. Equations (44) and (48) show that these limiting values do not depend on $v_0$.‡

*6. The Second Moments.* To obtain the second moments of the volume distribution as functions of time, simply replace $h_v$ by $h_v^2$ in equation (19) or $w_v$ by $w_v^2$ in equation (45). The case of dispersionless growth will be discussed in part II of this paper (Trucco and Bell, 1970). If, on the other hand, we assume linear volume increase with age, we can derive simple asymptotic expressions for the second moments as $t \to \infty$. The calculations are somewhat more involved, but basically not very different from those already given. For simplicity we shall consider only the case of birth volumes. Let $\mu_2(t)$ be the second moment for the distribution of volumes at birth, and denote by $m_2$ the second moment of the carrier density:

$$m_2 = 2\int_{x=0}^{\infty} x^2 e^{-cx} f(x)\,dx.$$

Then it can be shown that

$$\mu_2(\infty) = (r^2/3)(m_2 + 2\theta^2). \tag{50}$$

This also follows from multiplying both sides of equation (49) by $v^2$ and then integrating over $v$.

*7. Discussion.* It has been assumed up to this point that all cells with a given age and volume change their volume at the same rate, say $F(a, v)$. Actually, the quantity $F(a, v)$ must be understood as the mean rate of volume increase for such cells. In fact, Fredrickson *et al.* (1967) have shown that although the true rate of change may vary from cell to cell, it is only the average rate that appears in the final equations. A similar remark was made by Harvey *et al.* (1967, p. 616) after their derivation of the Collins-Richmond equation.

The assumption that all the cells in the population have age zero initially is fulfilled with good approximation if the cells are synchronized by selecting those in mitosis (as was done in the experiments of Sinclair and Ross). On the other hand, it will certainly not be true that every cell has the same initial volume, $v_0$.§ If $\varepsilon(v_0)\,dv_0$ is the fraction of cells with initial volumes between $v_0$ and $v_0 + dv_0$, so that

$$\int_{v_0=0}^{\infty} \varepsilon(v_0)\,dv_0 = 1,$$

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‡ It would appear from Sinclair and Ross' equation (5) that they chose $r = v_0/\bar{\tau}$. In that case, of course, the right-hand sides of equations (44) and (48) would be proportional to $v_0$: $\langle v(\infty)\rangle = v_0/(c\bar{\tau})$ and $\langle v_b(\infty)\rangle = v_0\theta/\bar{\tau}$.

§ Of course, Sinclair and Ross were well aware of this, and their $v_0$ should be understood as an average initial volume.

the actual mean cellular volume will be

$$\langle \bar{v}(t) \rangle = \int_{v_0=0}^{\infty} \langle v(t) \rangle \varepsilon(v_0)\, dv_0,$$

where $\langle v(t) \rangle$, which depends on $v_0$, is given by equation (19). For "dispersionless" growth, equation (23) is still valid with $v_0$ replaced by

$$\bar{v}_0 = \int_{v_0=0}^{\infty} v_0 \varepsilon(v_0)\, dv_0.$$

The calculations presented here show that the problem posed by Sinclair and Ross can be solved rigorously, but the resulting equations are rather unmanageable although they furnish valuable information on the asymptotic behavior of the mean cellular volumes for $t \to \infty$. It is difficult to estimate how closely Sinclair and Ross' treatment approximates the derivation of equation (19). Therefore, it might be of interest to use the methods of this paper and test whether equation (2) or equation (3) gives a closer fit to Sinclair and Ross' excellent experimental curves. Perhaps an even better procedure, however, would be to simulate the life histories of individual cells (including volume changes) with a Monte Carlo program, and obtain the mean cellular volume by taking averages over sufficiently large numbers of cells.

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RECEIVED 5-18-70