NORMAL DISTRIBUTION OF CELL GENERATION RATE[1, 2]

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THE description of the growth of a cell population and its variability requires a knowledge of the distribution of generation times for the individual cells. The generation time τ is the time between the birth by fission (inception) of a cell and its division (termination) to form two daughter cells. This definition is somewhat imprecise: nuclear division and effective autonomy may be established prior to cytoplasmic fission, which may be so irregular as to give rise to daughter cells which appear to be multinucleate to different degrees. In cells with more regular division, the generation time is a useful concept when the variability in time between nuclear division and cytoplasmic division (or other cytological endpoint being measured) is small compared to the variability introduced during the growth of the cell before division.

The distribution of generation times, also called the τ-distribution, was first studied by Kelly and Rahn [4]. Two extreme hypotheses were advanced. The first, Rahn's hypothesis [9], proposed that fission follows the duplication of a number of essential and independent entities, perhaps genes. If each of these has the same probability of being duplicated at any instant the generation times obey Yule's distribution [7],

$$dF = \frac{g}{m} e^{-\tau/m} (1 - e^{-\tau/m})^{g-1} d\tau. \tag{1}$$

The parameter g indicates the number of entities to be duplicated, and m is the probability of duplication in unit time. Using Rahn's hypothesis, Finney and Martin [2] analyzed the data of Kelly and Rahn, and found that some 25 essential structures would need to be duplicated.

Kendall's hypothesis [5] proposed that a series of g events takes place step-by-step in a definite order during duplication. The resulting distribution is chi-squared (Pearson Type III [7]) when the probability of completing a step at any given instant has the constant value m; for g steps,

$$dF = \frac{\tau^{g-1} e^{-\tau/m} d\tau}{m^g \Gamma(g)} \tag{2}$$

Kendall obtained a value for g of about 20 steps for Kelly and Rahn's data.

[1] This work was performed under the auspices of the U.S. Atomic Energy Commission.
[2] With the technical assistance of H. E. Bendigkeit.

These results provided no clear distinction between the two distributions. Furthermore, it was not possible to decide between them on the basis of goodness-of-fit. In a later study of six species of bacteria, Powell [7] also was unable to decide clearly between the two hypotheses on the basis of goodness-of-fit. A difficulty with his work, however, is that the mean generation time of a given strain of bacteria did not remain constant from day to day (personal communication). This variability, which also is evident in the data of Kelly and Rahn [4], obscures the true distribution. Nevertheless, Powell found a correlation between the generation times of sister cells, which provides evidence against both of these hypotheses. This correlation has been confirmed recently by Schaechter *et al.* and extended to include nuclear division [10].

This report presents further evidence against both hypotheses. The distribution of generation time appears to be the result of the normal distribution of its reciprocal, defined here as the *generation rate*.

MATERIALS AND METHODS

Inocula from 18-hr broth cultures of *Escherichia coli* B/r were deposited from a wire loop upon flat thin nutrient agar squares. These squares, about 1 cm on a side and less than 1 mm thick, were placed in depression slides specially constructed to give optical clarity. The depression slides were conventional microscope slides in which a $\frac{5}{8}''$ diameter hole had been drilled. A coverslip was sealed to the top with stopcock grease, the bacterial surface of a square was placed in contact with this coverslip, and the bottom was sealed with another coverslip. The slide was then placed on the stage (37°C) of a phase microscope and photographed (Cine-Kodak Super-X) at 1000 ×, usually at 10-sec intervals. One colony (1.1) was photographed at 6-sec intervals. Following development, the film was examined with a dissecting microscope.

Compared to the eye, the photographic technique has the disadvantage of poorer optical resolution, as Powell [7] pointed out. However, photography has a great advantage in the accuracy of timing, not available to the eye when some 30 cells of a rapidly growing strain are in close juxtaposition. In addition, photography gives a permanent record, allowing reduction of errors and the possibility of reexamination of the film for associated data.

Division was assumed to be marked by the appearance of a septum dividing the mother cell into two daughters. Since the cells are rod-shaped their septa are normal to the plane of the agar surface and therefore easily observable. The cells were coplanar through the fifth generation. During the sixth generation, the layer of cells began to buckle, and it was no longer possible to keep all of them within the depth of focus, which was less than 2μ. Later, secondary cell layers appeared. Thus measurements were limited to a maximum of five generations.

RESULTS

The clonal histories.—The complete observations on the clonal histories of 4 microcolonies are available in Table I. Colonies 2.1, 2.2, and 2.3 were located in the same field of view.

Increase of cell number.—One test of the constancy of experimental conditions is to compare the growth rate constant from experiment to experiment. Every experiment to determine the generation time distribution for cells in

TABLE I. *Observed generation times.*

This table shows the generation time, τ, in frames, of the cells of each generation, starting from the time of the division of each single parent cell. Values for daughter cells are recorded adjacent to that of their mother cell. The sign > ("greater than") indicates a minimum value: the cell was observed not to have divided by the frame number recorded. Colony 1.1 was photographed at 10 frames/min, colonies 2.1, 2.2, and 2.3 were in the same field of view and were photographed at 6 frames/min.

Colony 1.1 Generation					Colony 2.1 Generation				Colony 2.2 Generation					Colony 2.3 Generation				
1	2	3	4	5	1	2	3	4	1	2	3	4	5	1	2	3	4	5
				412									160					170
			139					> 102				94					154	
				197									167					148
		441					183				223					147		
				234									> 125					158
			140					> 102				145					144	
				222									> 125					122
	223					157				109					238			
				275									> 163					150
			259					> 125				155					151	
				273									> 163					155
		237					156				175					152		
				260									166					156
			207					> 125				153					161	
				264									148					94
226					200				321					188				
				211									> 157					176
			297					—				150					140	
				207									> 157					132
		232					> 270				151					138		
				231									> 164					108
			311					—				143					162	
				413									147					140

Table I continued:

Colony 1.1 Generation					Colony 2.1 Generation				Colony 2.2 Generation					Colony 2.3 Generation				
1	2	3	4	5	1	2	3	4	1	2	3	4	5	1	2	3	4	5
	231					172				144					207			
				273									177					166
			244					> 91				136					165	
				278									> 185					121
		214					179				137					174		
				217									> 174					174
			304					> 91				147					130	
				232									> 174					183
				234									176					152
			257					174				104					127	
				326									156					168
		214					146				141					108		
				479									150					178
			255					209				125					155	
				299									127					157
	177					152				259					191			
				220									148					170
			293					162				105					128	
				267									148					139
		208					139				186					142		
				247									> 131					118
			319					162				128					157	
				319									> 131					144
285					129				219					245				
				> 550									191					193
			269					114				156					117	
				328									212					171
		204					169				146					171		
				341									191					136
			319					124				132					142	
				281									214					179
	177					197				190					149			
				220									> 171					190
			299					> 164				173					119	
				222									> 171					151
		228					152				170					139		
				327									> 197					206
			307					> 164				147					163	
				208									> 197					175

the steady exponential growth phase implicitly assumes that cell numbers increase according to the mean growth law

$$N = N_0 e^{\lambda t} = N_0 2^{t/T}; \qquad (3)$$

N is the number of cells present at time t if N_0 cells were present initially with a growth rate λ or a cell mean doubling time T. This law is well-established experimentally for large populations.

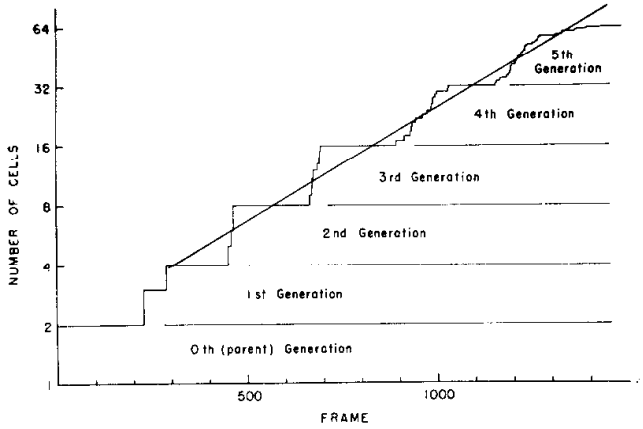

Fig. 1.

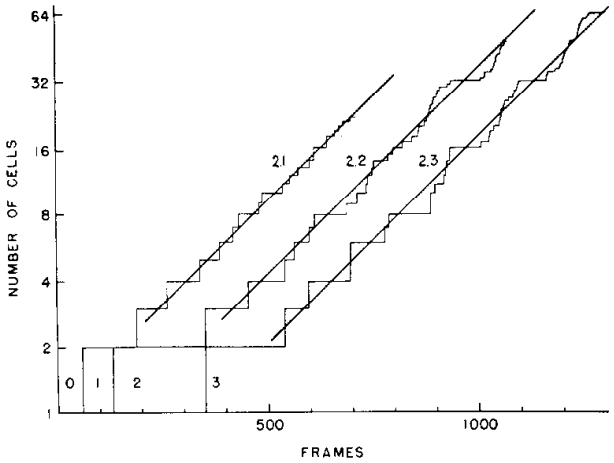

Fig. 2.

Fig. 1.—Growth curve from a single cell. *E. coli* B/r, 6 sec/frame, 37 C, on nutrient agar.

Fig. 2.—Growth curves from three bacteria in the same field of view. 10 sec/frame, 37 C, on nutrient agar.

The data of Table I provide a test of the extension of the numbers mean growth law to small numbers of cells. The numbers growth curves (Figs. 1 and 2) show an overall agreement with the mean growth law, although there are cyclic fluctuations of the kind observed by Prescott for *Tetrahymena* [8], in which cell division frequency first lags behind the mean and then surpasses it. In these figures the straight line approximations to the mean growth rate are visual estimates and not least squares fits of the data; such fitting would depend unduly upon the time at which data collection ceased. Values of T calculated from the slopes of these lines (Table II) agree closely for the four microcolonies: within observational errors, no difference can be demonstrated. The same table shows that the agreement between means, whether potentially biased values are removed or not, or between medians calculated from the values in Table I is much less satisfactory. The potentially biased values removed before calculation of the final two columns in this table are (1) all first generation data, since some of the colonies may not have reached exponential growth phase before the first division, and (2) the data of all generations in which two or more cells had failed to divide at the termination

of the photographic record. Powell [7] has discussed the bias introduced when incomplete data are used. The uniformity of the visual estimates are attributable to inclusion of information contained in the clonal lineage, that is, to the integration of successive divisions.

TABLE II. *Mean and median values of generation time, in seconds.*

Colony No.	Mean[a]	Mean[b]	Mean[c]	Median[c]
1.1	1580	1580	1390	1440
2.1	1600	1640	1700	1650
2.2	1600	1590	1560	1470
2.3	1590	1560	1540	1530

[a] Calculated from the slopes of the straight lines in Fig. 1 and Fig. 2.
[b] Calculated directly from data in Table I, omitting the incomplete (minimum) values.
[c] Calculated from data in Table I after removing potentially biased values as explained in text.

The τ-distribution.—The generation time distribution shown in Fig. 3 is constructed in two parts from all of the data of Table I. The area labeled *A* represents the contribution of generation times that were measured. The area labeled *B* shows the contribution expected from those cells that had not divided by the end of the experiment, and for which, consequently, only a minimal value of τ was available. For each of these cells the *A* distribution was used, as an approximation to the true distribution, to estimate the probability of division during the remainder of the cell's history. For example,

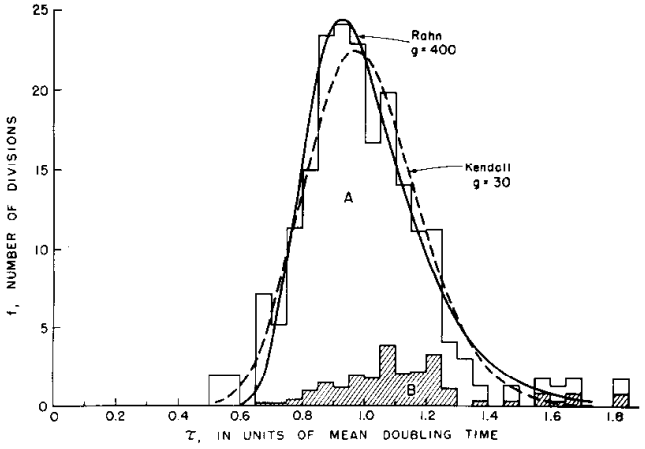

Fig. 3.—The generation time distribution for *E. coli* B/r. The smooth curves are the distributions predicted by Rahn's and by Kendall's hypothesis for the parameters given in Table III. The histogram is composed of two areas: A, values observed for individual cell generation times; *B*, the probability distribution calculated for those cells which failed to divide during the experiment.

a cell that had not divided by time $\tau = 1.2$ was assumed to follow the A probability distribution from that time onward. Although this procedure underestimates the proportion of divisions that occur at long generation times, it serves to illustrate more clearly the asymmetry of the distribution commonly found.

TABLE III. *Parameters and goodness-of-fit for Rahn's and for Kendall's hypothesis.*

Hypothesis	g	m	χ^2	n	$P(\chi^2)$
Rahn	400	0.16	8.3	13	0.82
Kendall	30	0.033	10.1	13	0.67

Both frequency functions, eqs. 1 and 2, can be made to fit the data well, as shown in Table III. On the basis of goodness-of-fit alone either of these hypotheses would be acceptable.

The validity of the τ-distribution shown in Fig. 3 depends not only upon obtaining complete sets of data for each generation, but also depends, as must all such determinations, upon (1) the resolution with which individual times of division could be determined, (2) the establishment of exponential growth under reproducible conditions, and (3) the homogeneity of culture conditions for all cells within a colony.

Independent observations by different observers of the frame during which the septum was completed give a resolution in these experiments of about ± 2 frames, considerably superior to previous visual measurements upon dividing bacteria.

The best evidence for the establishment of the same rate of exponential growth in the different colonies is presented in Fig. 1 and Fig. 2. On the basis of these figures there is little or no evidence that mean colony growth rate deviated from the mean growth law, even for first generation cells.

Homogeneity of culture conditions was tested by examination of a frame with 52 cells from colonies 2.2 and 2.3. Their relative locations, clonal relationships, and generation times are shown in Fig. 4. Cells with generation times greater than the mean of this group, 156 frames, appear to be distributed at random. Certainly, there is no obvious radial dependence with large generation times near the center, as might be expected for very large colonies with centrally depressed levels of nutrient and increased concentrations of metabolic products. As a further test, the 7 cells surrounded

by two or more rows of cells, indicated in the figure by crosshatching, have a mean generation time of 150 frames, not significantly different from the overall mean. This is also the case for the 31 cells on the perimeter which have a mean interdivision time of 157 frames. Thus no gradient of inhomogeneity can be demonstrated.

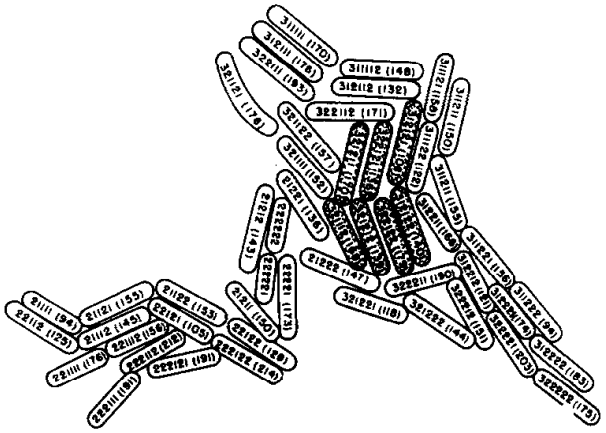

Fig. 4.—Generation times for bacteria in two continuous microcolonies. The generation time (in 10-sec frames) is given in parentheses after the cell number. The first digit indicates the original ancestral cell in the line, and the remaining digits indicate the cell lineage; the digits 1 and 2 were arbitrarily appended for the two sister cells formed at each division.

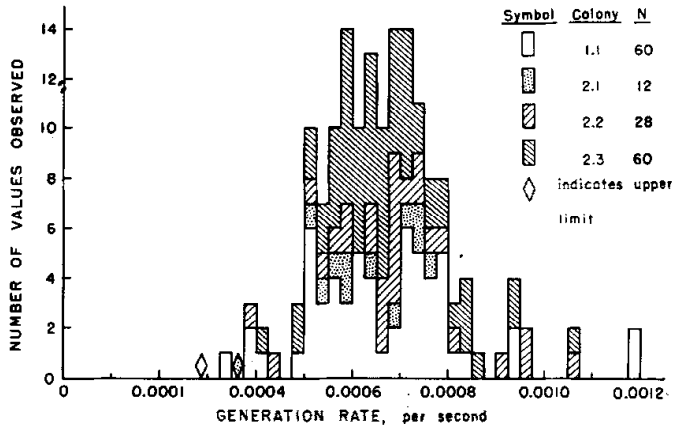

Fig. 5.—The generation rate distribution for *E. coli* B/r.

The generation rate distribution of E. coli B/r.—A more critical interpretation of the cell division data is made possible by examining the distribution of the reciprocal of cell generation time, termed the *cell generation rate*. The distribution of generation rates for *E. coli B/r* is shown in Fig. 5; potentially biased data are not included. To good approximation this distribution is normal, permitting calculation of the product moment correlation coefficients r_{ss} for sister cell pairs and r_{md} for mother–daughter pairs (Table IV). Since colonies 1.1 and 2.1 each still contained a cell that failed to divide within the

photographic record, the minimum and maximum values of the correlation coefficients were calculated on the assumption of the two extreme possibilities: (1) the cells would never have divided, or (2) division would have occurred by the next frame. Table IV shows that sister cell generation rates are in significant correlation, supporting the findings of Powell [7] and of Schaechter

TABLE IV. *Product moment correlation coefficients between sisters, r_{ss}, and between mother and daughter, r_{md}.*

Colony	N	r_{ss}	p	N	r_{md}	p
1.1	31	0.54–0.56	0.01	60	0.12–0.15	ns
2.1	7	0.01–0.22	ns	12	0.05–0.08	ns
2.2	15	0.72	0.01	28	0.16	ns
2.3	31	0.17	ns	60	0.07	ns
Total	84	0.44–0.45	0.01	160	0.10–0.12	ns

N indicates the number of pairs, p the level of significance (ns: nonsignificant). When two values of r are given they indicate the minimum and the maximum values possible for the correlation coefficient, arising because of the failure of one cell in colony 1.1 and one cell in colony 2.1 to divide during the experiment. For these colonies, correlation coefficients were calculated first under the assumption that the cell would never have divided, then under the assumption that division occurred immediately after the final observation.

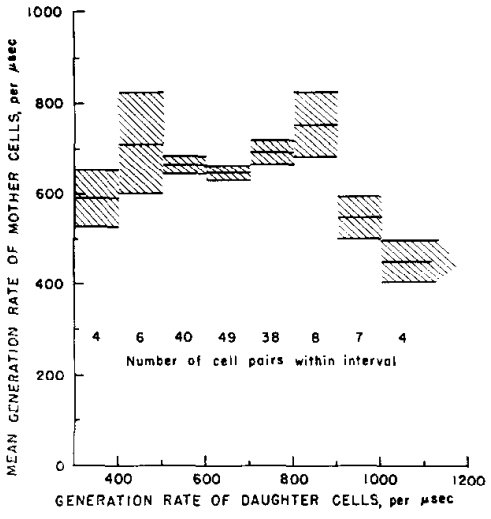

Fig. 6.

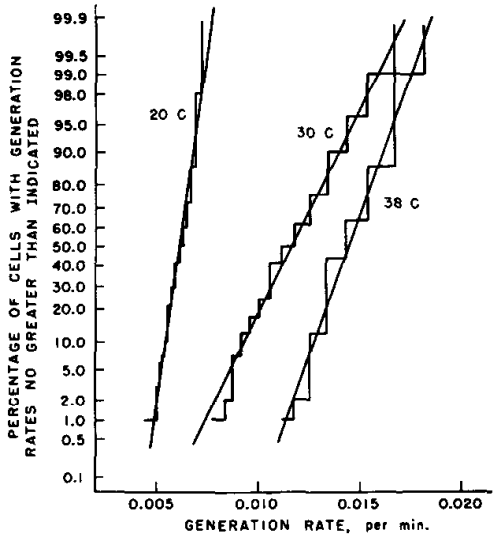

Fig. 7.

Fig. 6.—Grouped values of mother cell generation rates as a function of the generation rates of daughter cells in *E. coli* B/r.

Fig. 7.—The cumulative distribution of generation rates for *Saccharomyces cerevisiae* at three different temperatures. From data of Burns [1].

et al. [10]. The mean of the difference between sister cell generation rates is about two-thirds that for unrelated cells.

Although the *overall* correlation for mother–daughter pairs does not appear significant, a negative correlation exists between the generation rates of the most rapidly dividing cells and those of their mothers. In Fig. 6, which includes first generation data, the 11 most rapidly dividing cells were

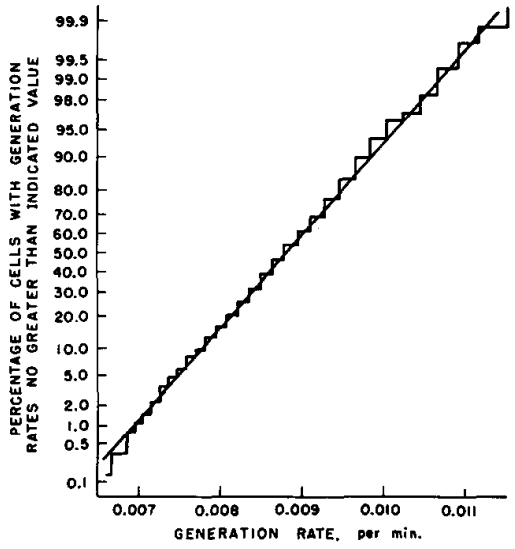

Fig. 8.

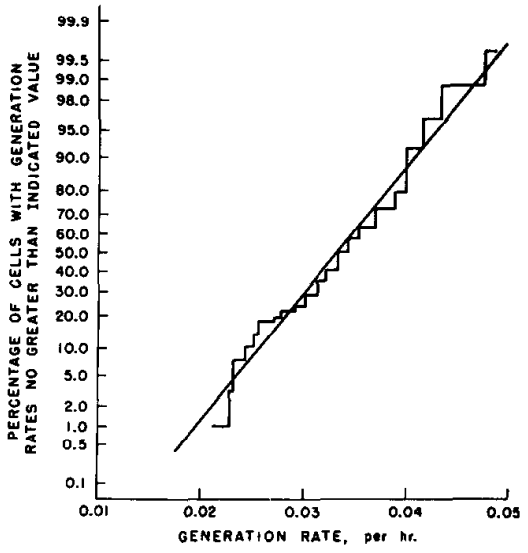

Fig. 9.

Fig. 8.—The cumulative distribution of generation rates for *Tetrahymena geleii* HS. From data of Prescott [8].

Fig. 9.—The cumulative distribution of generation rates for HeLa Cells From data of Hsu [3].

daughters of 9 different mothers, only one of which had a generation rate greater than the mean for the unbiased data, 638.1–643.5 μsec^{-1}. The mean value for these 9 mother cells, 524.8 μsec^{-1}, differs significantly ($t = 2.59 - 3.17$, $p < 0.01$). And even when first generation data are excluded, a significant decrease is observed: the 3 remaining mother cells, for which one or both daughters had generation rates greater than 1000 μsec^{-1}, had, themselves, a mean generation rate of 481 μsec^{-1} ($t = 2.11 - 2.57$, $p < 0.05$).

The normality of the generation rate distribution for other cells.—Cumulative generation rate distributions are constructed from published generation-time histograms for several other extremely different kinds of cells: for *Saccharomyces cerevisiae* (Fig. 7) from the data of Burns [1]; for *Tetrahymena geleii* HS (Fig. 8) from the data of Prescott [8]; and for HeLa Cells (Fig. 9) from the data of Hsu [3]. The criterion of fission was different in each case; for *S. cerevisiae* the initial appearance of a bud was scored; for *Tetrahymena*, cytoplasmic fission; and for HeLa cells, the time from anaphase to ana-

phase. Nevertheless, despite differences in criteria for fission end point, the generation rates in each case are in good agreement with a normal distribution. Furthermore, it is important to note that the agreement improves with increase in the number of cell divisions measured. Thus it would appear that the generation rate is the parameter of first interest, and that the generation time distribution may be computed on the assumption that generation rates follow a normal distribution for cells growing in a steady state in homogeneous culture (balanced growth).

DISCUSSION

It is convenient to think of cell generation rate as akin to the average metabolic rate of the cell from fission to fission. The normal distribution of this rate need not be inconsistent with what Mazia [6] calls "the principle of parallel pathways", which envisages the period between divisions as including a number of specific (and dissociable) preparations for division. But this principle does not reveal the mechanism of control of cell division. At one extreme, the generation rate of a cell could depend upon the individual rates of a large number of intermediate steps. At the other, the normal distribution might arise in an entirely different manner, through control by a single "bottleneck" step, say, through primary control of cell division by enzymatic induction or repression.

Furthermore, the correlations between cell generation times cannot provide a decision between these extremes, although they do rule out the original hypotheses proposed by Rahn and by Kendall, since these assumed independence of cell divisions. The sister–sister correlation indicates, instead, that the generation time is affected by inherited factors. How may we understand the lack of a corresponding overall mother–daughter correlation? Either (1) no division factor is carried over directly to daughter cells, or (2) carry-over occurs, but other strongly compensating factors must also be transmitted. The former is negated by the sister–sister correlation; the latter receives further support from the negative correlation between the rates of the most rapidly dividing daughters and their mothers.

The finding that generation rates appear to be distributed normally for widely different kinds of cells is the most intriguing result presented here. This would seem to be the first evidence of common mechanisms of control of cell division in these cells which are so different cytologically: mitosis has been firmly established only for HeLa cells, while division in the amicronucleate strain of *Tetrahymena* used by Prescott is known to be amitotic.

Cytologically, structure and cell division appear quite different in bacteria, in yeasts and in cells of higher organisms. For example, bacteria lack the kind of nuclear envelope usually found to separate nucleus and cytoplasm. Because of these cytological differences, it is commonly supposed that the mechanisms controlling cell division must reside in smaller structures or in biochemical reactions or both, if, indeed, common mechanisms exist. Evidence for a common mechanism is now provided by the normal distributions of generation rate.

SUMMARY

This study demonstrates a normal distribution for the generation rate (the reciprocal of generation time) of cells of *E. coli B/r* in a condition of balanced growth. A normal distribution is also found upon recalculation of data published for a yeast, for a protozoan, and for HeLa cells.

The observation of this common distribution is evidence for the basic similarity of the cell division process in these widely different kinds of cells.

The normality of the distribution of generation rate allows the calculation of correlation coefficients between the generation rates of sisters and between mother cells and their daughters: sister rates in *E. coli B/r* are correlated, mother and daughter are not, unless daughter cells divide unusually soon after birth. These daughter cells usually had mothers with lengthy generation times. The correlations imply that inherited factors govern the processes leading to cell division, but that their effects are reduced by compensatory processes. In addition, these correlations negate hypotheses like those of Rahn and Kendall, which assume independence of cell divisions.

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