Mathematical relations for the number of mature T4 bacteriophages, both inside and after lysis of an Escherichia coli cell, as a function of time after infection by a single phage were obtained, with the following five parameters: delay time until the first T4 is completed inside the bacterium (eclipse period, \(\nu\)) and its standard deviation (\(\sigma\)), the rate at which the number of ripe T4 increases inside the bacterium during the rise period (\(\alpha\)), and the time when the bacterium bursts (\(\mu\)) and its standard deviation (\(\beta\)). Burst size \(B = \alpha(\mu - \nu)\), the number of phages released from an infected bacterium, is thus a dependent parameter. A least-squares program was used to derive the values of the parameters for a variety of experimental results obtained with wild-type T4 in E. coli B/r under different growth conditions and manipulations (H. Hadas, M. Einav, I. Fishov, and A. Zaritsky, Microbiology 143:179–185, 1997). A “destruction parameter” (\(\xi\)) was added to take care of the adverse effect of chloroform on phage survival. The overall agreement between the model and the experiment is quite good. The dependence of the derived parameters on growth conditions can be used to predict phage development under other experimental manipulations.
The average PPB in the lysed bacteria at \( t \) is therefore
\[
\phi_2(t) = \int_{-\infty}^{t} p_2(t') m(t') \, dt'
\] (6)
where the integral measures PPB for the bacteria that burst between \( t \) and \( t + dt \). Using equations 4 and 5,
\[
\phi_2(t) = (\alpha \sigma^2/2 \sqrt{\pi}) \int_{-\infty}^{t} \text{ierfc}[(v - t')/\sigma] \exp[-(t' - \mu)^2/\beta^2] \, dt'
\] (7)
The average PPB in bacteria which have not lysed by the time \( t \) is given by
\[
\phi_1(t) = [1 - \int_{-\infty}^{t} p_2(t') \, dt] m(t)
\] (8)
where the relation in the brackets is the probability that the bacterium did not burst by \( t \) and \( m(t) \) (equation 2) is the PPB if the bacterium indeed did not lyse. Hence,
\[
\phi_1(t) = \frac{\alpha \sigma}{2} \left[ 1 - \frac{1}{2} \text{erfc} \left( \frac{\mu - t}{\beta} \right) \right] \text{erfc} \left( \frac{v - t}{\sigma} \right)
\] (9)
and the total PPB is
\[
\phi(t) = \phi_1(t) + \phi_2(t)
\] (10)
As mentioned before, the measured quantities are \( \phi(t) \) and \( \phi_2(t) \).
In order to check these relations, we use the asymptotic forms of the functions appearing here (1). Thus for \( t \gg v \), \( (v - t)/\sigma \), which we denote by \( -u \), is negative, and
\[
\text{ierfc}(-u) \rightarrow u(1 + \text{erfu}) + e^{-u^2}/\sqrt{\pi}
\] (11)
For \( u \rightarrow \infty \) we have \( \text{erfu} \rightarrow 1 \) and \( e^{-u^2} \rightarrow 0 \); therefore \( \text{ierfc}(-u) \rightarrow 2u \), and \( m(t) \) (equation 2) \( \rightarrow \alpha(t - v) \), as it should (Fig. 1). Hence,
\[
\phi_1(t) = \phi(t) \left(1 + \frac{t}{t_0}\right) \left[1 - \exp\left(-\frac{t}{t_0}\right)\right]
\]

(12)

where \( u = (t - \mu) / \beta \). And after some algebra,

\[
\phi_2(t) = \frac{\alpha(\mu - v)}{\beta(\mu - v/2)} \left(\text{erf}(\eta) - \eta \text{erfc}(\eta)\right)
\]

(13)

For \( t \gg \mu \), the second term \( \rightarrow 0 \) and \( \phi_2 \rightarrow a(\mu - v) \), as it should (Fig. 1). Similarly,

\[
1 - \frac{1}{2} \text{erfc}\left(\frac{\mu - t}{\beta}\right) \rightarrow 0 \quad \text{for} \quad t \gg \mu
\]

and thus by equation 9, \( \phi_1 \rightarrow 0 \).

**Methods.** The model was tested against results (20) obtained with wild-type T4 phage (5) infecting either E. coli B/r (H266) (36) or E. coli K12 (CR34, thr leu thy dmr) (35). Bacteria were cultured with vigorous shaking at 37°C in the shaking media (36): Luria-Bertani broth containing glucose (LBG) (0.4%; doubling time \( \tau = 23 \text{ min} \)); M9 minimal medium supplemented with casein hydrolysate (1%), tryptophan (50 \( \mu \)g/ml), and glucose (GC) (0.4%; \( \tau = 28 \text{ to 30} \text{ min} \)) or with 0.4% of either glucose (\( \tau = 48 \text{ min} \)), glycerol (\( \tau = 70 \text{ min} \)), or succinate (\( \tau = 90 \text{ min} \)). For the thyA strain of E. coli K12, 5 \( \mu \)g of thymine/ml was added, either with deoxyguanosine (100 \( \mu \)g/ml) or not (35). Upon achieving a steady state (18, 34) at a concentration of \( 10^9 \) cells ml\(^{-1} \), cells were infected with phage (or treated prior to infection as described previously [20]) at a multiplicity of 0.5 (to guarantee a single infection) in the presence of 2 mM KCN (to synchronize the infective process). Phage development was initiated 4 min after phage addition by dilution (10\(^{-4} \)) in the same medium to cease further infections and eliminate the bacteriostatic effect of the cyanide. Samples were withdrawn periodically and plated immediately and through chloroform (after evaporation) after appropriate dilutions, while for the chloroform titration it is only 90.3. To improve the procedure as described above yielded the following estimates for the parameters: \( \nu = 18.2 \pm 0.1 \); \( \sigma = 2.93 \pm 0.1 \); \( \mu = 24.11 \pm 0.1 \); \( \beta = 3.8 \pm 0.14 \); \( \xi = 0.82 \pm 0.02 \); and \( B = 250.4 \pm 5.2 \). The \( r^2 \) values of the fit, shown by the lines in Fig. 2, are however rather poor. For the regular titration, we get 95.2, while for the chloroform titration it is only 90.3. To improve these estimates, we used the moving average method (see, e.g., reference 8), which smooths out the results. The average of the first three points was calculated and taken as the second value, the average of the second to fourth points for the third value, etc. The parameters estimated following this averaging process were as follows: \( \nu = 18.3 \pm 0.3 \); \( \sigma = 3.37 \pm 0.2 \); \( \mu = 23.6 \pm 0.1 \); \( \beta = 4.3 \pm 0.15 \); \( \xi = 0.84 \pm 0.01 \); and \( B = 247.1 \pm 5.3 \) (Fig. 3), with an obvious improvement in \( r^2 \) (regular titration, 97.8; chloroform, 97.4). It is clearly seen that such a smoothing transformation is quite beneficial. In addition, all parameter values other than \( \beta \) remained essentially unchanged, indicating that they were quite robust and reliable.

Note that the results (Table 1) were obtained with the same six parameters used for both \( \xi \cdot \phi \) and \( \phi(t) \) in each case. The overall agreement is quite good (see the Statistical Analysis section below). The value of \( \xi \) that is larger than 1.0 cannot be simply explained; more data points in the plateau region may solve this apparent discrepancy.

The numerical data processing method used here was developed in the last decade. The data contain values of \( \phi \) and \( \phi_2 \) at \( t_1, t_2, \ldots, t_n \). The independent model parameters were sought in such a way as to minimize the error. An elaborate least-squares method was used here because the problem is complicated due to the functional form (an integral for which no analytic expression was found).

The programs used to evaluate the parameters were Minpack (Argonne National Laboratory, 1980) and Simusolv (Dow Chemical Co., 1990). The algorithm used in Minpack is a modified Newton one (27, 28), where an approximation is built for the Hessian of the Newton method. It can be noted that in many cases Minpack helps to find the global minimum, independent of the starting point. In Simusolv, the movement towards the minimum is accomplished by combining two subgroups, Search and GRG (Generalized Reduced Gradient).

In the processing performed here, the simpler program, Minpack, was used to obtain good first approximations. Note that Simusolv can fit several (here, two [\( \phi \) and \( \phi_2 \)] functions simultaneously and even functions given by their differential equations. The latter was used specifically for \( \phi_2 \).

**Results and discussion.** Equations 7, 9, and 10 constitute a system whereby the experimental results can be analyzed. The measurement of total PPB (\( \phi \)) is performed by applying chloroform. We have found (data not shown) that the addition of chloroform usually causes a reduction of phage ability to form plaques (plating efficiency) by 5 to 20%, resulting in an effective reduction of PPB which does not change with time. Hence, we assumed that the measured PPB is given by \( \xi \cdot \phi \), where \( \xi \) is the chloroform “destruction parameter.” Thus, altogether six independent parameters were calculated: \( \mu, v, \alpha, \beta, \sigma, \) and \( \xi \).

The model was tested against previously published results (20). Results appear in Table 1, together with the burst size \( B = a(\mu - v) \). The large variation in derived estimated parameters might have been caused by a small number of data points. To appreciate the problems in analysis, the classical LBG experiment (17) is presented at a higher measured accuracy (minute-by-minute intervals) (Fig. 2). Even under casual observation it is seen that the scatter of points in the chloroform titration results is quite large. Applying the Simusolv procedure as described above yielded the following estimates for the parameters: \( \nu = 18.2 \pm 0.1 \); \( \sigma = 2.93 \pm 0.1 \); \( \mu = 24.11 \pm 0.1 \); \( \beta = 3.8 \pm 0.14 \); \( \xi = 0.82 \pm 0.02 \); and \( B = 250.4 \pm 5.2 \). The \( r^2 \) values of the fit, shown by the lines in Fig. 2, are however rather poor. For the regular titration, we get 95.2, while for the chloroform titration it is only 90.3. To improve these estimates, we used the moving average method (see, e.g., reference 8), which smooths out the results. The average of the first three points was calculated and taken as the second value, the average of the second to fourth points for the third value, etc. The parameters estimated following this averaging process were as follows: \( \nu = 18.3 \pm 0.3 \); \( \sigma = 3.37 \pm 0.2 \); \( \mu = 23.6 \pm 0.1 \); \( \beta = 4.3 \pm 0.15 \); \( \xi = 0.84 \pm 0.01 \); and \( B = 247.1 \pm 5.3 \) (Fig. 3), with an obvious improvement in \( r^2 \) (regular titration, 97.8; chloroform, 97.4). It is clearly seen that such a smoothing transformation is quite beneficial. In addition, all parameter values other than \( \beta \) remained essentially unchanged, indicating that they were quite robust and reliable.

Note that the results (Table 1) were obtained with the same six parameters used for both \( \xi \cdot \phi \) and \( \phi(t) \) in each case. The overall agreement is quite good (see the Statistical Analysis section below). The value of \( \xi \) that is larger than 1.0 cannot be simply explained; more data points in the plateau region may solve this apparent discrepancy.
Figure 4 presents three comparisons between the model and actual experiments previously reported (20) (Table 1). The three examples displayed were selected to cover the whole range of burst sizes (between 9 and 720); thus, a semilog presentation was used. (For clarity, the corresponding experiments with chloroform were not included, and the points at early times were deleted.) Correlations between burst size and cell size were observed in early studies with the T-series bacteriophages (see, e.g., references 13 and 21), but it was too early for them to be accounted for by the physiological parameters of \textit{E. coli}, which emerged a decade later (25, 32).

Table 1. Parameters, at different growth conditions, calculated by the analysis from experimental data\(^a\)

<table>
<thead>
<tr>
<th>(E. \text{ coli} ) strain</th>
<th>medium/treatment</th>
<th>(\tau ) (min)</th>
<th>(\nu \pm \sigma) (min)</th>
<th>(\mu \pm \beta) (min)</th>
<th>(\xi)</th>
<th>(B)</th>
<th>(\alpha = B(\mu - \nu))</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/r, LBG/steady state(^b)</td>
<td>23</td>
<td>18.3 ± 3.4</td>
<td>23.6 ± 4.3</td>
<td>0.84</td>
<td>247.1</td>
<td>46.6</td>
<td></td>
</tr>
<tr>
<td>B/r, LBG/SI 6 min(^b)</td>
<td>23</td>
<td>22.7 ± 4.0</td>
<td>31.9 ± 5.0</td>
<td>0.91</td>
<td>197.0</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>B/r, LBG/SI 10 min</td>
<td>23</td>
<td>20.9 ± 4.7</td>
<td>27.5 ± 8.2</td>
<td>0.88</td>
<td>105.6</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>B/r, LBG/Pn</td>
<td>23</td>
<td>19.2 ± 4.0</td>
<td>21.8 ± 5.2</td>
<td>0.75</td>
<td>195.2</td>
<td>77.1</td>
<td></td>
</tr>
<tr>
<td>B/r, GC</td>
<td>28</td>
<td>21.0 ± 1.6</td>
<td>24.4 ± 4.0</td>
<td>0.89</td>
<td>143.7</td>
<td>42.3</td>
<td></td>
</tr>
<tr>
<td>K12-CR34, GC/dG</td>
<td>30</td>
<td>22.3 ± 3.0</td>
<td>41.1 ± 10.1</td>
<td>0.87</td>
<td>169.0</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>K12-CR34, GC/Thy-limited</td>
<td>30</td>
<td>19.5 ± 4.7</td>
<td>30.7 ± 4.1</td>
<td>0.92</td>
<td>444.1</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>B/r, glucose</td>
<td>48</td>
<td>27.5 ± 5.0</td>
<td>40.9 ± 11.1</td>
<td>1.06</td>
<td>57.8</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>B/r, glucose + Pn</td>
<td>48</td>
<td>22.2 ± 3.5</td>
<td>33.9 ± 13.3</td>
<td>0.86</td>
<td>264.3</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>B/r, small synchronous cells(^c)</td>
<td>48</td>
<td>23.1 ± 3.5</td>
<td>31.1 ± 7.9</td>
<td>1.00</td>
<td>32.9</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>B/r, large synchronous cells(^c)</td>
<td>48</td>
<td>21.9 ± 2.2</td>
<td>31.3 ± 5.3</td>
<td>0.83</td>
<td>59.9</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>B/r, glycerol</td>
<td>70</td>
<td>42.2 ± 13.4</td>
<td>51.5 ± 8.5</td>
<td>0.88</td>
<td>33.5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>B/r, succinate(^c)</td>
<td>90</td>
<td>35.5 ± 7.5</td>
<td>40.2 ± 12.0</td>
<td>0.83</td>
<td>9.5</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Data and the abbreviations are taken from Hadas et al. (20). For additional details, consult references 2, 10, 16, and 33.  
\(^b\) Data from the experiment shown in Fig. 3.  
\(^c\) Data from the experiments presented in Fig. 4.  
\(^d\) Data from the experiments shown in Fig. 5.

Figure 4 presents three comparisons between the model and actual experiments previously reported (20) (Table 1). The three examples displayed were selected to cover the whole range of burst sizes (between 9 and 720); thus, a semilog presentation was used. (For clarity, the corresponding experiments with chloroform were not included, and the points at early times were deleted.) Correlations between burst size and cell size were observed in early studies with the T-series bacteriophages (see, e.g., references 13 and 21), but it was too early for them to be accounted for by the physiological parameters of \textit{E. coli}, which emerged a decade later (25, 32).

Figure 5 presents the results of an experiment not previously described, with two samples of a glucose-grown synchronous culture obtained by the “baby machine” (22); one infected upon collection (babies) and the other infected after 40 min of growth (almost one mass doubling). The latter supported a slightly faster phage assembly and yielded more phages (burst size of about 60) than the babies (\(B \approx 33\)). The results are qualitatively consistent with the hypothesis proposed before (20) that the rate of phage synthesis and assembly is proportional to the size of the protein-synthesizing system (9) in the cell upon its infection. This hypothesis will be rigorously tested, and results will be published separately.

**Statistical analysis.** Since the number of points in each experiment is small (of the order of 40 for the chloroform \[f\] and nonchloroform \[f_2\] parts combined), the statistical signifi-
cance of our results is not expected to be excellent. As we presently show, however, some parameters have a higher statistical validity than others. We analyze here two cases (the LBG assay from Fig. 3 and the babies from Fig. 5), while other cases show similar statistical attributes. Consider first the percentage of variation explained, which is a measure equivalent to $r^2$ in our case. As mentioned, for the average LBG, an $r^2$ of ca. 97.5% was obtained, while for Fig. 5 the results were, for
seen that the covariance between by the correlation matrix of the baby cells (Table 2), where it is values of points at these regions are small, the inability to get exact the end of their increase. Since the numbers of experimental mined by the curvatures of the PPBs at the beginning and matrix) stems from the fact that these parameters are deter-
ers in baby cells, the latter are not really determined. The larger er-
errors in $\beta$ and $\sigma$ (and see the discussion below of the correlation matrix) stems from the fact that these parameters are deter-
mined by the curvatures of the PPBs at the beginning and the end of their increase. Since the numbers of experimental points at these regions are small, the inability to get exact values of $\sigma$ and $\beta$ seems obvious. This observation is enhanced by the correlation matrix of the baby cells (Table 2), where it is seen that the covariance between $\mu$ and $\beta$ is of the order of $-0.9$.

To summarize the statistical results, it seems that for the experiments carried out here, the validity of the model is fairly established. The parameters $\mu$, $\nu$, and $\varsigma$ have a higher statistical significance than $B$, and much higher significance than $a$ and $\beta$. The latter is not even robust. Higher accuracies for both were obtained with an increased number of measured points.

**Calculation of correlation matrix.** To calculate the correlation matrix, the following steps are taken (7, 27, 28, 31). (i) The log likelihood function is calculated by

$$
\Phi = \frac{1}{2} \sum_{j=1}^{r} \left( n_j [\ln(2\pi) + 1] + n_j \ln \left( \frac{1}{n_j} \sum_{i=1}^{n_j} \frac{(z_{ij} - f_{ij})^2}{f_{ij}} \right) \right) + \gamma_j \sum_{i=1}^{n_j} \ln f_{ij},
$$

where $z_{ij}$ is the measured value of the $j$th response of the $i$th data point, $f_{ij}$ is the predicted $j$th response of the $i$th data point, $\gamma_j$ is the heteroscedasticity parameter for the $j$th response, $r$ is the number of measured response variables and $n_j$ is the number of data points of the $j$th response.

(ii) The Hessian matrix $H_{\theta, \theta}$ is defined as the matrix of the second partial derivatives of $\Phi$ with respect to each pair of parameters. Here, the Gauss approximation is used:

$$
H_{\theta, \theta} = \sum_{j=1}^{r} \sum_{i=1}^{n_j} \left( \sum_{p=1}^{r} \frac{\partial^2 \Phi(\theta)}{\partial \theta_p \partial \theta_i} \right) \left( \sum_{p=1}^{r} \frac{\partial f_{ij}}{\partial \theta_p} \frac{\partial z_{ij}}{\partial \theta_i} \right) \left( \sum_{p=1}^{r} \frac{\partial f_{ij}}{\partial \theta_p} \frac{\partial \theta_p}{\partial \theta_i} \right),
$$

where $\theta$ is the vector of adjustable parameters.

(iii) The variance-covariance matrix $V$ is estimated from the inverse of the Hessian, $V = H^{-1}$.

(iv) The correlation matrix is given by the normalized variance-covariance matrix of the parameters estimated by

<table>
<thead>
<tr>
<th>Variable</th>
<th>% Standard deviation</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>8.1</td>
<td>1.000</td>
</tr>
<tr>
<td>$\nu$</td>
<td>5.3</td>
<td>-0.603 1.000</td>
</tr>
<tr>
<td>$\beta$</td>
<td>25.8</td>
<td>-0.886 0.374</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>270 (!)</td>
<td>-0.515 0.404</td>
</tr>
<tr>
<td>$\varsigma$</td>
<td>4.4</td>
<td>0.006 0.136</td>
</tr>
<tr>
<td>$B$</td>
<td>6.4</td>
<td>0.294-0.179</td>
</tr>
</tbody>
</table>

FIG. 5. PPB in synchronous E. coli B/r cells (22) pregrown in glucose-minimal medium, infected (at a multiplicity of 0.5) with bacteriophage T4. + and *, eluted “babies”; O and x, “old” cells, after 40 min of growth following elution. + and O, without chloroform; * and x, with chloroform.


