

## Selection for Lysis Inhibition in Bacteriophage

STEPHEN T. ABEDON

*Department of Microbiology and Immunology, University of Arizona,  
Tucson, AZ 85724, U.S.A.*

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For *Escherichia coli* cells that have been infected by T-even bacteriophages (phages T2, T4, and T6), the adsorption of a second T-even phage results in an increase in the length of the original phage infection and an associated increase in the number of phages produced by the same infected cell. This is a phage encoded response called lysis inhibition. In this study the ecological significance of lysis inhibition is explored. In particular it is argued that lysis inhibition is an adaptive response to environments containing high concentrations of infected cells and low concentrations of uninfected cells.

### Introduction

For T-even bacteriophages (phages T2, T4, and T6) of *Escherichia coli*, lysis inhibition aids in the production of high titer phage stocks (Hershey, 1946a; Doermann, 1948) and helps define the morphology of wildtype (wt) plaques (Hershey, 1946a). T-even phages lacking lysis inhibition are called rapid lysis (*r*) mutants (Hershey, 1946a). T-even phage *r* mutants were critical in important early phage studies (e.g. Hershey, 1946a, b; Benzer, 1955; Crick *et al.*, 1961) and many investigators have explored their physiology and genetics (see Singer *et al.*, 1983). The physiology of lysis inhibition has also been extensively studied (Hershey, 1946a; Doermann, 1948; Levinthal & Visconti, 1953; Rutberg & Rutberg, 1965; Bode, 1967; Josslin, 1970, 1971; Linder & Carlson, 1985). In spite of these efforts there has been little attempt to understand the ecological significance of lysis inhibition.

Lysis inhibition is induced in T-even phage infected cells for a finite period by the secondary adsorption of one or more T-even phages (Hershey, 1946a; Doermann, 1948; Rutberg & Rutberg, 1965; Bode, 1967; note, I will refer to T-even phages simply as “phages” and phage susceptible *E. coli* cells simply as “cells”). Lysis inhibition is expressed as a prolongation of the phage latent period (the length of a phage infection) and a magnification of the phage burst size (the number of progeny phages produced per infected cell). The induction of lysis inhibition delays the lysis of the infected cell and results in an approximate doubling of the length of the period of intracellular progeny production (Doermann, 1948; Rutberg & Rutberg, 1965; Bode, 1967). At concentrations of  $4 \times 10^7$  cells ml<sup>-1</sup> and greater (Bode, 1967), there may be repeated secondary phage adsorptions resulting in the continuous induction of lysis inhibition. Phage latent periods may be extended in this manner for hours (Hershey, 1946a; Doermann, 1948; Levinthal & Visconti, 1953; Rutberg & Rutberg, 1965; Bode, 1967).

Doermann (1948) suggested that the selective advantage of lysis inhibition may be the larger phage burst size. Though he may very well be correct, this explanation ignores a key question: If a longer latent period with a larger burst size is an advantage, why is lysis inhibition not constitutively expressed? Elsewhere, I have argued that the shorter latent period in the uninduced state is an adaptation to rapid growth in environments containing high concentrations of cells (Abedon, 1989). Here, that the induction of the lysis inhibition phenotype plays two additional roles in the lytic strategy of T-even phages will be argued: (i) at high host cell and free phage concentrations the extension of the phage latent period with lysis inhibition prevents mature intracellular progeny phages from being exposed to previously infected cells in the extracellular environment. Previously infected cells display superinfection immunity and are consequently restrictive to secondarily adsorbing phages (Cornett, 1974; Vallée & de Lapeyrière, 1975 and citations contained within these references). (ii) The larger burst size is advantageous given the dearth of uninfected cells that may arise following the exposure of a cell population to large numbers of free phages.

The three aspects of lysis inhibition, (i) its requirement for secondary adsorption, (ii) the extension of the normal phage latent period, and (iii) the increase in the phage burst size, are all adaptations to the three phases of normal T-even phage growth in environments containing high concentrations of cells. These include: (i) rapid growth of phage populations when lysis inhibition is not induced and uninfected cells are plentiful, (ii) retention of the infected state when infected cells are plentiful and free phages are therefore vulnerable to inactivation, and (iii) retention of the infected state and a maximization of the phage burst size when uninfected cells are scarce.

### Probability of Secondary Adsorption

Lysis inhibition is induced in a T-even phage infected cell by the adsorption to that cell of one or more additional T-even phages. The kinetics of secondary adsorption, thus, are directly relevant to the kinetics of the expression of lysis inhibition. In particular, the probability of expression of lysis inhibition is dependent on the multiplicity of secondary phage adsorption ( $M_s$ ).  $M_s$  is the number of free phages that, on average, adsorb to a cell during the interval of a normal phage latent period ( $t_l$ ). That is,  $M_s$  is the number of phages that adsorb to an infected cell following the adsorption of the initially infecting phage.

$M_s$  is dependent on the concentration of free phages at the beginning of a phage latent period ( $P_0$ ), the concentration of cells ( $N$ ), and the adsorption constant ( $k$ ).  $k$  is the probability of adsorption of one phage to one bacterium in 1 ml of solution in 1 min (Schlesinger, 1960; Stent, 1963). If free phages are not replenished following adsorption to host cells,  $M_s$  is defined as,

$$M_s = P_0(1 - e^{-kNt_l}) / N, \quad (1)$$

where the term  $e^{-kNt_l}$  is from the adsorption equation presented by Schlesinger (1960) and Stent (1963) and describes the fraction of free phages that have *not* adsorbed to a host cell after some period,  $t_l$ .

Over short periods, at low cell concentrations, or if free phages are replenished over time (e.g. as a result of the lysis of infected cells and phage progeny release), the decline in free phages that is taken into account in eqn (1) may be ignored. The result is that the term  $M_s$  may be considered to be independent of cell concentration,  $N$ ,

$$M_s = kP_0 t_l. \quad (2)$$

Thus,  $M_s$  may be described as a direct function of the concentration of free phages.

Assuming a Poisson distribution of phages adsorbing to susceptible cells, the probability that a cell will be secondarily adsorbed to and therefore lysis inhibited over the length of a phage latent period ( $A$ ) may be defined as

$$A = 1 - e^{-M_s}. \quad (3)$$

If, for instance,  $M_s$  is equal to 0.75, then  $A$  is equal to approximately 0.5. That is, the probability that a given infected cell will be secondarily adsorbed to and lysis inhibited over a period  $t_l$  minutes long is 0.5. If  $M_s$  is equal to 4.6, then  $A$  is equal to 0.99.

#### Extended Period of Lysis Inhibition

In an environment of sufficient infected cell concentration, the lysis inhibited state in the majority of infected cells can be maintained for hours (Hershey, 1946*a*; Doermann, 1948; Levinthal & Visconti, 1953; Rutberg & Rutberg, 1965; Bode, 1967; Fig. 1 of this paper). Presumably lysis inhibition is maintained over long periods by the repeated secondary adsorption of previously infected cells. The maintenance of a lysis inhibited state via repeated secondary adsorption implies that the probability of secondary adsorption approaches 1.0 during successive periods over the length of an extended infection. In other words, lysis inhibition can only be maintained by a mechanism requiring repeated secondary adsorptions if at least one secondary adsorption occurs before each finite period of induction of lysis inhibition passes (i.e. before the infected cell lyses).

The number of infected cells that must lyse per unit time in order to maintain an extended lysis inhibited state may be estimated. First, it is necessary to assume that secondary adsorption must occur at least once per some interval in order for the lysis inhibited state to be extended another such interval (an "adsorption interval"). The length of this adsorption interval is probably about 10 to 30 min given a normal phage latent period that is 30 min long (Doermann, 1948; Rutberg & Rutberg, 1965; Bode, 1967).

As noted above, if  $M_s$  is equal to 4.6, then  $A$  is equal to 0.99 [eqn (3)]. If it is assumed that nearly all cells in an environment are phage infected, then this implies that 4.6 free phages are lost to adsorption during a single adsorption interval for every cell in the environment given that 99% of the infected cell population is secondarily adsorbed to over one adsorption interval. Of course, with such high phage multiplicities, it is likely that nearly all cells in the environment will be phage infected.

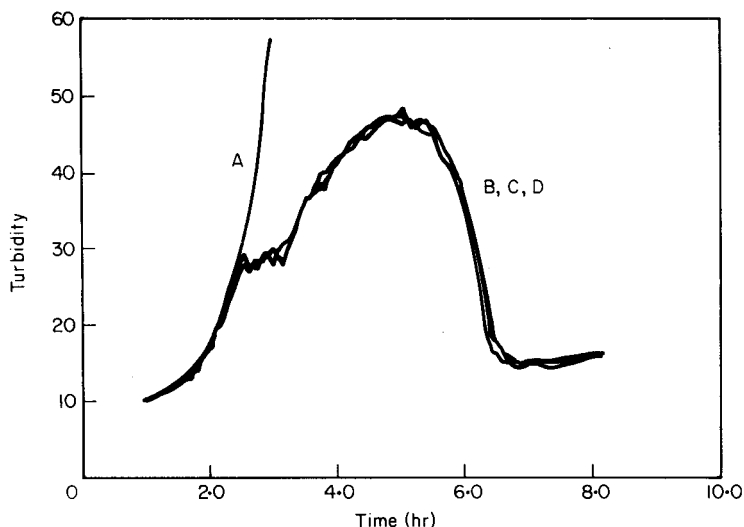


FIG. 1. A visualization of lysis inhibition and lysis as a function of the turbidity of host cells. Observations: (i) The period of lysis inhibition lasts for about 3 hr (from a point approximately 3 hr after the initiation of the experiment to approximately 6 hr into the experiment; a latent period of about 30 min is expected with no lysis inhibition under similar conditions). (ii) The lysis of the bulk of the infected cell population occurs over a 1 hr period, between 5 and 7 hr into the experiment. Method: 1.0 ml of a stationary phase *Escherichia coli* overnight culture was added to 125 ml of Hershey broth (Steinberg & Edgar, 1962). Of this (25 ml) solution was added to four flasks which were placed in a 37°C shaker at 0 hr. Cell growth was allowed until about  $10^7$  cells  $\text{ml}^{-1}$ . At this point (about 1.5 hr)  $10^6$  T4D phages (wt) were added to flasks B, C and D (in a volume of 0.1 ml), but not to flask A. The turbidity of these four cultures was determined in Klett units an average of six to seven times per hour. (Note: curves were drawn by connecting these individual determinations with their associated points omitted in order to enhance clarity.) Following what was perceived to be a post-lysis stabilization of the turbidity of the broth (at 8 hr), three drops of chloroform were added to flasks B, C and D to lyse any remaining cells. The same general experiment was first performed by Doermann (1948).

Approximately 100 phages are released by the lysis of a single T-even phage infected cell (Doermann, 1952). This means that approximately 4.6% of the infected cells in an environment must lyse per adsorption interval in order to replenish those free phages that have adsorbed to infected cells [4.6 phages/infected cell = (100 phages/lysed infected cell) \* (0.046 lysed infected cell/infected cell)] while lysis inhibition is maintained in 99% of the infected cells in an environment. Since one of the characteristics of lysis inhibited cells is an increased burst size, the actual fraction of infections that must lyse per adsorption interval in order to maintain this lysis inhibited state should decrease as the length of the lysis inhibited state is extended.

Of course, if 99% of the infected cells in an environment continue to be lysis inhibited, then 4.6% of them cannot be expected to lyse over that same period. A feedback mechanism, however, can be expected to operate to limit the lysis of infected cells and the release of free phages near some minimal level necessary to maintain the population-wide lysis inhibited state. That is, as free phages become scarce, secondary adsorption becomes less likely and infected cell lysis becomes

more likely. The lysis of infected cells increases the number of free phages in the environment thus increasing the probability of secondary adsorption. Secondary adsorption, however, inhibits infected cell lysis leading to a scarcity of free phages and the completion of the feedback loop. A scenario such as this could maintain some minimal number of free phages required for the secondary adsorption and the lysis inhibition of the majority of infected cells. Thus, it is reasonable to expect that a mechanism requiring repeated secondary adsorptions could operate to maintain a population-wide lysis inhibited state over long periods.

### Decline in Uninfected Cell Concentration

The majority of free phages that adsorb to infected cells fail to survive because infected cells display superinfection immunity. The fraction of uninfected phage susceptible cells ( $N/N_0$ ) remaining in an environment following  $t$  minutes exposure to a constant concentration of free phages is dependent on that concentration of free phages,  $P$ ,

$$N/N_0 = e^{-kPt}, \quad (4)$$

where  $N_0$  is the concentration of uninfected cells at  $t=0$ . Ignoring cell division, the half-life of uninfected cells,  $t_F$ , in an environment of constant phage concentration,  $P_0$ , can be approximated by setting  $N/N_0$  to 0.5, taking the natural log of the terms in eqn (4) and then solving for  $t$ ,

$$t_F = 0.69k^{-1}P_0^{-1}, \quad (5)$$

$t_F$  is equal to 3, 30, or 300 min with a constant free phage concentration of  $10^8$ ,  $10^7$ , or  $10^6$  free phages per ml and using a value of  $k$  of  $2.5 \times 10^{-9}$  ml min<sup>-1</sup> (Stent, 1963). Of course, with lower free phage concentrations the assumption that cell division can be ignored becomes less tenable. Nevertheless, high concentrations of free phages should lead to a rapid decline in the concentration of uninfected cells.

### Lysis Inhibition as a Mechanism of Burst Size Magnification

The various arguments presented in the above three sections can be summed up as follows: As free phage concentrations increase there is (i) a rise in the probability of secondary adsorption, (ii) a decline in the number of uninfected cells, and (iii) a rise in the number of infected cells. Lysis inhibition may be defined ecologically as a response to these three consequences of high free phage concentrations, especially when cell concentrations are also high. Below it will be argued that the burst size magnification phenotype of lysis inhibited cells is selectively beneficial at low concentrations of uninfected cells. To do this, several terms and relationships must first be defined:

(i) The phage latent period,  $t_L$ , is the sum of two periods that take place in the following order after phage adsorption to an uninfected cell (Doermann, 1952); the eclipse period and the progeny producing period ( $t_R$ ). Intracellular progeny phages are produced only during the progeny producing period. The number of progeny

phages produced per infection, the burst size ( $B$ ), is equal to the rate of phage production,  $C$ , multiplied by the length of the phage producing period,  $t_R$  (i.e.  $B = t_R C$ ). This linear intracellular increase in mature phage progeny with phage replication is consistent with that exhibited by T-even phage infections (Doermann, 1952; Levinthal & Visconti, 1953; McCarthy *et al.*, 1976). Lysis liberates free progeny phages and signals the end of the period of infection.

(ii) Free phages adsorb to phage susceptible cells. The fraction of free phages remaining unadsorbed following  $t$  minutes of exposure to cells ( $P/P_0$ ) is defined by Schlesinger (1960; see also Stent, 1963),

$$P/P_0 = e^{-kNt}, \quad (6)$$

where  $P$  is the concentration of free phages at time,  $t$ . This equation assumes that phages adsorb to both uninfected and previously infected cells with the same kinetics.

(iii)  $S_t$  is the fraction of free phages that adsorb to uninfected cells over some period,  $t$ .  $S_t$  is determined by multiplying the fraction of free phages that have adsorbed to cells over  $t$  min,  $[1 - P/P_0]$ ; eqn (6)], by the probability that a host cell is uninfected,  $U$ . Thus,

$$S_t = U(1 - [P/P_0]). \quad (7)$$

If it is assumed that the adsorption of a free phage to an infected cell results in the restriction of the adsorbing phage, a result of superinfection immunity, then  $U$  is also the probability of survival of a given free phage following adsorption to a given cell.

(iv) Elsewhere it is argued (Abedon, 1989) that T-even phages probably evolved a short latent period (keeping the rate of intracellular phage growth constant) in response to periodic growth in environments containing high concentrations of uninfected cells and low concentrations of phages. That is, at high cell concentrations phages with short latent periods more rapidly establish multiple parallel infections, rapidly monopolize available cells, and thus out compete long latent period phages. Conversely, when cell concentrations are low, phages with long latent periods are more fit than phages with short latent periods. This is because long latent period phages use their host cell resource more completely. That is, long latent period phages have a larger burst size than do short latent period phages.

These arguments were tested by computer simulation of phage T4 growth through several rounds of infection as it would occur in an environment that contains only some constant concentration of uninfected cells (Abedon, 1989). An environment that additionally contains high concentrations of free phages and infected cells may be modeled in an identical manner. This is done by assuming (i) that free phages die when they adsorb to previously infected cells and (ii) that the product of the total cell concentration (uninfected plus infected cells,  $N$ ) and the time free phages are exposed to infected cells ( $t$ ) is large. In such an environment the fraction of free phages that remain unadsorbed is small [i.e.  $P/P_0 \rightarrow 0$  as  $Nt \rightarrow$  infinity; eqn (6)] and the probability of free phage survival,  $S_t$ , becomes effectively equal to the probability that the cell to which a free phage adsorbs is uninfected,  $U$  [eqn (7)]. Similarly, the effective phage burst size (i.e. the fraction of a burst that survives

following adsorption to cells) is now equal to the product of the phage burst size ( $B$ ) and the fraction of phage susceptible cells that are uninfected ( $U$ ). The effective cell concentration remains equal to the concentration of uninfected cells,  $U$ .

Thus, regardless of the number of infected cells or free phages that are found in a given environment, phages that display a long latent period and a large burst size (i.e. lysis inhibited phages) should be more fit than phages that display a short latent period (i.e. phages that are not lysis inhibited) as long as the concentration of uninfected cells is low. Low concentrations of uninfected cells are, of course, one consequence of high concentrations of free phages [eqns (4) and (5)]. Lysis inhibition as a mechanism of burst size magnification, then, can be seen as a response to the low concentrations of uninfected cells that results from the exposure of a cell population to high concentrations of free phages.

### **Lysis Inhibition as a Mechanism of Latent Period Elongation**

Below it will be argued that the delay of lysis phenotype of lysis inhibited cells can be selected as a mechanism that limits the exposure of free progeny phages to the restriction that results from adsorption to previously infected cells and the expression of superinfection immunity by these cells.

The lysis of a population of lysis inhibited infected cells growing in rich broth occurs over a short interval (Fig. 1). The progeny of an infected cell which lyses before the lysis of most of the infected cell population would have a high probability of adsorbing to infected cells and dying if the concentration of infected cells is high and the concentration of uninfected cells is low [eqn (6)]. For example, in an environment containing  $10^7$  lysis inhibited infected cells per ml and no uninfected cells, 90% of the free phages released following the lysis of an infected cell would adsorb to infected cells, and, therefore, die after 100 min [eqn (6)].

Given the eventual lysis of the bulk of infected cells (i.e. Fig. 1), a delay in lysis could result in a significant increase in the survival of progeny phages. This is especially true if lysis is delayed until the point of lysis of most of the infected cell population. Thus, the extension of the phage latent period with lysis inhibition in an environment containing high concentrations of lysis inhibited infected cells and low concentrations of uninfected cells can be efficacious even without burst size magnification. An environment that is dominated by lysis inhibition-minus rather than lysis inhibition-plus phage infected cells, however, would derive less of a fitness advantage from latent period extension alone but a similar advantage from the associated burst size magnification.

### **Lysis Inhibition is a Response to Sensory Input**

The lysis inhibition signal is probably carried through the host cell envelope. This assumption is based on the following considerations: (i) phages, including secondary phages, adsorb to the cell envelope (Goldberg, 1983), (ii) exclusion of secondarily adsorbing phages probably acts at the cell envelope and prevents secondarily

adsorbing phage DNA from penetrating to the cell protoplasm (Cornett, 1974; Vallée & de Lapeyrière, 1975; Kao & McClain, 1980; Lu & Henning, 1989), and (iii) phage gene products, including *r* gene products necessary for lysis inhibition, have been located on or within the cell envelope (Peterson *et al.*, 1972; Pollock & Duckworth, 1973; Beckey *et al.*, 1974; Fletcher *et al.*, 1974; Huang, 1975; citations in Singer *et al.*, 1983). Since lysis inhibition is a response to an extracellular condition signaled through the secondary adsorption of a T-even phage, lysis inhibition can be regarded as a response to a signal that is sensed at or through the envelope of an infected cell. The host cell envelope, perhaps with phage specified modifications, thus may be considered to be a sensory organ by which infecting T-even phages monitor the concentration of free T-even phages in the extracellular environment.

### Discussion

Lysis inhibition is a phage encoded mechanism of latent period prolongation and burst size magnification (Hershey, 1946*a*; Doermann, 1948; Rutberg & Rutberg, 1965; Bode, 1967). It is initiated by the adsorption of a second T-even phage to a T-even phage infected cell. For the most part this second phage does not enter the cell, does not replicate, and does not express the lysis inhibition phenotype (Cornett, 1974; Vallée & de Lapeyrière, 1975; Rutberg & Rutbert, 1967). There are two key aspects of lysis inhibition that require explanation: (i) what, if any, is the benefit of the extended phage latent period and (ii) why is the expression of lysis inhibition tied to secondary adsorption?

A long latent period may be selected by conditions in which free phages are less durable than phage infected cells. An antagonist specific to released progeny and other free phages should therefore select for a longer latent period. Restrictive cells, ones that allow irreversible adsorption but not phage multiplication, are free phage antagonists. Cells which have been previously infected by T-even phages but have not yet lysed are restrictive to secondarily adsorbing homologous phages (Cornett, 1974; Vallée & de Lapeyrière, 1975). Thus, lysis inhibition may be selected by the cost of otherwise exposing progeny phages to high concentrations of previously infected cells.

The probability that a cell has been previously infected is a function of the free phage concentration as is the probability of secondary adsorption [eqns (1), (2) and (4)]. The rate at which free phages are lost to adsorption, however, is dependent on the concentration of cells. Thus, it may be concluded that lysis inhibition is an adaptation to high concentrations of both phages and cells. A second advantage of lysis inhibition, one that is dependent only on high free phage concentrations, is an increase in phage burst size. This reflects a more complete utilization of each host cell resource for progeny phage production when uninfected cells are scarce (Abedon, 1989). The selection for lysis inhibition and for long and short phage latent periods in general (from Abedon, 1989) are summarized in Table 1.

A strong competitive advantage of wt T-even phages over *r* mutants (lysis inhibition-minus phages) at high phage and cell densities was demonstrated by Hershey (1946*a*) in both wt and *r* mutant phage-rich environments. Hershey noted that the

TABLE 1

Summary of selection imposed upon *T-even* phages by various combinations of high and low phage and cell concentrations

	Different environments			
	i	ii	iii	iv
[Cell]†	high	high	low	low
[Phage]	low	high	low	high
[Uninfected cell]	high	low	low	low
[Infected cell]	low	high	low	low
<i>P</i> ‡ (free phage adsorption)	high	high	low	low
<i>P</i> (secondary adsorption)	low	high	low	high
<i>P</i> (free phage restriction§)	low	high	low	low
<i>S</i> ‡ (large BS‡)	yes <sup>  </sup>	yes	yes	yes
<i>S</i> (SLP‡ with small BS)	yes	no	no	no
<i>S</i> (LLP‡ with large BS)	no	yes	yes	yes
<i>S</i> (LLP without large BS)	no	yes	no	no
<i>S</i> (rapid lysis¶)	yes	no	no	no
<i>S</i> (lysis inhibition¶)	no	yes	no	yes

† Brackets, “[*x*],” denote concentration of “*x*”.

‡ “*P*” refers to probability. “*S*” refers to selection for a particular trait. “BS” = burst size. “SLP” = short latent period. “LLP” = long latent period.

§ Restriction is due to phage adsorption to infected cells.

|| “Yes” and “no” refer to whether this trait will be selected for in a given environment.

¶ The lysis inhibition phenotype is an induced long latent period with a large burst size; the rapid lysis phenotype is an uninduced short latent period with a small burst size.

burst size advantage of lysis inhibited wt phages over *r* mutants was not sufficient to explain the competitive advantage of wt phages compared with *r* mutant phages (Doermann, 1948, however, later suggested that, indeed, it was). Hershey suggested that a phenomenon known as mutual exclusion (Delbrück & Luria, 1942) might be responsible for this difference.

At cell concentrations of  $4 \times 10^7$  ml<sup>-1</sup> and greater (Bode, 1967), wt phage infected cells appear to be maintained in the lysis inhibited state for considerably longer periods than at lower cell concentrations (Hershey, 1946; Doermann, 1948; Levinthal & Visconti, 1953; Rutberg & Rutberg, 1965; Bode, 1965; Fig. 1 of this paper). This is probably due to the premature lysis of some infected cells leading to repeated secondary adsorption of the remaining infections (Hershey, 1946; Doermann, 1948; Levinthal & Visconti, 1953; Rutberg & Rutberg, 1965; Bode, 1965). I propose that the most of the progeny phages that are released in the presence of high concentrations of lysis inhibited cells are doomed to secondary adsorption and restriction [eqns (6) and (7)]. Thus, the extra advantage of wt phages over *r* mutant phages may result, in part, from superinfection immunity derived restriction of *r* mutant free phages by the much longer infecting lysis inhibited wt phages. To a degree this explanation is consistent with Hershey's suggestion that mutual exclusion between wt and *r* mutant phages was responsible for the unexplained fitness advantage of wt phages over *r* mutant phages. Considering that high *E. coli* cell concentrations

(i.e.  $4 \times 10^7$  ml<sup>-1</sup> and greater) have been routinely measured in the pre-fecal gastrointestinal tracts of normal, healthy mammals (Smith & Jones, 1963; Smith, 1965; Savage *et al.*, 1968; Lee *et al.*, 1971), a scenario such as this one could very well maintain lysis inhibition in nature.

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