CELL GROWTH AND DIVISION

V. Error Analysis of the Collins-Richmond Equation

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ABSTRACT Error propagation in the Collins-Richmond equation is analyzed in order to obtain the ratio of the fractional error in rate of cell volume increase to the fractional error in each experimental variable. Typical data are analyzed numerically for the total errors resulting from counting statistics, from spectral broadening, and from volume calibration shift. The measurement of 10^4 cells can give a precision of better than 10% in the volume growth rate with a volume resolution of 3%.

INTRODUCTION

A simple but powerful equation first derived by Collins and Richmond (1) provides a means of determining the volume growth rate of cells in balanced exponential growth to a precision and resolution superior to that of any other method. Such use of the equation requires knowledge of the volume distribution spectra of the total cell population and of subpopulations of dividing and newborn cells. It has been applied to bacteria (1, 2) and to mammalian cells in culture (3). Use of the equation has been criticized by Koch (4) and by Kubitschek (5), but both discussions are inconclusive since neither is based on quantitative considerations of the properties of the equation. The inherent accuracy of the equation can be determined only through a rigorous analysis of error propagation. The present paper presents such an analysis. Error coefficients are derived which give the ratio of fractional error in the calculated growth rate to fractional errors in experimental variables. These coefficients are completely general and are relevant for any source of error. We present numerical results of their application to selected examples of important errors.

Errors resulting from monlinearity of the Coulter spectrometer used to determine volume distributions are not considered specifically, since we believe these need not be of primary importance. For spherical objects such as mammalian cells, both theoretical and experimental analyses have indicated that a properly matched aperture and electronic system give accurate results (6–9). In addition, direct comparison of the growth rate deduced from the Collins-Richmond equation with that measured

for a synchronous culture gave excellent agreement (3). However, transducer limitations have produced serious spectral distortions in the past, and great care must be taken to verify that a given system does indeed have an acceptable small error, especially if nonspherical particles are measured. The error coefficients derived here should be applied to estimated spectrometer error to give the resultant error in growth rate for a specific instrument.

The errors we here calculate numerically include those due to random counting statistics, which are limiting when only a small number of cells are available for spectrometry. It is shown that 10^4–10^5 cells permit a precision of a few per cent with excellent volume resolution. Spectral broadening is shown to be relatively unimportant, but an invariant volume calibration between measurements is required and must be maintained to within a few per cent since a given fractional change produces a four- to sixfold larger error in growth rate.

THE COLLINS-RICHMOND EQUATION

The Collins-Richmond equation (1) is purely phenomenological; that is, it is not based on any particular model of the kinetics of cell growth and division but follows directly from conservation laws. Thus, for any cell population in balanced exponential growth, the rate of change in the number of cells having volumes less than V must be given by the difference between the rate at which cells enter the volume range 0 to V and the rate at which they leave it. For a general and rigorous derivation, see Bell and Anderson (10); here we give only the final result and its physical implications. One form of the equation is

$$\alpha N(V) = 2M(2V) - M(V) - f(V) \cdot n(V). \tag{1}$$

In this equation, α is the exponential rate constant for cell number increase and $N(V)$ is the number of cells of volume less than V in the total population. $2M(2V)$ and $M(V)$ are the number of cells which, per unit time, are newborn or which divide with volumes less than V. That is,

$$M(V) = \int_0^V p(V) \cdot n(V)\, dV, \tag{2}$$

where $p(V)$ is the probability that a cell divides with volume V. The term $f(V)$ is the rate of volume increase of cells of volume V, and $n(V)$ is the number of cells in the exponential population in unit volume increment at volume V.

Physically, the left side of equation 1 is the net rate of increase of cells of volume less than V which, in balanced exponential growth, is just the rate constant α times the number of cells. The right side contains all the relevant terms for the case in which cells divide exactly in half and there are no losses from the population. The first term is the rate at which daughter cells are produced by division; this term ex-

tends up to $2V$, since volume is halved. The second term is the rate at which cells are lost by dividing at volumes less than V. These two functions are identical for symmetric divisions, only the arguments differ.[1] The last term gives the flux of cells across volume V (i.e., the rate of loss of cells from the volume range due to volume increase). Setting $\alpha = 1$ (unit time is the e-folding time of the population) and solving for $f(V)$, we have the usual form of the Collins-Richmond equation:

$$f(V) = \frac{2M(2V) - M(V) - N(V)}{n(V)}. \tag{3}$$

If $f(V)$ remains finite for large V as $n(V)$ goes to zero, then the product $f(V) \cdot n(V)$ goes to zero. This gives the normalization condition for the two integral spectra [namely, $M(V) = N(V)$ for large V]. Operationally, the volume spectra of newborn or dividing cells will be measured with some different normalization. Thus, before a measured $M(V)$ is used in equation 3 it must be multiplied by a constant factor k to give the correct normalization, where

$$k = N(\infty)/M(\infty). \tag{4}$$

When the dividing cell spectra are multiplied by k at all values of V, equation 3 becomes[2]

$$f(V) = \frac{2k\,M(2V) - k\,M(V) - N(V)}{n(V)}. \tag{5}$$

The peculiar strength of this equation for the determination of cell volume growth rate is that it expresses the differential quantity $f(V)$ in terms of three integral volume distribution spectra and the differential spectrum at V. Being integral quantities, variables in the numerator are comparatively insensitive to many experimental errors. The denominator is measured directly when a differential multichannel pulse height analyzer is used and, therefore, $f(V)$ does not suffer the severe amplification of error which commonly results from attempting to determine an increment from the difference between successive values of a variable. When the error due to counting statistics is limiting, then to a fairly good first approximation (as we will see rigorously later) the rate of volume growth can be determined to about the same precision as the differential spectrum $n(V)$ can be measured (that is, to a few per cent).

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[1] Exact division in half is indicated for the cells we have studied (3). Should this not be the case, then $M(2V)$, which can be measured directly, is a different function from $M(V)$. In the following analysis, results for uncorrelated errors would not change but correlations would be different.

[2] If cell division is not symmetric, there will be two independently determined k's, one for the mitotic and one for the newborn spectrum.

APPROXIMATE ERROR ANALYSIS

We first evaluate the error in $f(V)$ caused by a small error in each of the experimental variables taken independently. Performing the corresponding partial differentiations on equation 5 and dividing by the variables, we obtain a series of six equations of the form

$$\frac{\partial f(V)}{f(V)} = \phi(V) \frac{\partial x(V)}{x(V)}, \tag{6}$$

which gives the error coefficient $\phi(V)$ relating a given small fractional change in an experimental variable $x(V)$ to the resulting error in $f(V)$.

Table I lists the coefficients $\phi(V)$ for each of the independent variables. The dependence of growth rate $f(V)$ on $n(V)$, the differential spectrum of the exponential culture, is particularly simple: the fractional errors are equal. For the integral spectrum variables $N(V)$, $M(V)$, and $M(2V)$, the coefficient depends on the ratio of the variable to the product $f(V) \cdot n(V)$. The remaining two variables $N(\infty)$ and $M(\infty)$ are the total numbers of cells in the exponential phase and mitotic spectra. The error coefficients are obtained by appropriate differentiation after substituting the ratio $N(\infty)/M(\infty)$ for k in equation 5.

Except for the case of $n(V)$, numerical values for the error coefficients can be calculated only when the volume distribution spectra are given. For the purpose of illustration, we choose a typical experiment with Chinese hamster cells (line CHO) growing in suspension or monolayer culture (3). The coefficient of variation of the spectrum of dividing cells was 18%, and the rate of volume growth $f(V)$, as deduced from the Collins-Richmond equation, was nearly exponential [$f(V) = V$] as shown in Fig. 1. The growth rate was calculated for two balanced exponential cultures: the monolayer culture (circles) from which the mitotic population was also derived by shaking (11) and a parallel suspension culture (triangles) which was apparently also balanced with the same doubling time (the possible significance of the difference is discussed below). The following numerical analysis uses the monolayer data [for the spectra of $n(V)$ and $m(V)$, see Fig. 4 of reference 3].

TABLE I

COEFFICIENT FOR THE FRACTIONAL ERROR IN $f(V)$ CAUSED BY UNIT FRACTIONAL ERROR IN A PARAMETER

Variable $x(V)$	Error coefficient $\phi(V)$
$n(V)$	-1
$M(2V)$	$2k \cdot M(2V)/f(V) \cdot n(V)$
$M(\infty)$	$-k \cdot [2M(2V) - M(V)]/f(V) \cdot n(V)$
$N(\infty)$	$k \cdot [2M(2V) - M(V)]/f(V) \cdot n(V)$
$N(V)$	$-N(V)/f(V) \cdot n(V)$
$M(V)$	$-k \cdot M(V)/f(V) \cdot n(V)$

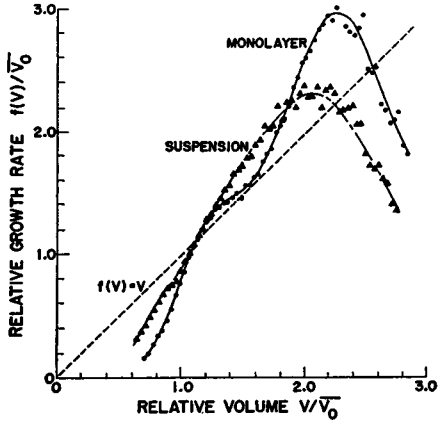

FIGURE 1

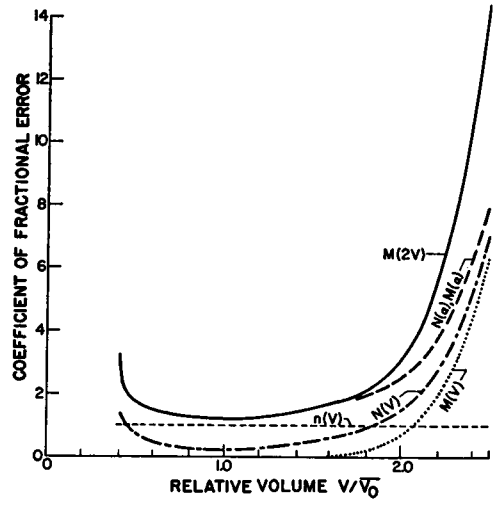

FIGURE 2

FIGURE 1 Volume growth rate as a function of volume for two related populations of CHO cells: (●) monolayer culture, and (▲) suspension culture. The dashed line labeled $f(V) = V$ corresponds to an exponential growth law. $\overline{V_0}$ is the average birth volume of the populations.

FIGURE 2 Absolute value of the coefficient of fractional error in $f(V)$ resulting from unit fractional error in each of the experimental variables as functions of volume. This numerical solution is for a typical set of data.

Results of using these spectra to calculate numerical absolute values for error coefficients of Table I as functions of volume are shown in Fig. 2. The largest error coefficient (but not the largest error contribution) is that of $M(2V)$, the cumulative spectrum of newly born cells. Over the range of principal interest and reliability, from $\overline{V_0}$ to $\overline{2V_0}$, this coefficient never exceeds 3. Total cell numbers $N(\infty)$ and $M(\infty)$ have identical error coefficients which are the same as that of $M(2V)$ until volumes at which $M(V)$ becomes significant, about 1.7 $\overline{V_0}$. At larger volumes, their coefficients are slightly lower. The coefficient of $n(V)$, which is a constant independent of V and of spectral shapes, is next largest while the coefficients of $N(V)$ and $M(V)$ are generally smaller—the latter, in fact, being zero at small volumes because of the very narrow spectrum of dividing cells. As we shall see below, the order of importance in contributing to the error in $f(V)$ may not be the same as the order given here if actual experimental errors in variables are taken into account. Since the coefficients are all of the order of unity over the range of interest, a 10% error in $f(V)$ can be obtained from primary data which have errors of several per cent. Near $\overline{V_0}$, a 5% error in the most sensitive variables, $M(2V)$, will produce no more than a 6% error in $f(V)$.

COUNTING STATISTICS

So far we have dealt with the ratio of error in $f(V)$ to the error in the experimental variable. In order to obtain numerical values for errors in $f(V)$, values must be ascribed to the errors in each measured variable. A case of particular interest is that in which these errors are random due to counting statistics. This case is important because it sets a lower limit on attainable error and because, in many practical cases (where a limited number of cells are available for measurement), the counting error may indeed be limiting. We have calculated the counting statistical error for a mammalian cell experiment in which a total of 245,000 cells from an exponential population and 94,000 cells from a mitotic population were analyzed (these measurements require 3–4 min each). The statistical error was taken to be Poisson (i.e., the variance of the number of counts in a volume channel is equal to the number of counts in that channel). The results are shown in Table II; in part A, the calculation was made at the average birth volume, $\overline{V_0}$. For each of the six experimental variables, assumed to be independent, the table gives in the second and third columns measured values (the number of cells counted = x) and resulting per cent standard error in the parameter $(100/\sqrt{x})$. The coefficient of error given in the fourth column is taken from Fig. 2 (which is based on the same data), and the last column gives the resulting per cent error contributed to $f(V)$ by each parameter. The primary importance of $n(V)$ in determining the error is apparent, and the reason for this is also clear: the other variables are integral functions and have much smaller fractional errors because the number of counts is larger. (A similar reduction in error by the integrating process would result in the case of any other random error but not necessarily in the case of systematic errors.) Thus, $n(V)$ contributes nearly twice the error of the next largest source $M(2V)$, and more than the sum of the next two largest errors. If all six errors are assumed to be uncorrelated and are combined as the square root of the sum of their squares, the calculated error in $f(V)$ is found to be 1.30%. This is compared with the value of 1.24% derived from the exact analysis given below which takes into account correlations between variables.

The second part of Table II is a similar calculation at $V = \overline{2V_0}$. The qualitative picture is the same in spite of the rather different error coefficients, $n(V)$ still making the predominant contribution to error in $f(V)$. The total error in $f(V)$ is now almost twice as large, but the effect of correlations is greater, reducing the error from 2.39 to 2.14% [for a complete presentation of the error in $f(V)$ as a function of V, see below].

The most important result of this analysis is to demonstrate that very precise values of $f(V)$ are obtainable without inordinate requirements on counting statistics. The errors of 1–2% given in Table II can be contrasted with, for example, the 40% difference between linear and exponential growth models at these volumes. More important, with this level of precision, one can abandon the empirical "fitting" of

TABLE II

NUMERICAL STATISTICAL ERROR CONTRIBUTIONS FROM EXPERIMENTAL PARAMETERS

Parameter	Measured value	Error in parameter	Error coefficient $\phi(V)$*	Error in $f(V)$
A. At $V = \overline{V_0}$		%		%
$n(V)$	9,145	1.05	1.00	1.05
$M(2V)$	41,447	0.49	1.23	0.60
$M(\infty)$	94,079	0.33	1.23	0.40
$N(\infty)$	244,605	0.20	1.23	0.24
$N(V)$	145,984	0.26	0.23	0.05
$M(V)$	0	. .	0	0
$f(V)$ From above, no correlations				1.30
From exact equation with correlations				1.24
B. At $V = \overline{2V_0}$				
$n(V)$	2,187	2.15	1.00	2.15
$M(2V)$	94,079	0.33	3.15	1.04
$M(\infty)$	94,079	0.33	2.46	0.81
$N(\infty)$	244,605	0.20	2.46	0.49
$N(V)$	226,102	0.21	1.46	0.31
$M(V)$	41,447	0.49	0.69	0.34
$f(V)$ From above, no correlations				2.39
From exact equation with correlations				2.14

* Calculated from measured value of the parameter (column 2) and the formula for the error coefficient from Table I.

arbitrary growth models and proceed to ask what the fine structural detail of the growth rate function may be. Note that these data are obtained at high volume resolution; in the example used, the primary data have a resolution of about 3% of $\overline{V_0}$.

The precision of the method remains impressive even when much smaller numbers of cells and, hence, much poorer counting statistics are assumed, since the statistical error varies inversely as only the square root of the number of cells counted. Thus, if one-tenth as many counts were recorded (i.e., totals of 24,000 and 9400, respectively, for the exponential and mitotic populations), all errors given in Table II would be increased by 3.2 and at $\overline{2V_0}$ the error in $f(V)$ would be only 6.8%. The usual process of numerical differentiation (determining dV/dt by difference of mean volume of synchronized cultures over a small time interval), on the other hand, can easily involve error coefficients of 10–100, depending on the volume resolution desired, and an uncertainty in $f(V)$ of 70% can result (12). Statistical precision can also be improved at the sacrifice of volume resolution by using wider channels.

EXACT ERROR ANALYSIS

As noted above, Table I and Fig. 2 apply to the case in which there is an error in only one of the experimental variables. Exact analysis, including the effect of corre-

lations among variables, is straightforward but somewhat tedious algebraically. In order to simplify the appearance of the equations, we drop the explicit notation of volume dependence so that, for example, $M(V)$ will be written M and $M(2V)$ becomes M_2. We also let a subscript zero denote the exact values of the functions so that, in the absence of any experimental errors, equation 5 becomes

$$f_0 = (2k_0M_{2_0} - k_0M_0 - N_0)/n_0 . \tag{7}$$

The corresponding experimental quantities are the true function plus an error such as $n = n_0 + \delta n$, etc. Thus, the equation corresponding to equation 7 for the experimental quantities is

$$f = \frac{[(k_0 + \delta k)(2M_{2_0} + 2\delta M_2 - M_0 - \delta M) - N_0 - \delta N_0]}{(n_0 + \delta n)} . \tag{8}$$

Subtracting equation 7 from equation 8 and calling the difference δf, we have

$$\delta f = \delta k \frac{[2M_{2_0} + 2\delta M_2 - M_0 - \delta M]}{n_0 + \delta n} - \delta n \frac{f}{n_0 + \delta n} + \frac{k_0(2\delta M_2 - \delta M) - \delta N}{n_0 + \delta n} . \tag{9}$$

In this equation,

$$\delta k = \frac{\delta N(\infty) - k_0 \delta M(\infty)}{M_0(\infty) + \delta M(\infty)} , \tag{10}$$

which follows from definition of the terms. So far no approximation has been made, and equation 9 holds for all errors—random or consistent, large or small. (The error coefficients of Table I can be derived from equation 8 by letting only one of the errors be nonzero and solving for fractional errors.) In equation 9 the errors δn, δN, and $\delta N(\infty)$ will be correlated, since some of the same experimental data (namely, the differential volume spectrum of the exponential population) are involved in each quantity. In addition, the errors δM and $\delta M(\infty)$ will be correlated, and both will be correlated with δM_2 if the same experimental data are used in determining M and M_2 (i.e., the volume spectra of dividing and of newborn cells).

As before, we now consider the special case in which errors in experimental variables are due to random fluctuations in the number of counts in each channel of the pulse height analyzer. The primary error distributions will then be Poisson, and using angular brackets to denote expected values,

$$\langle \delta x \rangle = 0, \tag{11}$$

$$\langle (\delta x)^2 \rangle = x, \tag{12}$$

where x is any of the experimental variables n, N, M, M_2, $N(\infty)$, and $M(\infty)$.

We note in passing that second-order terms in equation 9 will result in $\langle \delta f(V) \rangle$ being nonzero [i.e., the random primary errors produce a small systematic error in $f(V)$]. It can be shown that the expected value of this error, expressed as a fraction of $f(V)$, is approximately equal to $1/n(V)$, which is $\sqrt{n(V)}$ times smaller than the random error in $f(V)$ and, hence, negligible. Thus, it is necessary to consider only the variance $\langle [\delta f(V)]^2 \rangle$. Making the substitutions according to equation 11 and neglecting second-order terms, the result is

$$\langle \delta f^2 \rangle = \frac{f^2 + 2f}{n} + \frac{4k^2M_2 - 3k^2M + N}{n^2} + \frac{2M_2 - M}{n^2M(\infty)} \cdot [2fn + 2N + (k-1)(2kM_2 - kM)]. \quad (13)$$

In deriving this equation,[3] it is assumed that $\langle \delta n \delta N \rangle = n$, $\langle \delta M \delta M(\infty) \rangle = M$, etc. The first relation follows from expressing N as a sum of n over all experimental channels having volumes $\leqslant V$. Hence, if i is a volume channel index,

$$N_I = \sum_{i=1}^{I} n_i ,$$

and

$$\langle \delta n \delta N \rangle = \langle \delta n_I \sum_{i=1}^{I} \delta n_i \rangle = \delta n_I \equiv \delta n.$$

Other covariances are similarly obtained by considering the multichannel data. If errors in experimental functions were uncorrelated, then no cross products would be retained and

$$\langle \delta f^2 \rangle = \frac{f^2}{n} + \frac{4k^2M_2 + k^2M + N}{n^2} + \frac{(2M_2 - M_0)^2(k + k^2)}{n^2M(\infty)}. \quad (14)$$

Numerical solution of equation 13 gives the entries in Table II (above) ascribed to "exact equation with correlations." Equation 14 gives results identical with those obtained by taking the square root of the sum of squares of individual errors ("no correlations") in the same table. Using the same experimental data and extending the calculation over the volume range for 0.6–2.5 $\overline{V_0}$, equation 13 gives the results plotted in Fig. 3 for the coefficient of variation (in per cent) of $f(V)$ [i.e., 100

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[3] Equation 13 can be derived by considering f as a function of six random variables $f(X_1, \ldots X_6)$ where $(X_1, \ldots X_6)$ are n, N, M, M_2, $N(\infty)$, and $M(\infty)$ and using the approximate relation

$$\langle \delta f^2 \rangle \simeq \sum_{i=1}^{6} \left(\frac{\partial f}{\partial X_i} \right)^2 \langle \delta X_i^2 + 2 \rangle \sum_{j>i}^{6} \sum_{i=1}^{6} \frac{\partial f}{\partial X_i} \frac{\partial f}{\partial X_j} \langle \delta X_i \, \delta X_j \rangle.$$

Equation 14 would be obtained by assuming uncorrelated experimental variables (i.e., $\langle \delta X_i \delta X_j \rangle = 0$).

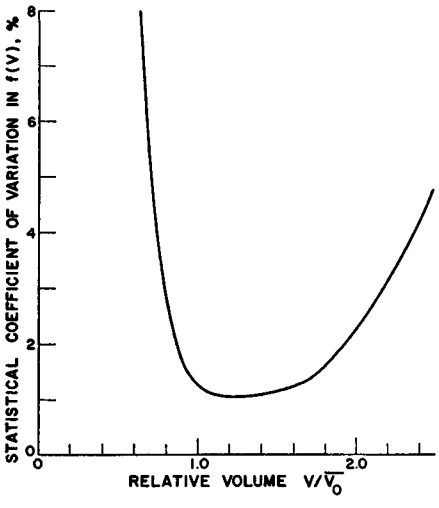

FIGURE 3 Statistical error (coefficient of variation) in $f(V)$ as a function of volume resulting from a typical determination in which 2×10^6 exponential and 10^6 mitotic cells were analyzed.

$\sqrt{(\delta f)^2}/f$] due to counting statistical errors The sharp rise in fractional error below $\overline{V_0}$ is due to the small value of the growth rate of these small cells. The slower rise above $\overline{2V_0}$ is due to increased statistical error as $n(V)$ becomes small. At the extremes of volume represented here, other sources of error also become important (i.e., primarily corrections to the spectra for interfering objects).

EFFECT OF SPECTRUM BROADENING

The effect upon $f(V)$ of instrumental dispersion of the volume spectra can be estimated by the following calculation. The measured spectra are processed by a computer code which redistributes the contents of each channel into adjacent channels according to a normal distribution function of adjustable width. Growth rate is then recalculated on the basis of the broadened spectra. Broadening functions with constant coefficients of variation (rather than constant standard deviations) were used. Effects upon the primary spectra are shown in Table III for coefficients of variation of 0.1 and 0.2.

Results of the calculation of $f(V)$ for the three cases show little effect. As would be expected, the primary effect of broadening is to reduce the amplitude of fine structure and to give a simpler curve; however, the magnitude of the change is surprisingly small. In spite of the fact that the additional broadening of 20% exceeds the initial measured width of the mitotic cell spectrum, the errors introduced in $f(V)$ are only 11% and 13% at $\overline{V_0}$ and $\overline{2V_0}$, respectively

Resolution of the Coulter spectrometer when applied to the measurement of mammalian cells has not been determined (for lack of a comparison method of comparable precision). However, general theoretical and experimental studies suggest that instrumental resolution is better than a few per cent. One can conclude, therefore, that it is unlikely to contribute significantly to the error in $f(V)$.

TABLE III

WIDTHS (COEFFICIENT OF VARIATION) OF PRIMARY SPECTRA AS A FUNCTION OF ADDITIONAL (GAUSSIAN) BROADENING

Measured spectrum	Additional broadening introduced		
	None	Coefficient of variation = 0.1	Coefficient of variation = 0.2
Exponential-phase culture $n(V)$	0.294	0.310	0.356
Mitotic cells $m(V)$	0.181	0.201	0.255

EFFECT OF VOLUME DRIFT

A potential source of serious error in $f(V)$ is a change in volume calibration of the system. If changes occur in a time short compared with a spectral measurement, the spectrum will be broadened and the results will be similar to those discussed above. If there is slow drift on a longer time scale, its time dependence can be evaluated by repeated measurements; interpolations to a common time would then eliminate the effect. (Such a problem has not been observed in our experience.) A remaining possibility, which is difficult to disprove, is that a consistent error may exist between measurements of the spectrum of mitotic cells and that of the exponential population. Such an error might result from biological causes such as trauma of removing the cells from monolayer (by shaking or trypsinization) or, when the total population is from a suspension culture, from a failure of the suspension and monolayer (the source of the mitotic population) to be in identical states of balanced growth. If exponential cultures from suspension and monolayer give identical spectra, this is suggestive evidence that neither is perturbed.

The effect of an assumed calibration shift can be calculated as follows. We assume the shift to be due to a change in gain of the system (although it could, in principle, be of biochemical origin) which occurs between measurement of the two spectra. Since the mitotic spectrum defines the volume scale in terms of average birth volume $\overline{V_0}$, it is correct by definition and the error is in the volume scale of the exponential spectrum. Differentiating equation 5 with respect to system gain g and noting that k is independent of gain, we have for the error coefficient $\phi(V)$

$$\phi(V) = \frac{\partial \ln f(V)}{\partial \ln g} = -\frac{1}{f(V)\, n(V)} \cdot \frac{\partial N(V)}{\partial \ln g} - \frac{1}{n(V)} \cdot \frac{\partial n(V)}{\partial \ln g}. \qquad (15)$$

For numerical calculation, we approximate the partial derivatives of $N(V)$ and $n(V)$ by the observed changes in these quantities between adjacent channels. Unit change in volume at volume V corresponds to $\partial \ln g = 1/V$, $\partial N(V) = n(V)$, and the above equation becomes

$$\phi(V) = V\left[-\frac{1}{f(V)} - \frac{\partial n(V)}{n(V)}\right]. \qquad (16)$$

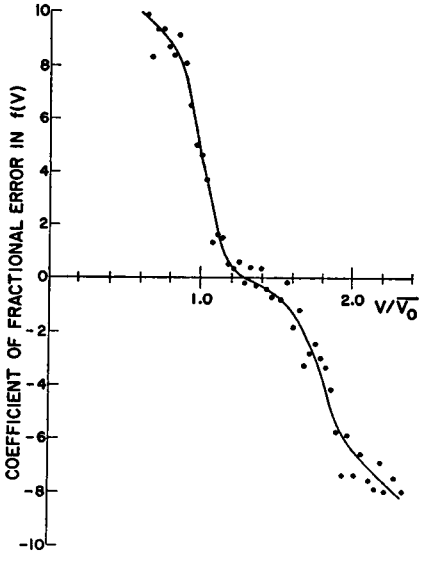

FIGURE 4 Coefficient of fractional error in volume growth rate resulting from unit error in volume calibration as a function of volume.

Results of solving equation 16 using the same pair of spectra analyzed previously are shown in Fig. 4. Over the range $\bar{V}_0 < V < 2\bar{V}_0$, the fractional error in $f(V)$ will vary between +4.4 and −6.4 times the fractional change in gain (or volume calibration). There is a region in the vicinity of 1.3 $\bar{V}_0$ in which $f(V)$ is independent of small changes in gain but, unfortunately, this is also the region in which $f(V)$ is least sensitive to changes in the growth law. One can conclude that, if an accuracy of 10% is required for $f(V)$, the gain must then be stable to about 2% for the above volume range.

BIOLOGICAL ERRORS

Our aim has been the rigorous analysis of error propagation to provide equations of general applicability which can be used to deduce the error in growth rate when individual contributing errors are known or can be estimated. The specific cases presented as examples were chosen because they can be factors limiting accuracy and because we feel that the resulting level of accuracy illustrated is attainable by proper transducer electronic systems for spherical particles. A general discussion of all possible errors is beyond the scope of this paper and would include the basic electric and hydrodynamic properties of the Coulter transducer and associated electronics, the techniques used in experimental manipulations, and some of the biological properties of the system measured. As indicated in the Introduction, we believe that conditions have been established under which the Coulter system gives an accurate measurement of volume distribution of spherical objects. (Careful attention to detail is necessary to achieve these conditions.) If the objects are nonspherical or are seriously deformed in the measurement process, then a general method is

not known to ensure accuracy, although special cases can be handled to useful degrees of approximation.

Among the biological sources of error it is of prime importance, of course, that the population measured be in a state of balanced exponential growth as required by the derivation of the Collins-Richmond equation. While this condition may not always be easily attained, it is relatively simple to prove when present. It is necessary and sufficient (in this context) that cell number increase at a constant exponential rate over at least one generation time and that volume distributions of the total population and of dividing cells be invariant over the same time span.

Another essential requirement is that the populations be monodisperse. While this may preclude use of the method with some populations, it is again a simple matter to demonstrate compliance. Visual scoring of the culture will give a direct measure of the fraction of multiples and serve to monitor the procedure used to disperse them. A number of effective methods are available (including gentle trypsinization, hydrodynamic shear, sonication, chemical change of the growth medium, etc.) which permit the measurement of some, but not all, difficult cases. The presence of multiples sometimes can be detected also as a secondary peak at large volumes in the Coulter spectrum. If sufficiently separated from the main peak, this can be removed by spectrum-stripping techniques. The magnitude of the error from this procedure can be estimated by numerical computation of $f(V)$ using a sequence of extrapolations of the primary spectrum.

Unidentified biological errors may, of course, remain. An example of the puzzling discrepancies sometimes encountered was given in Fig. 1. The two populations whose growth rates are plotted both apparently met the requirements for applicability of the equation. The monolayer had been aliquoted from the suspension culture and planted on glass to provide the source of the mitotic population for both calculations. A significant difference developed between the spectra of the two cultures, resulting in the difference in the growth rate shown. While it is possible that this difference is real, it seems more likely that the suspension results are in error. Other experiments (3) confirmed, for a different monolayer culture, a growth pattern very similar to that shown in Fig. 1. This confirmation was obtained by direct measurement of average volume as a function of time for a synchronized suspension culture derived from the monolayer by mitotic selection, thus demonstrating both that growth patterns can be the same on monolayer and in suspension and that the Collins-Richmond method gave the correct result.[4] If the discrepancy near the average division volume in Fig. 1 is not real but an artifact, it could be due to failure of the postmitotic cells of the suspension to separate promptly (they lack the "leverage"

[4] As pointed out in reference 3, the comparison between the Collins-Richmond growth rate of an exponential culture and the modal volume increase of a synchronized culture involves two quantities which need not be identical but which will approach one another if the age distribution of cells of volume V is narrow.

resulting from attachment to glass). The presence of too many cells in this region of the spectrum would cause an apparent depression in calculated growth rate. A definitive answer is not possible without further experimentation, but the example is introduced here as a warning against overconfidence in the calculated physical errors as a complete measure of accuracy of the entire method. However, note that even in this case the discrepancy is small compared with the errors associated with other methods of determining the growth rate and that the difference from linear growth [$f(V) = 1.44\,\overline{V}_0$ independent of V] is unmistakable.

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REFERENCES

1. COLLINS, J. F., and M. H. RICHMOND. 1962. *J. Gen. Microbiol.* **28**:15.
2. HARVEY, R. J., A. G. MARR, and P. R. PAINTER. 1967. *J. Bacteriol.* **93**:605.
3. ANDERSON, E. C., G. I. BELL, D. F. PETERSEN, and R. A. TOBEY. 1969. *Biophys. J.* **9**:246.
4. KOCH, A. L. 1966. *J. Gen. Microbiol.* **45**:409.
5. KUBITSCHEK, H. E. 1970. *J. Theor. Biol.* **28**:15.
6. GROVER, N. B., J. NAAMAN, S. BEN-SASSON, and F. DOLJANSKI. 1969. *Biophys. J.* **9**:1398.
7. GROVER, N. B., J. NAAMAN, S. BEN-SASSON, F. DOLJANSKI, and E. NADAV. 1969. *Biophys. J.* **9**:1415.
8. ANDERSON, E. C., and D. F. PETERSEN. 1967. *Biophys. J.* **7**:353.
9. HARVEY, R. J., and A. G. MARR. 1966. *J. Bacteriol.* **92**:805.
10. BELL, G. I., and E. C. ANDERSON. 1967. *Biophys. J.* **7**:329.
11. PETERSEN, D. F., E. C. ANDERSON, and R. A. TOBEY. 1968. *In* Methods of Cell Physiology. D. M. Prescott, editor. Academic Press, Inc., New York. **3**:347.
12. SINCLAIR, W. K., and D. W. ROSS. 1969. *Biophys. J.* **9**:1056.