Estimation of Growth Rate from the Mitotic Index

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The growth rate of a eukaryotic population dividing at a constant rate can be estimated from the equation, \( t_m/g \ln 2 = \ln (1 + R) \), in which \( t_m \) is the time required for mitosis, \( g \) is the generation time, and \( R \) is the fraction of cells undergoing mitosis. Values for \( t_m \) and \( R \) can be determined by direct microscope examination of the population. The validity of the derived equation has been checked with an exponentially growing culture of a prokaryote, Escherichia coli, in which chloramphenicol was administered to inhibit protein synthesis. Cells having enough protein completed the division process whereas the rest of the population was inhibited. From the plot of the growth curve before and after administration of chloramphenicol, \( t_m \) and \( R \) were estimated. The calculated and actual growth rates were almost identical.

A problem in ecological studies is to estimate growth rates in natural populations of microorganisms. Rates measured on pure cultures are of doubtful significance because the conditions do not exactly simulate those of the mixed population in the natural environment.

A method for estimating the growth rate of eukaryotic microbes growing at a constant rate in nature, discussed by Hughes (3), involved direct microscope examination of the natural population, and estimation of the fraction of cells undergoing mitosis. The time for mitosis was measured by continuous examination of individual cells. A formula, ascribed to Crick, for calculating growth rate from these two measurements was cited (3) as

\[
\frac{t_m}{g} \ln 2 = \ln \frac{1 + 2R}{1 + R},
\]

in which \( R \) is the fraction of cells showing mitosis, \( t_m \) is the time required for mitosis, and \( g \) is the time from formation of a new cell by division to its termination by division (interdivision time). This formula was used by Warner (10) and Scherbaum (7), and quoted by Brock (1), but we are not aware of a published derivation.

MATERIALS AND METHODS

Our derivations led to a different result, namely, the equation,

\[
(t_m/g) \ln 2 = \ln (1 + R),
\]

obtained also by Stanners and Till (8). We have derived it from the age distribution presented by Painter and Marr (5) and as follows:

Consider a population in balanced exponential growth such that the number of cells, \( N = N_0e^{kt} \), in which \( k \) is the specific growth rate constant. The age of the cells in the population, \( N_0 \), at the time of observation, \( t_0 \), is a function of the time at which the parent cell divided. These divisions occurred in the generation time, \( g \), preceding the observation at \( t_0 \). The value \( g \) is assumed to be the same for each cell in the population, i.e., undistributed. One generation time of history for a population is shown graphically in Fig. 1. The curve shows the change with time in the number of cells and in the number of old cells as each cell present at time, \( -g \), divides to form the population \( N_0 \) at \( t_0 \). The difference between the values for the two curves of any time is the total number of newly formed cells. The rate at which they are formed is 

\[
dN_{\text{new}}/dt = 2kN\text{.}
\]

A curve showing this rate of production of new cells will be as in Fig. 2. The value for \( dN_{\text{new}}/dt \) at any time, \( t \), is the rate of production of new cells at that time, and the value for \( (t_0 - t) \) is the age of such cells. The curve thus shows the age distribution of the population at \( t_0 \). The sum of all the cells produced during this time equals the population, \( N_0 \), as follows:

\[
\int_{-g}^{t_0} \frac{dN_{\text{new}}}{dt} dt = \int_{-g}^{t_0} 2kN dt
\]

\[
= \int_{-g}^{t_0} 2kN_0e^{kt} dt = N_0.
\]

Any measurable developmental stage occupying a constant fraction of the total generation time can be used to estimate the growth rate in an exponentially

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Fig. 1. Curves to show changes with time in the total number of cells and the number of old cells in an exponentially growing culture during the generation time preceding observation of the population at time zero.

growing population (7). The number of cells in the population showing a developmental stage starting at age a and ending at age b is

$$2kN_e \int_{-\infty}^{b-a} e^{kt} \, dt = 2N_e(e^{b-a} - e^{a-b}).$$

Eukaryotic cells showing stages in division are those between age t at which mitosis starts and age g at which cell division occurs. The total number of such cells in the culture is

$$2N_e \int_{-\infty}^{t-g} e^{kt} \, dt = 2N_e(e^{t-g} - 0.5),$$

when g is given the value of one.

The fraction, R, which such cells constitute of the total population is

$$R = \frac{2N_e(e^{t-g} - 0.5)}{N_e} = 2e^{t-g} - 1.$$

Since $k = \ln 2/g$,

$$R + 1 = 2e^{-t/g}$$

and

$$\ln (R + 1) = -t/g.$$

Substituting $t_m$ for $g - i$ gives equation II.

The validity of this equation for a prokaryotic organism was tested in an experiment in which chloramphenicol (200 µg/liter) was added to Escherichia coli ML306 growing aerobically in minimal salts (9) plus 0.2% glucose at 25 or 30 C (Fig. 3).

We consider the exponentially growing population as consisting of two classes, those which have completed all protein synthesis necessary for cell division and will divide after chloramphenicol is added, and those which have not completed synthesis and will not divide. The number dividing equals the net increase in cell numbers after drug addition until divisions cease. The fraction, $R$, that this net increase constitutes of the total population is

$$R = \frac{N_e \exp (Kt_m) - N_e}{N_e}.$$

In this experiment with a prokaryotic species, $t_m$ represents the time interval between completion of protein synthesis and division of the cell.

RESULTS

In Table 1 the generation times estimated from the slope of the curves in Fig. 3 are compared with the values obtained by application of equation II. The results confirm experi-

Fig. 2. Curve showing the rate of production of new cells during the generation time preceding the time of observation $t_m$. The area (integrated rate $X$ time) between $t = -b$ and $t = -a$ represents the number of cells in the population between ages b and a. The total area under the curve represents the number of cells in the population at time zero.

Fig. 3. Curves showing the rate of growth of E. coli ML306 at 25 C (●) and at 30 C (○), and the increase in cell numbers following addition of chloramphenicol.
mentally the validity of the equation and demonstrate its applicability to a prokaryotic cell in which the duration of processes leading to cell division cannot be estimated by microscope examination.

The fraction of the generation time, 25/50.5, occupied by the division process at 39 C is greater than the faction, 36/87.4, occupied at 25 C. The fact that \( t_m \) and \( g \) vary independently at the two temperatures emphasizes the importance of measuring \( t_m \) and \( R \) under the conditions for which a value for \( g \) is desired.

DISCUSSION

Although the derivations are based on the assumption that \( g \) and \( t_m \) are not distributed, equation (II) is a good approximation during constant growth even if \( t_m \) and \( g \) are distributed. When distributed, the actual mean generation time, \( \tau \), is always greater (5) than \( g \), but unless the generation time varies markedly, little error is involved in assuming an identity in the value for \( \tau \) and for the assumedly undistributed value, \( g \).

The ecologist using the procedure for estimating growth rates in natural populations would be well advised to make a sufficiently large number of determinations of \( t_m \) to obtain some idea of the distribution of this characteristic. The proportion of cells that will fail to divide at all must be very small if the equation is to be applicable to the remaining cells.

Recalculation of Warner's results (10) according to equation II gives an average doubling time for Entodinium in the rumen of 17 h rather than the 16 h calculated from equation I (4). Scherbaum's values (5) for the duration of mitosis in Tetrahymena become 18.8, 15.3, and 13.5 min rather than the reported values of 17.4, 14.3, and 12.4.

LITERATURE CITED

7. Scherbaum, O. 1957. The division index and multiplication in a mass culture of Tetrahymena following inoculation. J. Protozool. 4:257-259.

<table>
<thead>
<tr>
<th>Incubation temp (°C)</th>
<th>Time till division (t_d) (min)</th>
<th>( R ) (min)</th>
<th>( g ) Experimental (min)</th>
<th>( g ) Calculated (min)</th>
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<td>25</td>
<td>0.41</td>
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* The experimental \( g \) is from the slope of the curves in Fig. 3. The calculated \( g \) is from equation II, using for \( R \) the value, \( (N_t - N_0)/N_0 \); \( t_0 \), \( N_0 \) and \( N_t \) are read from the graph in Fig. 3. For the prokaryotic system, \( t_0 \), instead of representing the time for mitosis, represents the interval prior to cell division during which the processes leading to division are insensitive to chloramphenicol.
ERRATA

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Volume 25, no. 5, p. 779, column 1, line 5 from bottom: Change “(200 µg/liter)” to read “(200 µg/ml).”
Page 779, column 1, line 4 from bottom: Change “ML306” to read “ML30G.”
Page 779, column 2: Equation should read as follows:
\[ R = \frac{N_x^{t_2} - N_x}{N_x} \]

Evaluation of a Fluorescent Antibody-Enrichment Serology Combination Procedure for the Detection of Salmonellae in Condiments, Food Products, Food By-Products, and Animal Feeds
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Volume 26, no. 5, p. 753, Table 1: Change column headings to read as follows:

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<th>Product</th>
<th>No. of samples</th>
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<th>FA (Difco sera)</th>
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