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Letter to the Editor
Evidence for a Phage Proliferation Threshold?

Both experiments (5) and theory (3, 4) have suggested that for a population of phage to increase in numbers requires the host cell population to surpass a critical density termed the "replication threshold" or the "proliferation threshold." However, recently in the *Journal of Virology*, Kasman et al. (1) argued that no such threshold exists. Why this discrepancy? For a population of phage to increase in numbers, not only must phage from the initial dose replicate but also progeny phage must survive long enough to sustain further replication. This in turn depends on the density of remaining uninfected cells and on the rate of loss of free phage. The proliferation threshold is that cell density above which the probability of a progeny phage replicating is greater than the probability of that phage being lost (4). From this we identify three ways to reconcile the apparent inconsistencies between Kasman et al. (1) and Wiggins and Alexander (5).

First, the rate of phage loss in vitro is many times lower than in natural systems such as in sewage or in vivo. Consequently, the proliferation threshold is expected a priori to be much lower in in vitro experiments such as those of Kasman et al. (1) than in any in vivo system, maybe even too small to measure. Wiggins and Alexander (5) assessed different rates of phage loss, but they were not reported by Kasman et al. If relevant parameter estimates were available, then the proliferation threshold could be predicted using a formula derived from kinetic theory (4). Second, where Kasman et al. use an actual multiplicity of infection of 10, the bacterial infection rate is so high that there are effectively no uninfected cells left for progeny phage to infect: inundation by the initial phage renders any subsequent phage replication or density threshold irrelevant. Kinetic theory predicts that the proliferation threshold is manifested only if the initial phage dose is much smaller than the actual multiplicity of infection of 10 (technically, the phage dose must be less than the "inundation threshold" but more than the "failure threshold" [4]). Third, in natural systems of interest the host cell density typically increases with time. Thus, if the initial cell density is low, it takes a certain time before the proliferation threshold is crossed and thereby made observable. Wiggins and Alexander made this transparent using explicit time series, whereas Kasman et al. took measurements at a fixed time point, which would hinder detection of any time-dependent threshold.

Therefore, that Kasman et al. (1) saw no proliferation threshold probably does not mean that no threshold exists but rather is an expected result of their specific scenario. Kasman et al. mention the profound implications for bacteriophage therapy, but therapeutically what really matters is the possible presence of thresholds in vivo, where phage loss is high. Merril et al. (2) highlighted the importance of the rate of phage loss for the efficacy of bacteriophage therapy in a mouse model, and the kinetic theory (3, 4) clarifies why this makes most in vitro measurements of in vivo processes and outcomes so misleading.

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Authors' Reply

Although we appreciate the above comments of Payne and Jansen, we believe that the true source of disagreement results from the use of several different and nonequivalent definitions of the term "phage replication threshold" (RT). In our opinion, the clearest definition of this term in the papers of Payne and Jansen (2, 3), which can be traced back to the earlier paper of Wiggins and Alexander (4), is that the phage replication threshold is the cell density which must be surpassed in order for phage to increase in number. We interpreted this, as the name implies, to mean the cell density beyond which phage replication occurs. The letter makes it clear that this was not the meaning that Payne and Jansen intended. Instead, they make additional requirements restricting the ratio of the probabilities of initial and progeny phage replication or the ratio of the probability of a progeny phage replicating to the probability of its being lost. Although these definitions do not seem equivalent to us, we see how these requirements are related to the questions of a sustainable phage infection as raised in reference 4. However, they are not directly related to the question of phage numbers increasing, since a single round of phage replication will almost always lead to an increase in phage numbers in the short term. More importantly to the conclusions of our paper (1), these other considerations are not necessary for determining an initial phage dose capable of infecting the entire cell population at arbitrary cell concentrations.

Payne and Jansen suggest that our results differ from those of Wiggins and Alexander (4) because phage loss is greater in vivo than in vitro and phage loss was not taken into account. However, only in vitro experiments are reported in reference 4 and our paper (1), both using similar conditions and artificial media. It is also argued that an RT was not detected because the phage dose used was too high. However, all of the definitions of RT are independent of phage dose; therefore, we do not see the rationale for suggesting that an RT would exist for some phage doses and not others. Last, it is suggested that we did not observe an RT because measurements were taken at

only one time point. In our experiments, we used a nonreplicating transducing phage; therefore, a time course was not possible. Instead we simulated a time course by mixing a constant dose of phage with separate aliquots of cells, each containing a different cell density which represented a culture at different time points in its growth curve. Moreover, it should be noted that the conclusions of our paper (1) suggest that the model utilized by Payne and Jansen (2, 3) would not be valid over an extended period of time. In particular, by treating the "transmission coefficient" b as a constant, the important role of cell density, which our paper seeks to address, is not taken into account.

We did realize upon receiving this letter and rereading references 2 and 3 that we should have acknowledged Payne and Jansen for suggesting the possibility of using an inundation dose and passive (nonreplicating) phage therapy before us. We very much regret this omission.

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