Enlargement of *Escherichia coli* After Bacteriophage Infection

I. Description of the Phenomenon

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Escherichia coli B/r and B_s-1 ceased division and increased in mean cell volume soon after infection with T-even phage. The effect was obtained with wild-type or rapid lysis mutants, as well as with ultraviolet light-killed phage and with bacteriophage ghosts which lack deoxyribonucleic acid. The cell response did not require the presence of phage genetic material or the production of progeny phage. A Poisson distribution of the fraction of adsorbed phage at different multiplicities of infection indicates that one phage per bacterium will produce maximum increase in cell volume. T-even phage-resistant *E. coli* mutants showed no enlargement response, and phage T1, T3, and T7 elicited neither abrupt termination of cell division nor host cell enlargement. Infection with baseplate-defective T4D 12⁻ amber mutants, which bind reversibly to but do not penetrate the bacterium, also had no effect. In vitro restoration of normal baseplate function in these defective viruses allowed phage adsorption and penetration and caused host cell division arrest and enlargement. These findings indicate that arrest of division and increase in mean cell volume occur together when a sensitive strain of *E. coli* is infected with T-even phage that adsorb and penetrate normally.

The results of several investigators indicate that host bacteria sometimes enlarge after phage infection. Bayne-Jones and Sandholzer (1) demonstrated with motion photomicrography that *Bacterium coli* and *Bacillus megaterium* infected with "lytic filtrate" increased in volume up to 900% before lysis. Buller and Astrachan (3) obtained an increase in the packed cell volume of *Escherichia coli* K-12 (λ) cultures infected with phage T4 rII, and Luria and Human (14) presented micrographs of *E. coli* B infected with viable or killed T-even phage in which the host cells appeared swollen and partially vacuous.

Other investigations indicate that bacterial permeability changes after phage infection. Puck and Lee (15) reported that ³²P and ³⁵S effluxed from *E. coli* B infected with phage T2. This effect terminated shortly after infection and could be elicited by one phage per cell. Osmotic disturbances are also indicated by the leakage of ⁴²K and ²⁸Mg from infected bacteria into the growth medium (16). Other workers (11) report, however, that the appearance of β -galactosidase in the extracellular medium after infection of *E.*

coli B with wild-type and lysozyme-deficient mutants of phage T4 as well as phage ghosts resulted from a small fraction of lysed cells and not from generalized leakage.

Buller and Astrachan (3) reported a decrease in turbidity, starting 4 min after *E. coli* K-12 (λ) was exposed to T4 rII, under the same ionic conditions (high Na⁺, low Mg²⁺) as those in which packed cell volume increased. Earlier, Doermann (6) had reported an abrupt decrease in the turbidity of *E. coli* cultures infected with wild-type or rapid-lysis mutants of T-even phage. Cohen (5) interpreted such decreases in turbidity to reflect a loss of refractility of infected cells caused by ion leakage and cell enlargement. He offered the hypothesis that rapid osmotic swelling of an infected bacterium is an integral part of the infective process and that such swelling causes the termination of bacterial synthesis by breaking the genome of the host cell from its sites of membrane attachment. In view of the incomplete nature of the evidence suggesting host cell enlargement after bacteriophage infection, we have investigated the phenomenon, directly, by measuring cell volumes and culture titers electronically after exposure of sensitive and resistant *E. coli* cells to both viable and killed T phage.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* strains studied were B/r and B_{s-1}, which have been maintained for several years on refrigerated nutrient agar slants. These strains were selected because of their general sensitivity to infection by different T phage. The two strains are both derived from *E. coli* B but differ greatly in sensitivity to radiation and in ability to repair damage to deoxyribonucleic acid (DNA). An *E. coli* strain resistant to any one of the phage stocks was grown from a colony which appeared to have spontaneously reverted to phage resistance on an otherwise confluent lysed plate containing that particular phage stock. In all experiments, shake cultures at 37°C were maintained by repeated back-culture at the low cell titers (10^7 to 3×10^7 cells/ml) characteristic of balanced growth (4). At greater cell concentrations, growth is no longer balanced and the mean cell volume (MCV) decreases. The bacterial culture medium used was M9 (13) supplemented with 0.1% glucose and 0.5% vitamin-free Casamino Acids (Difco). M9 medium and glucose were sterilized separately by autoclaving. This medium was also used for the production of phage stocks, except that for the T4D phage L-tryptophan (20 µg/ml) and thymidine (2 µg/ml) were added.

Phage strains and procedures. The coliphage strains used were wild types T1-4, 6, and 7 from our own culture collection, the rapid-lysis mutants T2r and T4r II obtained from A. L. Koch, and the amber mutants T4D 12⁻ and T4D 23-27⁻ (18) obtained from W. S. Mason. The homogeneity of the phage stocks was verified by assay on sensitive and resistant hosts or on permissive and nonpermissive hosts. For production of phage stocks, exponential phase *E. coli* B/r cultures at a titer of 2×10^8 cells/ml were infected at a multiplicity of infection (MOI) of 0.1 to 0.5 and incubated for 2 to 6 hr at 37°C with vigorous aeration. The infected cultures were then exposed to chloroform, and the resultant lysates were freed from bacterial debris by two low-speed centrifugations ($2,500 \times g$, 10 min). Phage T4D 12⁻, an amber mutant in a genetic locus specifying baseplate function, was grown on the permissive *E. coli* strain CR 63, supplied by W. S. Mason, or on nonpermissive B/r. All phage stocks were titrated frequently on both B/r and B_{s-1} to test for viability and for the presence of host-produced virus modification. Identification of the various T-phage stocks was checked by plating each on known resistant strains of *E. coli* B, supplied by H. M. Krisch. All phage stocks were stored at 4°C until used.

Extracts for the in vitro restoration of base plate function in defective T4D 12⁻ (grown on B/r) were made from sonically disrupted B/r cells in the latent period of infection by phage T4D 23-27⁻, which contains amber mutations in both head and tail (but not baseplate) loci. Cell disruption was judged microscopically. The extracts were purified, checked for the presence of contaminating cells and phage, and stored frozen until used by the procedures of Edgar and Lielausis (9) and Simon et al. (18). In

vitro restoration of baseplate function was effected by incubation of extract plus defective T4D 12⁻ phage at 37°C for 30 min.

For ultraviolet light (UV) inactivation, phage lysates were dialyzed at 4°C against M9 salts solution to remove UV-absorbing components of the Casamino Acids. The phage were inactivated by exposure at 4°C to 254-nm light from a General Electric germicidal tube with a dose rate of 7,500 ergs per mm² per min as determined with a Blak-Ray UV meter. Plaque-forming ability was reduced in these stocks by a factor of 10^{-6} in 3 min, with no loss in ability to inhibit the division of the *E. coli* hosts.

T-even phage ghosts were prepared by the method of Duckworth (7). Ghost preparations were examined by electron microscopy; ghost heads were patent and tails were uncontracted. The plaque-forming units present as contaminants were, typically, 0.1% of the ghost titer. The ghost titer was estimated by using the lowest MOI giving complete inhibition of cell division as an indication that every bacterium had adsorbed at least one particle. From a Poisson distribution (Fig. 6), it is indicated that at MOI values of ~ 4 every bacterium has adsorbed at least one virus. Therefore, the lowest ghost MOI providing complete host-cell division-inhibition was taken to be ~ 4 . With this method to calculate ghost concentration, the efficiency of the osmotic shock used to prepare the ghosts was about 50%, as others have reported (8).

Counting and sizing bacteria. Samples from uninfected control cultures and from infected cultures were diluted 20-fold into 0.1 N HCl counting medium for determination of cell number and MCV. This treatment immediately halted cell growth and division. The modified Coulter counter-multichannel analyzer and the limitations of electronically sizing bacteria have been described (12). A comparison of counting medium, M9 salts solution, and water (later acidified) revealed no deleterious changes in the size distributions of control or of phage-infected cells diluted with 0.1 N HCl. Mean cell volumes were calculated from the multichannel analyzer frequency distributions by numerical integration or by geometrically approximating the cell-size distributions with triangles; the multichannel analyzer was calibrated with latex microspheres of known diameter. Frequency peaks occurred at integral multiples of the volume of a single microsphere ($1.16 \mu^3$) as two or more spheres passed simultaneously through the aperture of the Coulter counter (Fig. 1).

RESULTS

Figures 1a and b present typical sets of frequency distributions for cell volumes obtained from *E. coli* B/r infected with viable or with UV-killed rapid-lysis T2r phage. The MCV values, denoted by the arrowheads, increased with time after virus was added. In the 60 min after infection, the MCV values approximately doubled, whereas cell volumes of uninfected control cultures showed no change. Distributions of latex microsphere standards are included.

FIG. 1. Frequency distributions of the cell volumes of *E. coli* B/r at various times after phage infection, determined with a Coulter counter-multichannel analyzer. Balanced growing cultures were infected with viable (a) or with ultraviolet (UV) light-killed (b) rapid-lysis T2r phage, both at MOI values of 4. At increasing times after infection, 0.5-ml samples of the cultures were diluted 20-fold with 0.1 *N* HCl, and the cells were counted and sized electronically. The mean cell volumes (Δ) were determined by numerical integration of the frequency distribution curves, and the size abscissa was calibrated by measuring latex microspheres which provided frequency peaks at integral multiples of $1.16 \mu^3$.

Figures 2a and b are drawn from the average data of several experiments similar to Fig. 1 and include cell counts. Figure 2a shows that infection by T2r phage, whether UV-killed or viable, terminated the increase in culture titer after 20 min. The uninfected control culture continued to increase in cell number exponentially with a doubling time of about 25 min, whereas phage-exposed cultures increased only 20 to 30%. The decrease in cell number of cultures infected with viable phage reflected cell lysis, whereas no progeny phage were produced when cells were infected with UV-killed viruses. In Fig. 2b, the relative MCV values are plotted, also versus time after addition of phage. These volumes have been normalized to the starting MCV by dividing each subsequent volume by the value at time zero. Relative MCV values greater than 1 represent increases in cell size, whereas values less than 1 represent decreases. The relative volumes of the uninfected controls remained nearly constant, as expected for cells in balanced growth. During

this time, the UV-killed and the viable phage each elicited a large volume increase from the host cells. In both cases, the MCV increases were similar, nearly 80%.

Figures 3a and b summarize and compare the average changes in culture titer and host cell size caused by T-even and by T-odd phage. After infection, the T-even coliphage uniformly caused a host cell enlargement averaging 50%, whereas the T-odd phage initially caused no change in relative MCV and, in fact, ultimately produced a slight decrease in MCV (Fig. 3b). This decrease in MCV reflected a loss of large cells by lysis. The continued increase in MCV of cells infected with T-even phage occurred in the absence of cell division (Fig. 3a). Although the cell titer of uninfected control cultures increased exponentially, the cell titer of cultures infected with T-even phage slowly decreased as some early lysis occurred. This behavior is in contrast with that of bacteria infected with T-odd phage which showed no appreciable enlargement (Fig. 3b) and

FIG. 2. Changes in the relative cell concentration (a) and relative mean cell volume (b) of *E. coli* B/r infected with viable or with ultraviolet (UV) light-killed rapid-lysis T2r phage. T2r phage were exposed to 254-nm light for 3 min at a dose rate of 7,500 ergs per mm^2 per min, which reduced the plaque-forming ability of the phage by 10^{-6} . The relative values are obtained by dividing the cell titers or mean cell volumes found at increasing times after infection by the corresponding values at the time of infection. Relative values >1 indicate increases in cell titer or mean cell volume, whereas relative values <1 indicate decreases. Mean cell volumes and titers were obtained from frequency distributions similar to those in Fig. 1a and b. The data points are the averages from three to five experiments employing MOI values from 4 to 21.

which showed substantial, but more variable, increase in cell titer until 30 min after infection (Fig. 3a), whereupon lysis commenced. Confidence limits representing ± 1 standard deviation are shown for Fig. 3. Confidence limits for Fig. 2 and 4, not shown due to superimposed curves, are very similar in magnitude to those for T-even phage in Fig. 3.

The phage-produced effect seen in *E. coli* B/r exists to approximately the same extent in the radiation-sensitive strain, *E. coli* B_{s-1}, as shown in Fig. 4a and b. UV-killed phage T2r elicited

cessation of cell division (Fig. 4a) and increase in MCV (Fig. 4b) as efficiently as viable phage. Thus, the great difference in UV sensitivity of these two bacterial strains is not reflected in any strain difference in the cell enlargement phenomenon from UV-killed phage. The effects of UV-killed phage cannot be readily accounted for by host cell repair of UV damage to phage DNA, since neither B/r or B_{s-1} has been found to host cell-reactivate the plaque-forming ability of UV-irradiated T-even phage (10). Exposing the phage to very large doses of UV light did reduce their effectiveness on host cells (Fig. 5). At UV exposures greater than 2.2×10^4 ergs/ mm^2 , bacterial cell volumes did not increase substantially after infection, perhaps reflecting UV damage to the phage adsorption mechanism.

The phage MOI determined the degree of host cell enlargement (Fig. 6). Data were compiled from several different experiments, most of which used phage T2r and *E. coli* B_{s-1}. At MOI values

FIG. 3. Responses of relative cell concentration (a) and relative mean cell volume (b) of *E. coli* B/r infected by T-even and T-odd phage. Data points are the averages from five T-even and three T-odd experiments, all at MOI = 5. Data are treated as in Fig. 2a and b. Bars represent ± 1 standard deviation.

FIG. 4. Changes in relative cell concentration (a) and relative mean cell volume (b) of *E. coli* B_{s-1} infected with viable or with ultraviolet (UV) light killed rapid-lysis T2r phage. UV light exposure was as in Fig. 2 with a similar reduction in plaque-forming ability. These data points are the averages from four experiments employing MOI values from 3 to 7.

greater than 4, as seen in the graph derived from a Poisson distribution curve, more than 99% of the bacteria had adsorbed one or more phage. At such MOI values, the maximum degree of host cell enlargement was also seen, suggesting that only one phage per cell was required for host cell enlargement. Although it has not appeared necessary for a viable phage to infect a bacterium to elicit the increase in MCV, the presumptive host cell must be sensitive to the phage. If $B/r/4$ is exposed to T4, cell volume is unchanged, although the typical enlargement response occurs for sensitive *E. coli* B/r (Fig. 7). Ghosts of T2r phage that lack DNA also caused both a cessation of cell division as well as an MOI-dependent rate of increase in MCV (Fig. 8 a-e). As MOI increased, there was a progressively stronger effect on the division and size of infected cells. At MOI values that insured at least one ghost is adsorbed to each bacterium, division was blocked and enlargement ensued. There was a minimal contribution of viable phage to the infecting ghost titer.

The results presented here for T2r ghosts were also obtained for T2 and T4 ghosts (*unpublished data*).

Infection of nonpermissive *E. coli* B/r with baseplate-defective T4D 12^- led to continued cell division and no significant change in cell size (Fig. 9a and b). *E. coli* B/r infected with either T4D 12^+ or with nondefective T4D 12^- (grown on permissive *E. coli* CR 63) enlarged and ceased

FIG. 5. Effect of exposure to 254-nm ultraviolet (UV) light on the ability of rapid-lysis phage T2r to elicit an increase in mean cell volume of infected bacteria. Phage were irradiated at 4°C in M9 salts solution, and *E. coli* B/r and/or B_{s-1} were infected at MOI values from 3 to 7. The ability of the phage to elicit cell enlargement after 20 min is shown because the rate of enlargement becomes slower soon after this time with no clear end point. Plaque-forming ability was again reduced by 10^{-6} after 3 min of UV exposure.

FIG. 6. Role of MOI in eliciting an increase in host cell volume after T-even phage infection. The experimental curve (●), like Fig. 5, measures the percentage increase in mean cell volume after 20 min of exposure to phage, in this case as a function of MOI. At MOI ≥ 4 , the maximum increase in mean cell volume is observed. The theoretical curve (x), derived from a Poisson distribution and plotted to a different scale, shows that at MOI ≥ 4 nearly every bacterium has adsorbed one or more phage. Experiments are predominantly with *E. coli* B_{s-1} infected by phage T2r.

FIG. 7. Change in the relative mean cell volumes of sensitive and resistant bacteria exposed to T4 phage at an MOI of 9. *E. coli* B/r/4 was selected as a spontaneous revertant to phage resistance from a plate heavily seeded with B/r and T4 phage. Relative mean cell volumes were determined as in Fig. 2b.

division (*unpublished data*). The defective T4D 12⁻ phage, when complemented in vitro with gene 12 product from an extract of bacteria infected with the double amber mutant T4D 23-27⁻, caused cell division arrest and enlargement. Although the extract generally contained a very low concentration of viable phage contaminants, most likely amber revertants, the extract alone did not affect the cells (Fig. 9a and b). The MOI from these viable phage is 0.3 for the extract used in this experiment.

DISCUSSION

Based on direct electronic measurement of MCV and culture titer, the infection of a sensitive *E. coli* B/r or B_{s-1} by at least one T-even phage particle is sufficient to cause prompt, substantial, and prolonged increase in host cell size coupled with cessation of cell division. Because the greater part of the enlargement of the cell occurs well within the time required for a lytic cycle and because infection by killed phage or by phage ghosts which lack DNA and produce no progeny elicit the MCV increase, infected cell enlargement does not require the completion of viable progeny phage. The finding that T-even phage-resistant cells did not enlarge when exposed to the pertinent virus indicates that only attachment to and penetration of the bacterial surface elicit the cell volume increase and cell division arrest. This is supported by the results obtained with the baseplate-defective amber mutant T4D 12⁻. After

attachment by its long tail fibers to a host cell, the phage draws back from the cell surface when its tail sheath contracts (18). The wall of the would-be host is not breached. In these cases, there is no division arrest or cell enlargement, showing that reversible attachment alone does not alter bacterial size. The restoration of gene 12⁺ phenotype through in vitro complementation leads to phage with functional baseplates which produce the anticipated termination of host cell division and the initiation of MCV increase.

We found no significant difference in the effectiveness of wild-type and rapid lysis mutants of T-even phage in causing host cell enlargement. However, in the work cited in the introduction, Buller and Astrachan (3) reported that the rapid-lysis mutant T4r II was more effective than the wild-type phage T4r⁺ in causing an increase in

FIG. 8. Changes in relative cell concentration (●) and relative mean cell volume (○) of *E. coli* B/r after exposure to T2r ghosts at increasing ghost concentration. Effects on cell division and cell size increased with ghost concentration (a-e). The first appearance of complete inhibition of cell division (d) was taken to reflect an MOI of 4 as explained under Materials and Methods, and the other MOI values were calculated relative to this. The numbers in parentheses are the measured MOI values of viable phage present as contaminants in the ghost preparations. These data points represent the averages from two experiments.

FIG. 9. Effect of baseplate-defective amber mutant T4D 12⁻ phage on *E. coli* B/r with defective phage alone (○), restorative extract alone (△), or in vitro, extract-complemented phage (□). Defective phage were grown on the nonpermissive *E. coli* B/r, and the extract was prepared by the method of Edgar and Lielausis (9) from disrupted *E. coli* B/r in the latent period of infection by the double amber mutant phage T4D 23-27⁻. Baseplate function of defective T4D 12⁻ was restored after in vitro incubation with the phage-free, cell-free extract at 37°C for 30 min. The figure shows that the ability of the defective phage to inhibit host cell division and to increase host cell volume was also restored by the extract.

the packed cell volume of *E. coli* K-12 (λ) and that their results with T4r⁺ were very variable. One possible source of variation is the fact that our growth medium included a low but fixed concentration of Mg²⁺ (8.1×10^{-4} M), whereas their medium was not specifically supplemented with Mg²⁺. They reported that the effect was sensitive to Mg²⁺ concentration. In addition, their experiments used *E. coli* K-12 (λ) cells which were not in balanced growth when infection occurred, whereas our experiments used nonlysogenic strains of *E. coli* B/r and B_{s-1} which were in balanced growth. Our results agree with theirs to the extent that we both find that, under some experimental conditions, both wild-type and rapid-lysis mutants of T-even phage cause substantial increases in host cell volume.

In contrast to our results with T-even phage, we found that T-odd phage 1, 3, and 7 neither rapidly inhibit cell division nor cause cell volumes to increase. T-even phage are typically larger and contain more DNA than the T-odd phage we studied (2, 5, 20). The presence of contractile sheaths surrounding the T-even tails is of potential significance because infection by such phage has been shown to cause disruption of the host cell surface concomitant with tail-sheath contraction (17, 18). T1 has a noncontractile tail and both T3 and T7 have very short, possibly noncontractile tails (2). The restoration of baseplate function in T4D 12⁻ indicates that the cell enlargement response and division arrest follow active breaching of the cell wall by a representative of a phage class with contractile tails. The T-even phage all produce similar cytological changes in infected bacteria (14, 17).

There are several possible explanations for infected cell enlargement. First, in view of the evidence of ionic fluxes, a decrease in turbidity, and an increase in packed cell volume in an infected culture, it might be expected that phage penetration causes an osmotic equilibration between the host cell and its environment that allows an egress of cellular ions and an ingress of water. Electron micrographs have been obtained that show the distortion of the cell wall caused by phage infection (18). The reported decrease in optical density of an infected culture (6) suggests that enlarged, possibly depleted cells exist after T-even phage exposure. Also, a considerable MCV increase appears within 10 min, before phage completion has occurred intracellularly.

A second possible explanation for infected cell enlargement, consistent with cell growth without division and with a decrease in optical density, is that the cell wall or membrane, or both, continue to be synthesized after infection in the absence of host-cell synthesis of DNA, ribosomes, or proteins. Growth of the cell surface at a more rapid rate than the intracellular contents could cause the observed increase in mean cell volume and decrease in turbidity. In the following paper, we will present evidence to distinguish between the two mechanisms outlined here.

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