PSMU
Microbiology, Virology, and Immunology department

General virology.
Morphology and ultrastructure of viruses.
Cultivation of viruses.

For two-way communication

between the lecturer and students during the lecture,
please contact the following e-mail address:

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Viruses

- **Dmitry Ivanovsky -** microbiologist who, from his study of mosaic disease in tobacco, first detailed many of the characteristics of the organisms that came to be known as viruses. Although he is generally credited as the discoverer of viruses.

GENERAL CHARACTERISTICS OF VIRUSES

Definition:

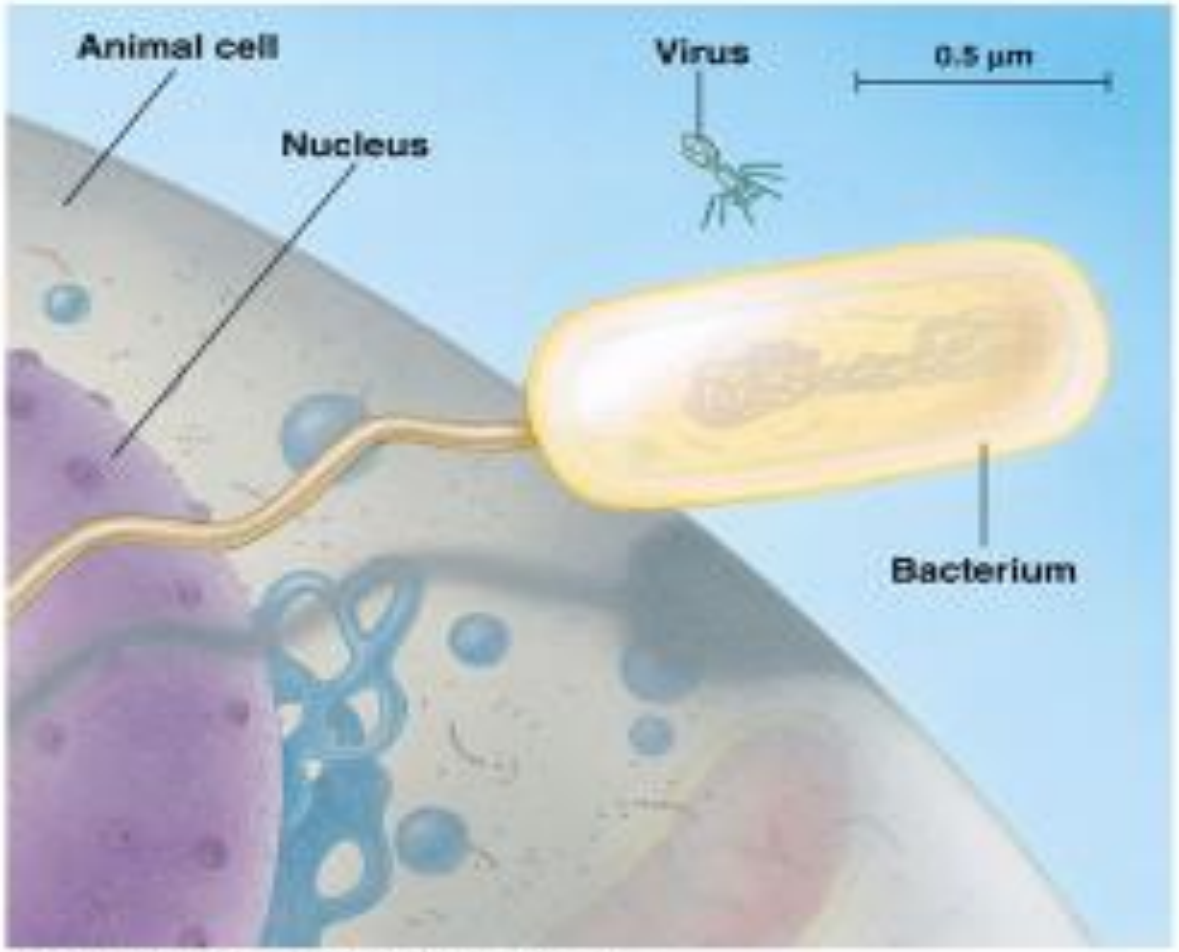
Viruses are the smallest known infectious agents, they can infect man, animals, plants, insects & even bacteria (Bacteriophage) .

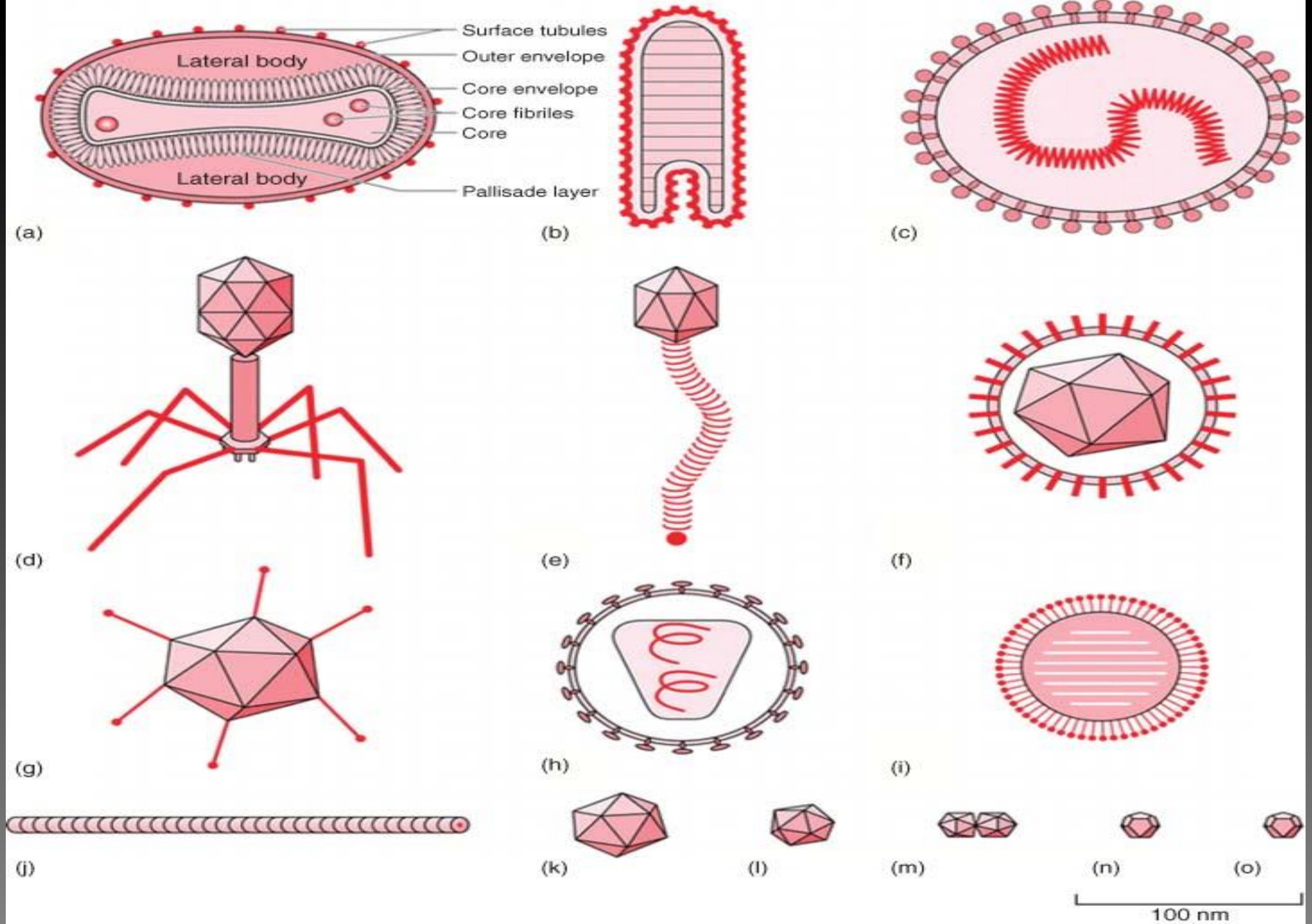
NB: The word (Virus) is a latin word meaning (Poison)

Viruses Are Obligate Intracellular Parasites

- Viruses do not have cellular organization
- They lack their own enzymes to perform metabolism and reproduction.
- They utilize the host's enzymatic machinery to accomplish these tasks.
- Viruses have a *host range or are host specific.*
  - Rabies infects more than one host
  - Eukaryotic viruses are usually tissue specific.
    - *Rhinoviruses, Adenoviruses, Herpes, HIV*

Compare the size of a Eukaoryotic cell, Bacterial Cell and a Virus



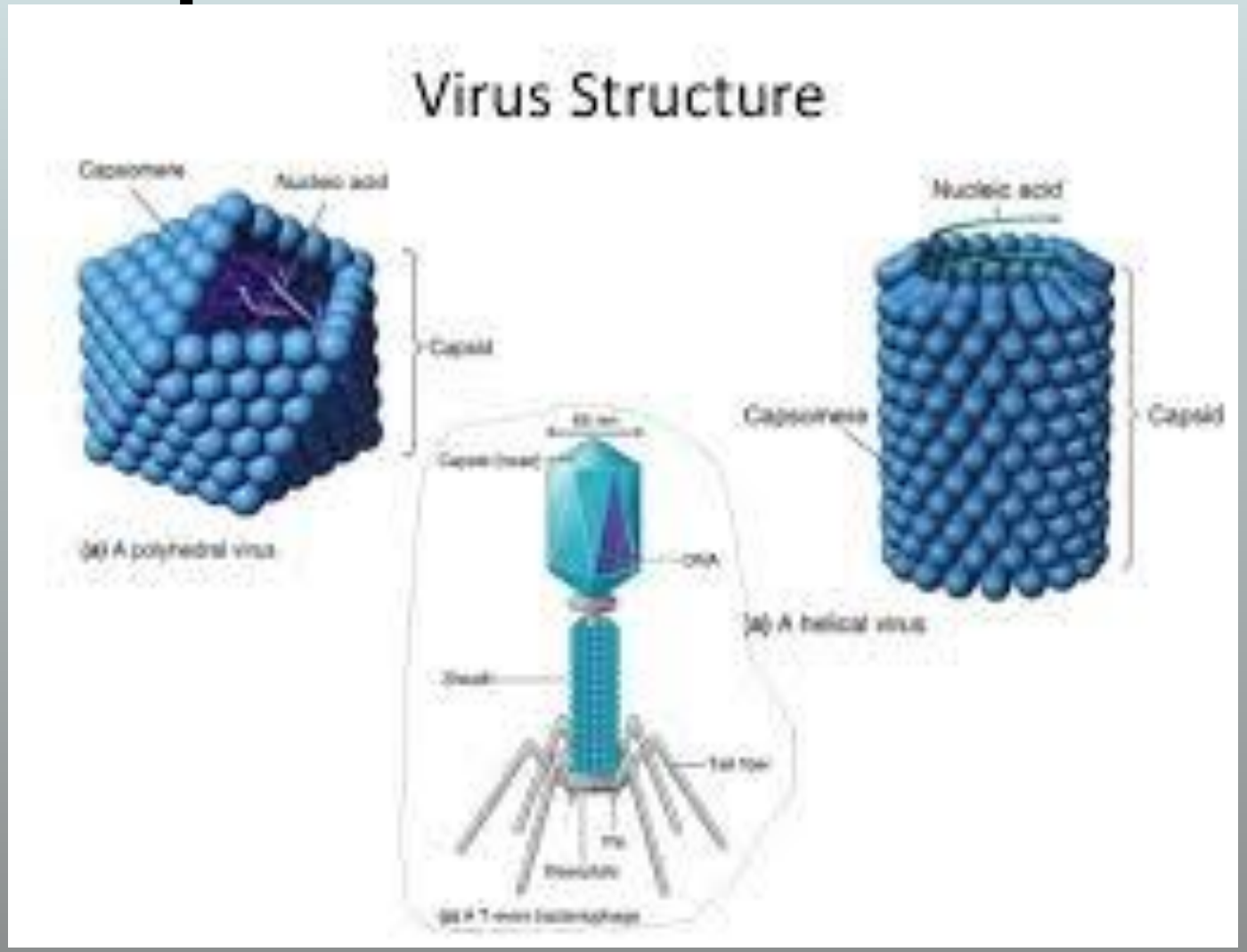

Surface tubules
Outer envelope
Core envelope
Core fibriles
Core
Pallisade layer
Lateral body
Lateral body
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
100 nm

Capsid

Three kinds of symmetry

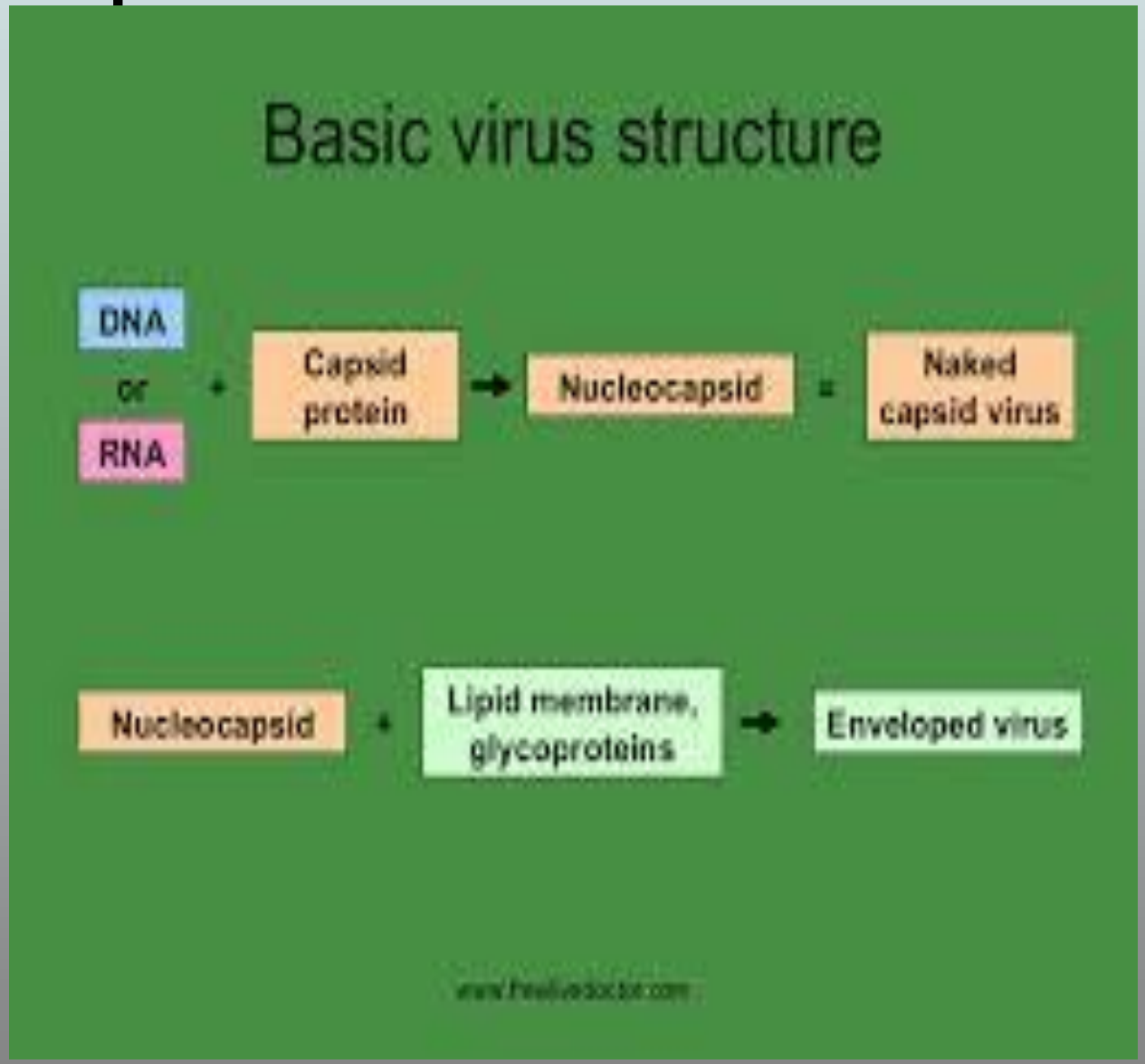
Icosahedral Cubical)

- - Helical
- - Complex

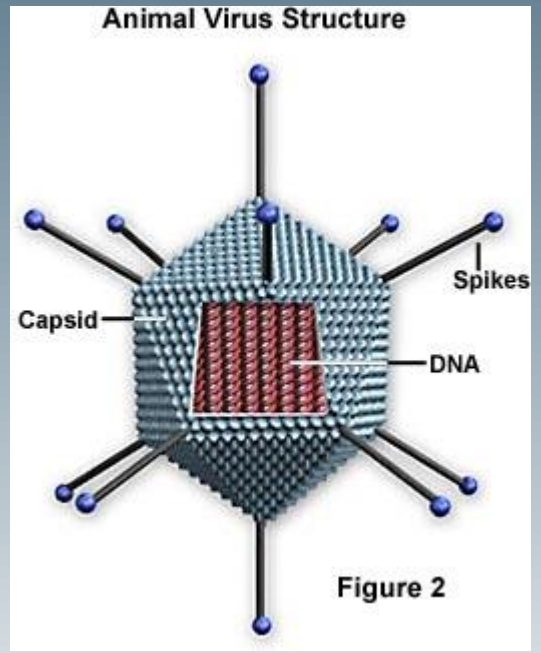


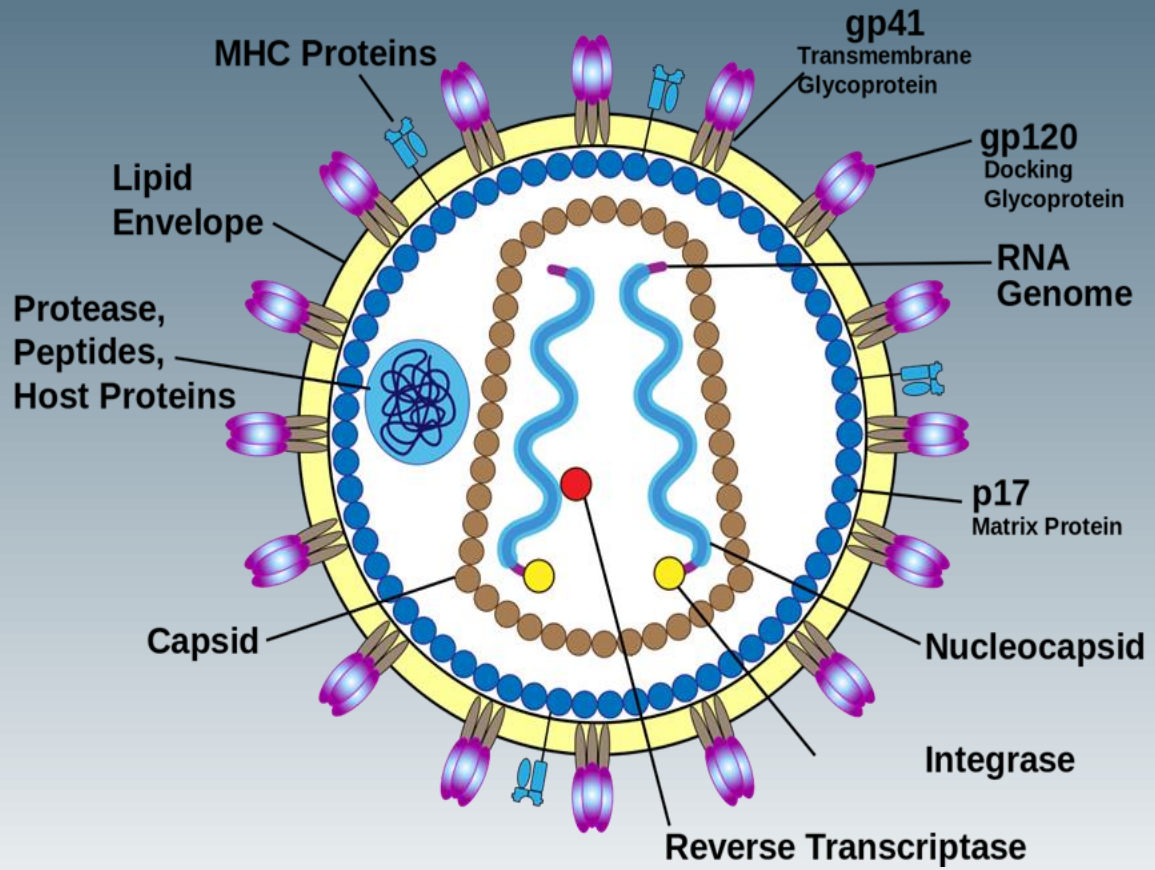
Viral Composition

- Capsid – protein coat (capsomers – monomers)
- Sometimes an envelope – glycoproteins
- Nucleic acid – DNA or RNA. Never both. Can be single or double stranded.
- Some have tail fibers – Bacteriophage T4



Non-Enveloped (naked) viruses Enveloped



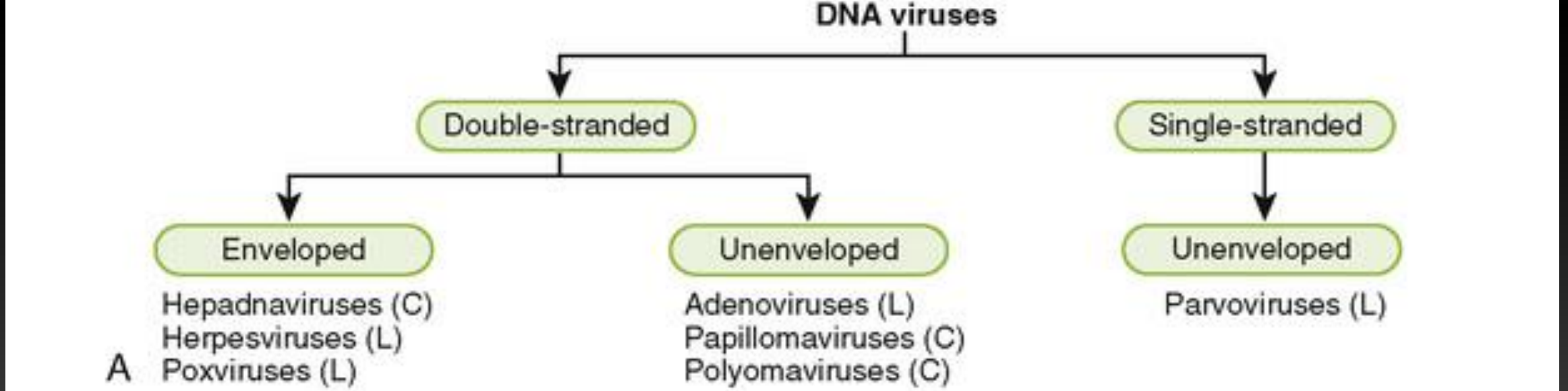


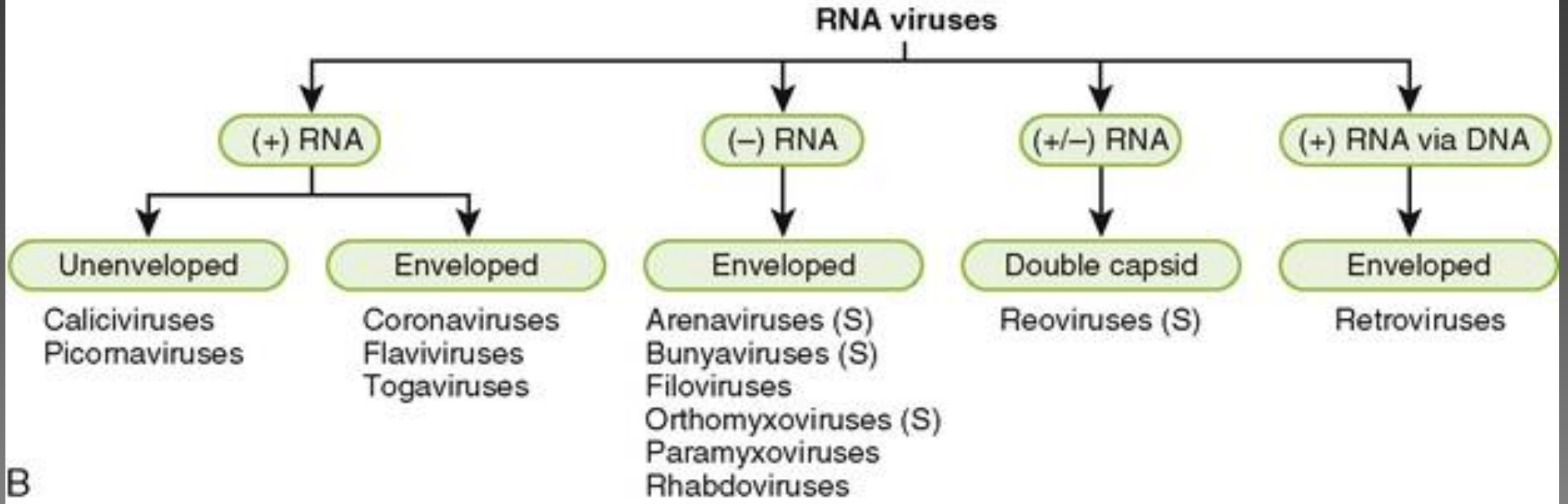
Virions. Envelope

- It is derived from the host cell membrane (lipoproteins)

- Protein subunits (peplomers) are virus coded:
- *Hemagglutinin*
- *Neuraminidase*

•Eclipse phase


DNA viruses
Double-stranded
Single-stranded
Enveloped
Unenveloped
Unenveloped
Hepadnaviruses (C)
Herpesviruses (L)
Poxviruses (L)
Adenoviruses (L)
Papillomaviruses (C)
Polyomaviruses (C)
Parvoviruses (L)
A


RNA viruses
(+) RNA
(–) RNA
(+/–) RNA
(+) RNA via DNA
Unenveloped
Enveloped
Enveloped
Double capsid
Enveloped
Caliciviruses
Picornaviruses
Coronaviruses
Flaviviruses
Togaviruses
Arenaviruses (S)
Bunyaviruses (S)
Filoviruses
Orthomyxoviruses (S)
Paramyxoviruses
Rhabdoviruses
Reoviruses (S)
Retroviruses
B

Virus nomenclature

The International Committee on Taxonomy of Viruses

- rules on classification and nomenclature
- does not accept Linnaean style binomial nomenclature
  (genus name followed by species name)
- recognizes taxonomic levels of Order, Family, Subfamily, Genus and Species with standardized Latinized endings
- includes host, symptom, and/or location in species names
- italicizes only a species name ending with “virus”

Classification of Viruses

1. **Taxonomic groups – family, subfamily, genus and species**
2. **The names of virus families (family) are italicized - End in Latin suffix –*viridae***
3. **The genera (genus) end in the suffix – *virus***
4. **The species – English common name**

Table 10.1 Taxonomic groups of viruses

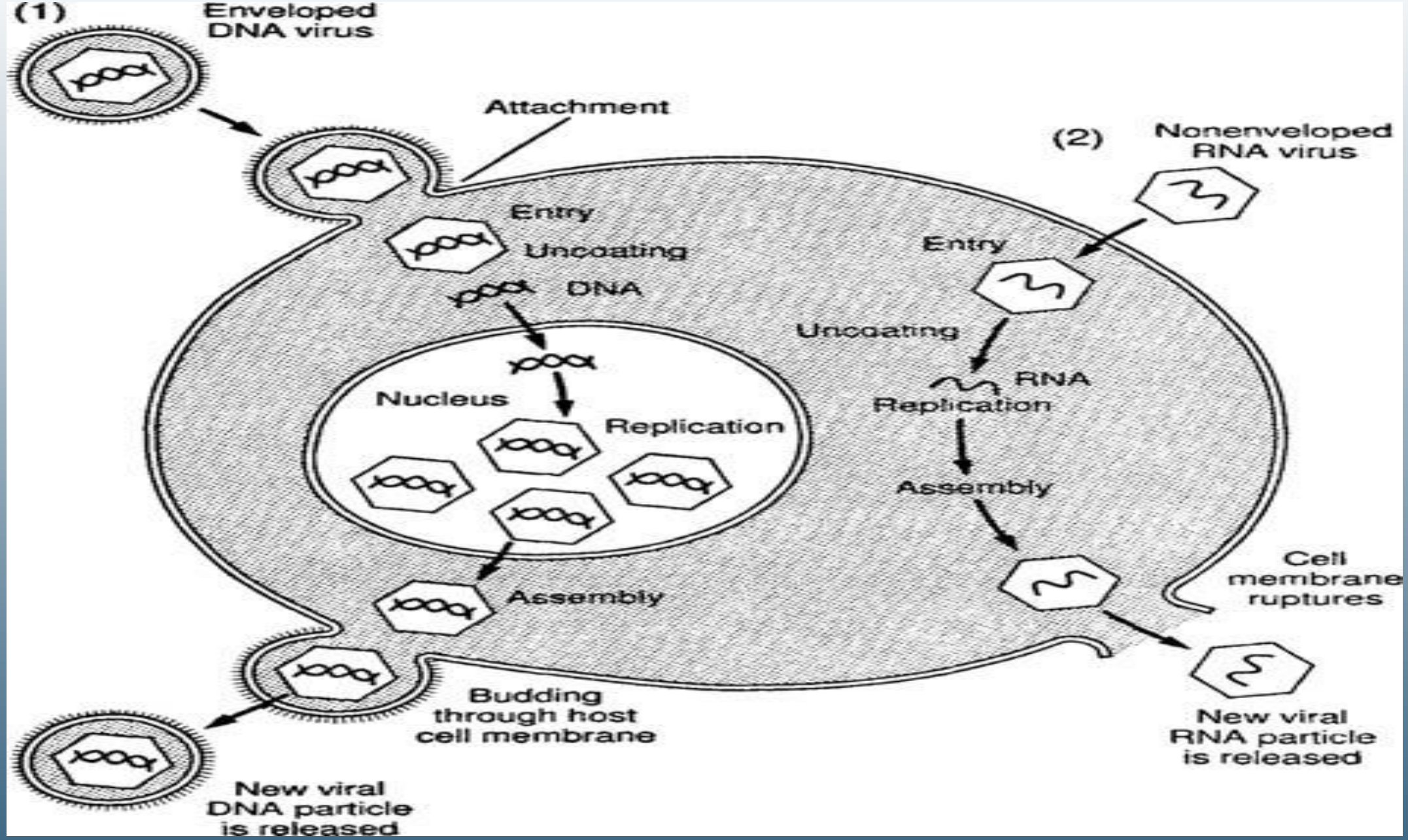
Taxonomic group	Suffix	Example 1	Example 2	Example 3
Family	*-viridae*	*Myoviridae*	*Paramyxoviridae*	*Coronaviridae*
Subfamily	*-virinae*	–	*Paramyxovirinae*	–
Genus	*-virus*	*T4-like viruses*	*Morbillivirus*	*Coronavirus*
Species	–	*Enterobacteria phage T4*	*Measles virus*	*Severe acute respiratory syndrome virus*

REPLICATION

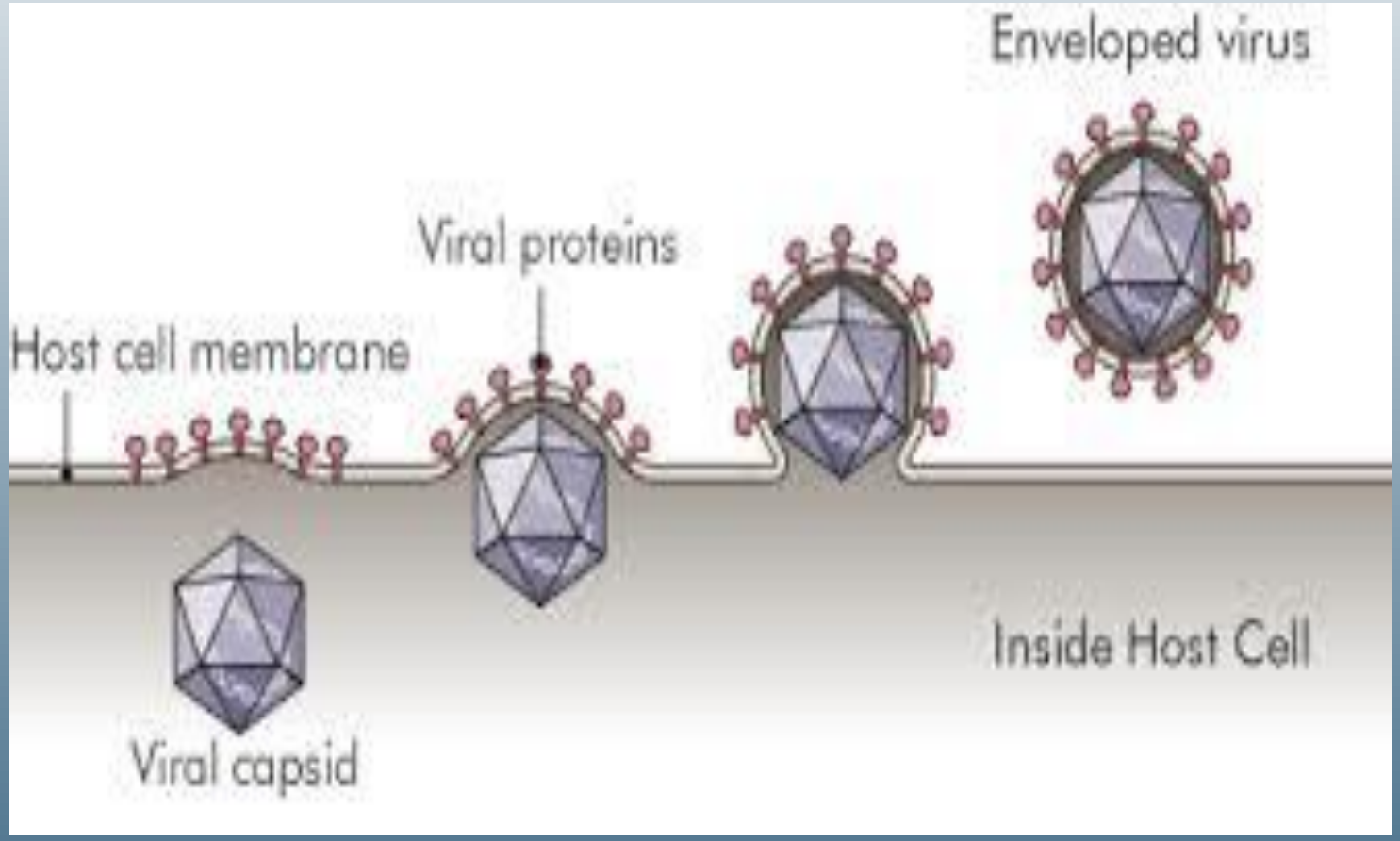
- All viruses depend on cells for reproduction and metabolic processes.
- By themselves, viruses do not encode for all of the enzymes necessary for viral replication.
- But within a host cell, a virus can commandeer cellular machinery to produce more viral particles.

Viral multiplication

- 1. Adsorption
- 2. Penetration
- 3. Uncoating
- 4. Biosynthesis
- 5. Replication
- 6. Maturation
- 7. Release

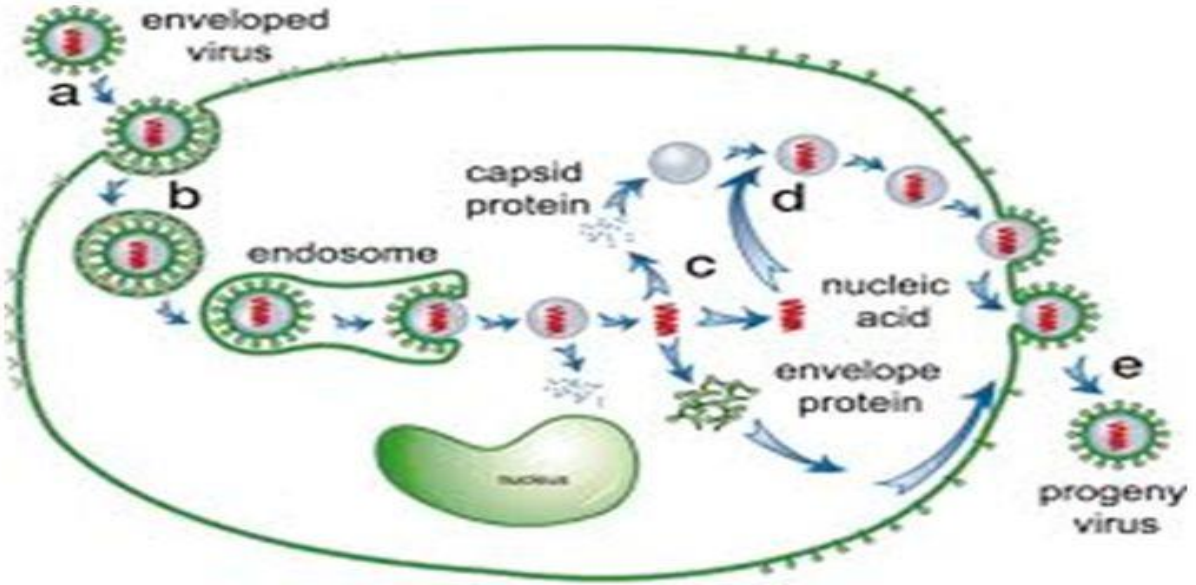

(1)
Enveloped DNA virus
Attachment
Entry
Uncoating
DNA
Nucleus
Replication
Assembly
Budding through host cell membrane
New viral DNA particle is released
(2)
Nonenveloped RNA virus
Entry
Uncoating
RNA
Replication
Assembly
Cell membrane ruptures
New viral RNA particle is released

Budding

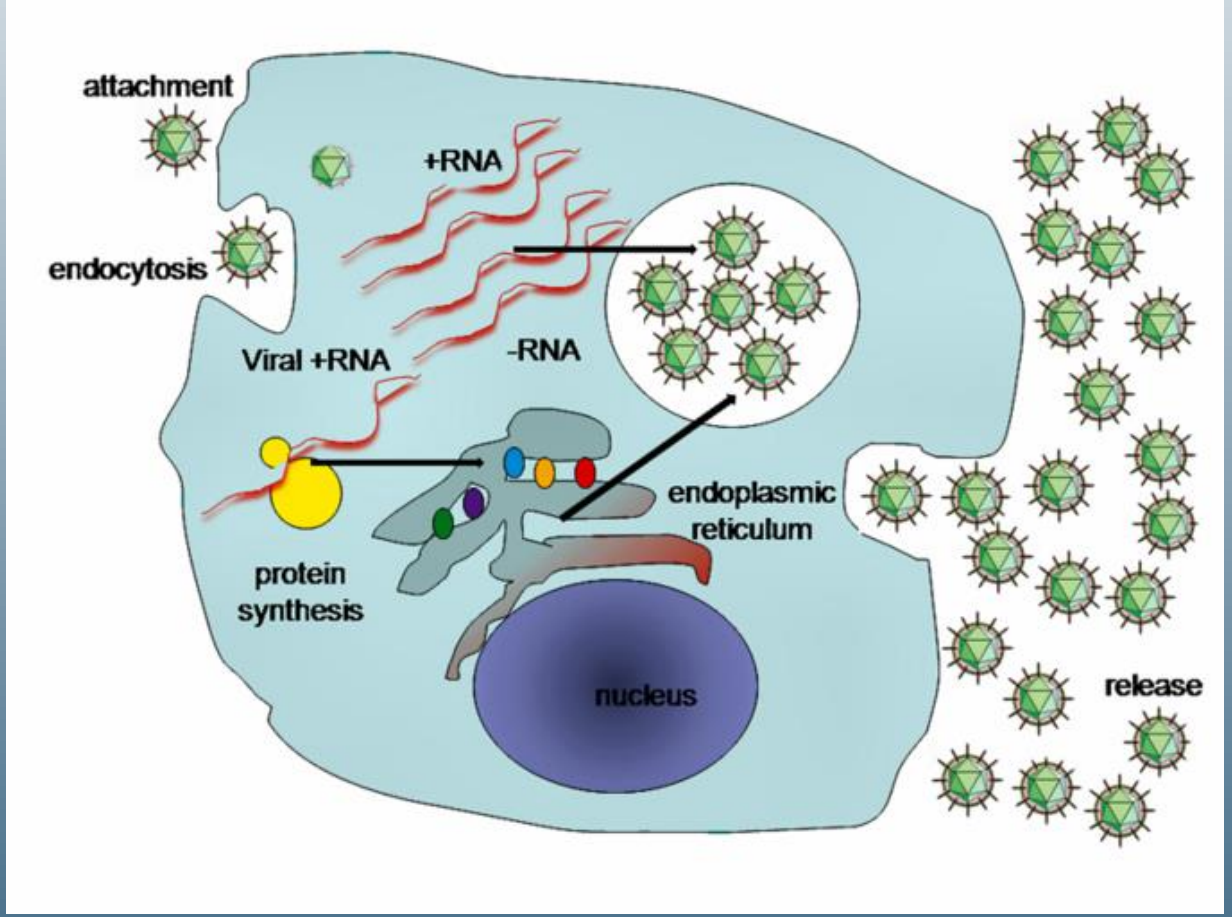

Enveloped virus
Viral proteins
Host cell membrane
Inside Host Cell
Viral capsid

Life Cycle of an RNA Virus

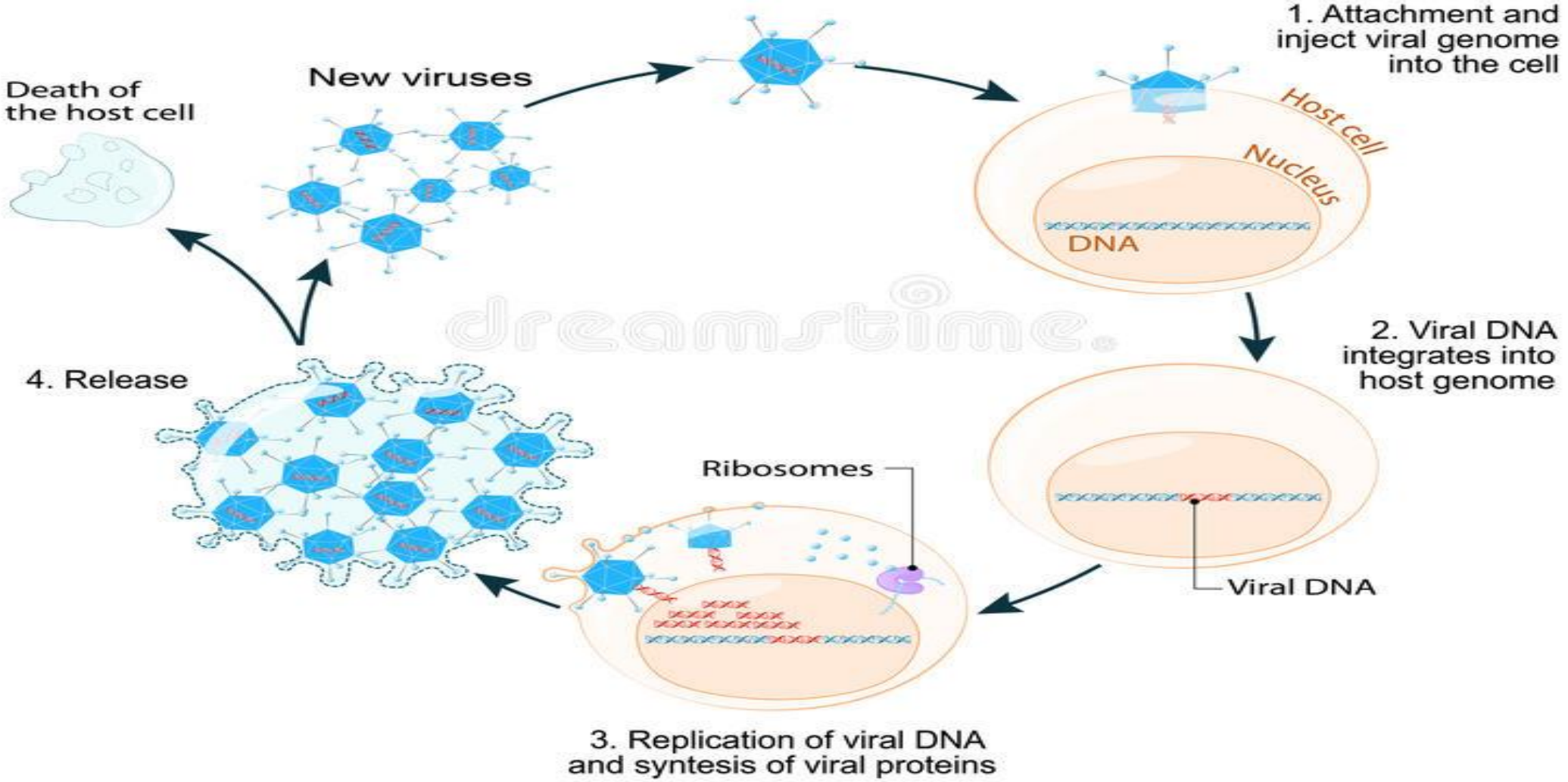
1) Attachment (a): Virus spikes bind to the surface receptor of its host cell (Also known as hijacking)
2) Entry (b): Envelope of the virus binds to the cell membrane of the host. Once fused the virus releases its contents into the cell.

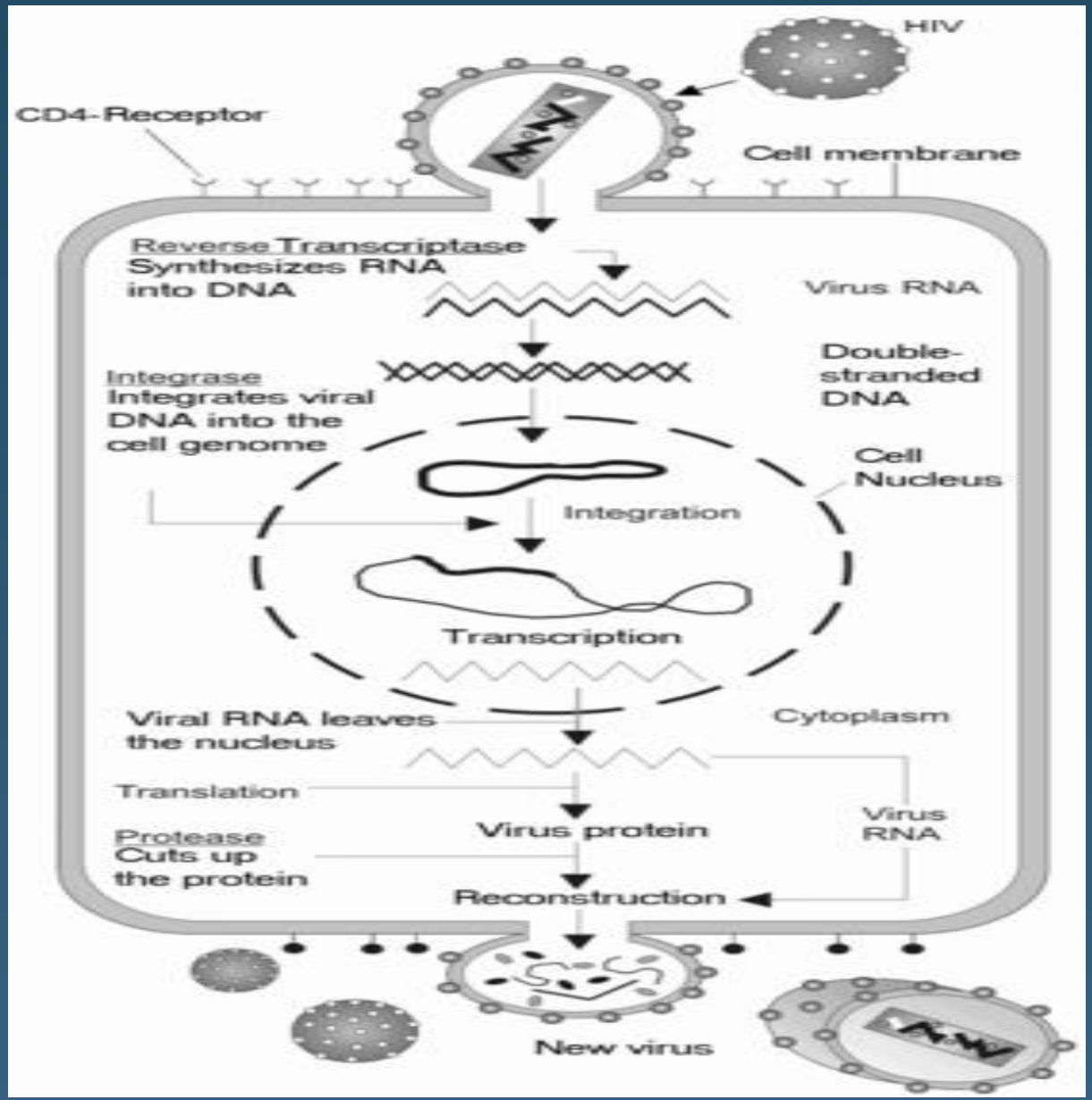


RNA+ and RNA- viruses

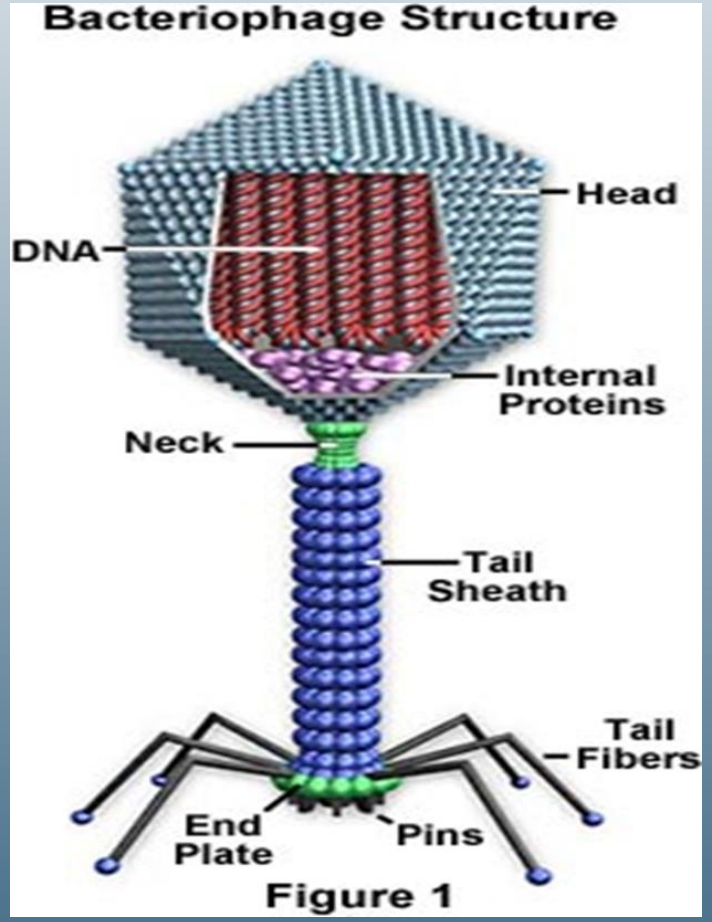


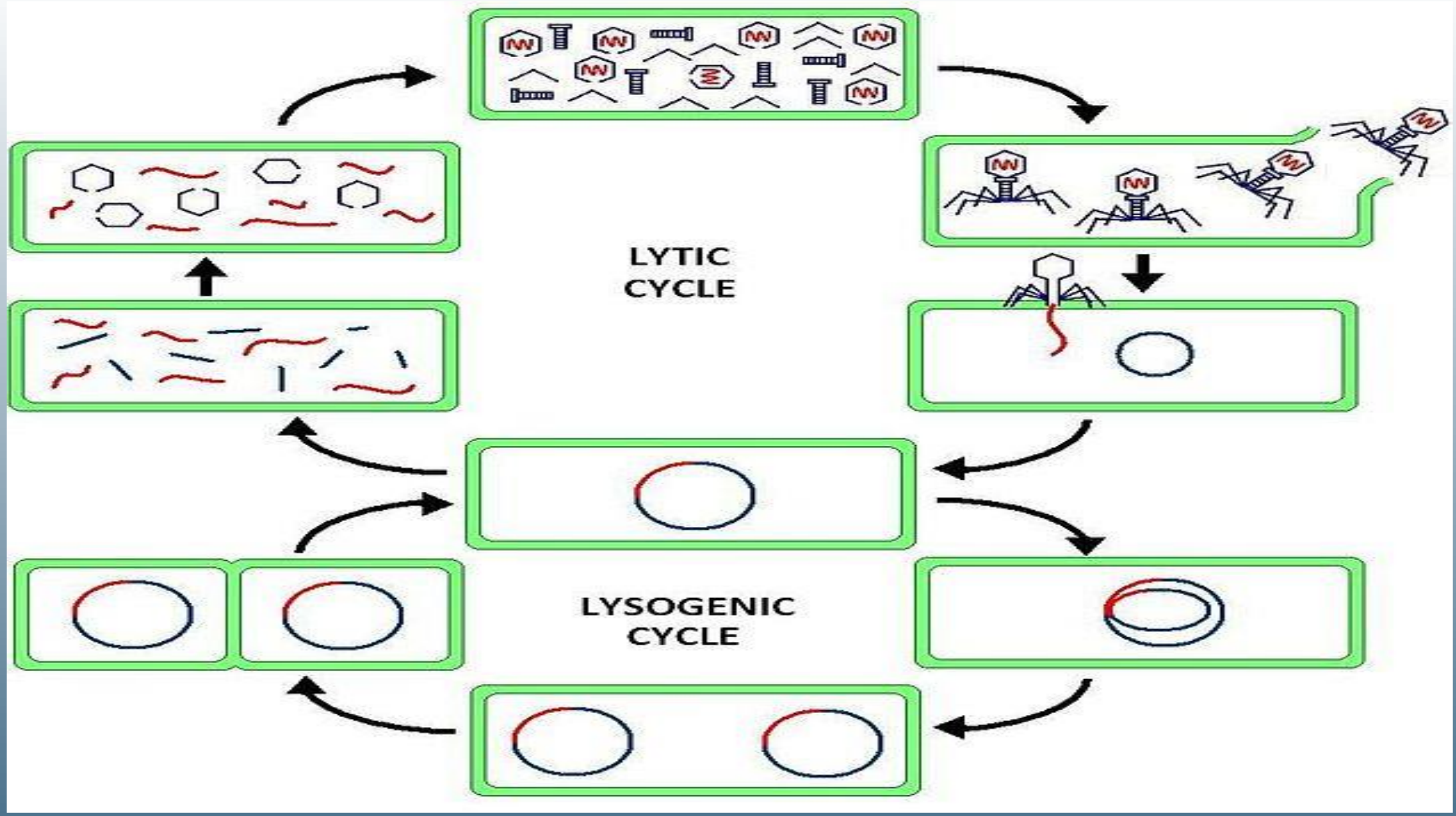
LIFE CYCLE OF VIRUSES




HIV
CD4-Receptor
Cell membrane
Reverse Transcriptase
Synthesizes RNA
into DNA
Virus RNA
Double-
stranded
DNA
Integrase
Integrates viral
DNA into the
cell genome
Cell
Nucleus
Integration
Transcription
Viral RNA leaves
the nucleus
Cytoplasm
Translation
Virus protein
Virus
RNA
Protease
Cuts up
the protein
Reconstruction
New virus

Bacteriophages




LYTIC
CYCLE
LYSOGENIC
CYCLE

Lysogenic Cycle Can Be Used For Cloning

- Viruses can infect without destroying the host cell.
- They integrate their DNA into the host cell and turn off their own genes.
- These types of viruses are called *temperate viruses.*
- Bacterial cells that possess these viral genes are called the lisogenic bacteria.
- Integrated viral DNA are called the *prophages.*
- Viral DNA can be replicated along with the host cell's DNA.

LAB. DIAGNOSIS OF VIRUSES

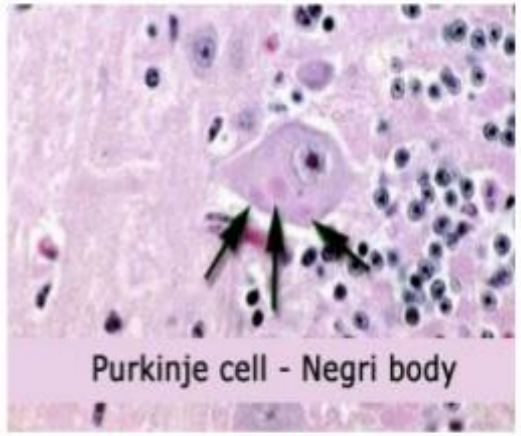
5 methods are used for diagnosis in the virology laboratory:

1. Direct microscopy
2. Cultivation of viruses
3. Serology
4. Detection of viral antigens
5. Detection of viral nucleic acids

MICROSCOPIC METHOD

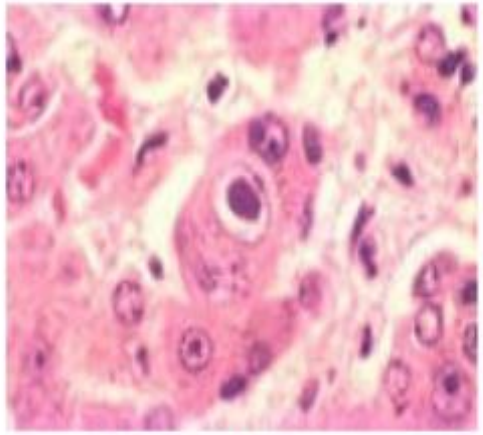
3- Inclusion bodies

- Site of **virus assembly** or **degenerative changes**
- Their location and appearance are **diagnostic** for a particular virus.



Purkinje cell - Negri body

a- **Intracytoplasmic:** e.g. **Rabies (Negri bodies)**



. b- **Intranuclear:** e.g **Herpes viruses**

c- **Both:** e.g. **Measles virus and CMV**

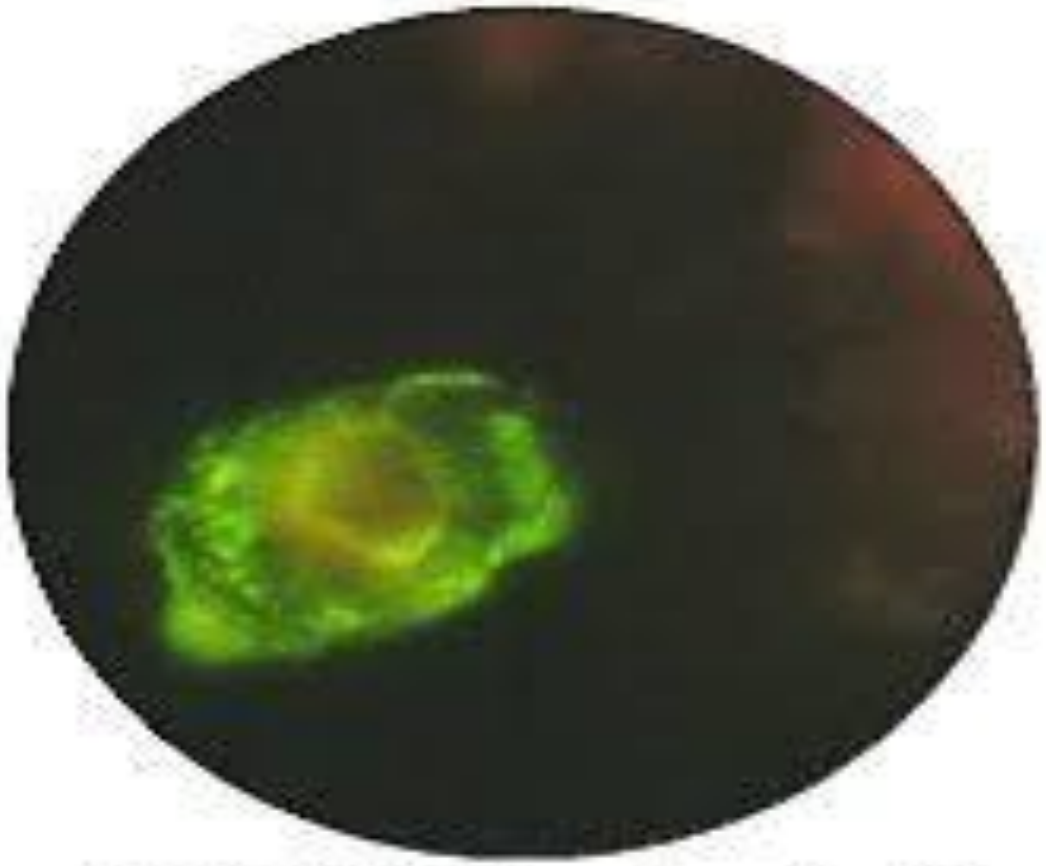


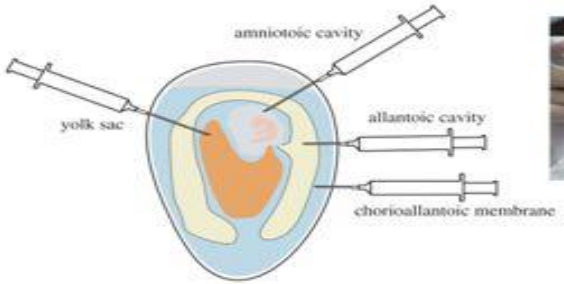
Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

Methods for Cultivation of Virus

- Animals are used for studying viruses which do not grow in cell cultures or eggs, and for testing vaccines
- Eggs support a fairly wide range of animal and human viruses – hence their importance in the diagnostic service
- Cell cultures; different types of cell lines will support different types of viruses

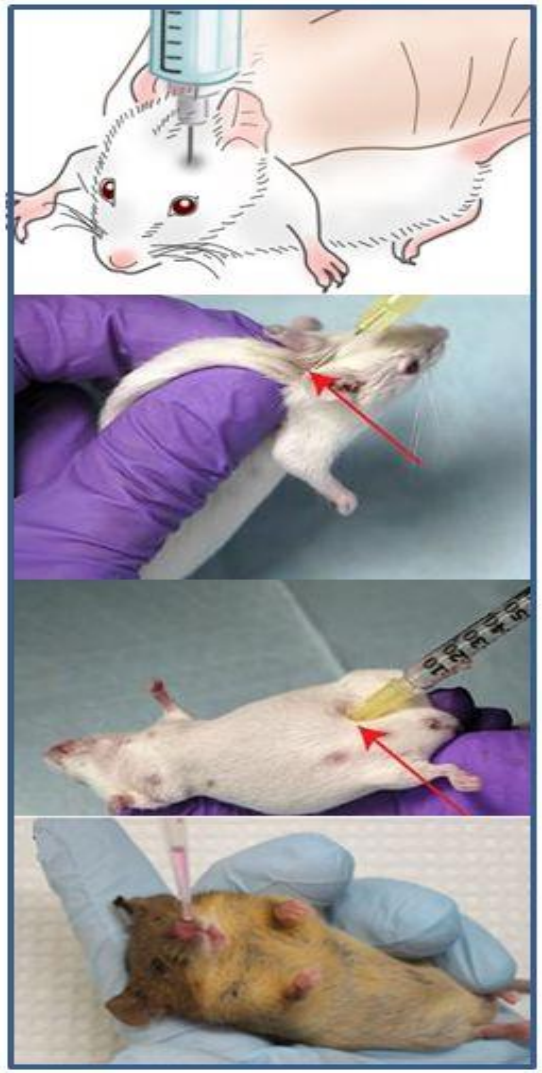
Methods for Cultivation of Virus

- Generally three methods are employed for the virus cultivation
  1. Inoculation of virus into animals
  2. Inoculation of virus into embryonated eggs
  3. Tissue culture



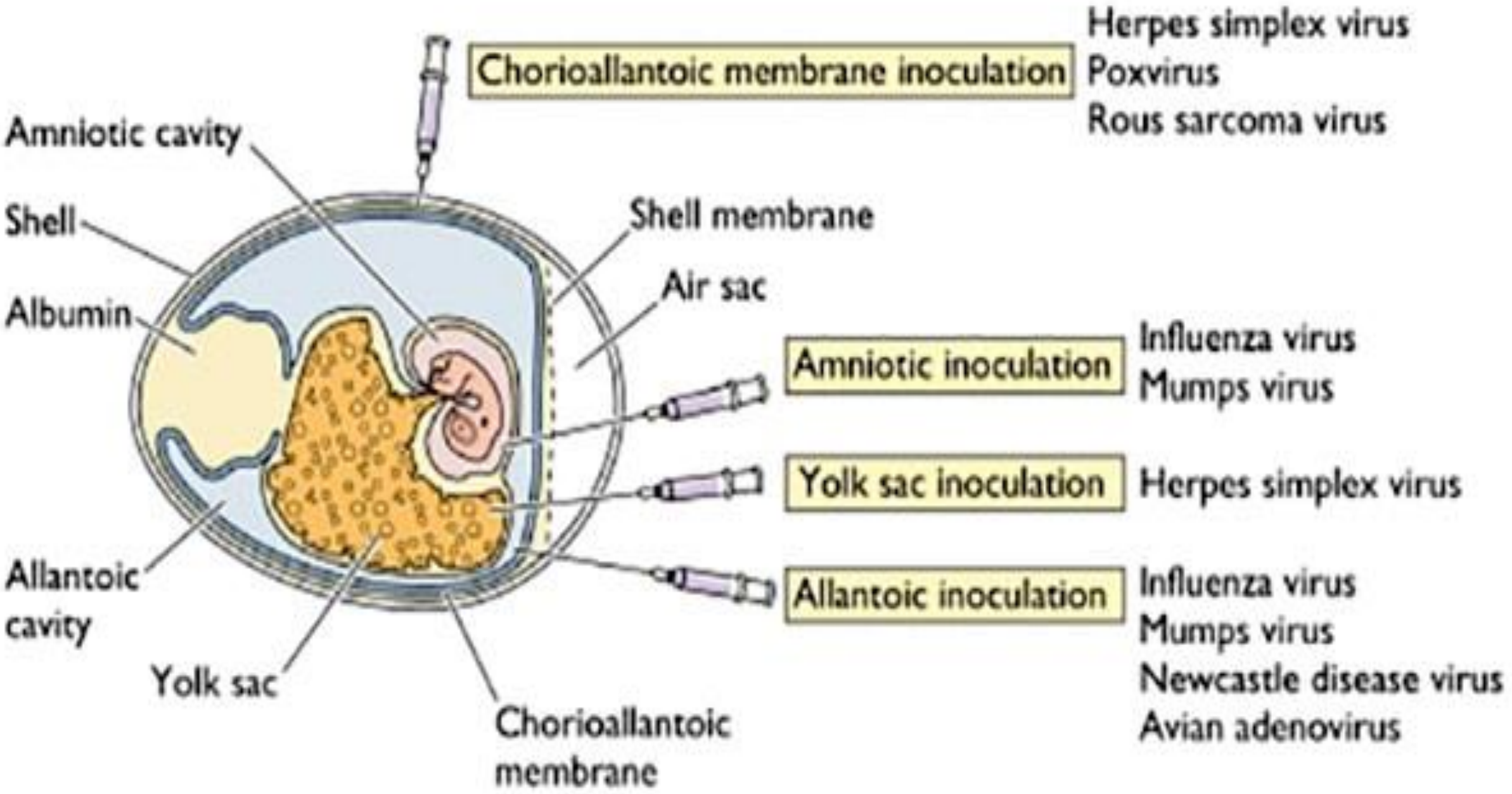
Inoculation of Virus in Animals

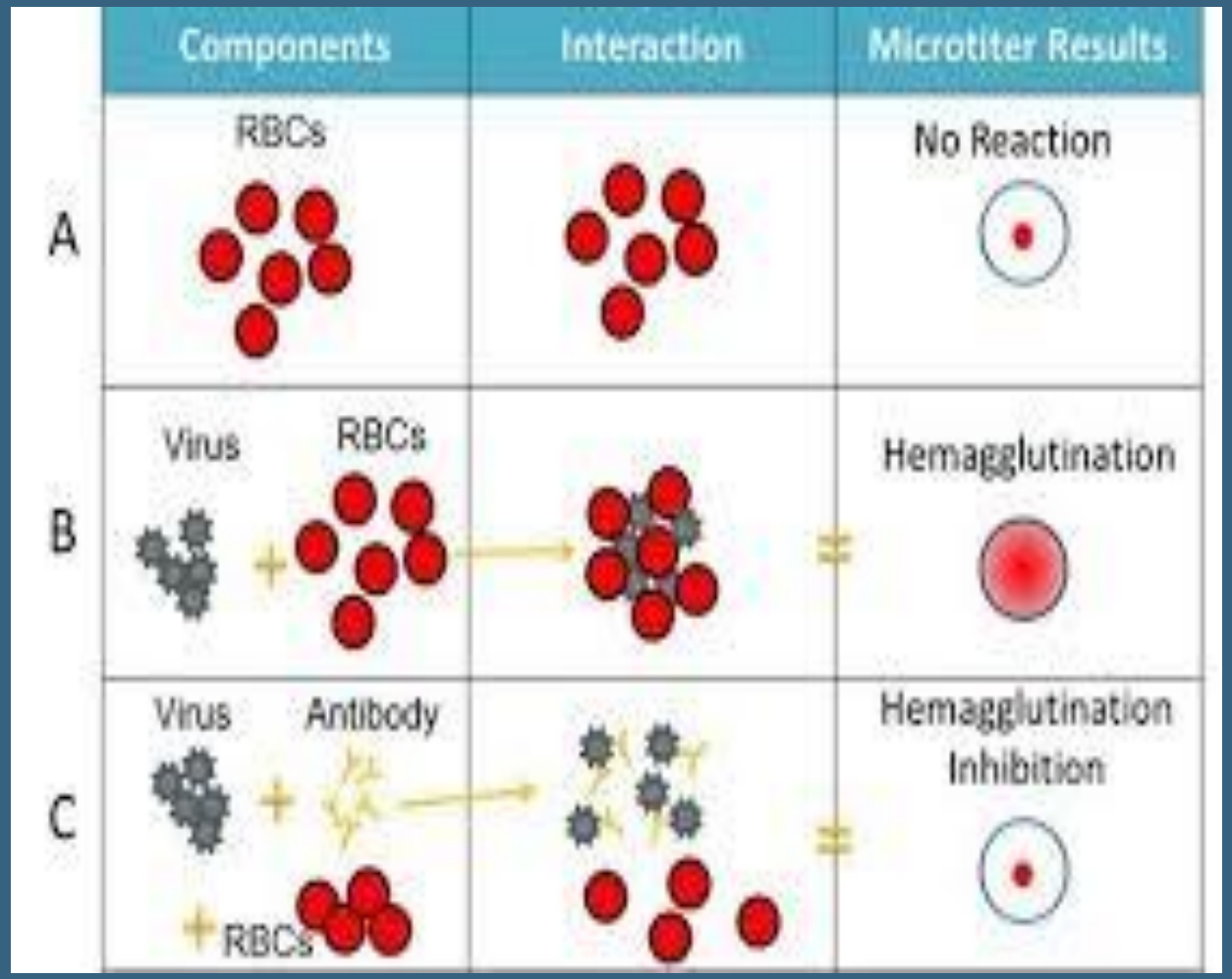
- The different routes of inoculation in mice are:
  - intracerebral
  - subcutaneous
  - intraperitoneal
  - or intranasal
- After the animal is inoculated with the virus suspension, the animal is:
  - observed for signs of disease
  - visible lesions
  - or is killed so that infected tissues can be examined for virus

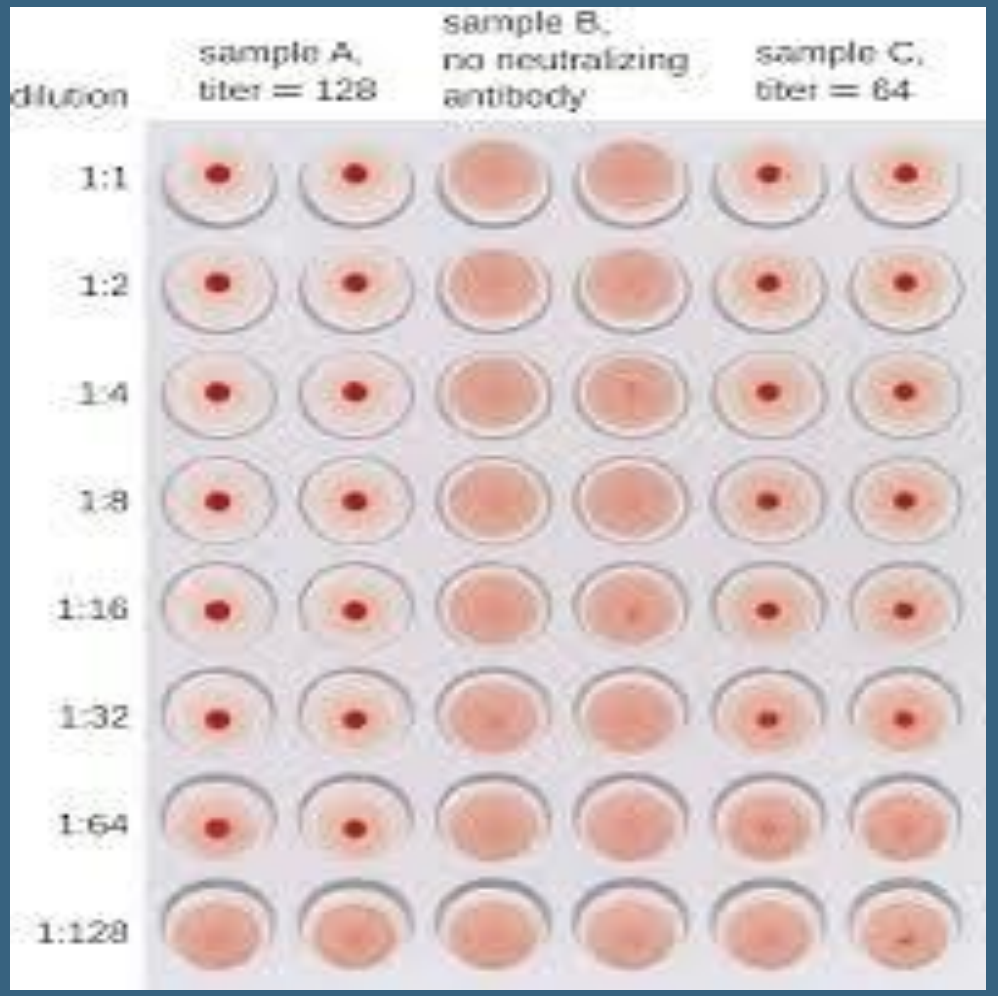


Advantages of inoculation into embryonated egg

- Widely used method for the isolation of virus and growth.
- Cost effective and maintenance is much easier.
- The embryonated eggs are readily available.
- They are free from contaminating bacteria and many latent viruses.
- Ideal substrate for the viral growth and replication.
- less labor is needed.
- Widely used method to grow virus for some vaccine production.
- Defense mechanisms are not involved in embryonated eggs.


Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Yolk sac
Chorioallantoic membrane

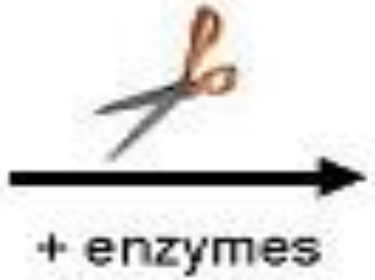

Components
Interaction
Microtiter Results
A
RBCs
No Reaction
B
Virus
RBCs
Hemagglutination
C
Virus
Antibody
RBCs
Hemagglutination Inhibition

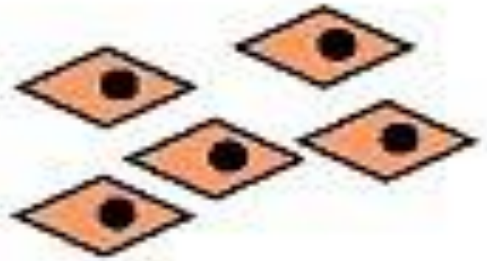

dilution
sample A,
titer = 128
sample B,
no neutralizing
antibody
sample C,
titer = 64
1:1
1:2
1:4
1:8
1:16
1:32
1:64
1:128

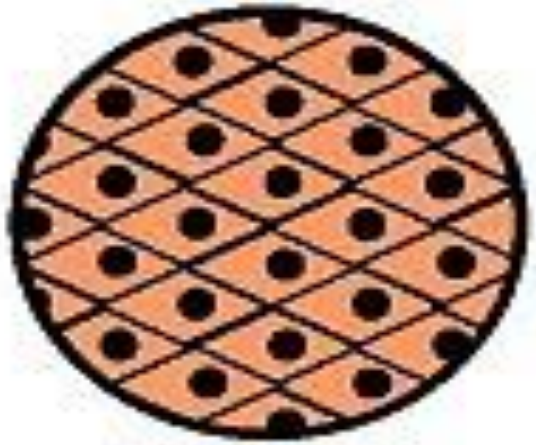
Cell cultures:

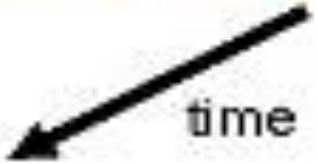
- 1. Primary cell cultures
- 2. Diploid cell strains
- 3. Continuous cell lines

Primary cell culture





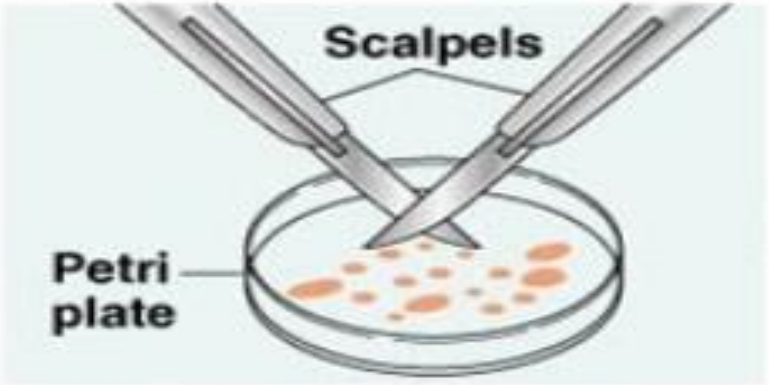




Cultivation of viruses

c. Cell culture (tissue culture)

The most widely used method



- Tissues + **trypsin** → separate cells

- Cells + **growth media** in flat-sided bottles

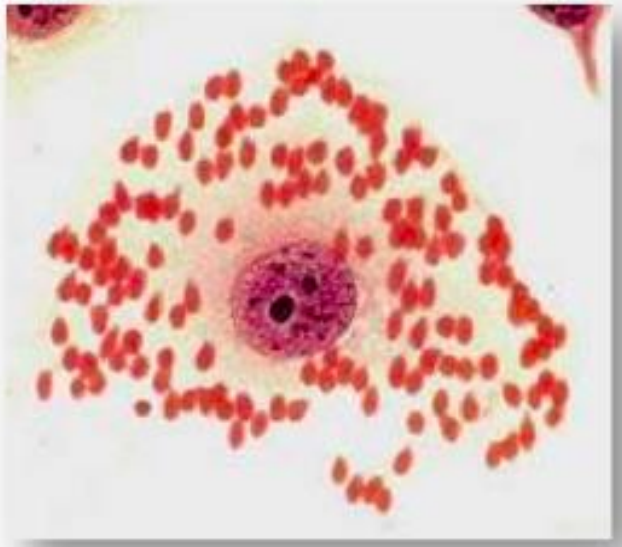
↓

monolayer culture

Detection of virus growth in cell cultures:

- Cytopathic effect:
  - crenation of cells;
  - degeneration of the entire cell sheet;
  - syncytium formation;
  - discrete focal degeneration;
  - granular clumps;
  - cytoplasmic vacuolation
- Metabolic inhibition
- Hemadsorption

Hemadsorption



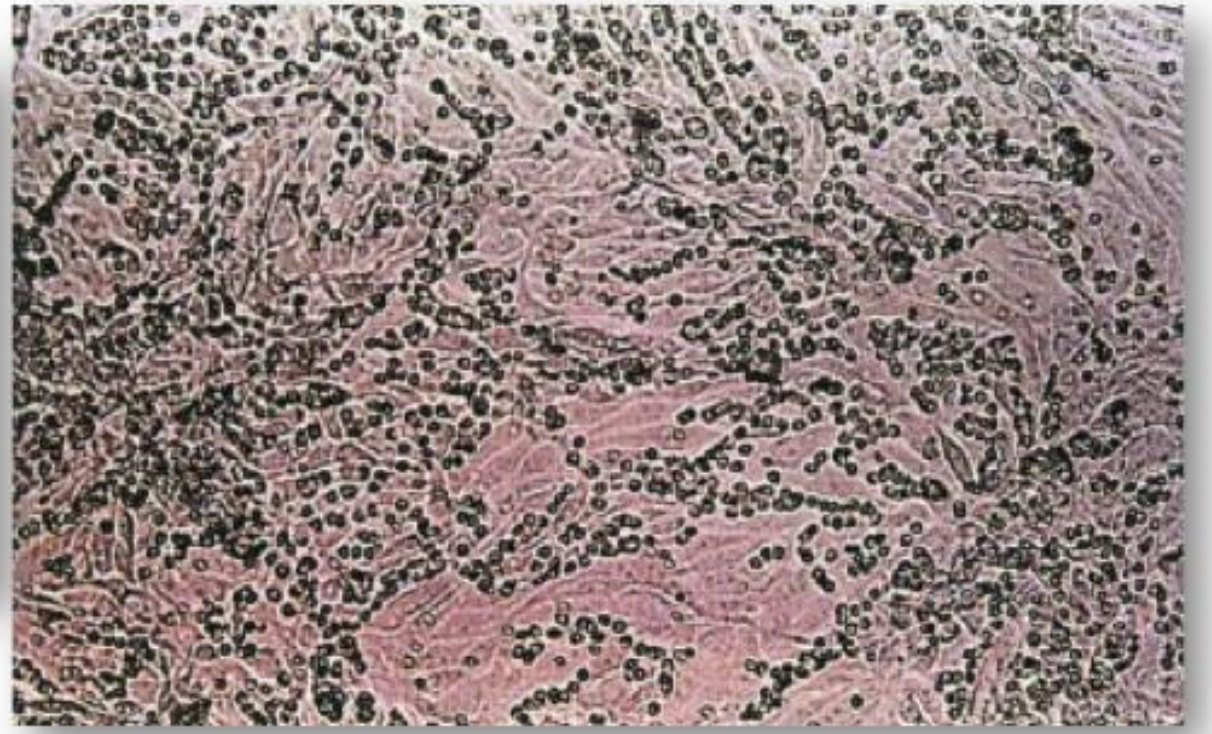
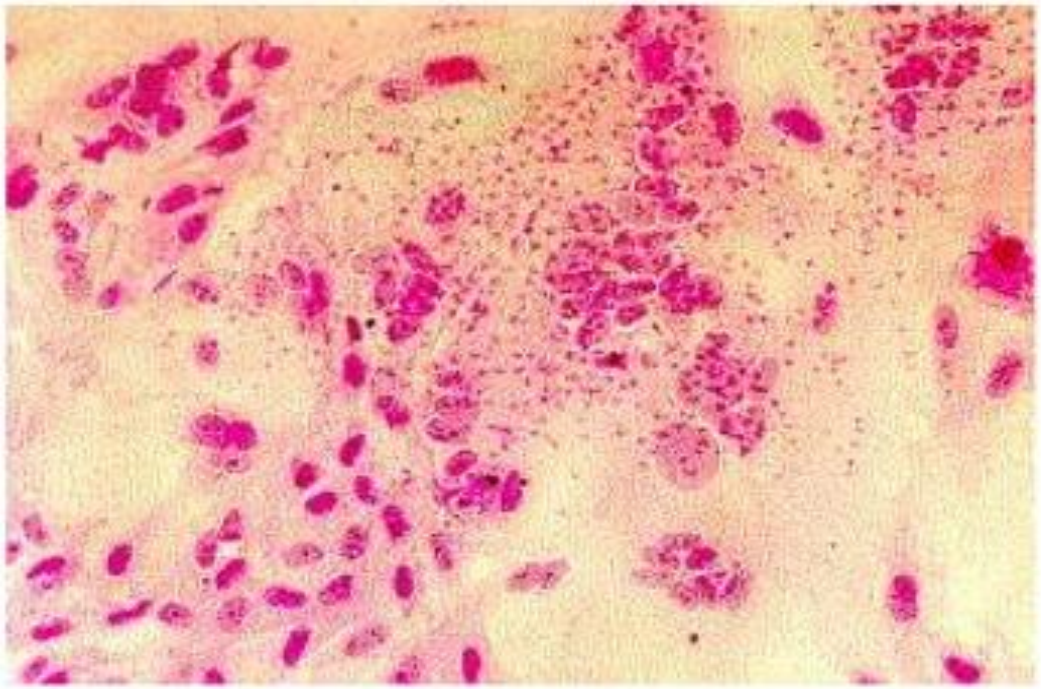


Figure 47-5: Hemadsorption of erythrocytes to cells infected with **influenza viruses**, mumps virus, parainfluenza viruses, or togaviruses. These viruses express a **hemagglutinin (HA)** on their surfaces, which binds to erythrocytes of selected animal species.

Haemadsorption

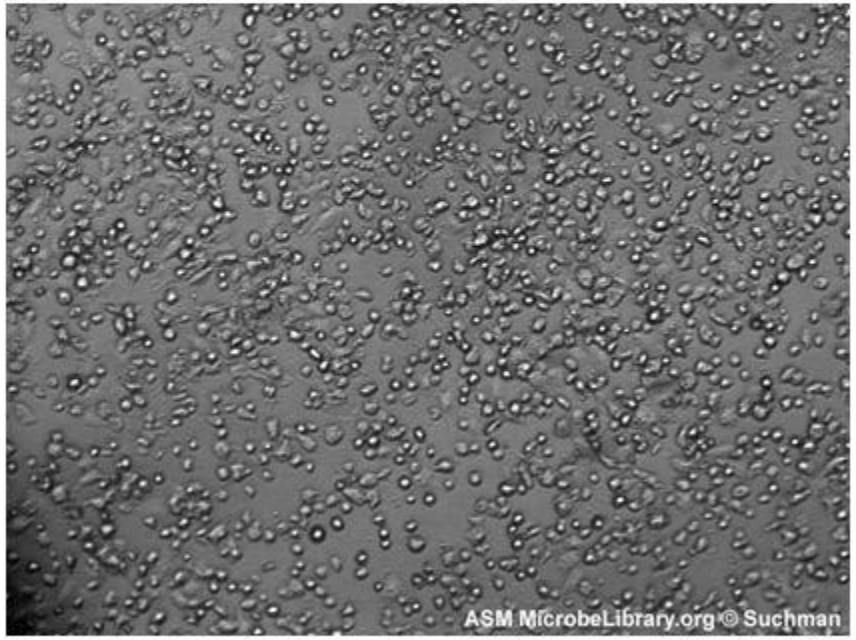


Syncytial formation caused by mumps virus and haemadsorption of erythrocytes onto the surface of the cell sheet.

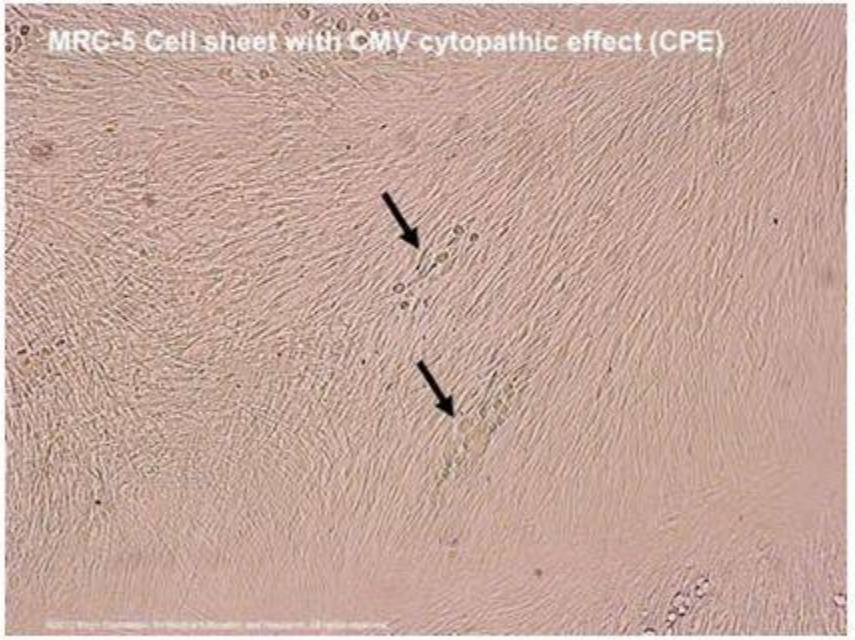
(courtesy of Linda Stannard, University of Cape Town, S.A.)

Swelling and Clumping

Adenovirus infected cells greatly enlarge and clump together in" grape-like" clusters.



Cytomegalovirus infected cells swell and round up



SEROLOGICAL METHOD:

- diagnostic titer
-technique of paired sera