One Step Multiplication Curve
Paper: Virology
Unit:2

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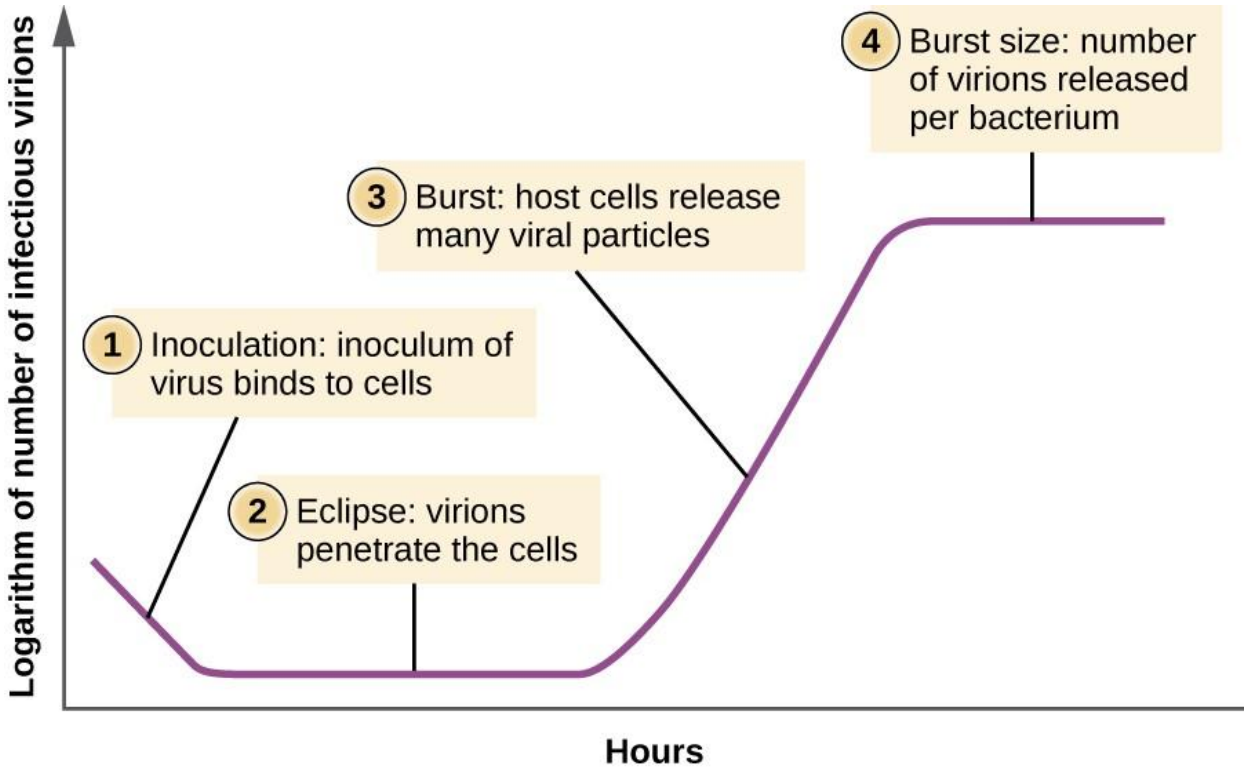
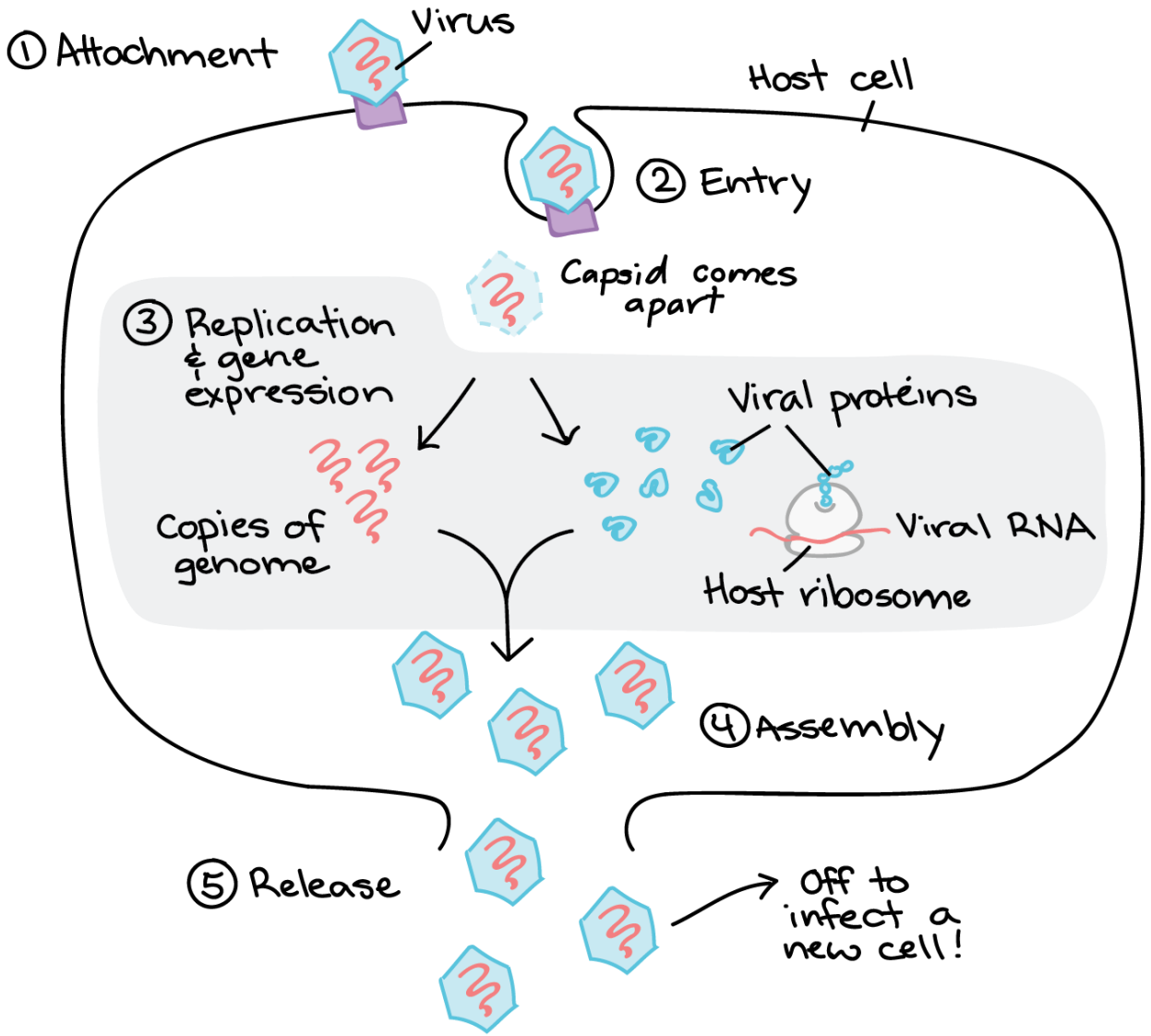


Outline

- General Graph of a virus life cycle
- Important definitions
- Study viral infection
- How may viruses are there?
- Introduction
- Multiplicity of Infection (MOI)
- Phases of one step growth curve

GENERAL DIAGRAM of a VIRUS LIFECYCLE

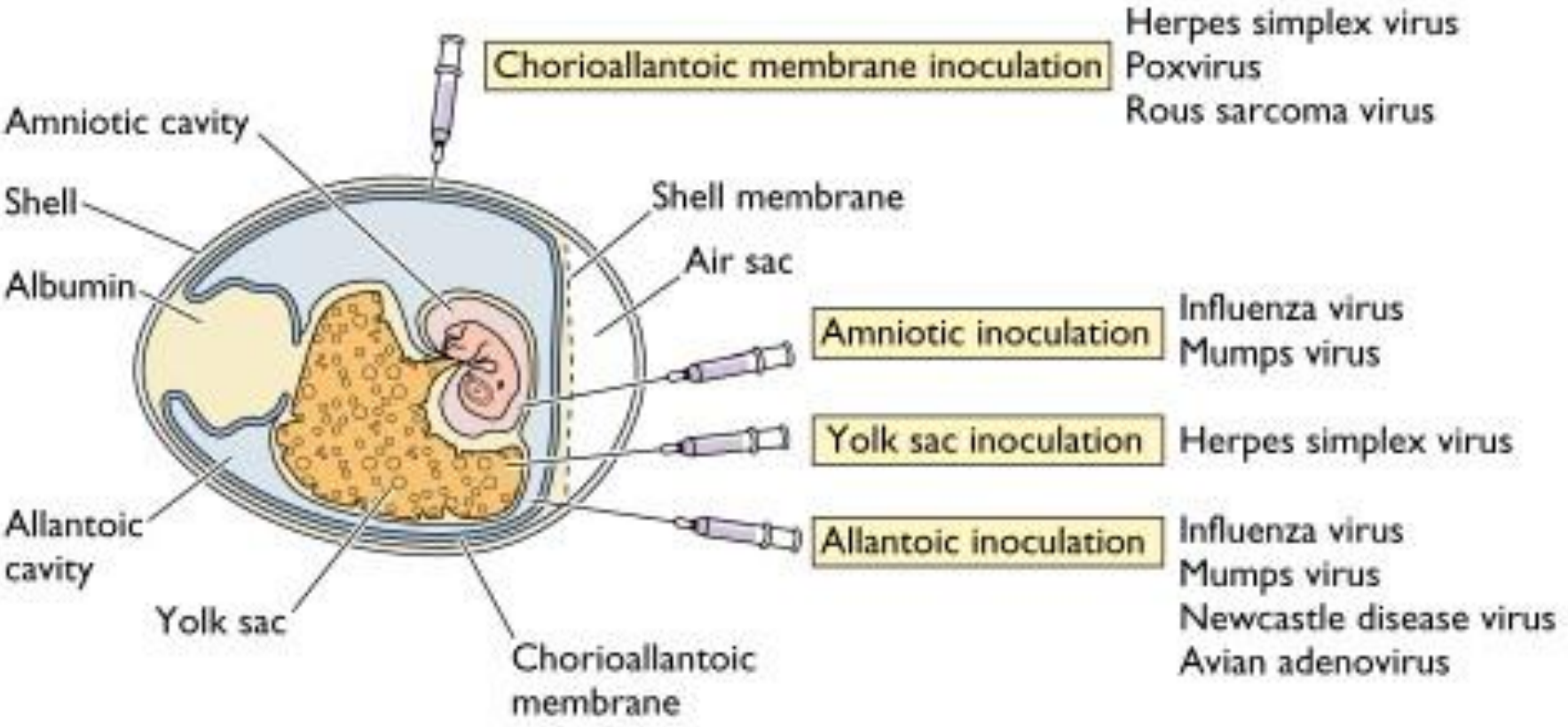


Important definitions

- A **susceptible cell** has a functional receptor for a given virus. The cell may or may not be able to support viral replication.
- A **resistant cell** has no receptor- it may or may not be competent to support viral replication
- A **permissive cell** has the capacity to replicate virus- it may or may not be **susceptible**
- A **susceptible and permissive cell** can take up a virus particle and replicate it

Study viral infection

- Virologist could not study animal and human viruses well in the lab before tissue culture methods were developed by **John F. Enders**, **Thomas H. Weller** and **Frederic C. Robbins** in the late 1940s.
- Before their work, viruses were injected into animals and tissues were analyzed for pathological signs of viral infection.
  - Experimental animals difficult to work with and expensive to maintain.
  - Animals were not very permissive to infection with human viruses due to the species barrier and the animal immune response



12 day post fertilization chicken egg

Studying the infectious cycle in cell

- Not possible before 1949
- Enders, Weller and Robbins won the nobal prize in Physiology or Medicine for the **cultivation of poliovirus in nonnervous tissue** culture (Human embryonic and muscle cells) in 1954.
- Played a monumental role in the development of vaccines against poliovirus in the 1950s (Salk vaccine) and 1960s (Sabin vaccine)

A _____ and _____ cell is the only cell that can take up a virus particle and replicate it (fill in the blanks)

1. Naive and resistant
2. Primary and permissive
3. Susceptible and permissive
4. Susceptible and naive
5. Continuous and immortal

How may viruses are there?

- Infectivity
- Physical- virus particle and their component
    - Plaque assay (1930)

Introduction

- One-step growth curve, developed by **Max Delbriick** and **Emory Ellis (1939)** using Escherichia coli-T_4 bacteriophage system**,** marks the starting of modern bacteriophage research.
- An experiment by which molecular events during reproduction can be observed in a single replication cycle of a virus.
- It reveals the fundamental nature of virus replication.
- In this only single or one cycle of virus growth is observed hence it is called as One-step multiplication curve

Multiplicity of Infection (MOI)

- Monolayers (or cell suspension in liquid medium) of tissue culture cells such as monkey kidney cells are allowed to adhere and form monolayers on the bottom of plastic dishes.
- The monolayers of cells subsequently infected with the viruses of choice.
- They are infected at a high multiplicity of infection (MOI) to ensure that every cell of monolayer is infected simultaneously.
- The MOI is the average number of viruses/ cell. Hence classic one-step growth experiments usually use an MOI of 10 (10 viruses/ cell).

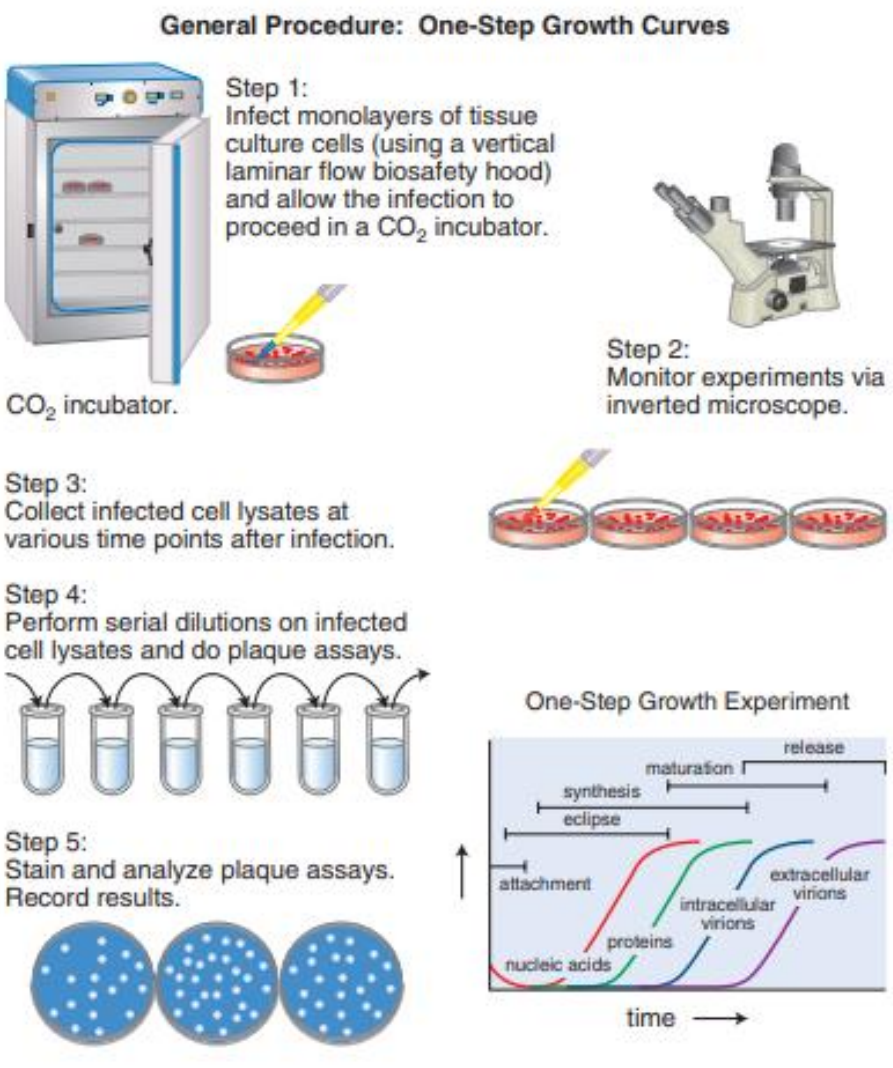
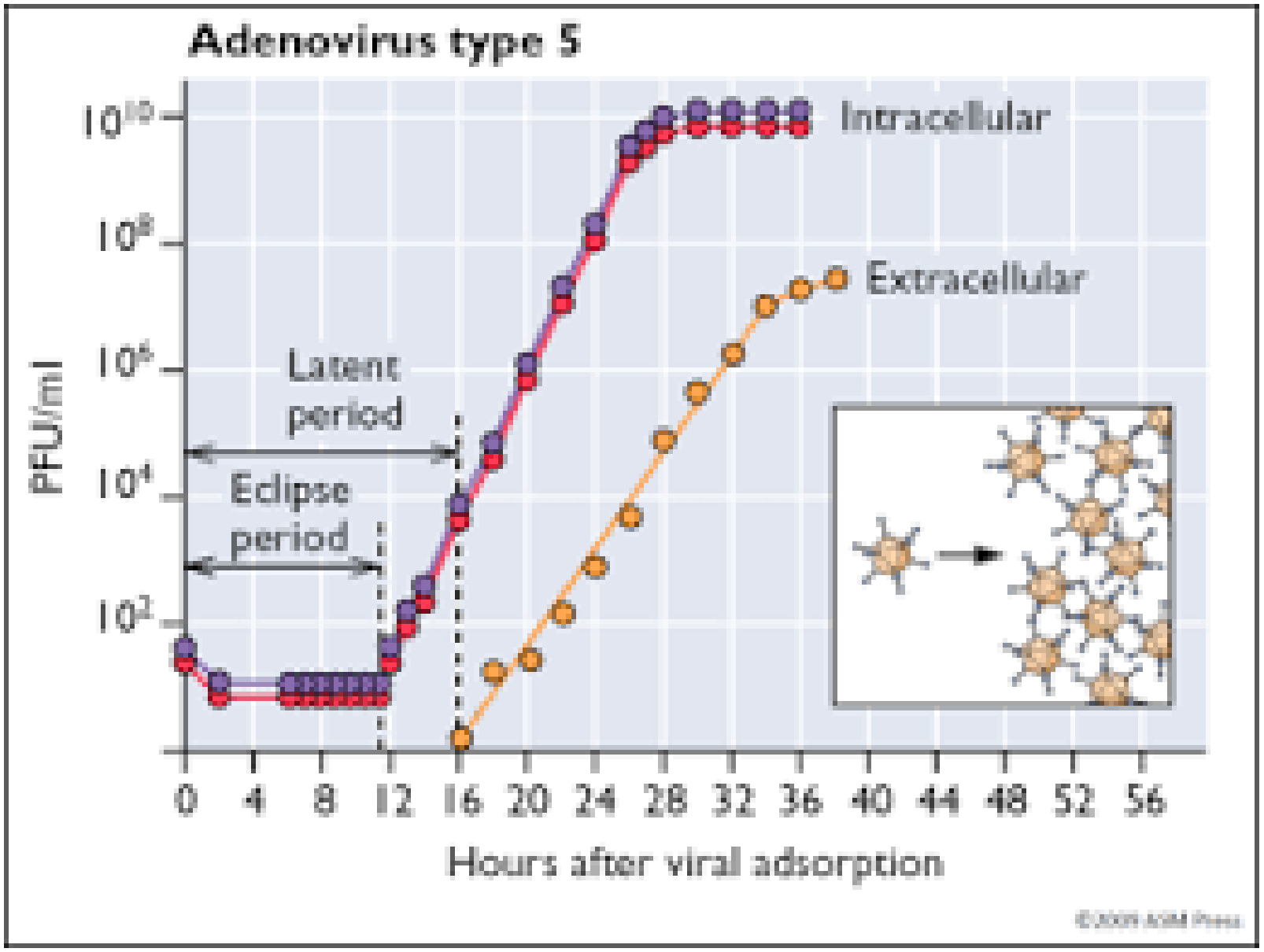


FIGURE 4-1 The diagram briefly outlines the steps involved in performing one-step growth experiments. Step 5 includes a photograph of viral **plaques** (clearings where the virus destroyed the cell monolayer). The plaque assay is a quantitative assay used to determine the number of viruses present in a given sample. The results of these assays can be used to generate a one-step growth curve for a particular virus. For more details about virological methods see Chapter 5, Laboratory Diagnosis of Viral Diseases and Working with Viruses in the Research Laboratory.


Adenovirus type 5
PFU/ml
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
Intracellular
Extracellular
Latent period
Eclipse period
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56
Hours after viral adsorption

Phases of one step growth curve

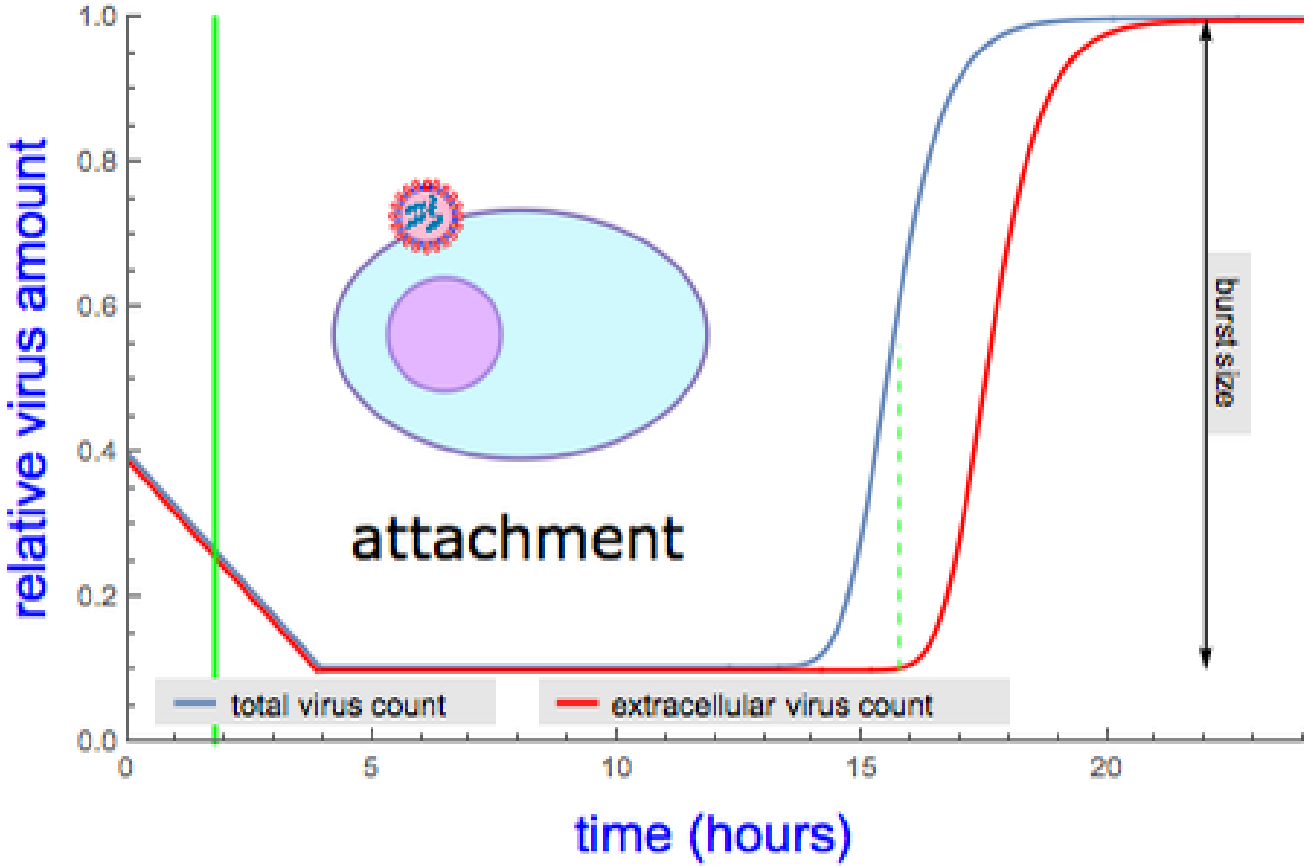
- log no. of plaque forming unit/ml plotted against time, a curve is obtained, termed as one step growth curve
- It has three distinct phases
  1. Latent period
  2. Burst or Rise period
  3. Plateau period

1. Latent Period

- The latent period is described as the time **period prior to the release of infection particles or appearance of extra cellular phages**.
- In the latent period, attachment, entry, replication, transcription, translation and assembly of progeny phages occur.
- During this period there is no release of new phage particle therefore plaque count remains constant
- Latent period can be divided into two phases

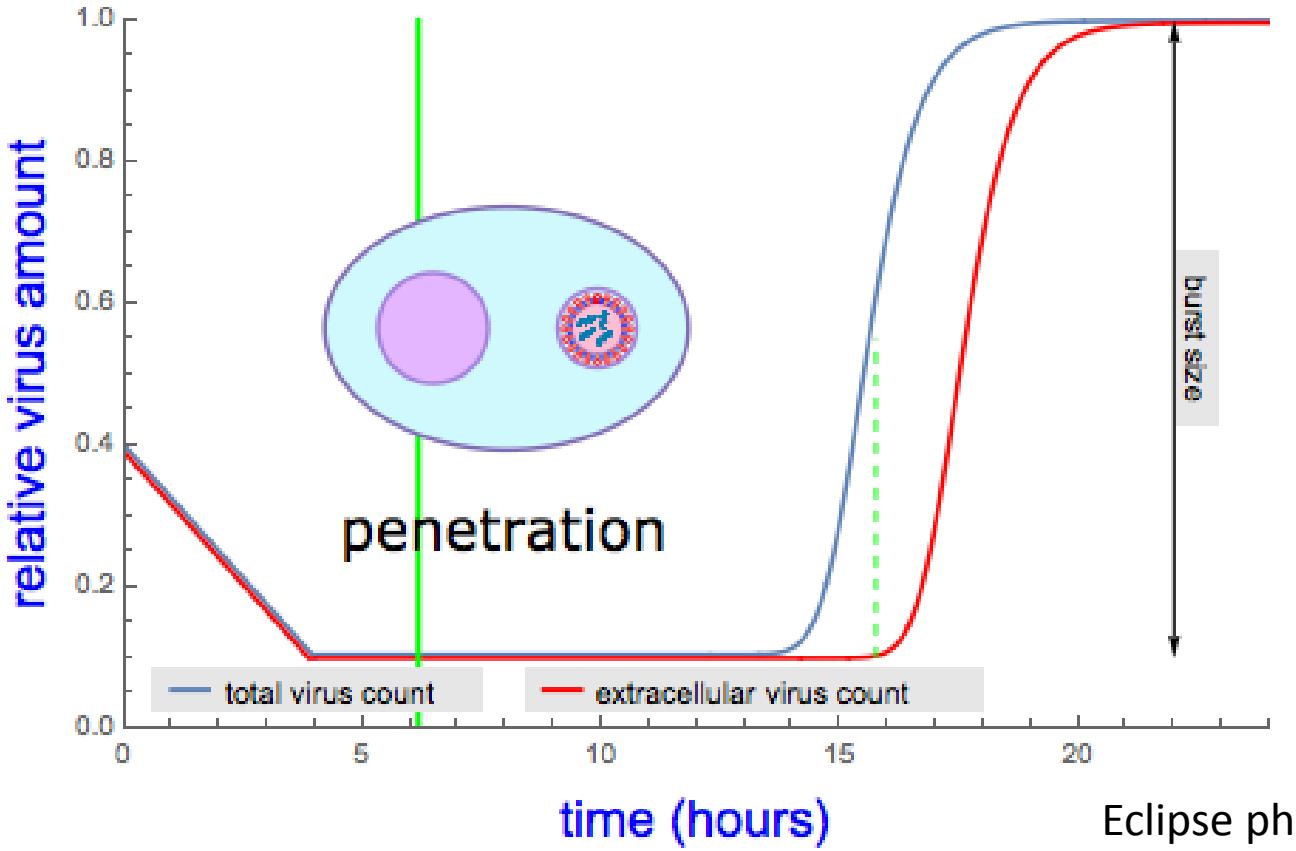
a. **Eclipse:** This period immediately follows the penetration of viral particles into the host cell. Eclipse is characterized by the incapacity to detect free virions since viruses are actively transcribing and replicating inside the host. The eclipse usually lasts from minutes (bacteriophages) to hours or days (animal or plant viruses).

b. **Intracellular accumulation**: During the eclipse period all structural proteins and viral genomes have been produced and massively accumulated in the cytoplasm of the host cell. Both components self assembly to form new viral particles that accumulate intra cytoplasmatically



phase: inoculation

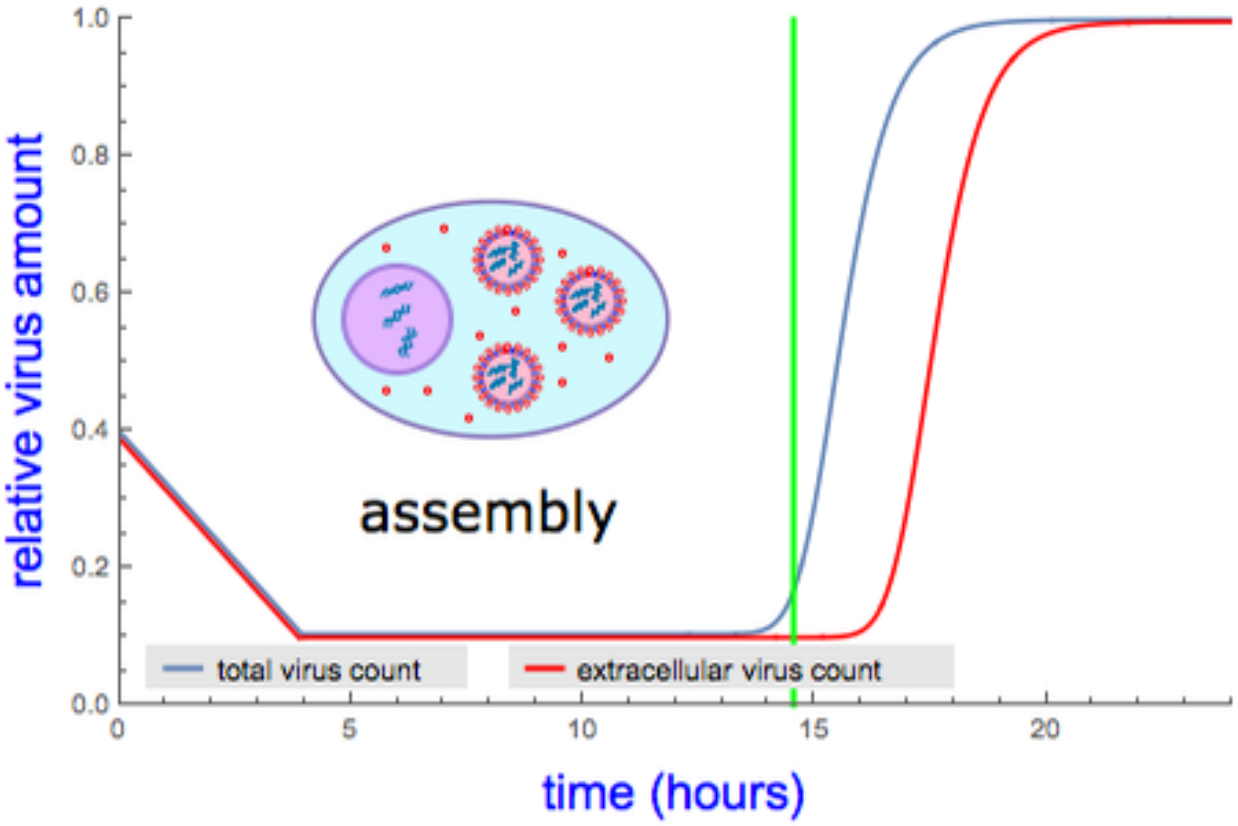
Inoculation phase: A host (cells, bacteria, etc.) is inoculated with a virus. During this phase, the virus undergoes the first step of the *viral life cycle*: **attachment** (to host cells). Sometimes, the amount of virus decreases because the virions attached to host cells are not yet considered viruses. After some time, the culture medium is diluted or antibody is added to prevent new virion attachment to host cells, thereby freezing the number of virions for the rest of the experiment.



Eclipse phase for T_4 is 11.5 min

phase: eclipse

Eclipse phase: Viruses are now being manufactured within host cells. During this phase, viral contents enter the cell during the **penetration** step of the viral life cycle. After genetic material is **uncoated** (the capsids removed), genetic material is copied and viral components are manufactured during the **biosynthesis** step. Typically, a combination of viral enzymes packaged in the original virus and machinery and materials already present in the cell is used during biosynthesis.



phase: maturation

Maturation phase: After synthesis of capsids, enzymes and other materials, new virus particles (virions) are formed during the **assembly** step. Total virus count increases before extracellular virus count increases: there is a lag while virions are being created, but not enough have been created for **release** to occur. Non−enveloped viruses accumulate in cells until cell lysis. Enveloped viruses assemble near cell membranes and "bud" off cell membranes via exocytosis.

2. Rise Period

- In this period lysis occurs and librated a crop of new virus particle hence extracellular phages appear
- During this phase, phage particle increase in their concentration rapidly.

T_4 rise period = 10 min

3. Plateau Period

This period represents the end of all infected host cell lysis.

The newly liberated phage particles fail to meet uninfected host cells due to high dilution.

Therefore during this phase, the plaque count remains constant.

Burst Size: Average yield of infectious virus per cell is called burst size.

Burst Size = pfu/ml at plateau/ pfu/ml at latent period

There is much variation in bursts size between different kind of cells. (range between 20-3000 pfu/ml)

Burst size for T_4 = 100

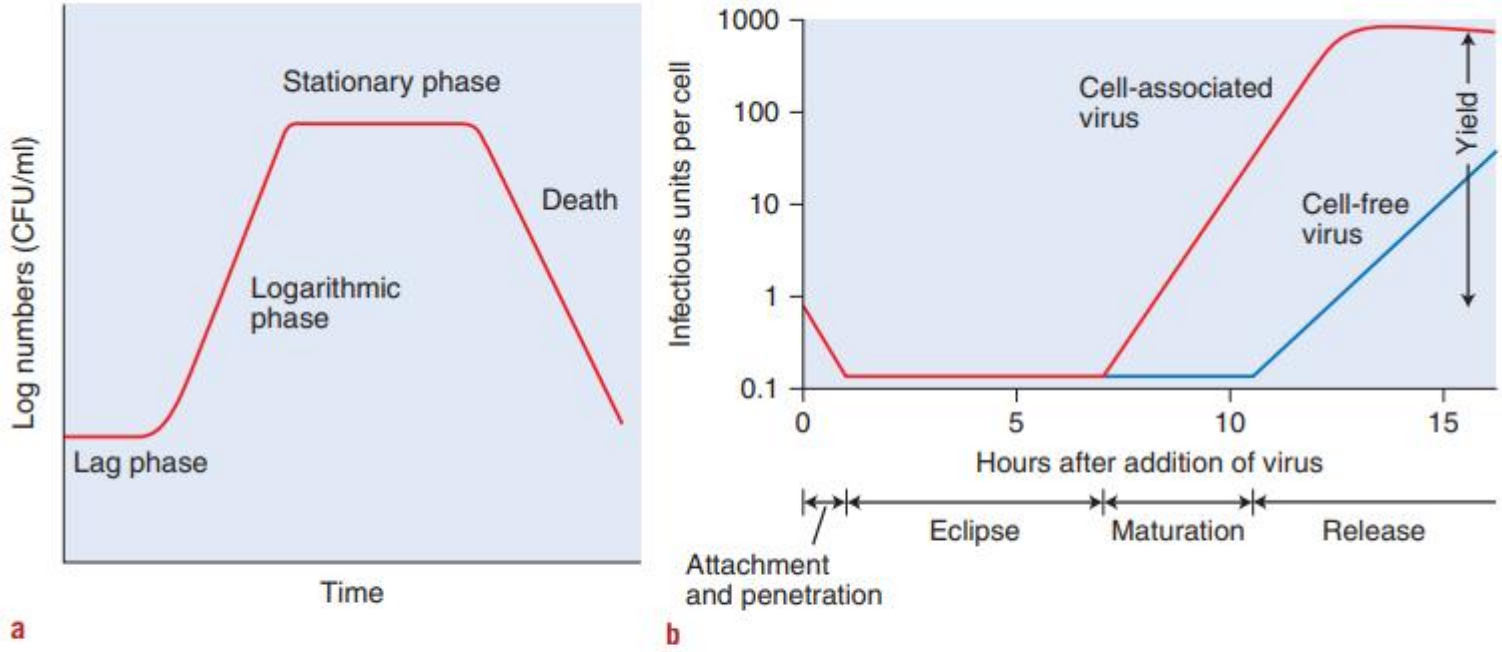


FIGURE 4-2 Typical bacterial growth versus a one-step growth curve of a naked virus. **(a)** Bacterial growth generally proceeds in a series of phases: lag, log (exponential growth in which the rate of multiplication is most rapid and constant), stationary, and death. Viruses require host cells for growth and reproduction. CFU/ml = colony forming units per milliliter. Modified from an illustration by H. Douglas Goff, Ph.D., University of Guelph. **(b)** Viruses are assembled from preformed "parts" when enough of the preformed parts have been made. Adapted from White, D. E., and Fenner, F. J. *Medical Virology,* Fourth Edition. Academic Press, 1994.