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# Bacteriophage burst size during multiple infections

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**Abstract.** A significant positive correlation was observed between multiplicity of infection and burst size of mycobacteriophage I3. During multiple infections, the average contribution of each infecting phage to the burst size was inversely correlated with multiplicity of infection even when bacterial resources were not limiting. We conclude that the efficiency of phage-coded functions rather than the extent of bacterial resources determines the burst size.

**Keywords.** Bacteriophage; mycobacteriophage I3; burst size; multiple infections.

## Introduction

The last few decades have witnessed phenomenal advances in our understanding of the detailed mechanism of phage growth and reproduction (Lewin, 1977). Bacteriophages inject their DNA or RNA into a sensitive host bacterium and utilize the bacterial machinery to produce a number of progeny phage particles. The number of phages liberated by an infected bacterium at the end of one cycle of phage growth designated as the burst size is characteristic of a given phage-host system. Questions such as why a particular phage should have a certain burst size or to what extent the phages utilize the nutritional resources in the bacterium are still unanswered.

When a phage infects a bacterium, several phage and host factors come into play to produce a crop of phage particles. The actual number of phage particles produced may be determined by: (a) nutritional status of the bacterium if the phage utilizes the bacterial machinery maximally for its reproduction or (b) phage-coded functions such as polymerases and regulatory proteins essential for phage production; their inherently low efficiency may not permit the phage to make full use of bacterial resources and consequently the burst size may be limited. In this paper we describe results of experiments designed to distinguish between alternatives (a) and (b) and thus elucidate the factors which might be responsible for the burst size of a bacteriophage.

## Materials and methods

*Organisms*

*Mycobacterium smegmatis* SN2 and a clear plaque mutant (Gopinathan *et al*., 1978) of its phage I3 (Sunder Raj and Ramakrishnan, 1970) were used.

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*Methods*

The experiment consisted of four parts (figure 1): I. Desired numbers of bacteria (*A*) and phage (*B*) were mixed in nutrient broth. The number of bacteria in each ex-

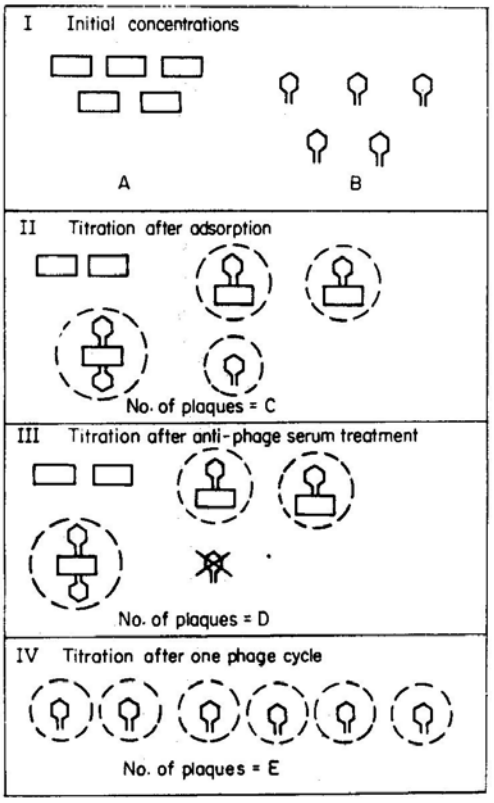


**Figure 1.** Schematic representation of the experimental protocol
Bacteria and phage are shown schematically. Each circled entity gives rise to one plaque when titrated with indicator bacteria.

periment was $10^7$. The number of phages was varied from experiment to experiment, II. At the end of 30 min of adsorption at 37°C in the presence of 3 mM KCN to synchronize infection and thus avoid any interference (Hutchison and Sinsheimer, 1966), the phage-bacteria mixture was titrated with indicator bacteria. Let the number of plaques obtained be *C* which equals the number of infected bacteria plus the number of free phages remaining. Each infected bacterium gives rise to only one plaque irrespective of the number of phages infecting it. III. Free phages were inactivated by the addition of antiphage serum and the titration was repeated. Let the number of plaques obtained now be *D* which equals the number of infected bateria. IV. The mixture was diluted $10^3$-fold and incubated in a rotary shaker for one cycle of phage growth (4 h) (Gadagkar, 1979), lysed by the addition of chloroform and titrated again with indicator bacteria. Let the number of plaques obtained now be *E* which equals the total number of phages liberated at the end of one cycle

of growth. Then, *B/A=multiplicity of infection* defined as the ratio of phage: bacteria added; *C–D*=number of unadsorbed free phage; *B–(C–D)*=number of phages adsorbed;

$$\frac{B-(C-D)}{D} = \text{mean number of phages adsorbed perinfected bacterium.}$$

called the *effective multiplicity of infection*; *E/D=burst size*, defined as the mean number of phages liberated per infected bacterium and *E*/[*B*–(*C–D*)]=mean number of phages liberated per phage adsorbed, called the *effective burst size*. The experiment at each multiplicity of infection from 1-6 was repeated four to six times. The data were statistically analyzed by the student's *t* test and the level of significance is indicated in each case (Bailey, 1959).

**Results**

*Correlation between multiplicity of infection and burst size*

It can be seen from figure 2 that there was a significant positive correlation between multiplicity of infection and the burst size. This can be interpreted to mean that at low multiplicity of infection, the phages did not utilize the entire resources available in the bacterium and hence the burst size was limited by the inherent capacity of the

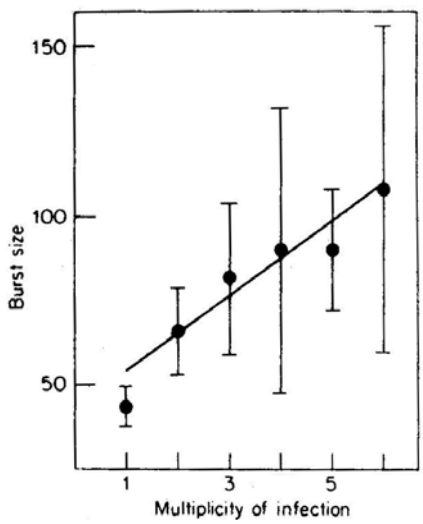


**Figure 2.** Correlation between multiplicity of infection and burst size
Mean ±S. D. from 4-6 experiments for each point are shown. The solid line is the least squares fit. Correlation coefficient = +0.58, significant at $P \leq 0.01$. Multiplicity of infection is defined as the ratio of phage: bacteria added and burst size is defined as the mean number of phages liberated per infected bacterium.

phage for reproduction. This does not imply that the nutritional status of the bacterium had no effect on the burst size but simply that under the given conditions, the phage did not make full use of the resources in the bacterium. In fact, the burst size is further reduced if the bacteria were growing in a synthetic minimal medium instead of nutrient broth (Gadagkar, 1979).

*Correlation between multiplicity of infection and effective multiplicity of infection*

As the multiplicity of infection was increased, the effective multiplicity of infection (defined as the mean number of phages adsorbed per infected bacterium) also increased significantly (figure 3). At any given multiplicity, the effective multiplicity of infection was often higher than the initial value myself.

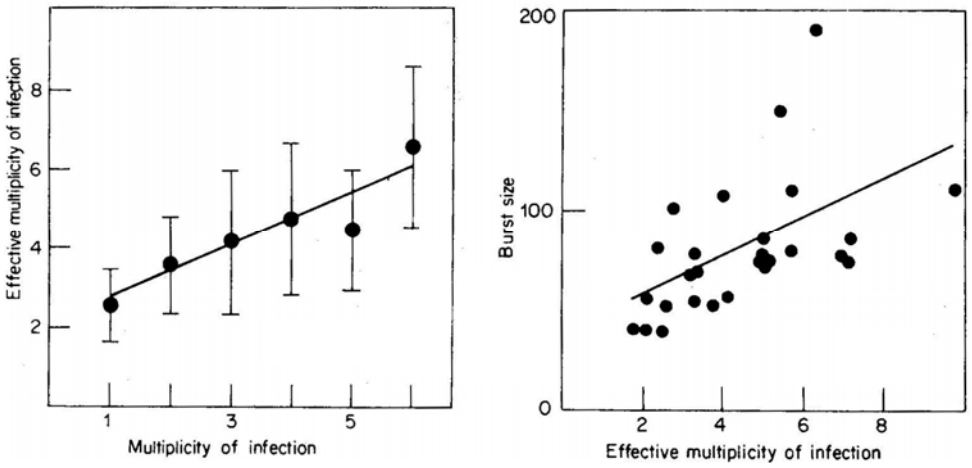


**Figure 3.** Correlation between multiplicity of infection and effective multiplicity of infection
Mean ± S.D. from 446 experiments for each point are shown. The solid line is the least squares fit. Correlation coefficient= +0.62, significant at $P < 0.001$. Effective multiplicity of infection is defined as the number of phages adsorbed per infected bacterium.

**Figure 4.** Scatter diagram showing correlation between effective multiplicity of infection and burst size
The solid line is the least squares fit. Correlation coefficient= + 0.56, significant at $P < 0.01$.

*Correlation between effective multiplicity of infection and burst size*

In spite of the fact that effective multiplicity of infection was often slightly higher than multiplicity of infection, there was a significant positive correlation between effective multiplicity of infection and burst size (figure 4). This result confirms that as the number of phages infecting a bacterium increased, phage production also increased.

*Correlation between effective multiplicity of infection and effective burst size*

Since the burst size increased during multiple synchronous infections (the phrase 'multiple infection' is used throughout to represent the simultaneous infection of a bacterium by more than one phage), it was interesting to see how the phages interacted with one another and contributed to the total phage production. For this reason we have computed the effective burst sizes (mean number of phages liberated

per phage adsorbed). Figure 5 shows that there was a significant negative correlation between the effective multiplicity of infection and effective burst size. Therefore, during the multiple infections, the contribution of each phage to the total burst size decreased with increasing number of phages infecting a bacterium. The negative correlation between effective multiplicity of infection and effective burst size does not mean that the bacterial machinery becomes limiting because the burst size is positively correlated with effective multiplicity of infection. This suggested that although the bacterial machinery was not limiting, there was some kind of interaction among the phages during multiple infection which reduced the effective burst size.

It must be pointed out that despite the apparent scatter, all the correlations are highly significant as indicated by students' *t* test ($P<0.01$).

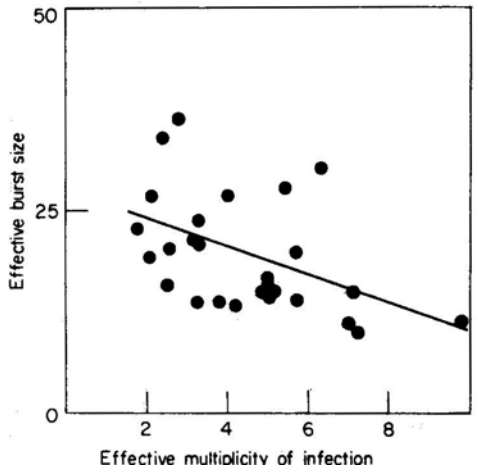


**Figure 5.** Scatter diagram showing correlation between effective multiplicity of infection and effective burst size
The solid line is the least squares fit. Correlation coefficient= –0.49, significant at $P<0.01$. Effective burst size is defined as the mean number of phages liberated per phage adsorbed.

## Discussion

Delbruck and Luria (1942) working with phages $\alpha$ and $\beta$ infecting *E. coli* observed that the burst size during multiple infections was higher than during single infections but that there was no correlation between multiplicity of infection and burst size. In contrast, it is known in a number of systems that if lysis of the infected bacterium is delayed, there is an increase in the burst size. This has been achieved in the case of T-even $r^+$ phages by superinfection within a few minutes of the primary infection (Doerman, 1948) or by the inhibition of protein synthesis in the middle of the latent period (Hershey and Melechen, 1957) and in the case of $\phi$X174 by infection with a lysozyme defective mutant (Hutchison and Sinsheimer, 1966). Doerman (1948) has made a detailed study of the mechanism of lysis inhibition by T-even phages. Although not explicitly mentioned, it is clear from his data that even at a multiplicity of infection which did not bring about an extension of the latent period by delaying lysis, there was a positive correlation between multiplicity of infection and burst size.

Hutchinson and Sinsheimer (1966) working with a lysis defective mutant of the phage $\phi$X174 have shown that maturation of phages continued longer than in the case of wild type infection leading to a 10-fold increase in the burst size. This suggests that in wild type infections, maturation of phage particles within a bacterium is not a synchronous process and that lysozyme is produced much before all the potential progeny mature. It may be reasonable to conclude that multiple infections result in a situation similar to that of lysis inhibition and the increase in burst size observed under these conditions may be due to similar mechanisms. For example, if maturation of phages is controlled by limiting concentrations of one or more phage-coded catalytic factors, multiple infections can lead to increased phage production before the onset of lysis even in wild type conditions. This is because there would be an increased supply of those limiting factors. However; during multiple infections, the threshold concentrations of lysozyme needed to lyse the cell also might be reached somewhat earlier so that each phage produces less progeny than in single infections and yet the total phage production in one bacterium can be higher than in single infections.

Thus it could be a general property of phages that they utilize a small proportion of the bacterial resources in the course of their reproductive cycle. While the reasons for this are not obvious, it suggests that the efficiency of phage-coded regulatory factors involved in phage development and reproduction determine the burst size. That the burst size can still be reduced by decreasing the nutritional content of the bacteria (Gadagkar, 1979) suggests that phages have evolved to utilize only a small proportion of whatever resources are present in the bacterium One possible reason for this is that in nature it may be very important for the phage to lyse the bacterium as quickly as possible. This may be because of a certain finite probability of bacterial death which would increase with time. However, going through more generations rather than utilizing all the resources available in one bacterium may help leave more progeny behind. Thus the burst size may be adapted to adjust to such factors as availability of hosts, probability of death of hosts and the time and energy required to channel the host towards phage production. This would explain why phages could have evolved to utilize only a small proportion of whatever resources are available in a bacterium and also why maturation of the progeny is asynchronous leaving the actual time of lysis as the flexible control point.

The present study indicates that more detailed investigations on the molecular mechanisms involved in resource utilization by bacteriophages are needed. Similar experiments could then be extended to pairs of different mutants of a bacteriophage or even to different bacteriophage species with a common host. This should lead to an understanding of the molecular basis of ecological isolation (Hardin, 1960), that is, those subtle differences in the requirements of different bacteriophages with a common host, that have made it possible for them to coexist.

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**References**

Bailey, N. T. J. (1959) *Statistical methods in biology*, (The English University Press).

Delbruck, M. and Luria, S. E. (1942) *Arch. Biochem*., **1,** 111.

Doerman, A. H. (1948) *J. Bacteriol*, **55,** 257.
Gadagkar, R. R. (1979) *Physiological and biochemical studies on mycobacteria phage 13*. Ph.D. thesis, Indian Institute of Science, Bangalore.
Gopinathan, K. P., Saroja, D., Gadagkar, R. and Ramakrishnan,T. (1978) in *Genetics of actinomycetales*. eds. E. Freekson, I. Tarmok and J. H. Thumin, Proc. Int. Colloq. held at Forschungsinstitut, Borstel, Gustav, Verlag, Fisher, p. 227.
Hardin, G. (1960) *Science*, **131,** 1292.
Hershey, A. E. and Melechen, N. E. (1957) *Virology*, **3,** 207.
Hutchison, C. A., III and Sinsheimer, R. L. (1966) *J. Mol. Biol*, **18,** 429.
Lewin, B. (1977) *Gene expression*, (New York: John Wiley), Vol. 3.
Sunder Raj, C. V., and Ramakrishnan, T. (1970) *Nature (London)*, **228,** 280.