Programme: Bachelor of Science (Third Year)

Subject: Microbiology

Course Code: MID 105

Course Title: Virology

Unit II: Bacteriophages

Module Name: One step multiplication curve

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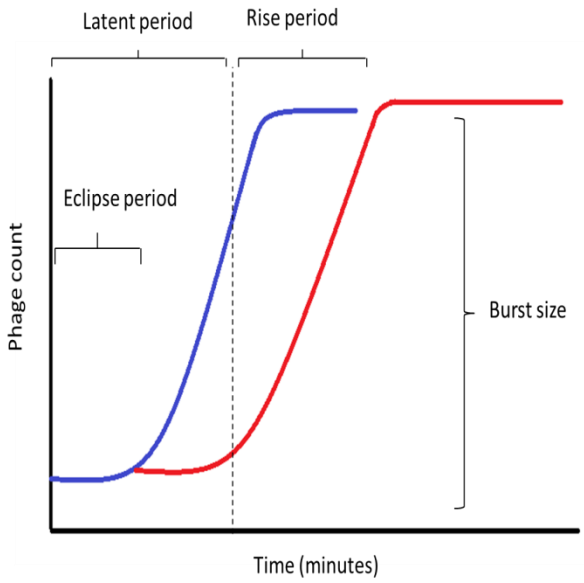
Notes

One step multiplication curve is also known as one-step growth curve. A one-step growth curve refers to the infection of susceptible bacteria by bacteriophages. The idea that one-step growth analysis can be used to study the single-cell life cycle of viruses originated from the work on bacteriophages by Emory Ellis and Delbrück. They added virus particles to a culture of rapidly growing *E. coli* cells. These particles got adsorbed quickly to the cells. The infected culture was then diluted to preventing further adsorption of unbound particles and also effectively synchronizes the infection. Samples of the diluted culture were then taken every few minutes and analyzed for the number of infectious bacteriophages. The results did not resemble the growth curves of bacteria or cultured cells. After a short lag, bacterial cell growth becomes exponential (i.e., each progeny cell capable of dividing) and followed a straight line. Exponential growth continues until the nutrients in the medium are exhausted. Numbers of new viruses do not increase in a linear fashion from the start of the infection. There is an initial lag, followed by a rapid increase in virus production, which then plateaus. This single cycle of virus reproduction produces the "**burst**" of virus progeny. Immediately after dilution, there was a complete loss, or **eclipse**, of infectious virus for 10 to 15 min [input virions disappeared and no new phage particles were produced during this period]. The loss of infectivity is a consequence of the release of the genome from the virion, to allow for subsequent transcription of viral genes. Particle infectivity is lost during this phase because

the released genome is not infectious under the conditions of the plaque assay. Next, new infectious particles were detected inside the cell, before they were released into the medium. These were newly assembled virus particles that had not yet been released by cell lysis. **1. Eclipse period-** the phase in which infectivity is lost when virions are disassembled after penetrating cells. **2. Latent period-** the time it takes to replicate, assemble, and release new virus particles before lysis (20 to 25 min for *E. coli* bacteriophages). **After latent period-** there is a sudden rise in phage forming units (PFU) in the medium and is called as burst size. "Burst" size - the average of many different bursts from individual cells. And calculated from the **total virus yield** [cell-associated virus + released virus]. Cell-associated virus decreases as cells are lysed. This classic experiment shows that phages develop intracellularly. The entire growth cycle here takes around 30 minutes.

- During the initial stage, an inoculum of virus causes infection
- In the eclipse phase, viruses bind and penetrate the cells with no virions detected in the medium.
- The chief difference that next appears in the viral growth curve compared to a bacterial growth curve occurs when virions are released from the lysed host cell at the same time
- Such an occurrence is called a burst, and the number of virions per bacterium released is described as the burst size.
- In a one-step multiplication curve for bacteriophage, host cells lyse, releasing many viral particles to the medium, which leads to a very steep rise in viral titer (number of virions per unit volume).
- If no viable host cells remain, the viral particles begin to degrade during the decline of the culture

In the initial part of the latent periods, the eclipse period, the host cells do not contain any complete, infective virions During the remainder of the latent period, an increasing number of infective virions are present, but none are released. The latent period ends with the host cell lysis and rapid release of virions during the rise period or burst. In the below figure the blue line represents the total number of complete virions.



The red line is the number of free viruses (the unadsorbed virions plus those released from host cells). When E. coli is infected with T2 phage at 37 degree C, the growth plateau is reached in about 30 min and the burst size is appx. 100 or more virions per cell. The eclipse period is 11-12 min and the latent period is around 21-22 min.

- The amount of cell-associated virus is determined by taking the cells pelleted from the medium, disrupting them, and assaying for virus infectivity as before
- The fact that virus appears inside the cells before it appears in the medium demonstrates the intracellular nature of phage replication
- The kinetics of appearance of intracellular phage particles are linear, not exponential
- This is consistent with particles being produced by assembly from component parts, rather than by binary fission