SIMULATION OF SYNCHRONOUS GROWTH

—ON KOCH AND SCHAECHTER'S MODEL FOR THE CELL DIVISION PROCESS—

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1. Introduction

Koch and Schaechter [1] have proposed a model for statistics of the cell division process in bacteria for explaining the experimentally observed skewed nature of the life-length (interdivision time) distribution, the positive sister-sister life-length correlation and the negative mother-daughter life-length correlation. The assumptions on which the model is based are: (i) that growth at the cellular level is deterministic; (ii) that the mean size of a cell at division is under cellular and environmental control; (iii) that the distribution of sizes of cells at division has a small coefficient of variation, and is independent of the size at previous divisions; (iv) that the cell divides nearly into equal halves. This model is referred to as Koch-Schaechter's model hereafter.

The principal postulate of this model where the basic process of biosynthesis of cellular protoplasm is considered to be deterministic radically differs from that of the previous models which presume a stochastic process.

We have attempted the statistical approach on the bacterial cell division process based mainly on data from synchronously dividing cultures. The expected growth curve based on the assumption that the life-length of a daughter is independent of that of the mother was found to provide a rather poor fit to the experimentally observed growth curves of synchronous cultures. And a simple model best fitting to the observed data was Koch-Schaechter's model including the negative mother-daughter life-length correlation compatible with the deterministic process hypothesis.

Some measures employed in our study are different from those in Koch and Schaechter's. In the present experiments the criterion for the cell at division is the presence of a septum, while Koch and Schaechter considered a cell whose adjoining ends appeared to be fully hemispherical

as the cell at division. Both of them possibly introduce a systematic error in scoring the cell length at division because cell division probably terminates either after the time assigned by us or before the time assigned by Koch and Schaechter. And the above criterion adopted by us may give rise to a rather systematic error also in scoring the length of a daughter cell. Using these measures, fittingness of the model to experimental data, not only obtained by us but also appearing in literatures, was analysed by the Monte-Carlo method.

Furthermore, it is pointed out that in the model the life-length distribution will be calculated from the observed distributions of cell lengths at division and of ratios of lengths of two daughters derived from a mother cell.

2. Brief description on the experimental methods

A detailed description on the experimental methods and materials appeared in our previous paper [6]. Here is only a brief description.

2.1 Synchronous culture

A logarithmic phase culture of *E. coli* B* in nutrient broth on a shaker was filtered through Milipore-filter of 1.2μ. The filtrate was cultivated on the shaker again immediately after the filtration.

2.2 Measurement of the number and size of cells

Electronic counting and sizing of bacterial cells were made by means of the Coulter Particle Counter, Model B (Coulter Electronics Inc., U.S.A.) with an aperture of 50μ in diameter. Besides, the ordinary microscopical measurement was carried out.

We expressed the size of bacterial cells measured by means of the Coulter Counter in terms of length because it is unlikely that this expression introduces any essential bias in the analysis of the present experimental system where the length of the bacterial cell can be proportional to the volume.

3. The model and the experimental results

3.1 The model and notation

Let the length of a cell at time 0 be y_0, and that of a growing but non-dividing cell at time t

$$y=y_0e^{\lambda t} \tag{1}$$

* The strain was received by courtesy of Dr. R. Nakaya of the National Institute of Health, Tokyo.

where λ is a common parameter to all cells of a strain. As stated in [1] the experimental data ([2], p. 425) are in accordance with the notion that each cell has the same growth rate constant.

Let the length of a cell at division be c, which is a random variable following the $g(c)$ distribution. $g(c)$ is the frequency function of the distribution of the lengths of cells at division.

Let the ratio of the length of one daughter to that of the mother be p, and the ratio of the length of the other daughter to that of the same mother $1-p$. And let the frequency function of the distribution of p be $h(p)$. The lengths of a pair of daughters are cp and $c(1-p)$, which both correspond to y_0. Each of them increases in length, in accordance with the equation (1), to reach c_1 or c_2 where the cell divides. c_1 and c_2 are independently distributed with the fr. f. g(c). Each cell is assumed to grow and divide in this way and the fr. f. of the life-length distribution is denoted by $f(t)$.

The notations, $g(c)$, $h(p)$ and $f(t)$, are in accordance with those of Koch and Schaechter [1].

The effect of the parameter λ on the mean life-length is essential. Let us here briefly compare the effect of the parameter λ where λ is assumed to be a constant λ_0 for all individuals (in fact, we assume so throughout this paper) with that of the growth rate λ where λ is assumed to be a random variable having the mean λ_0 and the coefficient of variation γ. It may be considered that γ is much smaller than 1. If we assume that the variation of λ is independent of either the starting size or the size at division, then $m_\lambda = m_{\lambda_0}(1+\gamma^2)+0(\gamma^2)$ and $\sigma_\lambda^2 = \sigma_{\lambda_0}^2+\gamma^2(3\sigma_{\lambda_0}^2+m_{\lambda_0}^2)+0(\gamma^2)$, where m_λ and σ_λ^2 (in the case λ varies) or m_{λ_0} and $\sigma_{\lambda_0}^2$ (in the case $\lambda=\lambda_0$: constant) are the mean and the variance of life-length distribution, respectively.

3.2 Estimations of $g(c)$ and $h(p)$

It is rather difficult to measure c and p in the strict sense. As stated in the above, the length of cells with a septum was adopted as an alternative to c, and the ratio of length of one side of the septum to the whole length of the cell as an alternative to p (see Fig. 1). It should be noted that the measurement of g(c) on samples from a synchronous culture will result in a wrong figure because there is a bias in the size distribution. There are evidences demonstrating that a large portion of dividing cells were rather smaller at the early stage of the dividing phase of the population in a synchronous culture than at the late stage, as shown in Figs. 2.1 and 2.2. Therefore, the observation of $g(c)$ was performed on 200 cells with a septum from an exponentially growing mass culture on a shaker, which is presented in Fig. 2.3. Fig. 3 presents an example of the distribution of p, measured on an aliquot

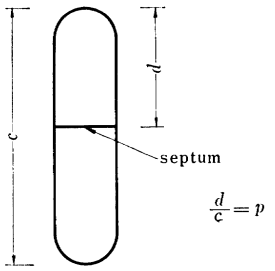
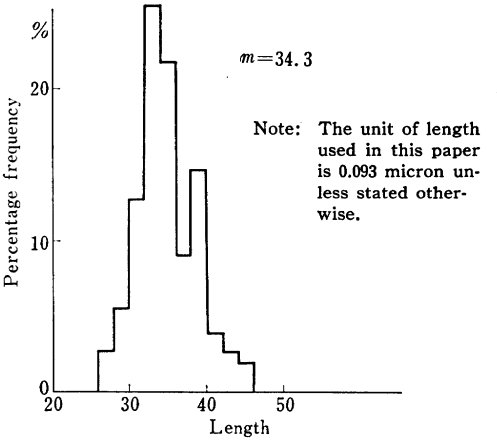


Fig. 1. Diagrammatic presentation of c and p



Note: The unit of length used in this paper is 0.093 micron unless stated otherwise.

Fig. 2.1. Distribution of lengths of cells with a septum (early stage of the dividing phase of a synchronous culture)

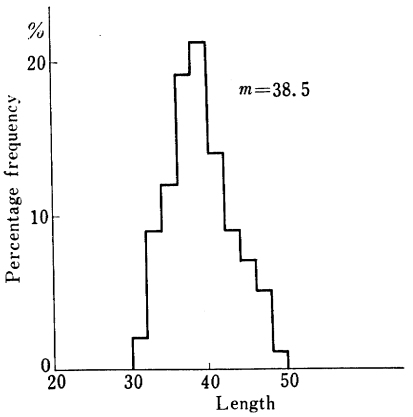


Fig. 2.2. Distribution of lengths of cells with a septum (late stage of the dividing phase of a synchronous culture)

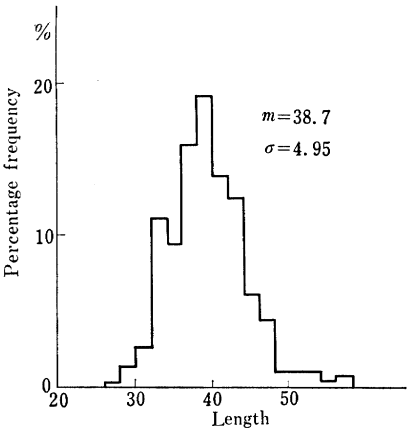


Fig. 2.3. Distribution of lengths of cells with a septum (exponentially growing nonsynchronous culture)

from a balanced growth culture, which shows that the distribution is fairly close to a normal distribution.

There are two evidences, on which the reason is based why the distribution of lengths of cells with a septum was reasonably adopted as an alternative to the $g(c)$ distribution: (i) the mean lengths of cells with a septum and their coefficients of variation in the present experiments were fairly close to those of Schaechter et al. [2] (Table 1); (ii) the ratio of the number of cells with a septum to the total number of cells at each observation time was parallel fairly with the rate of increase in the total number of cells at the time when the number of cells with a septum was measured (Table 2).

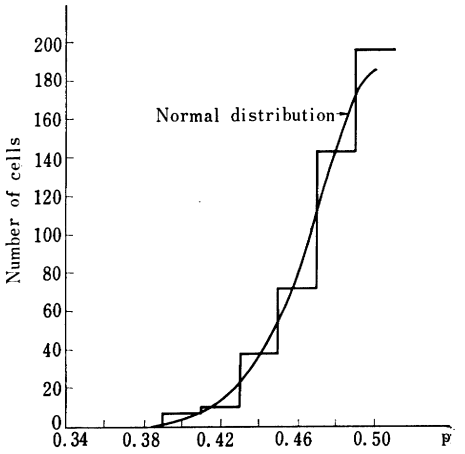


Fig. 3. Observed distribution of p in a balanced growth culture, exhibited in the form of a histogram, together with a fitted normal distribution curve

Table 1. Mean length of cells at division and the coefficient of variation

Source of data	Mean (micron)	C. V.	Sample size
Schaechter et al. [2]	5.1	0.085	37
〃	4.3	0.089	22
The present authors (Fig. 2.3)	3.6	0.128	299

Table 2. Rate of increase in the number of cells and ratio of the number of cells with a septum to the total of cells

	Rate of increase in the number of cells	Ratio of the number of cells with a septum to the total cells
Early stage of the dividing phase	0.157	0.132
Late stage of the dividing phase	0.056	0.030

3.3 Elongation of individual cells

In their model Koch and Schaechter have tentatively assumed, based on their data in [2], that individual cells increase exponentially in length. If this assumption always holds true, the total cell length of a population in a synchronous culture will increase in an exponential curve from the biginning of growth. Then the total cell length S_t at time t is $S_0e^{\lambda t}$. Let the number of cells at time t be N_t and the mean length be L_t. Then,

$$S_t = N_t L_t \,.$$

Thus, for an estimate of S_t we use $n_t l_t$, where n_t and l_t are the observed values for N_t and L_t, respectively. If the sample to estimate N_t and the sample to estimate L_t are drawn independently, then

$$\sigma^2(n_t l_t) = N_t^2 \sigma^2(l_t) + L_t^2 \sigma^2(n_t) + \sigma^2(n_t)\sigma^2(l_t) \,.$$

The vertical segments in Fig. 4.1 are $[n_t l_t - 2\sigma(n_t l_t),\ n_t l_t + 2\sigma(n_t l_t)]$. ($\sigma^2(*)$ denotes the variance of $*$.) As shown in Fig. 4, the data from synchronous culture experiments approximately fit the above hypothesis.

3.4 Comparison of the results from the electronic and the microscopic sizing

To determine the distribution of cell lengths at various situations of culture, the microscopic measurement as well as the electronic were made of two aliquots from the population of cells in a synchronous culture and of an aliquot from an exponentially growing nonsynchronous culture. The results are summarized in Table 3. The ratio in several measurement between the mean cell length measured by the Coulter Counter and that by the microscope were approximately constant (c.f. [3], p. 523).

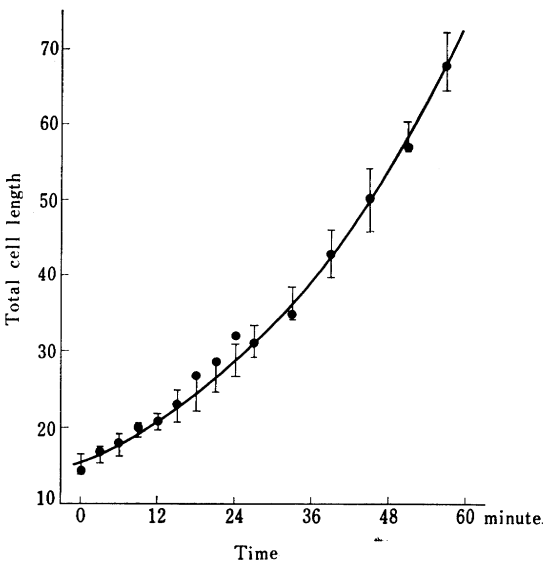


Fig. 4.1. Measurement error of total cell length in a synchronous culture

Solid circle: observed total cell length
Vertical segment: see the text
Smooth curve: expected curve based on the exponential growth hypothesis

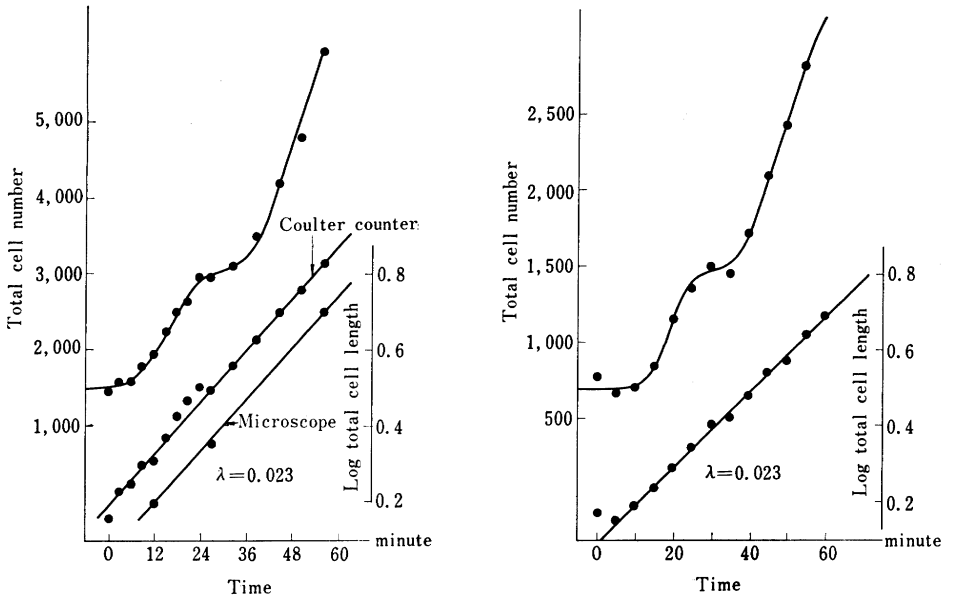


Fig. 4-2. Change in total cell length during the synchronous growth
A solid circle represents an observed value.
The smooth line is a fitted curve for the total number of cells (above) or for the total cell length (below).

Table 3. Comparison of electronic and microscopic measurements of cell length

Phase	Electronic		Microscopic		Sample size	B/A	D/C
	Mean: A	s.d.: C	Mean: B	s.d.: D			
Balanced growth phase*	10.41	2.33	25.61	7.07	500	2.46	3.03
Early stage of the dividing phase**	10.55	2.24	24.07	4.74	500	2.28	2.11
Late stage of the dividing phase**	11.34	2.74	25.84	5.70	500	2.28	2.08

Note: Based on these results, the transformation of the scale of sizing by the Coulter Counter (x) to the length in terms of the micrometer (y) was $y=2x+5$, for convenience.
* Nonsynchronous culture
** Synchronous culture

4. Simulation of cell division process

4.1 The data for simulation

Based on the data presented in the above and according to Koch-Schaechter's model, a simulation of cell division process was carried out. The following are the actual figures used in the simulation.

$g(c)$: See Fig. 5.

$h(p)$: See Fig. 6.

These distributions were obtained by smoothing and interpolation of the distributions in Fig. 2.3 and Fig. 3.

Elongation: $y=y_0 \exp(0.023\,t)$.

Initial distribution of cell lengths (at time 0): See Fig. 7. The distribution of cell lengths at time 0 calculated from the data obtained by the Coulter Counter was so modified that the expected rate of increase in the number of cells approached to the actually observed data, because the expected growth curve calculated from the unmodified data by the

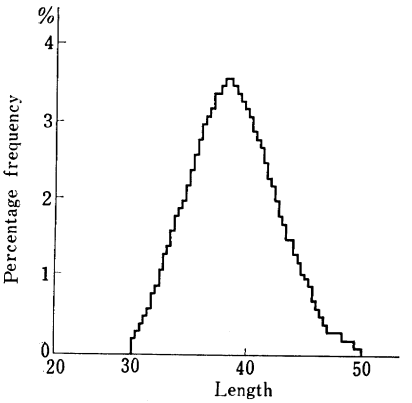


Fig. 5. $g(c)$ for simulation

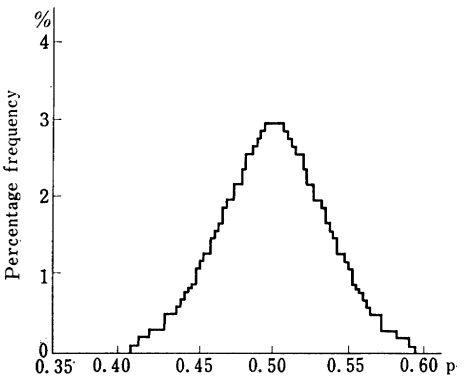


Fig. 6. $h(p)$ for simulation

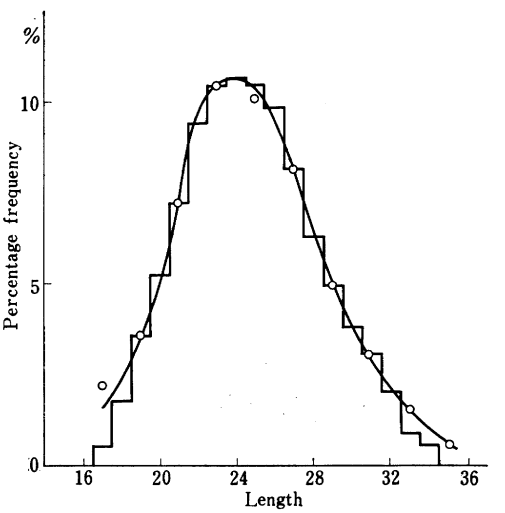


Fig. 7. Initial distribution of cell lengths in simulation (histogram) or in electronic sizing (empty circle with a fitted smooth curve)

Coulter Counter showed clear deviations from the observed growth curve in synchronous cultures.

4.2 Process of simulation

(i) Fifteen sample values $x_1, x_2, \cdots, x_{15}$ from the distribution with the fr. f. $g(c)$ were assigned to each of the 15 cells defined in Fig. 8, respectively.

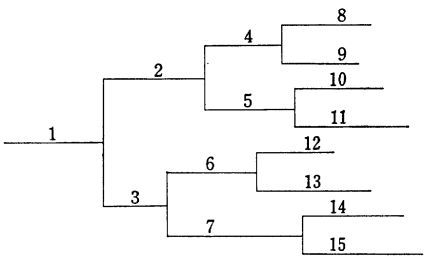


Fig. 8. Diagrammatic presentation of the multiplication of cells, by the binary division, starting with a cells, cell-1. The cells belonging to this clone are numbered successively, and referred to as cell-2, cell-3 and so on.

(ii) Seven sample values from the distribution with the fr. f. $h(p)$ $p_1, p_2, \cdots, p_7$ were assigned to each of 7 cells, from cell-1 to cell-7, in Fig. 8. And the lengths of daughter cells of each of the mother cells, from cell-1 to cell-7, were determined in turn by x_1p_1, $x_1(1-p_1)$, x_2p_2, $x_2(1-p_2), \cdots$.

It is to be noted that in the present case $\max c \cdot \max p$ was smaller than $\min c$.

(iii) The lengths of cell-1 at time 0 were so determined that the frequency was proportional to the given initial distribution (Fig. 7).

(iv) The life-lengths of 14 cells, from cell-2 to cell-15, were calculated. As to the cell-1, the length of time till the cell starts to divide was calculated.

As the simulation started with about 400 cells, the above calculations were repeated 400 times on each of the cells, from cell-1 to cell-15. And the distribution of cell lengths of the population which started with 400 cells was calculated at every 3 minutes interval, that made 20 observation times in total.

(v) The distribution of life-lengths of the cells, from cell-2 to cell-15, of the population which started with 400 cells was determined.

(vi) The correlation of the life-lengths of the cell-2's and those of the cell-4's and the correlation of the life-lengths of the cell-2's and those of the cell-3's were calculated.

4.3 Examination of random numbers

There were no significant differences between the distributions of random numbers employed in the simulation and the given distributions in Figs. 5 and 6 (cf. $\chi^2=8.63$ with 11 degrees of freenom for $g(c)$ and $\chi^2=10.96$ with 14 degrees of freedom for $h(p)$).

4.4 Explanation of the results

The life-length distribution resulting from the simulation is presented in Fig. 9. Sister-sister correlation of life-lengths was 0.248, and daughter-mother correlation of life-lengths was -0.382.

The growth curve resulting from the simulation when the distribution of the lengths of cell-1's was so modified as given in Fig. 7 is shown in Fig. 10.

The cell-length distributions resulting from the simulation did not always fit the observed data by means of the electronic sizing (cf. Fig. 13). The most striking discrepancy was whether two peaks appeared

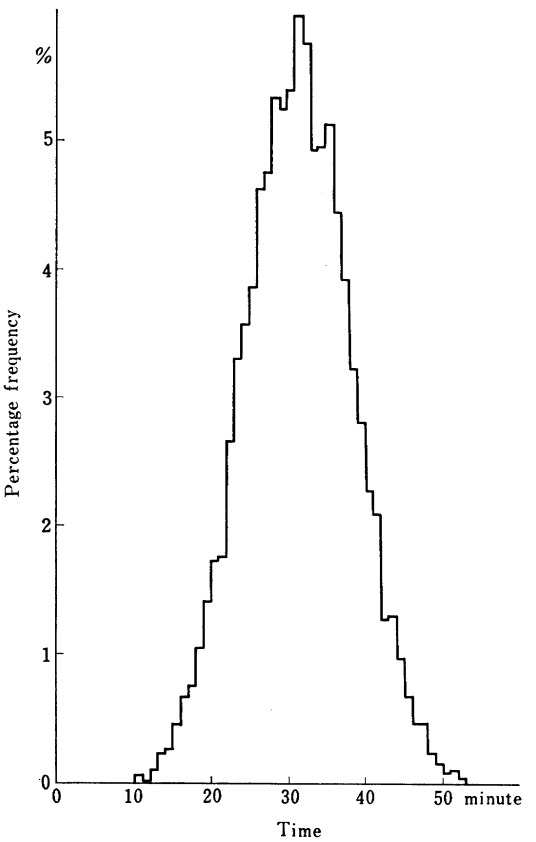


Fig. 9. Life-length distribution resulting from the simulation

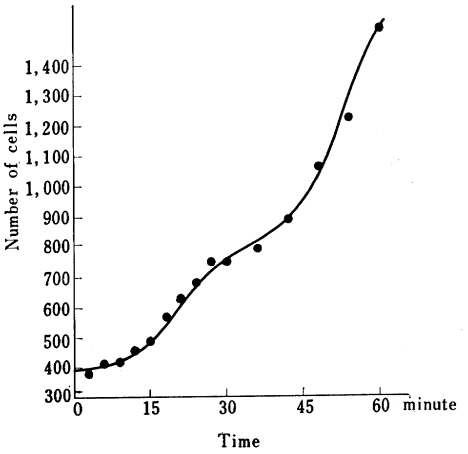


Fig. 10. Growth curve resulting from the simulation (smooth line) compared with the observed value (solid circle)

or not during the dividing phase. The distribution resulting from the simulation displayed two peaks, one of which was corresponding to the cell population going to divide and the other to the cell population having divided, while the observed data did not show two clearly discernible peaks.

5. Discussions

(1) Degree of synchrony

There was not much difference between the life-length distribution which was calculated by simulation from our data presented here in accordance with Koch-Schaechter's model and the life-length distribution actually observed by Schaechter et al. [2].

If it is assumed that the life-length of a daughter cell is independent of that of mother cell, no clearly discernible interdivision phase will appear between the first and second division phases in the expected growth curve, even if all the cells at the starting point of the culture were so completely synchronous that the cells were all of the same age of life, as shown in Fig. 11.1, in which the expected growth curve was calculated with the life-length distribution obtained above and under the assumption that all the cells at the starting point had age 0. The expected growth curve obtained from the life-length distribution presented by Schaechter et al. [2] under the same assumption showed a similar pattern if we assumed no life-length correlation between the daughter and the mother (Fig. 11.2).

On the other hand, under the assumption of the negative daughter-

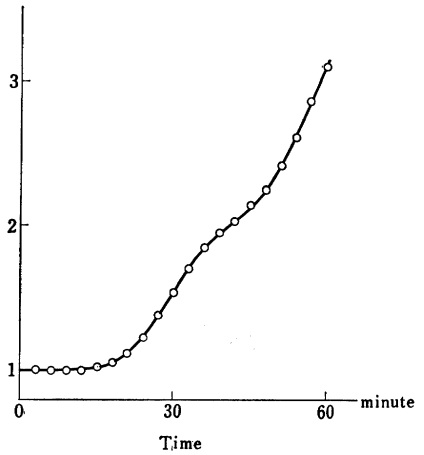


Fig. 11.1. Expected synchronous growth curve when no daughter-mother life length correlation is assumed, where all the cells at time 0 have age 0

Data: the present authors

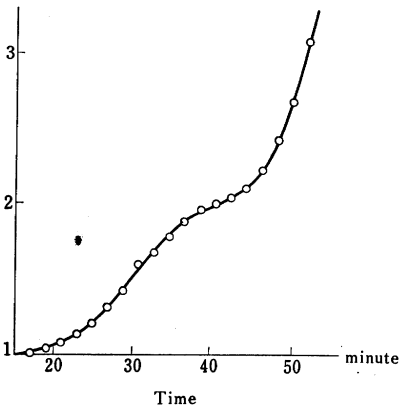


Fig. 11.2. Expected synchronous growth curve when no daughter-mother life length correlation is assumed, where all the cells at time 0 have age 0

Data: Schaechter et al. [2]

mother life-length correlation the expected growth curve showed a clear discernible phase between the first and second division phases as shown in Fig. 12.

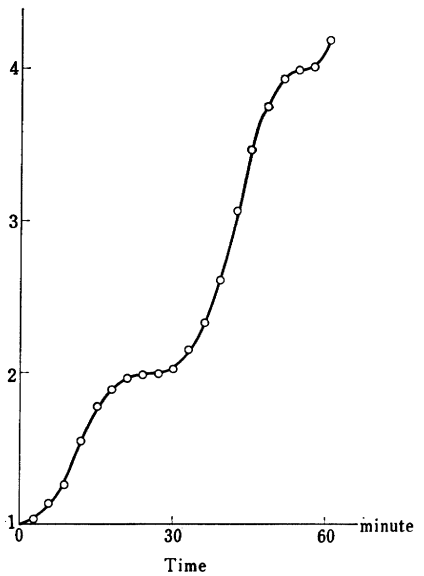


Fig. 12. Expected synchronous growth curve when negative daughter-mother life length correlation is assumed, where all the cells at time 0 have age 0

Data: the present authors

(2) Life-length distribution

Compared with the results from the simulation and those of Schaechter et al. [2], fairly coincident values are seen for the mean of the life-length distribution, the coefficient of variation, and the skewness (see Table 4).

Table 4. Comparisons of the life-length distribution resulting from the simulation (Fig. 9) and that of Schaechter et al. [2]

	Source of data	
	The present authors (Fig. 9)	Schaechter et al. [2]
Mean (min.)	30.3	28.4
s. d.	7.13	5.93
Skewness	−0.004	0.005

$\chi^2=7.80$, with 6 degrees of freedom

In the case there exists an intergeneration life-length correlation, the definition and estimation of the life-length distribution must be made carefully. In the present model, however, there will be no problem in this point, as noticed in 4.2 (ii).

(3) Correlation coefficients of life-length

The observed correlation coefficients between the life-lengths of sisters and between the life-lengths of daughters and mothers of *E. coli* B, (See [2]) were 0.183, 0.628, 0.795, 0.544 between sisters and −0.539, −0.542, −0.195, 0.008 between daughters and mothers, while the values resulting from the simulation were 0.248 between sisters and −0.382 between daughters and mothers.

When one compares the values of Schaechter and Koch with ours, the following three points must be kept in mind:

1) the values of [2] may possibly contain considerable sample variation, as those were obtained from a sample of size about 30. Therefore an assay for significance is needed.

2) The difference in the experimental conditions between [2] and ours may influence on the values. If so, one can not expect a meaningful conclusion from the comparison between the results obtained from the experiments different from each other.

3) The correlation coefficient between the life-lengths of sisters will be biased towards value +1/2 and that between daughters and mothers −1/2 by the errors contained in the direct measurement of the time of division, if one calculates the correlation coefficient from the data at the division time which are observed directly.

This may be deduced from the fact that there exist the following relationships between the true population correlation coefficient and the correlation coefficient including the measurement errors:

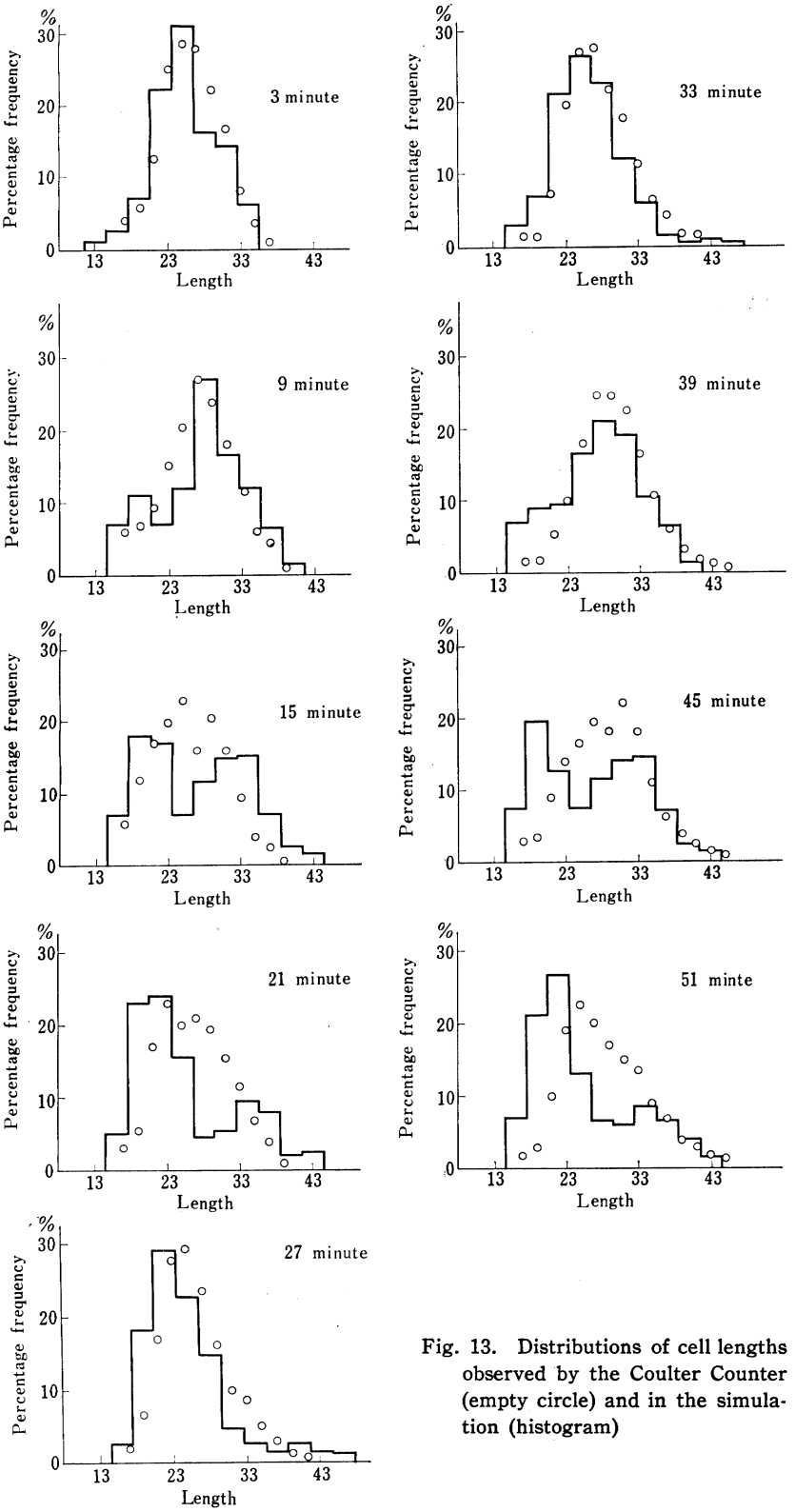


Fig. 13. Distributions of cell lengths observed by the Coulter Counter (empty circle) and in the simulation (histogram)

$$r_H=\frac{\rho_H-\gamma}{1+2\gamma}, \qquad r_S=\frac{\rho_S+\gamma}{1+2\gamma},$$

where r_H and r_S are the latter correlation coefficients between sisters and between daughters and mothers, respectively, ρ_H and ρ_S the former correlation coefficients between sisters and between daughters and mothers, respectively, and γ the ratio of the variance of observation error at the division time to the variance of the population life-length distribution.

The detailed descriptions concerning this point are presented in [4]. The values reported in [2] are more or less affected by these errors, while ours are not affected, because, in the calculation of the correlation coefficient, we did not use the data which were obtained by the direct measurement of the time of division.

As to values, there are other problems, what effects were given in the calculation by the unavoidable errors in the measurements of the number and lengths of cells with a septum and of the length of each side of the septum.

Considering those situations, it is not necessary to think that our data are inconsistent with those of [2].

(4) On the distribution of the cell-length

The discrepancy in the distribution of the cell-length between the observed data and the results from the simulation may be due to that the electronic sizing does not exactly reflect the cell-length. Otherwise, there may be some problems in the assumptions in the model. The resolution of the discrepancy needs further investigations.

(5) A few other problems still to be investigated

Can the distribution of lengths of cells with a septum be reasonably used as an alternative to $g(c)$ or $h(p)$? As stated in the introduction, the cell division will be completed some time after the septum becomes visible. And as the measurement of the number of cells is made on the cells completely separated, there must be a time lag between scoring of the length of cells at division, as defined in the present experiments, and scoring of the number of cells at division. The point is the magnitude of possible systematic errors due to the time lag.

The exponential elongation rate hypothesis is a problem also to be confirmed.

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