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Inhibition of Host Protein Synthesis During Infection of *Escherichi coli* by Bacteriophage T4

III. Inhibition by Ghosts

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Deoxyribonucleic acid (DNA)-less T2 "ghosts" were prepared by osmotic shock and purified by KBr density gradient centrifugation. *Escherichia coli* B was treated with these ghosts in inorganic salts-glycerol medium to see which features of phage infection could be elicited by ghosts. At a multiplicity that was just sufficient to block induction of β-galactosidase (EC 3.2.1.23), 89% of the bacteria were killed and the rates of ribonucleic acid (RNA) and DNA synthesis were about 10 to 15% of normal. However, protein synthesis was almost completely blocked but resumed after 30 min. During this period, it was possible to induce messenger RNA (mRNA) from the lactose operon, although this mRNA could not be translated into active β-galactosidase. These results suggest to us that the viable cells surviving ghost infection synthesize nucleic acids at close to a normal rate but are temporarily blocked in protein synthesis. The continued formation of untranslated host mRNA mimics the pattern of bacterial synthesis just after whole-phage infection, and is consistent with the interpretation that the immediate block in the initiation of host translation by these viruses is due to their attachment.

Infection of *Escherichia coli* by T-even bacteriophages results in a number of activities, any one of which would be sufficient to arrest host-cell growth. These include breakdown of the host chromosomes (32, 33), the inhibition of ribonucleic acid (RNA) transcription and its translation into protein (see references in 14 or 15), and leakage of certain cations (27). It is clear that these diverse effects do not occur simultaneously; some changes occur immediately upon phage adsorption and others appear later.

Deoxyribonucleic acid (DNA)-less phage ghosts might help distinguish between those processes affected by the adsorption itself and those mediated by the expression of phage gene products, since all syntheses subsequent to attachment are, of necessity, host-specific. It has been known for some time that ghosts are capable of causing rapid changes in the host cell as well as variable killing of the population (3, 8, 10, 11, 19). These early papers did not always agree with respect to the extent of killing or inhibition, probably because of the relatively unpurified preparations used. We have reexamined the "ghost effect" with more purified material and conclude that most cells are killed and become metabolically inert after ghost attachment. In those cells not killed, the initiation of host protein synthesis is blocked, whereas the slower shut-off of host transcription and chromosome replication is dependent on phage gene products.

MATERIALS AND METHODS

Bacteria and growth conditions. *E. coli* B was grown at 37 C in M9 minimal medium supplemented with 0.2% glycerol. The mass doubling time was 60 min. Overnight cultures were diluted, incubated for four generations, and then infected at a cell density of 5×10^8/ml.

Preparation of phage ghosts. (The following procedure is a slightly modified version of one kindly suggested by Robert Dyson of Oregon State University, Corvallis.) Ghost stocks were prepared by osmotic shock of bacteriophage T2. Equal volumes of 100% glycerol and a phage stock of high titer ($>10^{13}$ plaque-forming units/ml) were placed together for 30 min in a beaker with a capacity at least 400 times the phage volume; the suspension was diluted quickly by addition of 40 volumes of 0.001 M tris(hydroxymethyl)-aminomethane buffer (*p*H 7.4). $MgSO_4$ was added to a final concentration of 0.01 M. The shocked phage solution was then incubated for 30 min at 37 C with 10 μg of deoxyribonuclease per ml (Worthington Biochemical Corp.). Finally, 0.53 g of KBr crystals per ml of ghost suspension (to give 4.5 M KBr) was added and solubilized by gentle shaking at 37 C. (Recently, we have sometimes obtained inactive ghost stocks with this procedure. We traced the inactiva-

tion to the mixing of the KBr crystals in the ghost suspension. Therefore, we first concentrate ghosts by a 1-hr centrifugation at 31,000 $\times$ *g*; they are then resuspended by standing overnight in 4.5 M KBr.)

The mixture was centrifuged at 200,000 $\times$ *g* for 24 hr in an International B-60, 45 fixed-angle rotor. Sharp ghost bands which formed in the middle third of the centrifuge tubes were observed as visible bluish bands. They were collected and then were dialyzed against a phage diluting solution, consisting of 3.0 g of NaCl, 1.0 g of Bacto peptone, and 0.15 g of $MgSO_4$ (anhydrous) per liter of water. It is apparent in Fig. 1 that no labeled DNA is associated with the ghost fraction in these KBr gradients. The number of plaque-forming units per ghost particle after this preparation was less than 10^{-5}.

The number of ghost particles in a stock was estimated by counting the radioactivity of ^{3}H-leucine-labeled T2 ghosts. ^{3}H-leucine was incorporated into T2 protein during phage development, and the resultant radioactivity per plaque-forming unit of the mature phage measured. From this value, the number of ghost particles per milliliter derived from the ^{3}H-labeled T2 could be established after osmotic shock of these phage.

Other procedures. Induction of β-galactosidase (EC 3.2.1.23) with isopropyl thio-β-D-galactoside (IPTG) at 5 $\times$ 10^{-4} M and its assay, measurement of radioactivity, preparation of RNA for sucrose gradient centrifugation, preparation of RNA and DNA for hybridization, and the hybridization procedures have been described (14). Messenger RNA (mRNA) from the lactose (lac) operon was estimated by hybridization to DNA from the phage ϕ80 dlac, derived from *E. coli* CA5004 (kindly provided by Helen Revel, Massachusetts Institute of Technology). The denatured DNA (25 μg) was trapped on a nitrocellulose filter (15).

RESULTS

Relationships of cell killing, the inhibition of enzyme induction, and the multiplicity of ghost infection (MOI), Each preparation of ghosts was titered by determining the volume of ghosts per bacterium that would just block the induction of β-galactosidase. As shown in Fig. 2, the assay was sufficiently sensitive to detect about 2% of the noninfected level of enzyme after 12 to 15 min of induction. This multiplicity was used in all subsequent experiments with that stock. Results were reproducible from one ghost stock to another provided that the MOI used was just sufficient to block enzyme synthesis. Duckworth and Bessman (7) showed that the degree of

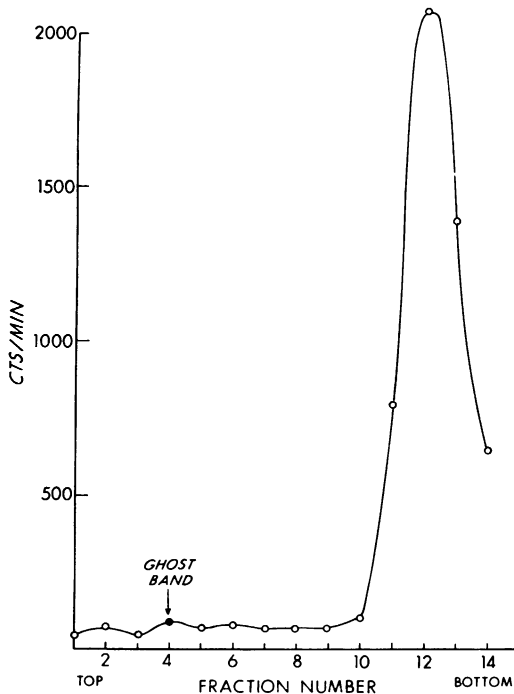

FIG. 1. *Potassium bromide density gradient of shocked bacteriophage T2 from T2 containing ^{3}H-labeled DNA (preparation and centrifugation of ghosts are described in Materials and Methods).*

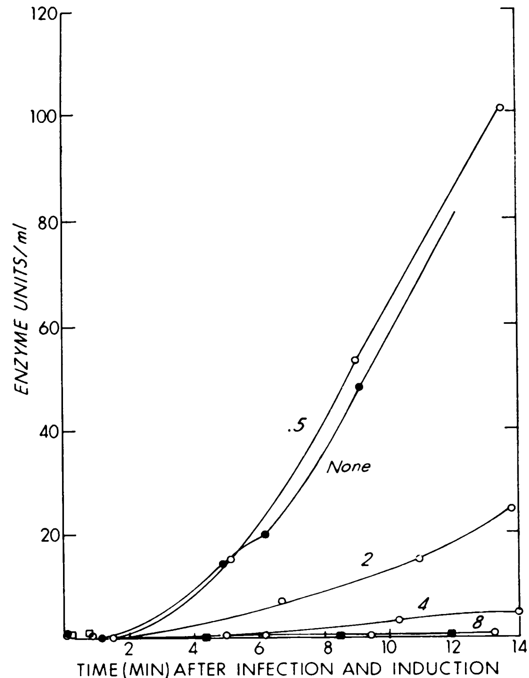

FIG. 2. *β-Galactosidase induction with variable multiplicities of ghost infection. Cells were induced by IPTG at the time of infection. Symbols: ●, noninfected controls; ■, infected with whole T2 (MOI of 8); ○, infected with different multiplicities of T2 ghosts.*

inhibition of β-galactosidase induction was proportional to ghost MOI. Our ghost stocks varied to some extent with respect to the approximate number, estimated from carrier ^{3}H-leucine-labeled phage ghosts required for a given degree of inhibition. Presumably, the shocking procedure may produce variable damage so that some particles do not adsorb or have lost some critical component. The relationship of multiplicity to enzyme inhibition for one stock is shown in Fig. 2. In this and all subsequent experiments, effects of whole-phage infection and ghost infection were compared in parallel cultures.

Killing by ghosts is also multiplicity-dependent (3, 11). This is shown in Fig. 3a, where the rates of enzyme synthesis, derived from Fig. 2, are also plotted (same stock in both cases). This parallel plot shows a very important aspect of ghost infection: the induction of enzyme is blocked in many cells that survive to produce progeny. For example, enzyme induction is reduced to 50% of normal with an MOI that kills only 5% of the bacteria, and when enzyme inhibition is complete 11% of the cells are still viable. This means that some cells have been reversibly inhibited. Indeed, French and Siminovitch (8) reported that the viable count begins to increase about 80 min after ghost infection.

The data of Fig. 3a and similar experiments are plotted on a semilog scale in Fig. 3b, which shows that there is a certain statistical probability that attachment of a ghost particle will kill or inhibit enzyme synthesis. Since both curves extrapolate to 100% on the ordinate, the attachment of one ghost particle can be sufficient to either inhibit or kill (or both). That attachment of one ghost is sufficient to kill had been shown earlier (3, 11). As shown, the probability for inhibition is greater than that for killing.

Recovery of the capacity to synthesize β-galactosidase occurred after 30 min (Fig. 4). The difference in times of recovery, as measured on the one hand by viable-cell count (80 min; 8) and on the other by enzyme synthesis (30 min), probably reflects the recovery of metabolic processes at 30 min followed by renewed cell division at 80 min. This recovery occurs at the same time (30 min) regardless of the initial MOI and the resultant degree of inhibition. Furthermore, the rate of enzyme synthesis in recovery is proportional to the number of cells that survive ghost infection (viable count). These observations

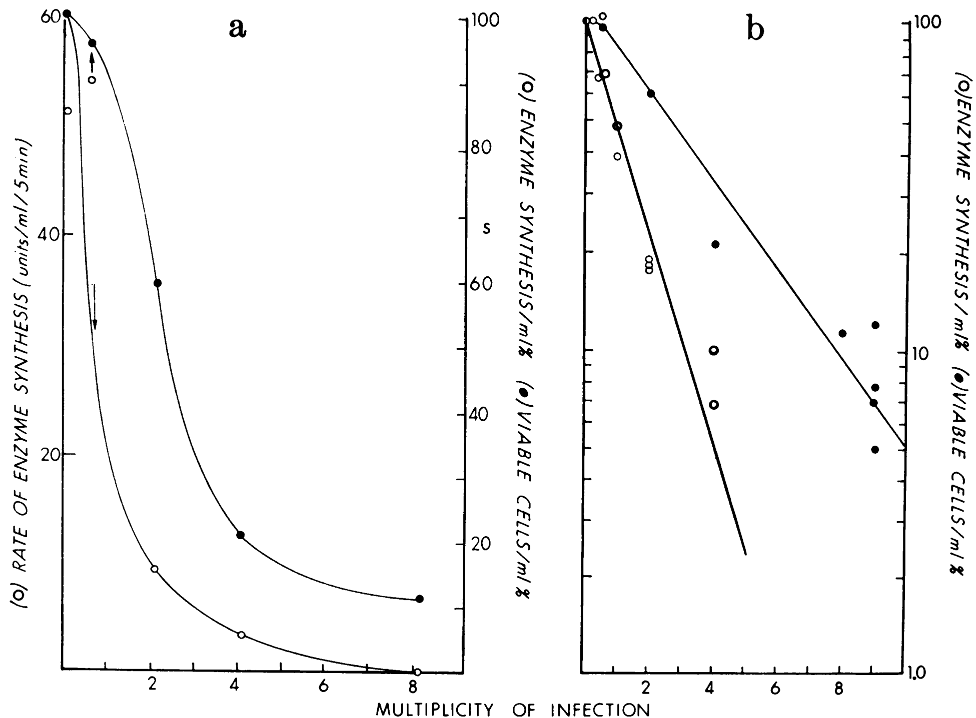

FIG. 3. *Rate of β-galactosidase synthesis measured between 6 and 11 min after induction (○) and cell viability measured 6 min after infection (●) as a function of ghost MOI plotted on (a) linear coordinates and (b) semilog scale. When 5% of the cells are killed (upper arrow in frame a), enzyme synthesis is inhibited 50% (lower arrow).*

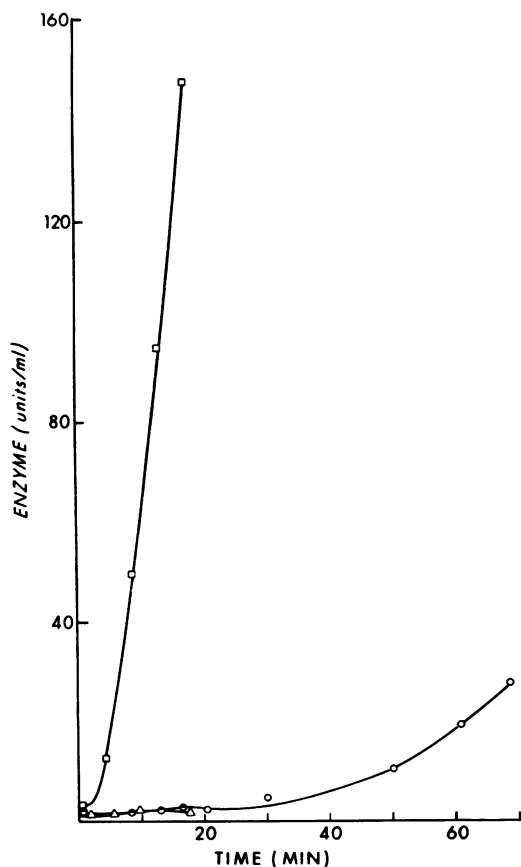

FIG. 4. *Resumption of β-galactosidase synthesis after ghost infection* (○). *The MOI was just sufficient to inhibit enzyme induction in this and following figures.* (□) *Noninfected controls;* (△) *infection with T2 (MOI of 8).*

suggest that the recovery is not a function of the degree of inhibition in single cells but rather depends on a subpopulation (see Discussion).

Nucleic acid and protein synthesis during ghost infection. The incorporation of uracil, thymidine, or leucine in uninfected, T2-infected, and ghost-treated bacteria is shown in Fig. 5. In ghost-treated cultures leucine was incorporated at 2 to 4% of the rate in uninfected cells, indicating an almost complete cessation of protein synthesis. However, after an initial 3- to 6-min lag, RNA and DNA synthesis, as measured by uracil and thymidine incorporation, respectively, continued at 8 to 20% of the uninfected rate for at least 20 min. This short lag was seen with the incorporation of any of these exogenous precursors and could reflect either an inability to concentrate the compounds into the pools of biosynthetic precursors or a brief cessation of all macromolecule synthesis.

The rate of incorporation of nucleic acid precursors is not always proportional to the rate of nucleic acid synthesis. In cells growing in different physiological states, the relative contributions from endogenous synthesis plus reutilization of turnover products versus exogenuously supplied base may be very different (16). However, we have been able to record a net accumulation of RNA in ghost infection. Bacteria were labeled for several generations with ^{3}H-uracil and infected with ghosts in the presence of ^{3}H-uracil of the same specific activity. At various times, small samples from the culture were precipitated with 5% trichloroacetic acid to determine the total ^{3}H-RNA/ml (Fig. 6). After an initial loss of about 3% of the cellular RNA, there was a net increase of about 3% per hr. The small early loss probably reflects leakage plus hydrolysis and masks biosynthetic activity that does occur during the initial 10-min period (Fig. 5). The continuing synthesis leads to an accumulation of RNA at about 10% of the rate in uninfected bacteria. In another experiment, this value was 15%.

From these results, we conclude that ghost infection inhibits protein synthesis almost completely. There is an initial lag of a few minutes in precursor incorporation before RNA and DNA syntheses continue at rates that are consistent with normal synthesis by the nonkilled population (see Discussion). These conclusions are also consistent with the observations of French and Siminovitch, who measured ^{32}P and ^{35}S incorporation during ghost infection (8).

Synthesis of ribosomal and transfer RNA during ghost infection. The preceding results showed that RNA synthesis continues during ghost infection. It is possible that the molecules being made are incomplete fragments. We looked for the synthesis of 16 and 23*S* ribosomal RNA (rRNA) and 4*S* transfer RNA (tRNA). As shown in Fig. 7, stable RNA molecules of this size are made during ghost infection. We do not know whether these species become functional.

Induction of mRNA synthesis during ghost infection. One of the major purposes of these studies was to determine whether ghost-treated bacteria can be induced to form new mRNA. Bacteria were induced with IPTG at various times after infection, and their RNA was purified for hybridization analyses (Table 1). As is the case in infection by whole T4 (15), lac mRNA could be induced during the first few minutes of T2 infection but not at a later time. However, in infection by T2 ghosts, significant levels of lac mRNA could be induced even at much later times. This occurred, of course, in the complete absence of any detectable induced enzyme activity (Fig. 2).

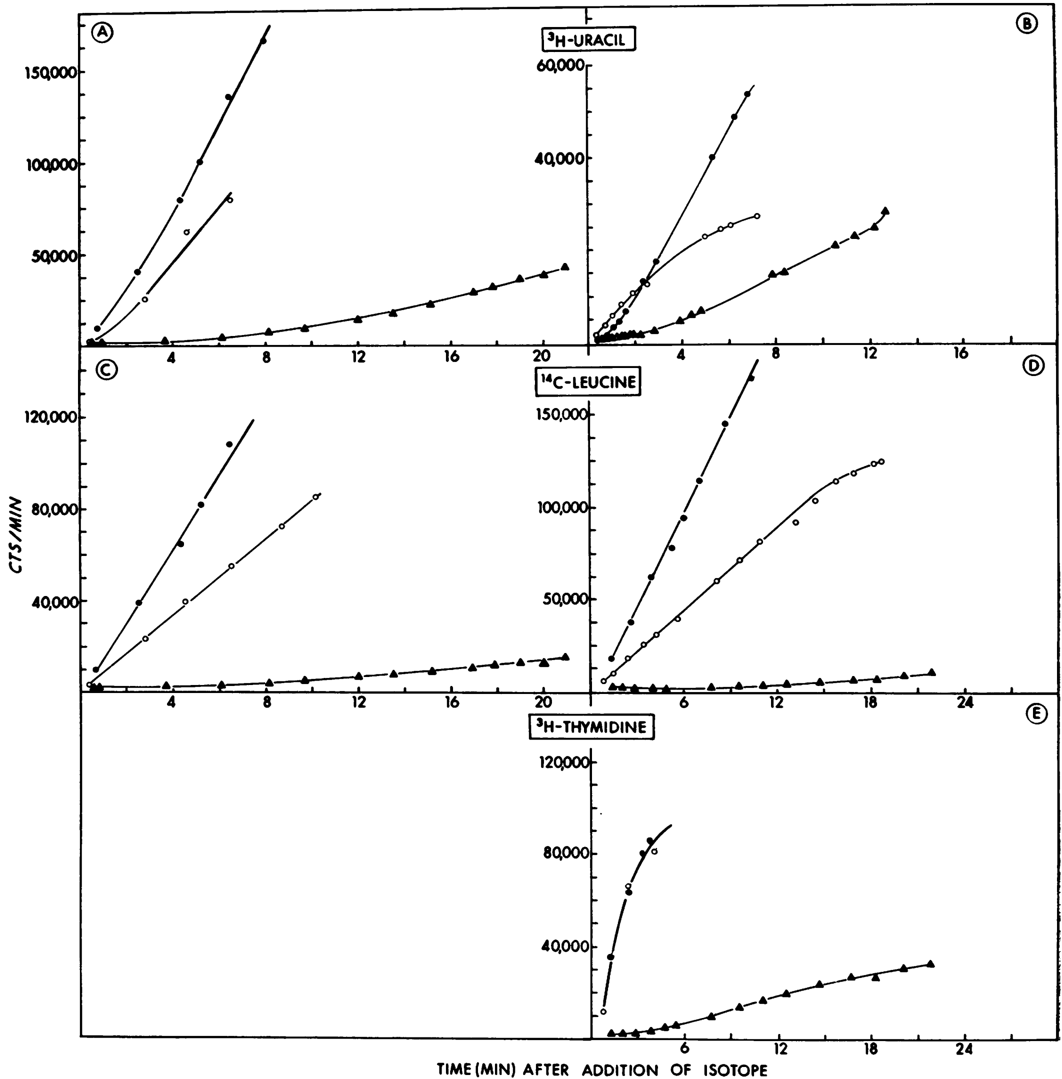

FIG. 5. *Incorporation of uracil, leucine, and thymidine during ghost or phage infection. Bacteria were (●) uninfected, (▲) infected with T2 ghosts, (○) or infected with whole T2 (MOI of 8). Labeled precursors were added 1 min after infection in A and C and 6 min after infection in B, D, and E. No detectable β-galactosidase activity was observed after phage or ghost infection.*

The fraction of RNA that is lac-specific and the total accumulation of RNA account for an amount of lac mRNA made during 20 min of ghost infection that is equal to the amount made in more than 1.6 min of exponential growth of an uninfected induced culture. However, no enzyme activity could be observed during this period even though less than 30 sec worth of normal enzyme induction could be detected easily (14). Thus, lac-specific RNA continues to be synthesized after adsorption of ghosts but it is not translated into enzyme.

DISCUSSION

Evidence for two populations of ghost-infected bacteria. β-Galactosidase synthesis, protein synthesis, RNA synthesis, and viability all decreased with increasing multiplicity of ghosts. At the MOI that just blocked (> 98%) enzyme induction, the rates of RNA and DNA synthesis as well as the viable count were about 11% of normal (Fig. 3, 5, and 6) Further increase in the MOI resulted in a proportional decrease in both viability and rates of nucleic acid synthesis. Two

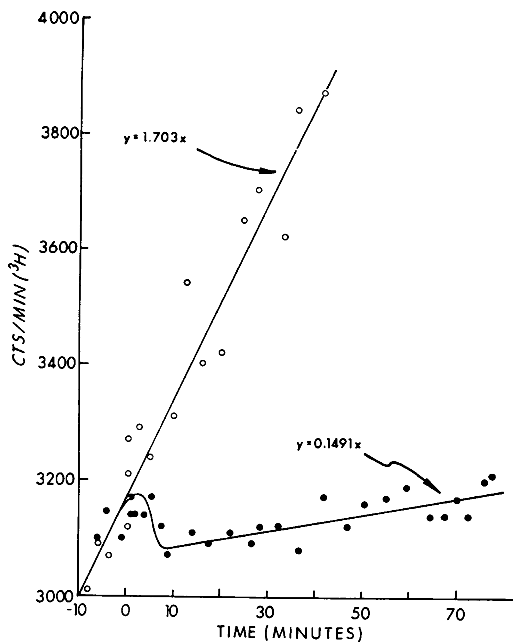

FIG. 6. *Accumulation of RNA during ghost infection. Bacteria were labeled for three generations with ^{3}H-uracil (5 μCi/ml, 20 μg/ml) and pulse-labeled with ^{14}C-leucine (0.02 μCi/ml, 4.7 ng/ml). ^{12}C-leucine (100 μg/ml) was added 1 hr before infection with T2 ghosts. Samples were acidified at various times after infection, and precipitable ^{3}H per milliliter was determined after normalization to a constant amount of ^{14}C per milliliter (^{14}C-protein is not lost during infection). The line was computed from a least-squares best fit. (○) Noninfected cultures; (●) infected with T2 ghosts.*

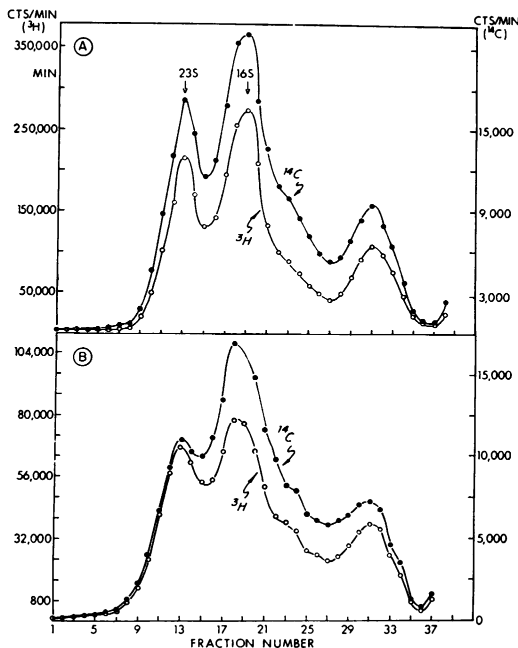

FIG. 7. *Sedimentation rates in 5 to 20% sucrose gradients of stable RNA synthesized before or during ghost infection. Bacteria were exposed to a very small amount of ^{14}C-uracil for three generations (0.75 μCi/ml, 0.2 μg/ml) before the experiment; the ^{14}C-uracil was only present in stable RNA at time of infection. (A) Noninfected bacteria (control) were exposed to ^{3}H-uracil (6 μCi/ml, 31 ng/ml) for 1 min and then to ^{1}H-uracil (100 μg/ml) for 10 min. (B) ^{3}H-uracil was added at 6 min after T2 ghost infection, and ^{1}H-uracil was added from 7 to 17 min. Centrifugation was at 5 to 10 C for 6.5 hr at 37,000 rev/min in a SW50 Spinco rotor.*

other inhibitions should be mentioned. (i) Several investigators have observed exclusion by T2 ghosts of superinfecting phage (3, 4, 9, 11), whereas French and Siminovitch (8) reported that exclusion by ghosts was reversible. We have examined this phenomenon and will present the details elsewhere. However, of significance here is our observation that only a fraction of ghost-infected bacteria exclude. At the MOI that just blocked enzyme induction, this fraction was 85 to 90%. (ii) French and Siminovitch (8) observed that the rate of respiration was also proportional to the fraction of viable cells remaining after ghost infection, although Lehman and Herriott observed a small reduction (19).

These relationships suggest to us that there may be two populations in ghost-infected bacteria, as proposed by French and Simonovitch earlier (8). One population (85 to 90% of total) is not synthesizing protein or nucleic acids, does not support superinfecting phage, and does not recover to reproduce; the bacteria are probably physiologically inert. The second population (10 to 15% of the total) is not synthesizing protein but does recover this function in 30 min; it is synthesizing nucleic acids and respiring at close to normal rates, does support superinfecting phage to a normal level (after a 30-min lag), and does recovery and reproduce.

The inert cells might be those bacteria which are unable to recover from leakage of critical molecules required for macromolecule synthesis. They could represent the entire population of bacteria either when the cells are infected at a very high MOI, or when ghost infection occurs in media in which permeability losses are so great as to be lethal. This may explain some of the apparently contradictory results from different laboratories. Recently, Duckworth (5) reported that ghost infection completely inhibits all RNA, DNA, and protein synthesis, and that most of the soluble pool of nucleotide derivatives is com-

TABLE 1. *Hybridization analyses of RNA*[a]

Expt	DNA	RNA hybridized (%)					
		No T2	T2 ghosts			T2 phage	
			30 sec	6 min	15 min	30 sec	6 min
1	Lac	0.597	0.356	0.261	—	0.456	0.108
	Lac	0.631	0.429	0.310	—	0.533	0.139
	Calf	0.085	0.048	0.092	—	0.091	0.055
	Calf	0.064	0.044	—	—	0.054	0.076
	E. coli	22.1	17.7	15.4	—	12.6	0.743
	T2	0.042	—	—	—	20.5	45.0
2	Lac	0.779	—	0.482	0.775	—	—
	Lac	1.260	—	0.464	0.710	—	—
	Calf	0.209	—	0.146	0.144	—	—
	E. coli	35.0	—	19.8	23.5	—	—
	T2	0.308	—	0.289	0.190	—	—
3	Lac	0.713	—	0.277	0.219	—	—
	Calf	0.049	—	0.039	0.029	—	—
	E. coli	24.7	—	—	—	—	—
	T2	0.079	—	—	—	—	—

[a] Bacteria were induced with IPTG at 30 sec, 6 min, or 15 min after ghost or whole-phage infection. "No T2" are noninfected bacteria. ^{3}H-uracil was added 30 sec after induction. Bacteria were iced after 2 min of induction. RNA was purified and hybridized to the species of DNA shown in the first column (see 15). Between 50,000 and 100,000 counts/min and 10 to 40 μg of RNA were annealed to the following amounts of denatured DNA: ϕ80 dlac, 25 μg; *E. coli*, 100 μg; calf, 5 and 50 μg; T2, 50 μg. Thus, excess complementary DNA sites were available for all RNA except total *E. coli*; *E. coli* values should be multiplied by about 3 to give the expected yields with excess *E. coli* DNA present.

pletely lost to the surrounding medium. However, Duckworth purposely worked with broth-grown cells so that the population would be homogeneous with respect to its inability to recover, and 97 to 98% of the bacteria were killed. Thus, we would guess that her conclusions apply to the physiological state of the bacteria that are killed (85 to 90%) in our experiments. Duckworth has since confirmed that there is nucleic acid synthesis when ghost infection occurs in a synthetic medium (6). Bacteria that are killed probably are simply "washed out" (for practical purposes, lysed); as such, their presence obscures the specific inhibitory effects of ghost attachment on the bacteria that are not destroyed outright. Another possibility that cannot be excluded at this time is that cells that survive do so because they are in a certain stage of the cell cycle, and that a smaller fraction are in this stage in broth-grown cultures.

This rapid killing can explain at least one puzzling observation. Lac mRNA induced just *prior* to whole-phage infection is translated into β-galactosidase during the first minutes of infection (13, 14, 24). When repeated with ghosts instead of phage, there was a barely detectable amount of enzyme made. This result was reproducible, and was quite disturbing because it seemed to imply that the early inhibition of induction by ghosts might be via an entirely different mechanism from that resulting from phage infection. However, it is clear that this is what one would expect given the two populations described above. If 85% of the bacteria are totally stopped metabolically by the attachment process, only the remaining 15% could make enzymes. In this case, 60 sec of preinfection induction would produce only 9 sec of enzyme; this would be barely detectable.

Similarity of T2 ghosts and certain colicins. Colicins are bactericidal substances that share many characteristics with virulent phage ghosts. The review on colicins by Nomura (21) can be consulted for references and details. Colicins and ghosts are composed of proteins, and a given kind of colicin or ghost adsorbs to a limited number of different genetically determined receptor sites on the bacterium. In both cases, killing of a population is multiplicity-dependent; a single particle can kill a cell and does so with a certain probability. However, colicins are more like products of lysogenic phage in that bacteria can carry their genetic determinants (col factors), and when they do so they are immune to their specific colicins. One class of colicins, in particular (E3),

causes effects very similar to those seen after adsorption of T2 ghosts: inhibition of protein synthesis while DNA and RNA synthesis, respiration, adenosine triphosphate production, and potassium transport are not affected. E3 adsorption is believed to cause an alteration in the "23*S* core" of the 30*S* ribosomal subunit (17).

Relationship of ghost inhibitions to whole-phage infection. The bacteria that are not killed by ghosts do not synthesize proteins for 30 min. We believe that this prolonged inhibition results from the same process that leads to the immediate incapacity to induce new enzymes after whole-phage infection.

First, the early effects of phage infection on transcription and translation are similar to those resulting from ghost attachment. The block in enzyme induction occurs within seconds; on the other hand, there is only a partial inhibition of host transcription. Second, infection in the presence of chloramphenicol identifies inhibitions that are dependent on phage protein synthesis. With this inhibitor present during whole-phage infection, certain aspects of host metabolism are similar to those observed in ghost-infected cells: host transcription goes on (23), the bacterial chromosome is not degraded (22), and its replication continues (23). Similarly, early cytological evidence revealed that the host chromosome does not break down in ghost infection (3) and, as shown in this paper, transcription and replication continue (see Results).

These observations suggest that the attachment of T-even phage blocks the induction of new host enzymes; however, attachment has little effect on the host translations in progress and does not interfere with the transcription of host DNA [except for possible competition for RNA polymerase (see below)] or its replication. These latter inhibitions require phage gene products.

Summary of events contributing to takeover of E. coli by T-even phage. Since this is the last in a series of papers on the early effects of T-even infection on host metabolism, we should relate this early block in host protein synthesis to other known effects of phage infection. Effects on host protein synthesis can be grouped conveniently under transcriptional or translational controls.

Transcriptional control. (i) Host RNA synthesis continues for the first few minutes of phage infection at a progressively declining rate (14, 18). By 7 min, it has virtually ceased (14). (ii) Recent in vitro studies suggested that the RNA polymerase from uninfected *E. coli* transcribes a genome equivalent of T4 DNA about four times faster than a cellular equivalent of *E. coli* DNA (29, 31). (iii) When protein synthesis is prevented by chloramphenicol (23) or amino acid starvation (25), T-even phage infection inhibits host nucleic acid synthesis only partially and to an extent that is multiplicity-dependent. As shown in this paper, adsorption of ghosts probably has little or no effect on transcription in the nonkilled cells.

Translational control. (i) Infection causes an *immediate* block in the capacity to induce host enzymes (1, 2, 20, 26). (ii) However, *E. coli* induced to synthesize β-galactosidase just prior (10 to 30 sec) to T4 infection makes a small amount of enzyme during the first several minutes of infection (13, 14, 24). (iii) In contrast to their effects on transcription, T2 ghosts completely block the capacity to induce host enzymes. (iv) A recent study by Hsu and Weiss (12) showed that a heat-labile factor from ribosomes of 12-min T4-infected cells causes a selective decrease in the in vitro translational yield from MS2 RNA or *E. coli* RNA templates. Furthermore, the production of this factor is prevented by the presence of choramphenicol during infection. However, ribosomes from bacteria that were infected for only 3 min did not show this changed specificity.

These observations suggest the following sequence of events after a virulent phage attacks *E. coli*. Attachment of the virus causes an immediate block in initiation of translation of new host proteins, rather than of translation as such. The phage DNA, injected into the cell, competes favorably with the host DNA for RNA polymerase molecules as they are released upon completion of transcription of molecules of mRNA. Transcriptions in progress at the time of infection probably are completed because preinduced β-galactosidase mRNA continues to be transcribed and translated for several minutes.

The preferential transcription of T4 DNA may be of minor significance in vivo. RNA polymerase is probably present in excess amounts in a bacterium; this is implied by the rapidity with which bacteria can increase RNA synthesis upon changed environmental conditions. More significant, during T4 infection in chloramphenicol, about half the RNA being made from 8 to 10 min is bacterial. This was observed at an MOI of 14, which would give an average of several T4 genomes per cell (23). However, this would explain why the inhibition of host transcription in chloramphenicol is multiplicity-dependent; more copies of the phage DNA are injected per cell to compete with the bacterial DNA for RNA polymerase. In contrast, the inhibition is multiplicity-dependent in ghost infection because

more cells are killed at high multiplicities and only the viable ones are synthesizing RNA.

The complete cessation of host transcription that occurs at about 7 min must result from other factors. These could include the progressive breakdown of the host DNA by phage nucleases (32, 33), and possibly alterations in the specificity of the RNA polymerase (28–30). However, it cannot be accounted for by an increasing pool of phage DNA to compete for RNA polymerase, since phage DNA synthesis is just getting underway by 7 min, at which time host transcription is nil.

The capacity to initiate translation of host proteins is blocked immediately by phage attachment. However, several minutes later, a specific phage protein alters the ribosomes so that they translate host mRNA very inefficiently in vitro. This latter alteration may seem superfluous in view of the fact that host protein synthesis is already blocked; probably it is superfluous under most laboratory conditions of infection. Nonetheless it may have played an important role in evolutionary selection because the inhibition resulting from phage attachment is reversible and host protein synthesis recommences after 30 to 35 min. In earlier progenitor strains, the latent periods may have been much longer, and phage strains developed which had this second mechanism for inhibiting host protein synthesis before the host could recover.

One thing is clear from these various studies. There is no *one* mechanism by which a host cell is shut off by a virulent phage. Evolution seems to have resulted in a whole variety of mechanisms, any one of which would suffice to terminate host protein synthesis. This would account for our failure, and the failure of others, to select mutants of T4 that do not inhibit host protein synthesis.

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