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Effect of Glucose on the Capacity of *Escherichia coli* to Be Infected by a Virulent λ Bacteriophage

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ABSTRACT

HOWES, WILLIAM V. (Massachusetts Institute of Technology, Cambridge). Effect of glucose on the capacity of *Escherichia coli* to be infected by a virulent λ bacteriophage. J. Bacteriol. **90**:1188–1193. 1965.—A substrate-dependent phenotypic resistance to phage λ_{vir} was observed among cells of *Escherichia coli* W3350 and C600 grown aerobically on glucose. Similar cells grown on glycerol were sensitive. When cells of W3350 grown on glycerol were transferred to glucose, the rate of appearance of the resistant fraction was proportional to the growth rate and became zero after 5.5 to 6.0 generations. Cells grown on glucose, upon transfer to glycerol, became sensitive within one generation. P^{32} studies with W3350 indicated that the resistant cells did not adsorb phage. Furthermore, among the sensitive cells approximately the same number of particles adsorbed to each cell. Among five strains of *E. coli* K-12 investigated, three did not exhibit this aerobic phenotypic resistance. W3350 grown anaerobically on glucose was 100% resistant. Anaerobic growth had no further effect on C600. These inconsistent effects of anaerobiosis probably involved a mechanism different from that of aerobic growth.

During preliminary investigations of biochemical modifications of *Escherichia coli* W3350 subsequent to infection by a virulent mutant of phage λ (Lederberg and Lederberg, 1953; Jacob and Wollman, 1954), it was observed that no plaque-forming particles could be detected when the phage was titered on plates enriched with 1.0% glucose and 0.5% yeast extract.

Weissbach and Jacob (1962) have observed, during the study of a clear mutant of phage λ (Weigle, 1953; Kaiser, 1957), that a reduced number of phage particles were attached to *E. coli* K-12S and C600 growing in the presence of glucose as compared to these strains growing in the presence of glycerol. These workers suggested that an alteration in the sugar content of the cell wall of the host could be responsible for the phenomenon. However, they were unable to demonstrate, chromatographically, such differences in cell-wall fractions of their strain when it was grown on glucose and on glycerol.

These two observations would indicate a modification of the host-virus interaction in the presence of glucose, which resulted in both fewer phage attached and fewer cells killed.

A possible explanation is that a significant fraction of the cells grown on glucose is resistant and thus survives the exposure to phage. Such behavior of cells grown in complex medium has been observed by Fry (1959), who reported that a fraction of the bacteria in cultures of *E. coli* K-112 gave neither a lytic nor a lysogenic response after exposure to temperate phage λ_{22}. He termed these bacteria "refractory" and assumed they were resistant. It is, of course, questionable whether these isolated observations involving virulent, clear, and temperate λ phages and various growth media can be compared. The following studies were undertaken in an attempt to elucidate specifically this effect of glucose with respect to the capacity of *E. coli* to be killed by a virulent mutant of phage λ.

MATERIALS AND METHODS

Media. Tryptone (Difco) broth consisted of (grams per liter of distilled water): tryptone, 10.0; NaCl, 2.5. Tryptone agar contained the same ingredients plus 11.0 g of agar. Enriched glucose-agar consisted of (grams per liter of distilled water): tryptone, 10.0; yeast extract, 5.0; NaCl, 5.0; agar 15.0. Before autoclaving, 1.0 ml of 1.0 M NaOH was added to each liter of medium. After autoclaving, sterile glucose was added to produce

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1.0%. Soft agar consisted of (grams per liter of distilled water): dehydrated nutrient broth, 8.0; NaCl, 5.0; agar 6.5. Routinely, 0.01 M $MgSO_4$ was added to the soft agar.

Chemically defined phosphate medium consisted of (grams per liter of distilled water): K_2HPO_4, 10.0; $MgCl_2 \cdot 6H_2O$, 0.2; Na_2SO_4, 0.2; NaCl, 5.0; sodium citrate, 0.2; $FeSO_4$, 0.001; $CaCl_2$, 0.001. A phosphate solution was prepared at twice the above concentrations, adjusted to *p*H 7.0 with concentrated HCl, and added, immediately before autoclaving, to an equal volume of the other ingredients at twice the specified concentrations. Ammonium sulfate, 0.1%, was added before autoclaving. Carbon sources were added aseptically after autoclaving at 0.2%. This medium was used for growth of all bacteria unless otherwise specified. All dilutions for viable counts and for titering of phage were made in this medium without an added carbon source. Adsorption medium consisted of (grams per liter of distilled water): KH_2PO_4, 0.3; $Na_2HPO_4 \cdot 7H_2O$, 0.6; $MgSO_4$, 1.2. The phosphates and the $MgSO_4$ were prepared and sterilized separately at 100 times the above concentrations, and added aseptically to sterile water.

Bacteria and bacteriophage. The bacterial strain used for production of phage, as the indicator, and as the host was *E. coli* strain W3350, a derivative of W3110 unable to ferment lactose (*lac*$^-$) or galactose (*gal*$_1^-$, *gal*$_2^-$) and resistant to streptomycin (*str*^{r}) (Fraser, 1962). The double mutant, *lac*$^-$ *str*^{r}, was derived from the original W3350, *lac*$^+$ *str*^{s}, by selection for *str*^{r}. The phage was a virulent mutant of phage λ (λ_{vir}), producing clear plaques on both lysogenic and nonlysogenic strains. Strain W3350 resistant to λ_{vir} was selected from an area of confluent lysis on tryptone agar and was purified by restreaking. The survival of this resistant strain, when exposed to λ_{vir}, was 100%.

Other streptomycin-sensitive strains of *E. coli* used for comparison studies were: W3110, an F$^-$ prototroph; C600, with requirements for threonine, leucine, and vitamin B_1 (Appleyard, 1954); 3.000, a Hayes Hfr with a requirement for vitamin B_1; and a sensitive K-12 prototroph designated K-12. Strain W3350 was obtained from D. K. Fraser. All other strains were obtained from the collection of S. E. Luria, as was the phage stock.

Cultural conditions. Aerobic conditions were obtained on a rotary shaker. Forced aeration was performed in a tube (25 by 230 mm), the entire bottom of which consisted of a coarse fritted-glass disc. Air, saturated with sterile water, was forced vigorously through 30 ml of medium. Anaerobic conditions were obtained by sweeping the inoculated medium with sterile oxygen-free nitrogen, followed by stationary incubation. All cultures were grown at 37 C.

Production of bacteriophage. Phage stocks were prepared by multiple lytic passage in tryptone broth, purified by differential centrifugation, and suspended in adsorption medium.

P^{32} phage particles were produced in strain 3.000 by lytic passage in a low-phosphate tryptone medium containing P^{32}-orthophosphate (0.1 mc of $Na_3P^{32}O_4$ per ml, Iso/Serve, Cambridge, Mass.). The particles were purified by differential centrifugation, and suspended in and dialyzed against adsorption medium. The specific activity of the suspension, based on the counting techniques described herein, was 6×10^{-4} counts per min per plaque-forming unit.

Exposure. Logarithmic-phase cultures (2×10^8 cells per ml) were harvested by centrifugation, washed once, and resuspended in adsorption medium. The cell suspensions were starved aerobically at 37 C for 30 min, except as indicated. To 1.0 ml of the cell suspension was added an equal volume of phage stock in adsorption medium such that the desired multiplicity was obtained. After 20 min at 37 C, 0.1 ml of λ antiserum ($K = 10\ min^{-1}$) was added. The exposed cells were diluted after 10 min and plated on enriched glucose-agar by use of a spreading technique. During spreading, one drop (about 0.04 ml) of antiserum was added to the surface of the plate. Original viable counts were performed on the starved bacteria by use of an identical spreading procedure, except the antiserum was omitted. Multiplicities were based on cell counts performed in a Petroff-Hausser bacteria counter. The fraction of the cells surviving the exposure will be indicated by R_0. The time of exposure (the elapsed time from the addition of phage to the addition of antiserum) will be expressed, in minutes, as a superscript. Thus, the fraction surviving a 20-min exposure will be indicated by R_0^{20}.

As suggested by Fry (1959), the numerical ratio of the phage particles to the bacteria in a given infection mixture will be termed multiplicity of exposure (MOE).

Adsorption of P^{32} phage particles. Logarithmic-phase cultures were harvested by centrifugation, washed, and suspended in adsorption medium to 1.5×10^8 cells per milliliter. All suspensions were starved aerobically at 37 C for 30 min except the anaerobic and samples mixed with an equal volume of P^{32} phage particles in adsorption medium (10 phage per cell) for 20 min at 37 C. The cells were sedimented, resuspended in tryptone broth for 10 min at 37 C, chilled for 20 min at 0 C, sedimented, and resuspended in adsorption medium. A sample of each suspension was collected on a membrane filter (Millipore, HA), washed with phosphate medium, and counted in a low-background Geiger counter (Nuclear-Chicago Corp., Des Plaines, Ill.; model C110B).

RESULTS

Preliminary observations. The growth rate of the host strain and burst size when infected with λ_{vir} in various media were determined. Generation times were 105, 61, and 33 min for cells growing on glycerol, glucose, and tryptone broth, respectively, with corresponding burst sizes of 43, 75, and 130. The growth rates were determined by turbidimetric procedures.

The minimal latent period for λ_{vir} in phosphate medium was 50 to 60 min at 37 C and was independent of the carbon source.

Attempts to enrich the tryptone agar used for titering of phage with either glucose or yeast extract usually resulted in much smaller plaques, at times so completely overgrown as to be indiscernible. With overnight cultures of indicator bacteria grown on tryptone broth, as much as 0.2% glucose could be added to the tryptone agar with little effect on the plaque size; however, if the indicator bacteria were grown on glucose, no plaques were evident. This behavior suggested a modification by growth on glucose of the capacity of the indicator cell to be lysed by λ_{vir}.

Comparisons of initial viable-cell counts, before exposure, with viable-survivor counts and infective-center counts indicated that 85 to 100% of all cells which were nonviable after 20-min exposure at a multiplicity of 20 phage per cell produced infective centers independent of the carbon source used for the initial growth of the cells.

Effect of starvation of the host. With the R_0 value as an indication of phage infection, the effect of aerobic starvation in the presence of $MgSO_4$ at 37 C was investigated. Cells were grown aerobically, exposed at a MOE of 10, and R_0^{20} was calculated for various starvation times. No significant difference in R_0^{20} values was observed from 0 to 180 min of starvation for either carbon source. However, for all subsequent studies, a 30-min starvation was used to minimize any effects due to catabolite repression (Nakada and Magasanik, 1964), and to reduce endogenous stores.

"Infectious heterogeneity" of cells grown on glucose. To investigate phage infections as a function of time, cells grown on glucose and on glycerol were starved as described and exposed for increasing time intervals, and the R_0 values were calculated. The logarithm of the R_0 values was plotted versus time (Fig. 1). R_0 was constant at 0.23 after 40 min for cells grown on glucose and at 0.0015 after 30 min for cells grown on glycerol. Thus, cells grown on glucose exhibited a marked heterogeneity with respect to the capacity to be infected by λ_{vir}. This phenomenon will be termed "infectious heterogeneity."

In a separate experiment, cells grown on glucose were exposed at a MOE of 17 for 60 min. An R_0^{60} value of 0.27 was observed. A sample of these cells could not be further infected when treated a second time by use of an identical procedure.

A second sample of the cells was diluted and plated with antiserum on glucose-minimal agar. Typical colonies were transferred to glycerol and

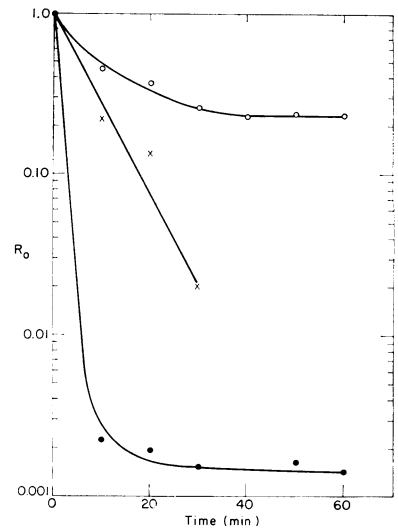

FIG. 1. *Effect of time of infection on the fraction of viable survivors, R_0, of phage infection. Host cells grown on glycerol (●) were infected at a MOE of 35 and cells grown on glucose (○), at a MOE of 24. Lambda antiserum was added to samples of the infection mixture at the times indicated, and the fraction of viable survivors was determined as described. A subtraction of the 40- to 60-min portion from the 0- to 30-min portion of the curve representing cells grown on glucose results in the linear curve, ×.*

to glucose medium. The cells were exposed as described at a MOE of 20, and R_0 values were calculated. Values of 0.003 and 0.25 were obtained for cells grown on glycerol and on glucose, respectively. Thus, the cells were essentially indistinguishable from cultures never exposed to phage.

In view of this behavior, the survivors of a 60-min exposure at a MOE of approximately 20 can be considered to be noninfectable unless subcultured. If the R_0 value corresponding to the 40- to 60-min interval is subtracted from the curve representing the cells grown on glucose in Fig. 1, the linear curve results. This curve indicates a $\Delta R_0(R_0^{20}$ to $R_0^{60})$ of 0.07.

The ΔR_0, thus defined, is an indication of the fraction of the population, capable of being infected, which is not infected during a 20-min exposure.

The effect of the MOE on the fraction of viable survivors was determined for cells grown on glycerol (Fig. 2). A similar study for cells grown on glucose was performed on a sample of the suspension used to study R_0^{20} as a function of time (Fig. 1, $R_0^{60} = 0.23$). Figure 2 indicates little change in R_0^{20} for a MOE greater than 20 for cells grown on glycerol.

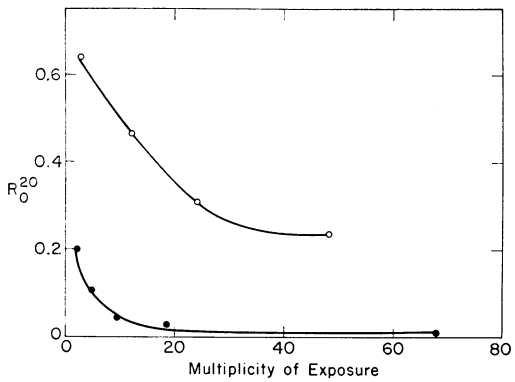

FIG. 2. *Effect of the multiplicity of exposure on the fraction of viable survivors, R_0^{20}, of phage infection. Host cells grown on glycerol (●) and on glucose (○) were infected at the MOE indicated. At 20 min, λ antiserum was added, and the fraction of viable survivors was determined as described.*

TABLE 1. *Effect of aeration on viable survivors of phage infection*

Run	Cultural condition	MOE	Glucose			Glycerol
			R_0^{20}	R_0^{60}	ΔR_0*	R_0^{20}
1	Aerobic	24	0.33	0.23	0.10	—
2	Aerobic	21	0.28	—	—	0.0024
3	Aerobic	26	0.58	0.48	0.10	—
4	Aerobic	1,000	0.52	0.45	0.07	—
5	Anaerobic	16	1.0	—	—	—
6	Forced aeration	16	0.20	0.14	0.06	—

* The difference between R_0^{20} and R_0^{60} is defined as ΔR_0.

Effect of aeration on "infectious heterogeneity." These data are summarized in Table 1. From a comparison of runs 5 and 6, it is evident that aeration greatly affected phage sensitivity, because cells grown anaerobically on glucose could not be infected to any measurable extent. Runs 3 and 4, performed in parallel with samples of the same cell suspension, indicated that high multiplicities of exposure had little effect. The R_0^{60} value for these cells was the largest ever observed.

"Infectious heterogeneity" in other strains. To determine how widespread this phenomenon was among *E. coli* sensitive to λ_{vir}, several strains were grown aerobically and anaerobically on glucose and aerobically on glycerol. These data (Table 2), clearly indicate that "infectious heterogeneity" is not unique to strain W3350, nor is it a phenomenon common to all strains of *E. coli* sensitive to λ_{vir}.

Adsorption of P^{32} phage particles. The survivors of phage exposure may have been resistant to phage adsorption, resistant to injection of the phage genome, or immune, i.e., the injected genome had no discernible effect on the host. To determine whether the phage particles adsorbed to the survivors, host cells were grown aerobically on glycerol and aerobically and anaerobically on glucose. These cells were exposed to P^{32} phage particles as was a resistant strain grown aerobically on glycerol. These data are summarized in Table 3. It will be assumed that the radioactivity associated with equal numbers of bacteria can be related to the multiplicity of infection. The radioactivity associated with the resistant strain probably reflects nonspecifically adsorbed phage or phage not removed by the washing procedure. It is evident that a greater total number of phage were adsorbed to cells grown aerobically on glycerol than to similar cells grown on glucose.

If it is assumed that 30% of the cells grown aerobically on glucose do not adsorb phage, the net value of 14.6×10^4 count/min would be corrected to 24.4×10^4 count/min for 100% sensitivity. This value is in good agreement with 23.2×10^4 count/min for cells grown aerobically on glycerol. The validity of this correction is strengthened by the low number of phage attached to the anaerobic cells. Thus, it would seem that among those cells which adsorbed phage an equivalent average number of particles was adsorbed to each cell.

Kinetics of appearance and disappearance of the resistant fraction. To investigate the kinetics of appearance, host cells were grown on glycerol, washed, and transferred to glucose medium. Samples were withdrawn at intervals, and the resistant fraction was determined. Figure 3 presents a plot of the logarithm of R_0 values versus time. The change in R_0^{20} was approximately paralleled by the change in R_0^{60}. On a basis of cell count, a generation time of 67 min was observed with a period of 30 min preceding any detectable growth. The rapid increase in R_0 values during the phase of adjustment reflects only a small change in the absolute number of resistant cells and is exaggerated in Fig. 3 owing to the exponential plot. In several extended experiments the rate of change to the R_0 values became zero between 5.5 and 6.0 generations.

To investigate the kinetics of disappearance, the reverse transfer was performed (cells grown on glucose to glycerol). For this study only R_0^{20} was determined. As shown in Fig. 4, the cells became sensitive within one generation.

TABLE 2. *Comparison of viable survivors of phage infection in various strains of Escherichia coli*

Strain	Cultural conditions	Medium supplements*	Glucose		Glycerol R_0^{20}
			R_0^{20}	R_0^{60}	
C600	Aerobic	*leu*, *thr*, B_1	0.18	0.14	0.0006
	Anaerobic	*leu*, *thr*, B_1	0.14	—	—
K-12	Aerobic	None	0.019	0.009	0.006
	Anaerobic	None	0.009	—	—
3.000	Aerobic	B_1	0.025	0.006	0.004
	Anaerobic	B_1	0.21	—	—
W3110	Aerobic	None	0.004	0.0003	0.0002
	Anaerobic	None	0.22	—	—

* L-Leucine and L-threonine were added at 10 μg/ml; vitamin B_1, at 1 μg/ml. MOE was between 20 and 40.

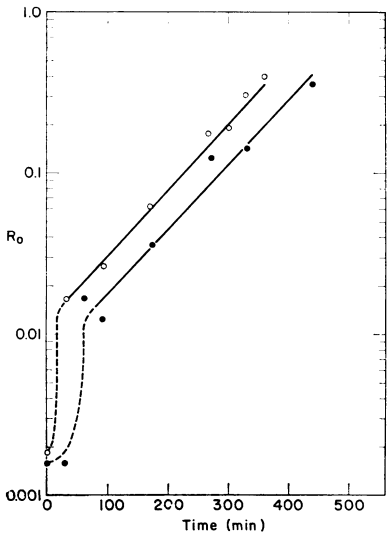

FIG. 3. *Effect of carbon source transfer on the fraction of viable survivors of phage infection. Host cells were grown aerobically on glycerol (5 × 10⁸ cells per milliliter), harvested, and resuspended in carbon-free phosphate medium to 10⁸ cells per milliliter. At zero-time, glucose was added to produce 0.2%. Samples of 3 × 10⁸ cells, based on cell count, were withdrawn at specified intervals, collected and washed on a membrane filter (Millipore, HA), infected in parallel at a MOE of 15 to 20, and the fraction of viable survivors, R_0^{20} (○) and R_0^{60} (●), was determined. The cell density was maintained between 10⁸ and 5 × 10⁸ cells per milliliter by dilution into prewarmed, preaerated medium.*

TABLE 3. *Adsorption of P^{32} phage particles to sensitive and resistant host strains*

Host strain	Growth conditions	Substrate	Count/min × 10^{-4} per ml	
			Initial	Net*
Sensitive....	Aerobic	Glycerol	26.7	23.2
Sensitive....	Aerobic	Glucose	18.1	14.6
Sensitive....	Anaerobic	Glucose	5.4	1.8
Resistant....	Aerobic	Glycerol	3.5	0.0

* Net values have been corrected for radioactivity associated with the resistant strain. All values are expressed in terms of the original suspensions.

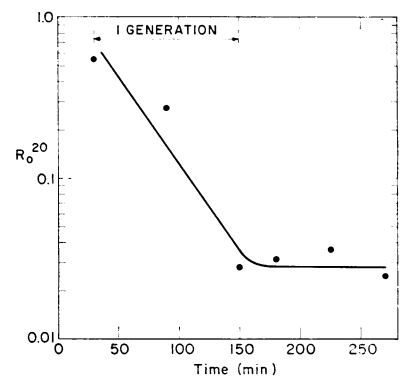

FIG. 4. *Effect of carbon source transfer on the fraction of viable survivors of phage infection. Host cells were grown aerobically on glucose (5 × 10⁸ cells per milliliter), harvested, and resuspended in carbon-free phosphate medium to 10⁸ cells per milliliter. At zero-time, glycerol was added to produce 0.2%. The remainder of the experimental procedure is described in the legend to Fig. 3, except that R_0^{60} was not determined.*

DISCUSSION

The phage stocks used in these studies were prepared in tryptone broth and resuspended in buffered $MgSO_4$. Exposure of the phage to either glucose or glycerol did not occur. The bacteria

were washed free from the culture medium and also resuspended in buffered $MgSO_4$. The mixing of samples of these suspensions of bacteria and of phage then afforded an infection environment essentially free from carbon and nitrogen. Any behavioral differences in a given bacterial strain with respect to the capacity to be infected by λ_{vir} must reflect the history of the bacterial strain prior to exposure to the phage particles.

Adsorption studies with P^{32} phage particles suggest that the survivors of phage exposure resist adsorption. Furthermore, transfer studies suggest that the increase in the fraction of resistant cells is related to growth. This behavior cannot be explained by a simple dilution of adsorption sites, for the sensitive fraction of the heterogenous population seems to have the same number of sites on each cell as a totally sensitive population. It is possible that a segregation of resistant cells occurs. Regardless of the mechanism by which the resistant fraction appears, the resistance is lost within one generation upon transfer to glycerol. This rapid loss suggests that the moiety responsible for the appearance of resistance is rapidly metabolized (or inactivated) in cells growing on glycerol. Simple aerobic starvation of cells grown on glucose has no effect on the resistant fraction. It is reasonable to assume that the appearance and disappearance of resistant cells involves the synthetic machinery of the host, specifically, a structural alteration in the region of the phage adsorption sites.

In conclusion, the data clearly indicate that the production of a resistant fraction among cells grown aerobically on glucose is a substrate-dependent phenotypic response. The capacity to produce this phenotypic response, however, is genotypic. The increased resistance of the anaerobic cells probably involves a different mechanism. Preliminary studies have suggested capsular formation.

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