# VIROLOGY

- Living chemicals

  Can be crystalised (Non living)

  Nucleic acid extracted are infective & causes multiplication (Living)
- No synthesis of their own constituents (No enzyme for protein & nucleic acid synthesis)
- Multiply by complex process ( Not binary fission)
- Able to cause disease from common cold to Ca / AIDS

Smallest known infective agent <10nm – 300nm
(Bacteria - 100nm, RBC – 7500nm)
No cellular organisation
Not strictly cellular (Cellwall enclosing cell absent)
Has either RNA / DNA
No ribosomes / protein synthesizing apparatus
No metabolic activity outside host cell
Multiplies only inside living cells.Not on inanimate media
In host cells, virus redirects cell's synthesizing machinery to synthesize virus components
(Transcription of virus genome into virus specific mRNA which directs host cell to produce new virus particle)

## Classification

I) DNA virus – Pox viridae
Herpes viridae
Adenoviridae
Papoviridae
Parvoviridae

II) RNA Virus – Picorna viridae
Orthomyxoviridae
Paramyxoviridae
Rhabdoviridae
Reoviridae
Retroviridae
Bunyaviridae
Arenaviridae
Coronaviridae
Caliciviridae

## MORPHOLOGY

10 – 300 nm

Passes through filters which can hold back bacterias

Ultramicroscopic

Core with nucleic acid – Genome

Sorrounded by protein coat – Capsid

Protects genome from adverse environmental factors (Nuclease in blood)

Chemical polypeptide molecule (Capsomere) arranged symmetrically forms the capsid

Capsid is antigenic which stimulates production of Ab
Capsid absorbs readily on cell surface

Some viruses are sorrounded by a lipoprotein envelop (Derived from host cell)– Enveloped virus
Envelop has chemical, antigenic & biological properties
Enveloped viruses are susceptible to lipid solvents like bile salt, ether, chloroform etc
Projecting subunits may be seen as spikes made of protein on surface of envelop - Peplomere

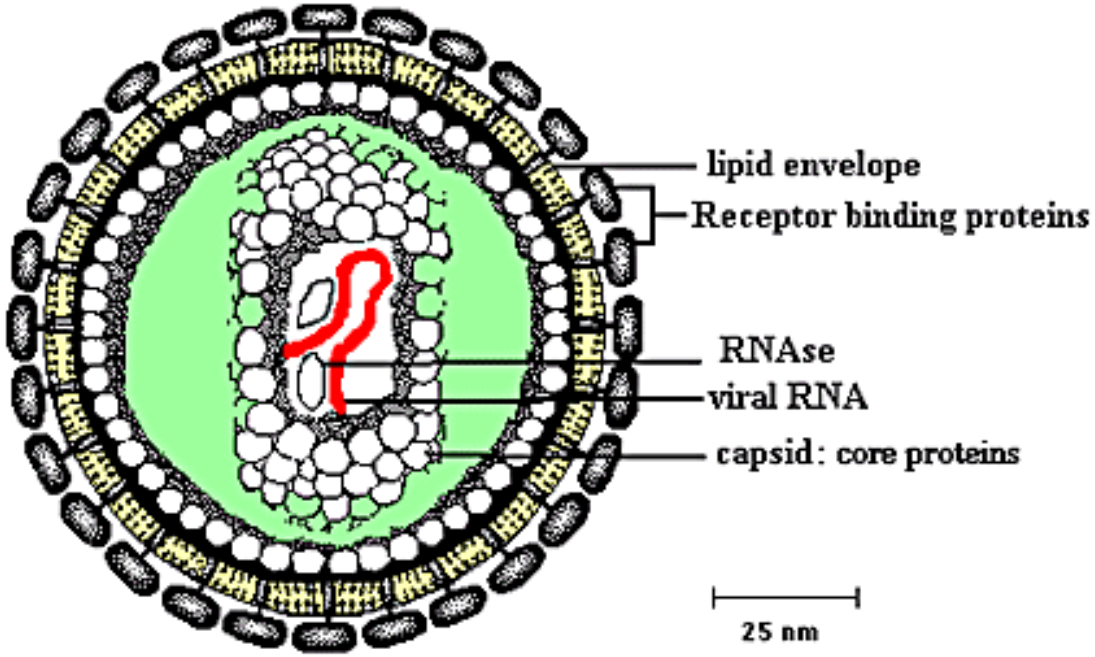
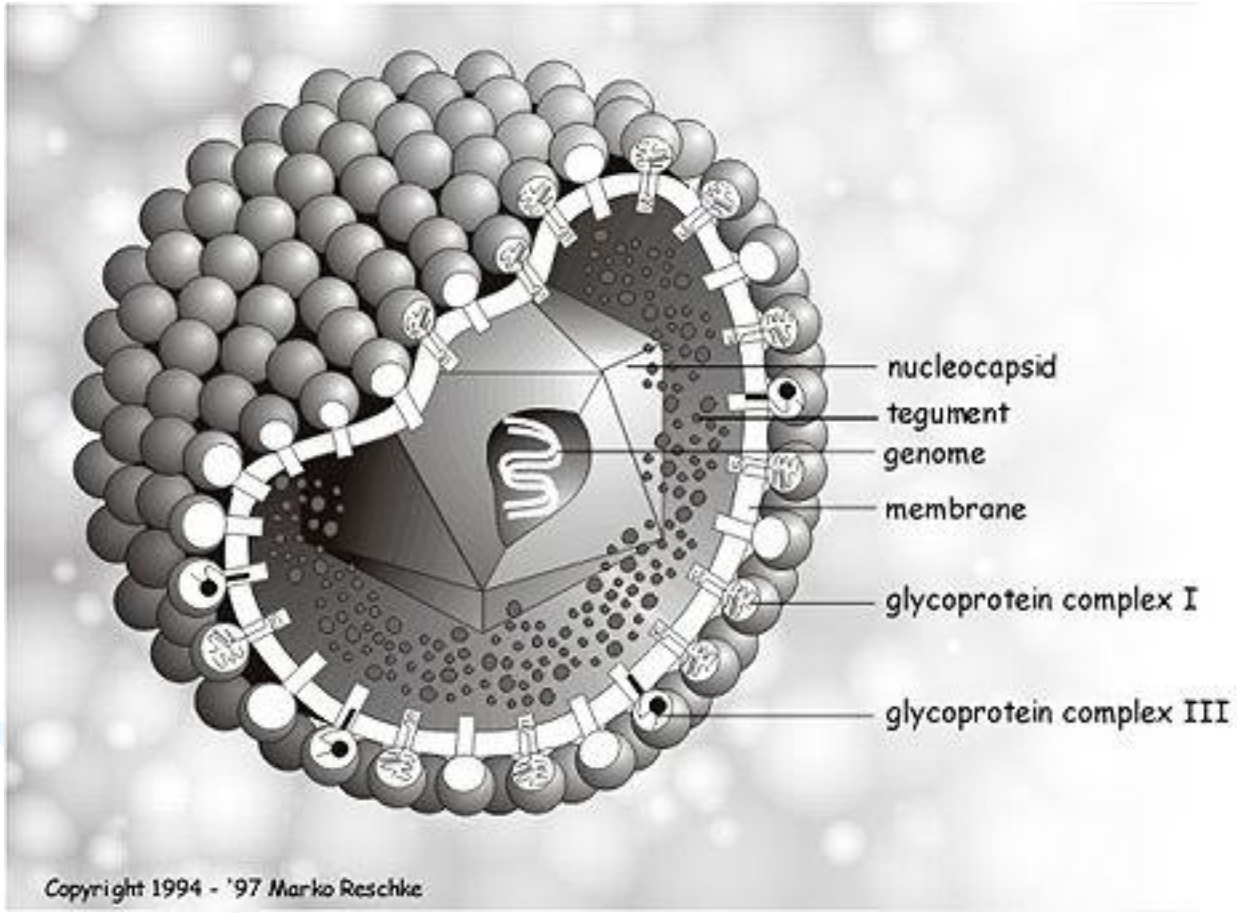
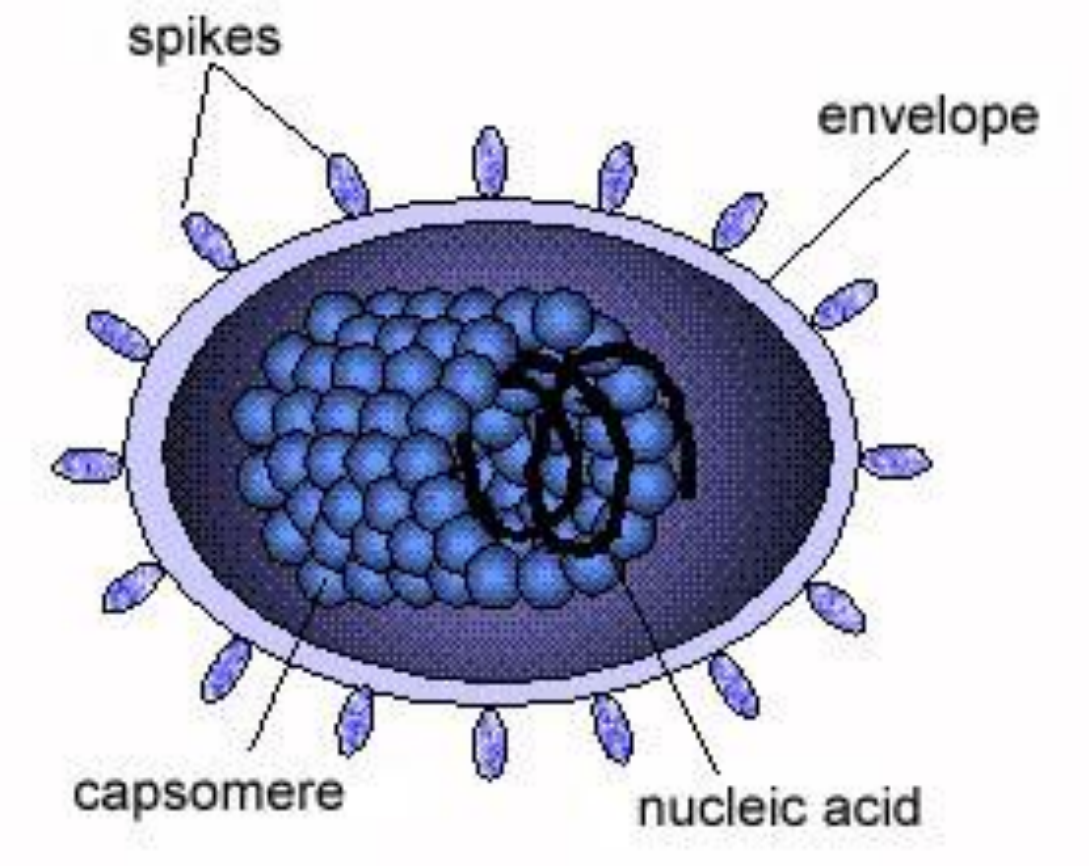



**Diagram of a Retrovirus**


nucleocapsid
tegument
genome
membrane
glycoprotein complex I
glycoprotein complex III
Copyright 1994 - '97 Marko Reschke


spikes
envelope
capsomere
nucleic acid

## Shape

Most look Spherical

Some pleomorphic

Icosohedral - (12 vertices & 20 faces)

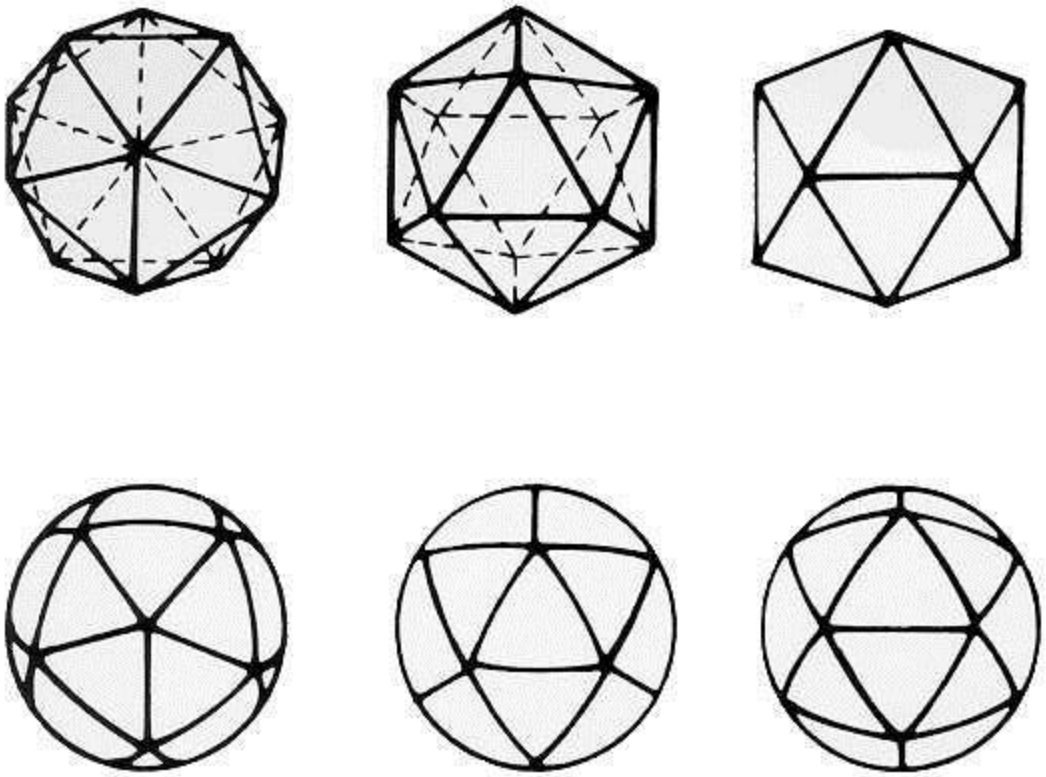
Hellical - (Nucleic acid & capsomere wound together to form hellical /Spiral)

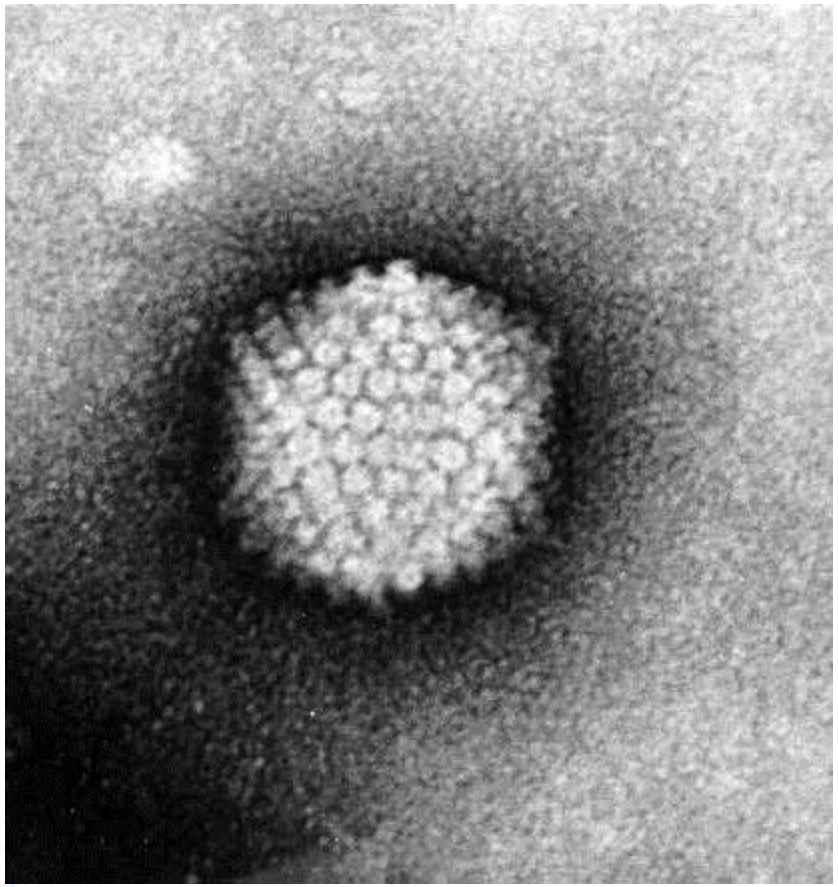
Complex – Neither cubical / Hellical

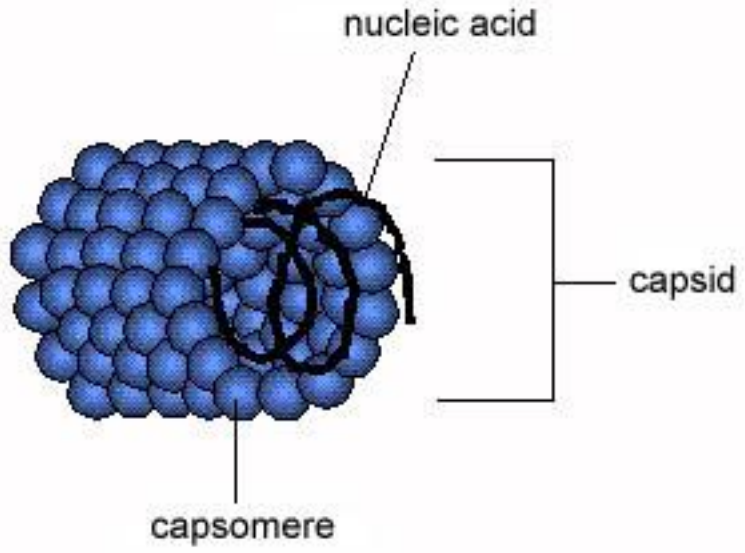
Rabies – Bullet shaped

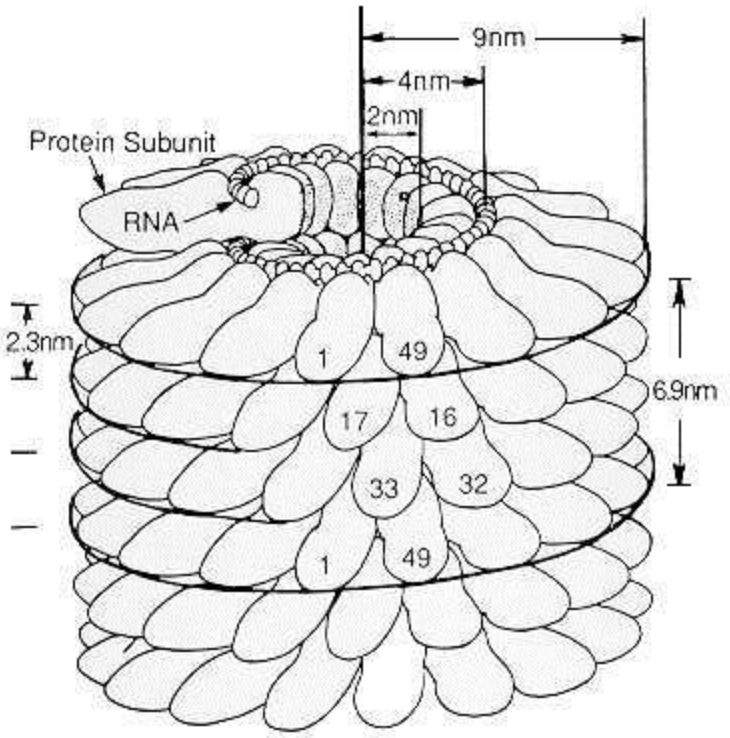
Pox – Brick shaped

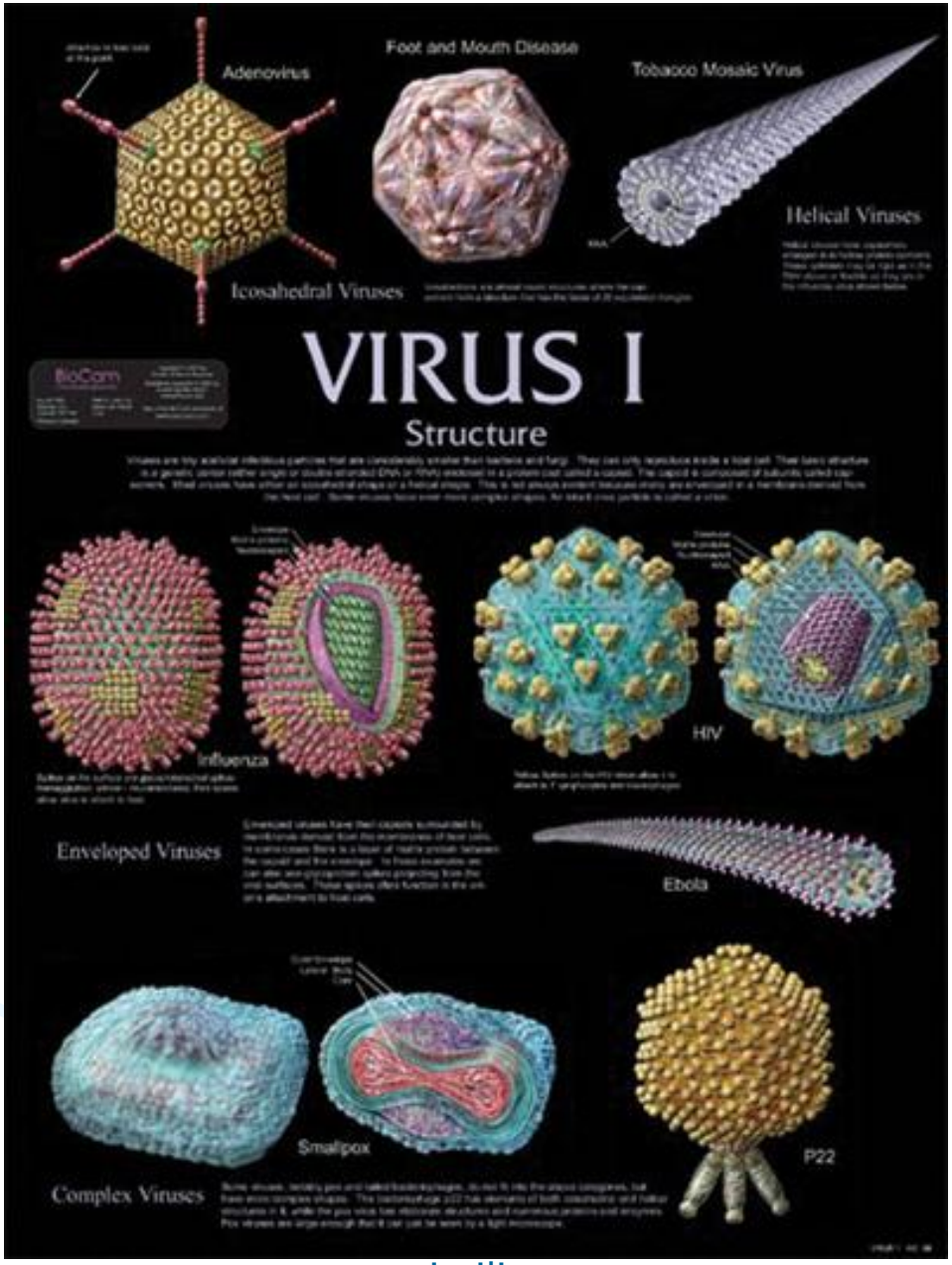
Tobacco mosaic – Rod shaped)

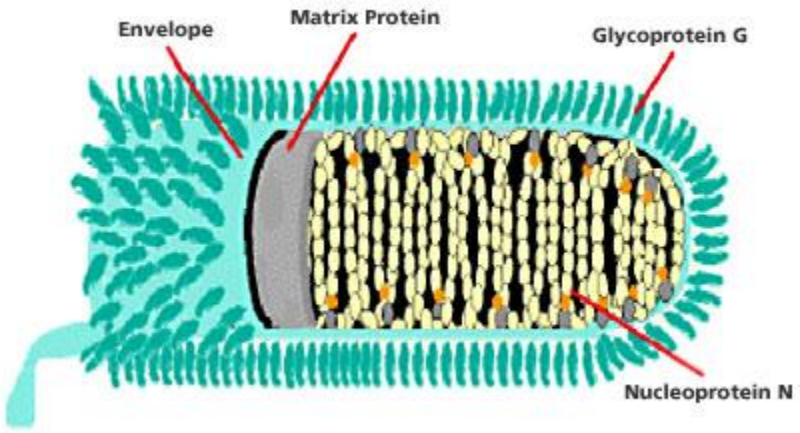





nucleic acid
capsid
capsomere


9nm
4nm
2nm
Protein Subunit
RNA
2.3nm
6.9nm
1
49
17
16
33
32
1
49


Adenovirus
Foot and Mouth Disease
Tobacco Mosaic Virus
Helical Viruses
Icosahedral Viruses
VIRUS I
Structure
Influenza
HIV
Enveloped Viruses
Ebola
Smallpox
P22
Complex Viruses


Envelope
Matrix Protein
Glycoprotein G
Nucleoprotein N

## Resistance

Generally heat labile with exceptions

Inactivated at 56 C, with in seconds at 37 C with in minutes & 4 C in days

Stable at low temperature

Long term storage at -70 C

Drying frozen virus under vaccum – Lyophilisation

(For prolonged storage. Can be activated by adding water)

Disrupted in alkaline medium

Vary in resistance to acidity

Inactivated by sunlight, UV & ionising radiation

Resistant to chemical disinfectants

Glycerol & Saline act as preservative for some

$H_2O_2$, $KMnO_4$, Hypochlorites – Active antiviral

Formaldehyde, Beta propiolactone, I, Cl compounds kills most virus ( Except Hepatitis & Polio)

Ether, Chloroform, Bile salts – Kills enveloped

Antibiotics - Ineffective

# CULTIVATION OF VIRUS

**Animal inoculation** – Mice rabbit, monkey, Guinnea pig

Death /Disease / Visible lesions

Helps to study pathogenesis, immune response, epidemiology & oncogenesis

Immunity of the animal / latent virus harboured by the animal may interfere

**Embryonated eggs**

Chick Embryo – Provides different sites (Chorioallantoic membrane, Allantoic cavity, Amniotic sac, Yolk sac) for cultivation & lesions are visible (Pox vesicle)

Duck egg – Bigger & long IP

**Tissue Culture** /– Cultivation in living & metabolising bits of tissue / organ of human / animals, grown in artificial favourable environment

a) Organ Culture – Small bits of organ in vitro preserving original architecture & function
(Monkey kidney)

b) Explant culture – Fragments of minced tissue embeded in plasma clot / suspension
(Adenoid tissue explant)

c) Cell culture

Tissues dissociated into component cells by action of proteolytic enzymes (Trypsin) & mechanical shaking.

Washed & counted cells are suspended in growth medium with 13 essential amino acid, 9 vitamins, salts, glucose, buffer, calf serum, Antibiotics etc

Cells multiply within 24 – 48 hrs which is dispensed in bottles, tubes / petridish

Cells adhere to glass surface & on incubation divide to form a monolayer sheet of cells covering the surface within a week

Primary – Normal cells freshly taken from body & cultured

Limited growth & cannot be serially cultured

For isolation of virus & vaccine preparation

Monkey kidney, human embryonic kidney, human amnion & chick embryo cell

Diploid cell strain - Single type of cell that retain original diploid chromosome number & karyotype during serial subcultivation for limited number of times

Virus isolation & vaccine preparation

Human fibroblast

Continuous cell lines – Cells of single type capable of continuous serial cultivation indefinitely

Human cancer cells maintained & stored at -70 C

Studies can be done on

Cytopathic effect – Morphological change in cells due to virus can be observed by microscopic examination ⟶ Identification of virus

(Crenation – enterovirus, Syncitium – Measles, Focal degeneration – Herpes, Large granular clumps – Adenovirus)

Metabolic inhibition – Indicator is added. No acid formation (Normal metabolism produces acids)

Haemadsorption – Addition of guinnea pig erythrocytes to the culture. it will be adsorbed to cell surface in presence of haemagglutinating virus (Influenza)

Interference – A known cytopathogenic virus will be inhibited by an unknown one. (Test growth of non cytopathogenic virus in cell culture)

Transformation – Oncogenic virus induced cell transformation (loss of contact inhibition & grow in piled up fashion – microtumours)

Immunoflourescence – Virus infected cells stained with flourescent antiserum & observed under UV microscope for presence of virus Ag.

## Multiplication

Genetic information for replication is in the nucleic acid. But lacks biosynthetic enzymes & depend on synthetic machinery of host cell

Adsorption – Virus attach to cell surface
with specific receptor site
Best at 37 C. Slow at 4 C
Enhanced by Mg & Ca
Penetration – Whole virus / nucleic acid enters
by mechanism similar to phagocytosis – Viroprexix
Enveloped fuse with cell membrane & release capsid into cytoplasm

Uncoating – Stripping of capsid to release nucleic acid into cell (Lysosomal enzyme of host cell)

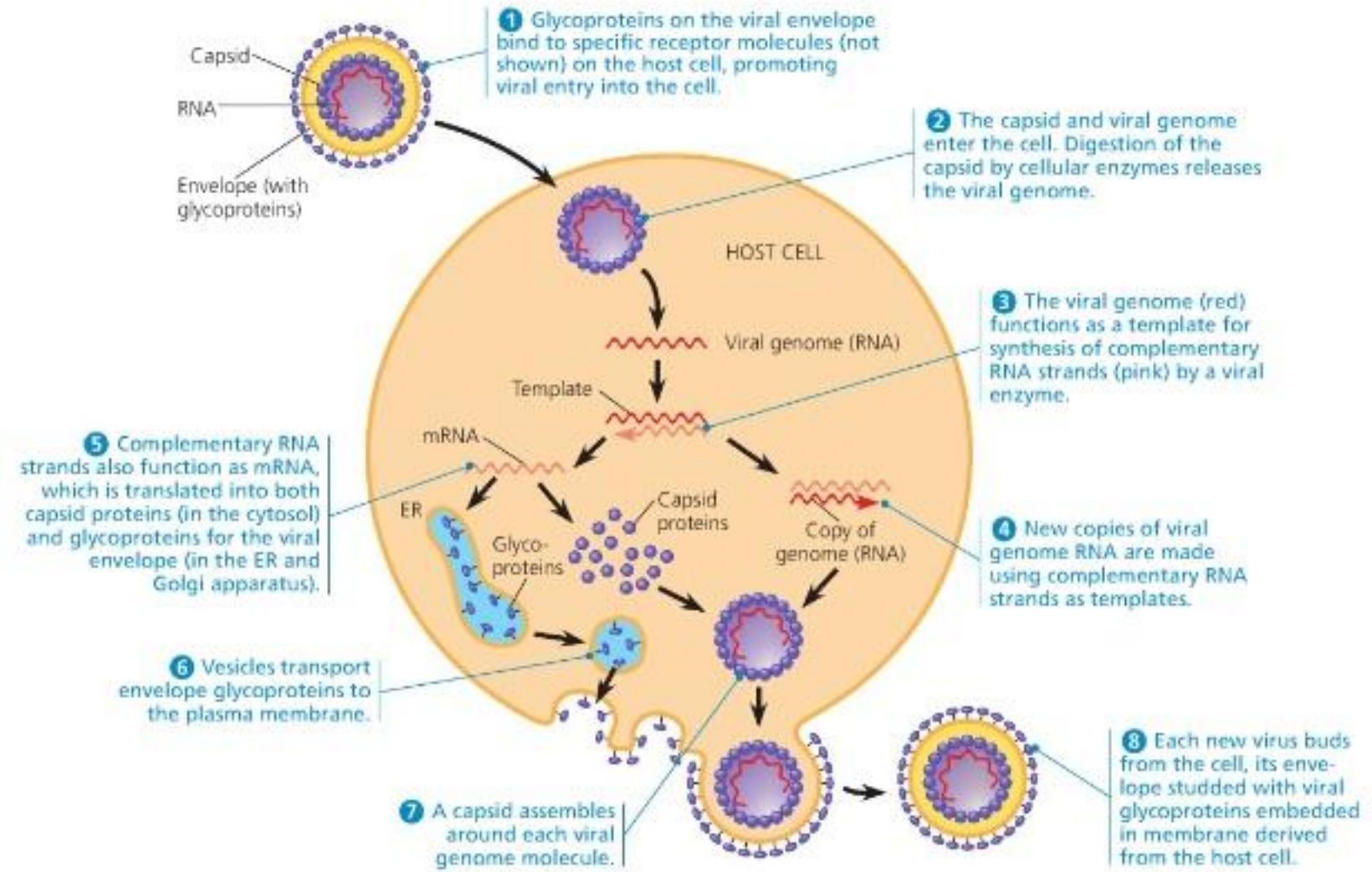
Transcription – Viral mRNA are produced from viral Genome

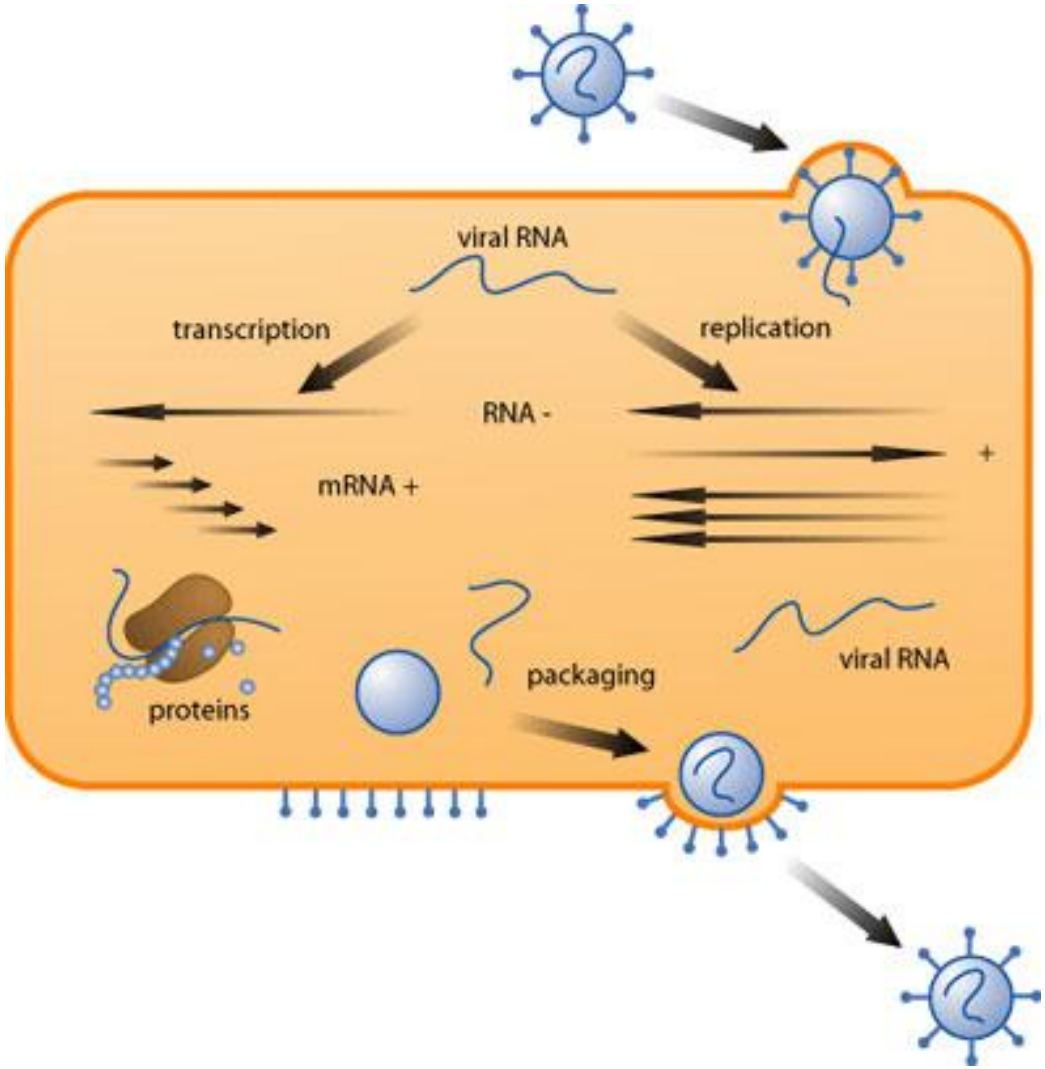
Biosynthesis – Synthesis of material necessary for nucleic acid, capsid & necessary enzymes.
Normal cellular metabolism of host cell is shut down

Maturation – All proteins are assembled to form capsid & nucleic acid to form fully developed virions
(Enveloped are not fully developed)

Release – Either by rupture causing cell lysis/ by budding thro' cell membrane of host cell without lysis
(Enveloped get envelop from host cell membrane on budding)

From stage of uncoating to mature virions, they cannot be demonstrated in a host cell – Eclipse phase


Capsid
RNA
Envelope (with glycoproteins)
1 Glycoproteins on the viral envelope bind to specific receptor molecules (not shown) on the host cell, promoting viral entry into the cell.
2 The capsid and viral genome enter the cell. Digestion of the capsid by cellular enzymes releases the viral genome.
HOST CELL
Viral genome (RNA)
3 The viral genome (red) functions as a template for synthesis of complementary RNA strands (pink) by a viral enzyme.
Template
mRNA
5 Complementary RNA strands also function as mRNA, which is translated into both capsid proteins (in the cytosol) and glycoproteins for the viral envelope (in the ER and Golgi apparatus).
ER
Glyco-proteins
Capsid proteins
Copy of genome (RNA)
4 New copies of viral genome RNA are made using complementary RNA strands as templates.
6 Vesicles transport envelope glycoproteins to the plasma membrane.
7 A capsid assembles around each viral genome molecule.
8 Each new virus buds from the cell, its envelope studded with viral glycoproteins embedded in membrane derived from the host cell.


viral RNA
transcription
replication
RNA -
+
mRNA +
proteins
packaging
viral RNA

## Pathogenesis

Virus enters thro' respiratory tract /GIT / GUT

Multiply at the site of entry ⟶ Localised infection (Influenza)

Lymphatic / Haematogenous spread ⟶ Systemic disease / Target organs

Skin / Conjunctiva - Multiply at the site of entry ⟶ Localised infection (Papilloma, Conjunctivitis)

Vertical transmission ⟶ Localised infection ⟶ Abortion / Maldevelopment / Neonatal disease

Incubation period – Time taken by virus to travel from site of entry to target organs & multiply ⟶ Lesions

Short – Site of entry & target organ are same / Blood injected

Long – Target organ is away from site of entry, Slow multiplication, slow rate of movement to target organ

## Pathological effect

Cell death / Cell lysis

Cell proliferation (Papilloma, CP)

Shut down of host protein & DNA synthesis

Accumulation of viral particles distort cellular architecture ⟶ Cell injury

Alteration of cellular membrane permeability releasing lysosomal enzymes

Fusion of adjacent cell membrane

Virus coded Ag on cell membrane on cell surface ⟶ new properties of cells

Haemagglutination

Damage to chromosomes

Malignant transformation

## Host Response

Depends on

Virulence / Strain

Resistance offered by host

Both humoral & cell mediated immunity acts

IgG, IgM (Blood & tissue spaces) IgA (Mucosal surface) – Prevent adsorption to cell receptors / enhance virus degradation / prevent release of progeny from infected cells

Complement – Surface damage of enveloped virus & cytolysis

Ab may cause complement dependent injury to cell pathogenesis (Ab passively acquired from mother Resp viral infection in early infancy which becomes milder in older Children who have no Ab,
Viral haemorrhagic Immunological thrombocytopenia,
Serum hepatitis)

Maternal Ab & passively administered gammaglobulin prevent viral infection.
Killed virus vaccine protects due to their ability to induce humoral Immunity

Deficient cellular immunity shows high susceptibility to viral infection
Contribute to tissue damage
Macrophage ⟶ Clearing (Leukopenia & Neutropenia)
Delayed hypersensitivity after vaccination / infection (mumps) is due to CMI

Some viral infection causes suppression of immune response

In general virus infection is followed by solid immunity to reinfection which may be life long

Re infection may be due to antigenically different virus

## Non immunological response

Body Temp – Most viruses are inhibited above 39 C (Fever)

Hormones – Corticosteroids enhance virus infection (Therapeutic treatment, Pregnancy)

Dipression of immune response & inhibition of interferon synthesis

Malnutrition ⟶ Complication

Age – Common & dangerous in extreme ages

# INTERFERON

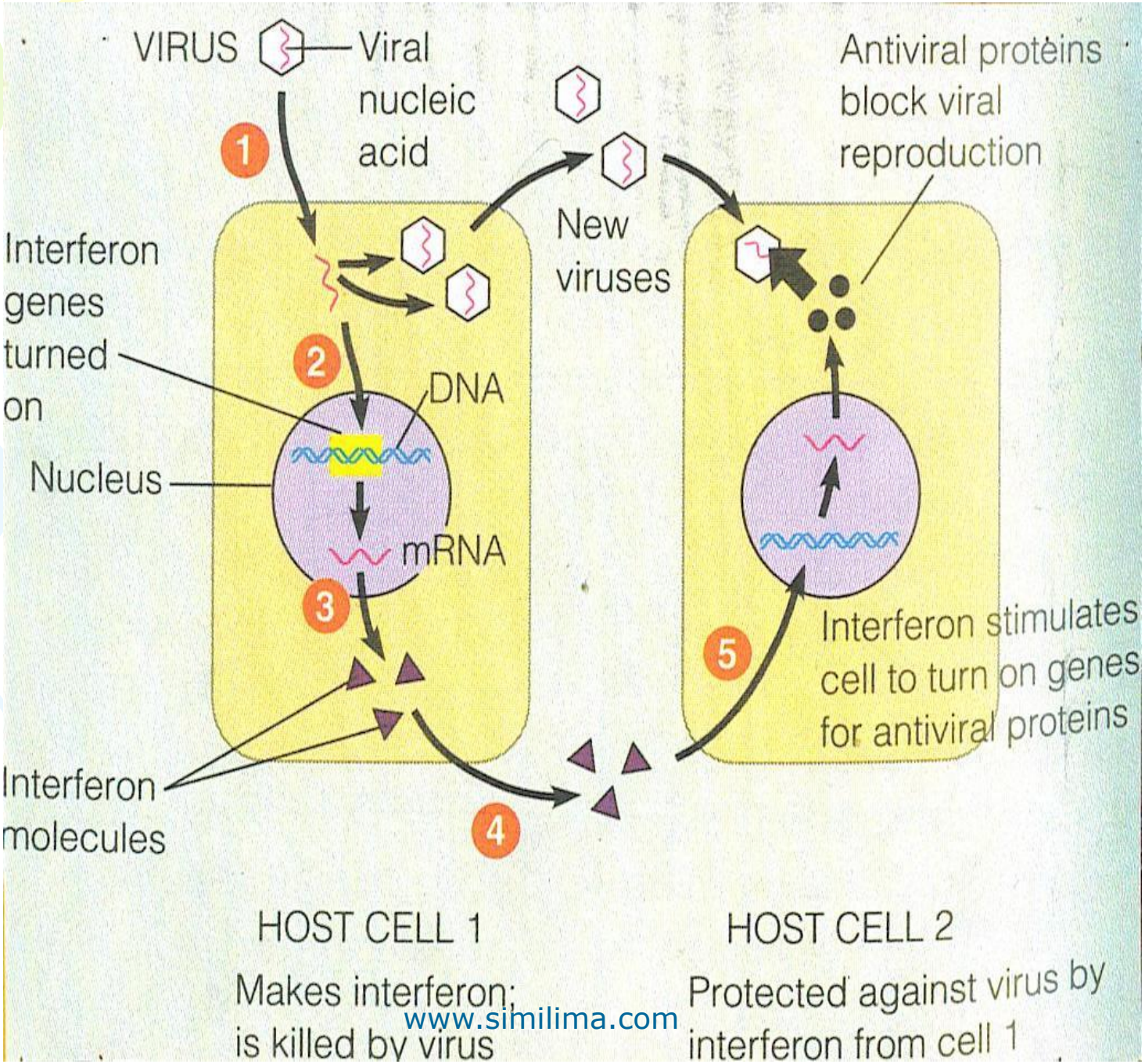
- Antiviral substances produced by vertebrate cells against virus infection as a natural defense mechanism
- Glycoproteins
- No direct action on virus
- Acts on other cells of same species preventing them from virus infection
- Species specific & not virus specific

(Cells exposed to interferon produce a protein –Translation inhibiting protein which inhibit translation of viral mRNA without effecting cellular mRNA
Also inhibits viral transcription)

- Production increases with temperature (Fever)
- Inhibited by steroids & oxygen tension
- Synthesis begins in ½ an hour of induction & reaches high in 6 – 12 hrs
- Inactivated by proteolytic enzyme. But uneffected by nucleases & lipases
- Can withstand heating at 56 – 60 C for 30 – 60 mts & are stable at Ph 2 – 10
- Non toxic
- Non antigenic
- Diffuse freely in body
- Non dialysable, non sedimented on centrifugation

Based on various characters & cell of origin, they are
Alpha – Produce by B lymphocytes & Macrophages
Beta – Fibroblast & epithelial cells
Gamma – T lymphocytes
Inhibits cell growth, cell proliferation, DNA synthesis & Protein synthesis
Enhances cytotoxic activity of NK cells & T lymphocytes
Activation of macrophages

Viruses vary in susceptibility to interferon & capacity to introduce interferon


VIRUS
Viral nucleic acid
1
Interferon genes turned on
2
DNA
Nucleus
mRNA
3
Interferon molecules
4
New viruses
Antiviral proteins block viral reproduction
5
Interferon stimulates cell to turn on genes for antiviral proteins
HOST CELL 1
Makes interferon; is killed by virus
HOST CELL 2
Protected against virus by interferon from cell 1

## Lab diagnosis

Microscopy ( Electron) – Virus particle / inclusion bodies

Virus isolation – Animal inoculation

Ag Ab detection ( Serum / Ds products )

CFT,

ELISA

Haemagglutination

Gel precipitation

Immunoflourescence

Electrophoresis

Radioimmunoassay

## INCLUSION BODIES

Structures with distinct shape, location & staining properties which can be demonstrated in virus infected cells (Histological)

Situated either in cytoplasm / nucleus / both

Usually acidophilic seen as pink structure with E & H stain

Few basophilic – Adenovirus

May be crystalline aggregates of virions / virus Ag / degenerative changes produced by virus

Type A – Variable in size & granular

(Herpes, Yellow fever)

Type B – Circumscribed & multiple

(Adeno, Polio)

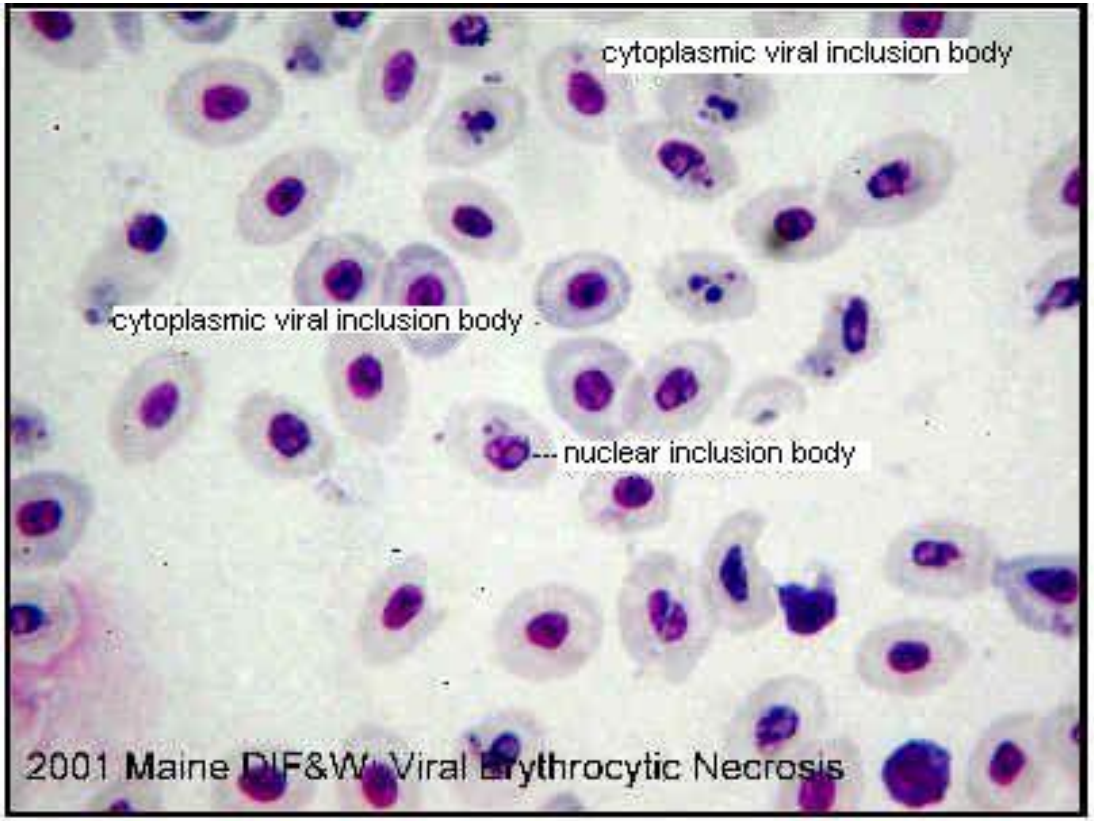
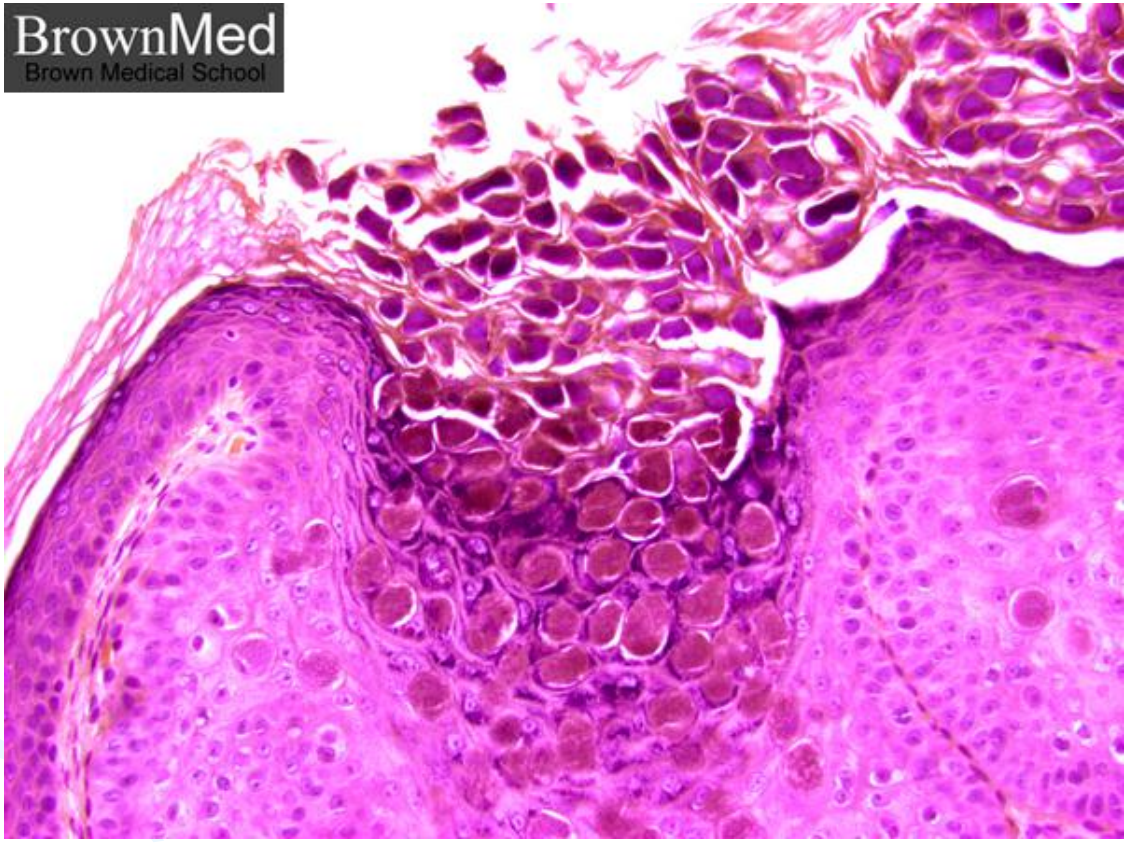



*Figure 2. Blood smear from cod with classical viral inclusion bodies in red blood cell cytoplasm and nucleus.*


BrownMed
Brown Medical School

## Negri bodies

Inclusion bodies seen in nerve cells infected with rabies

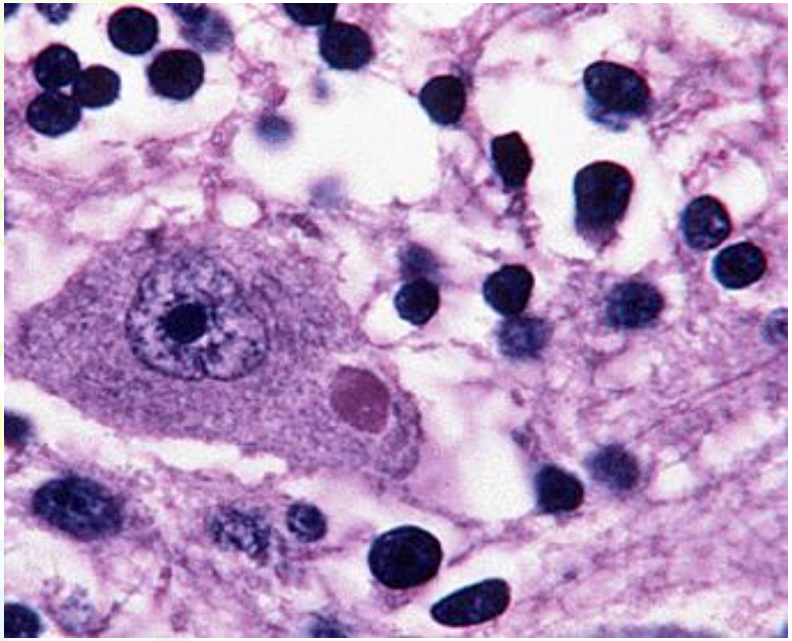
Eosinophilic, sharply outlined, intracytoplasmic

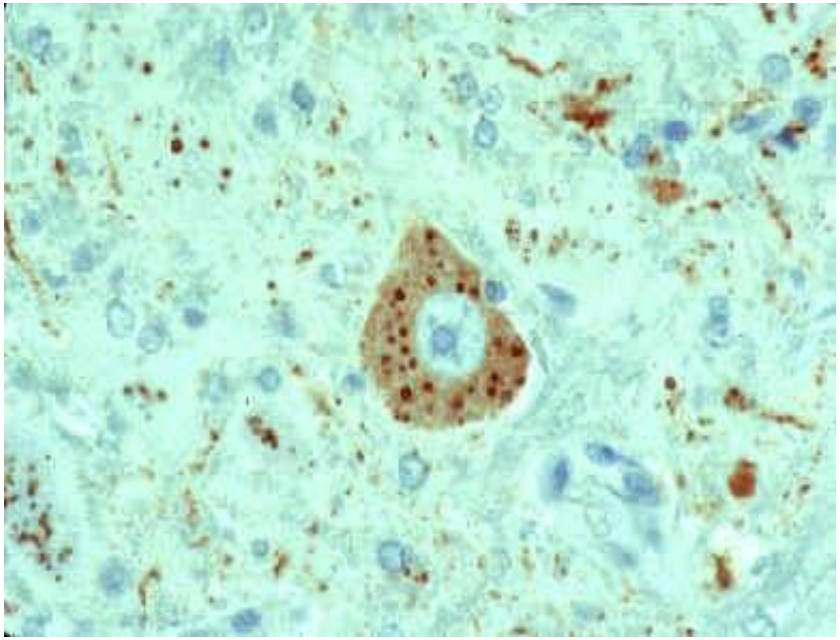
(Purplish pink with basophilic inner granule)

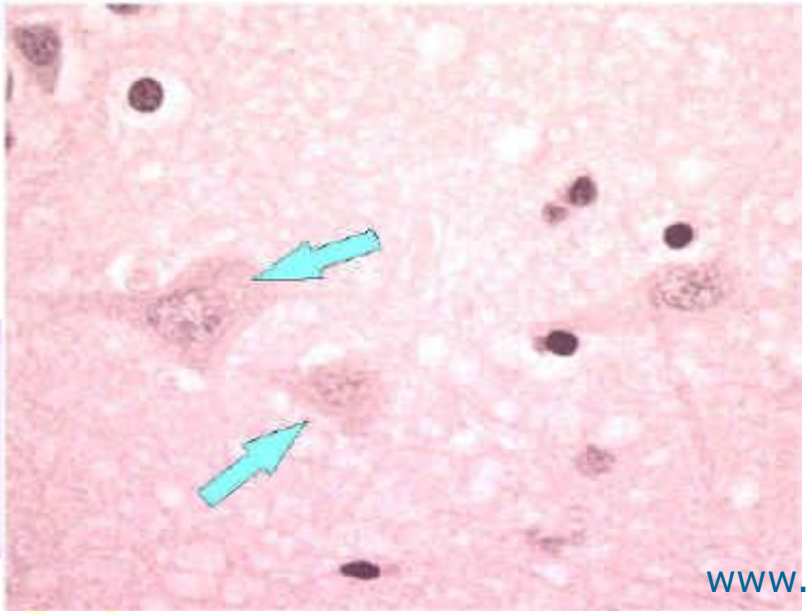
2 – 27 micro meter in diameter

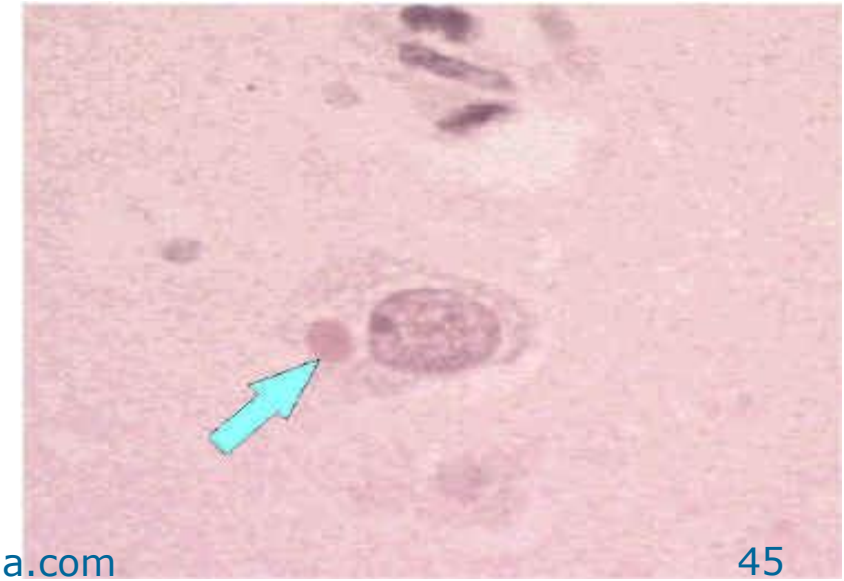
Round / oval

Used as lab diagnosis procedure by detecting it in the hippocampus of the animal infected with rabies









# BACTERIOPHAGE

Virus that infect bacteria

Tadpole shaped

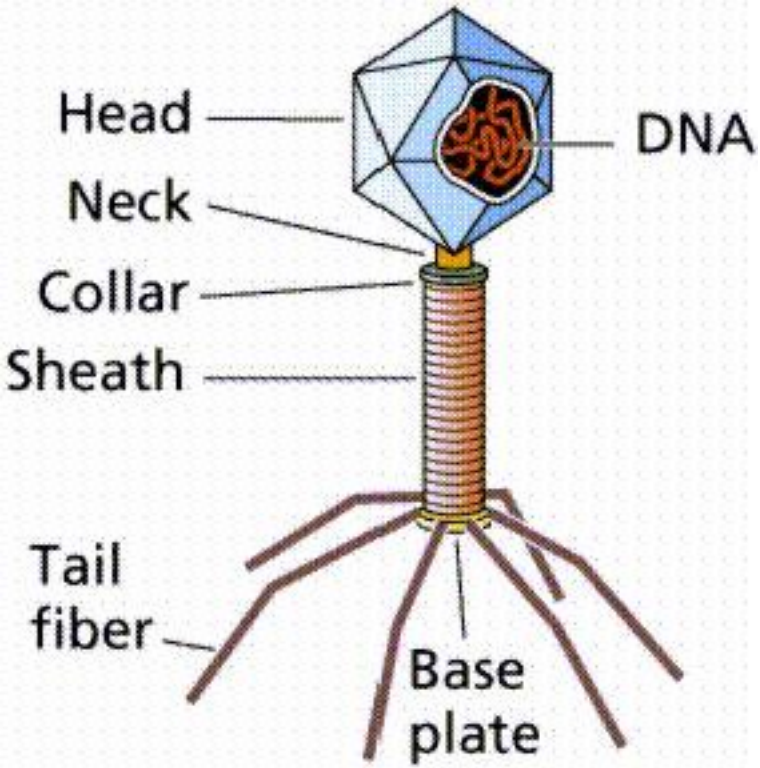
Hexagonal head & cylindrical tail

Head – Tightly packed core of nucleic acid ( double stranded DNA)

Sorrounded by capsid

Tail – Narrow core, Contractile sheath sorrounding core, Terminal base plate with tail fibres / prongs

Spherical / Filamentous with single DNA / RNA are also seen


Head
DNA
Neck
Collar
Sheath
Tail
fiber
Base
plate

Lytic / Virulent cycle

Attaches to surface of susceptible bacteria by its tail

Base plate & tail fibres held firmly against wall

Hollow core pierce cell wall

Contractile sheath contracts & injects DNA through core into bacteria

( Multiple holes produced on cellwall by large number of phage ⟶ Leakage of cell contents ⟶ Bacterial lysis)

Phage components are synthesised shutting down bacterial metabolism

Maturation

Lysis of bacterial cell & release

Lysogenic Cycle

Phage nucleic acid gets integrated with bacterial chromosomes & acts like a segment of host chromosome & replicate synchronously with it

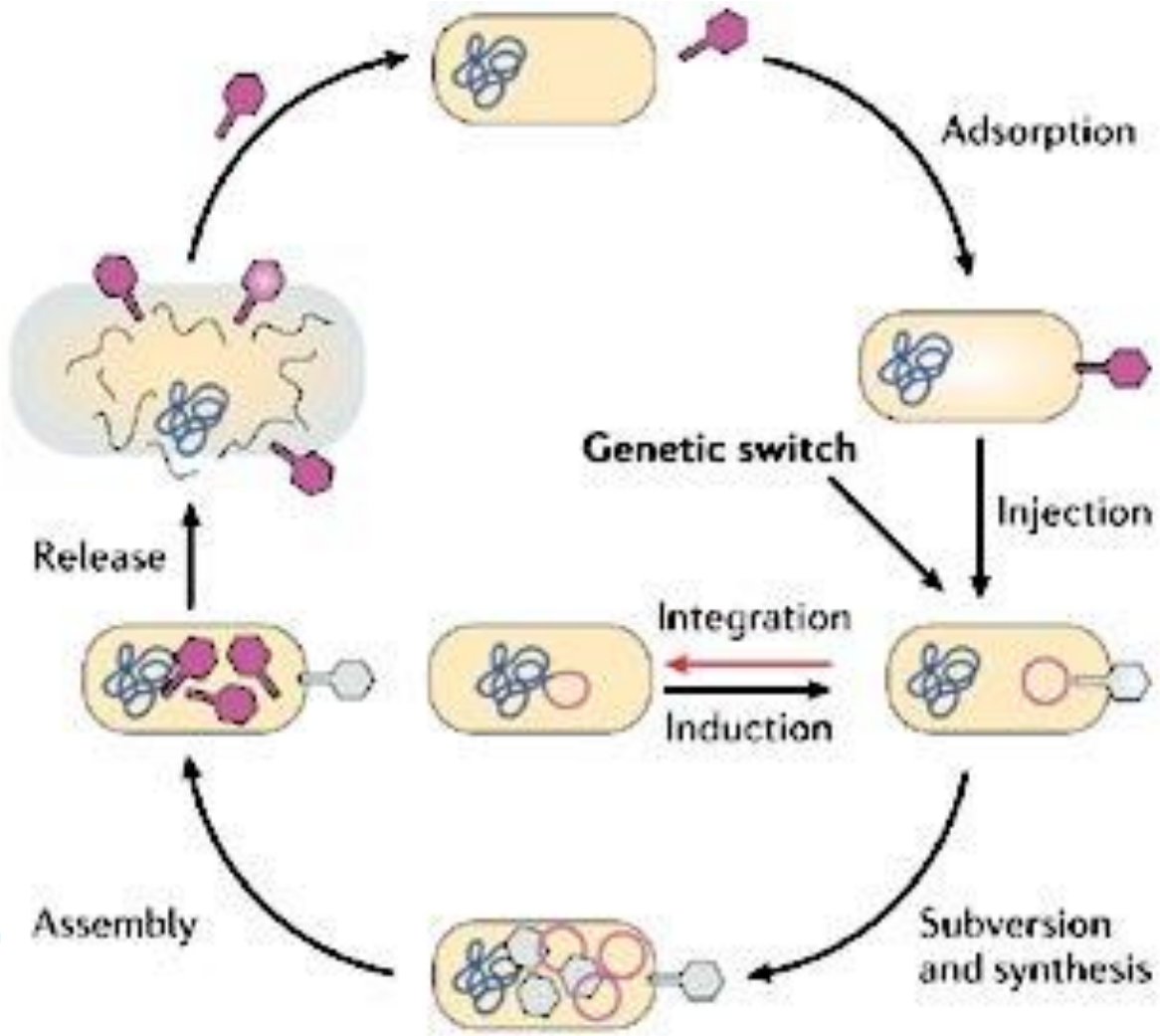
(Symbiotic relation without destroying host / upsetting bacterial metabolism)

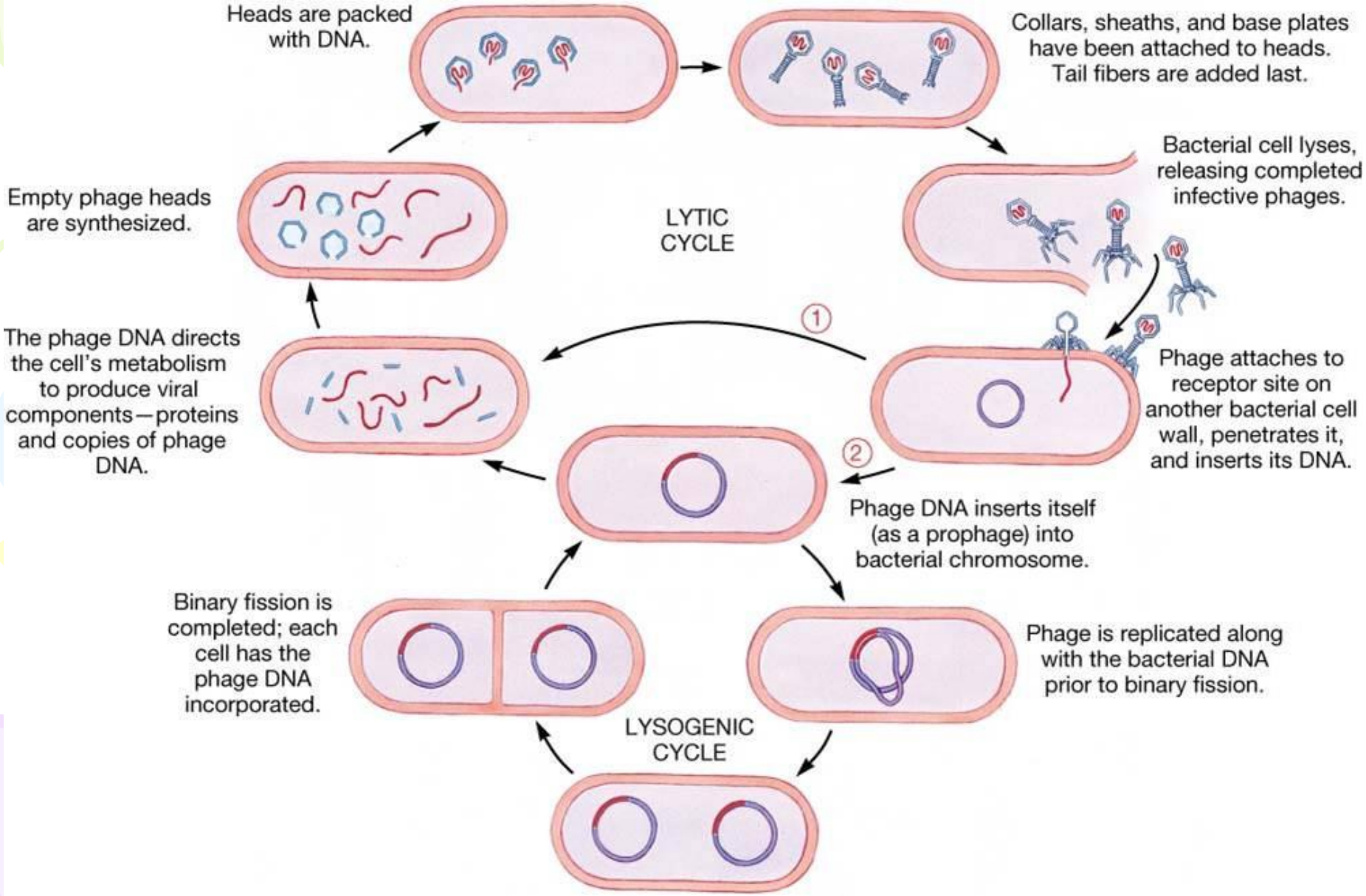
Prophage may give new properties to bacteria –

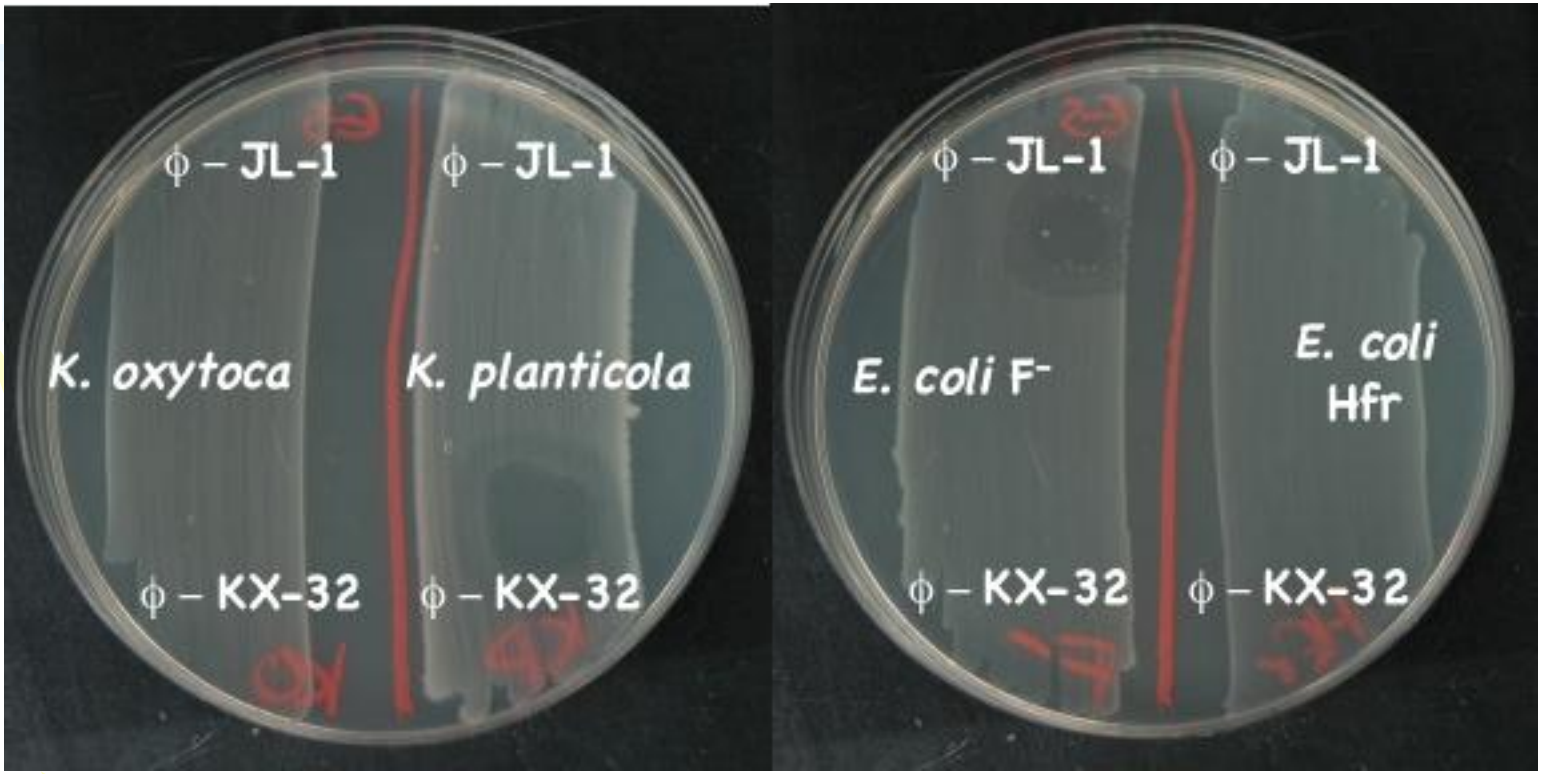
Lysogenic conversion / Phage conversion

Eg: Corynebacteria loses its toxigenecity by phage conversion

Phage typing – Identification of bacteria using phage as they have different degree of host specificity




Heads are packed with DNA.
Collars, sheaths, and base plates have been attached to heads. Tail fibers are added last.
Bacterial cell lyses, releasing completed infective phages.
Empty phage heads are synthesized.
LYTIC CYCLE
The phage DNA directs the cell's metabolism to produce viral components—proteins and copies of phage DNA.
1
Phage attaches to receptor site on another bacterial cell wall, penetrates it, and inserts its DNA.
2
Phage DNA inserts itself (as a prophage) into bacterial chromosome.
Binary fission is completed; each cell has the phage DNA incorporated.
Phage is replicated along with the bacterial DNA prior to binary fission.
LYSOGENIC CYCLE


ϕ – JL-1
ϕ – JL-1
K. oxytoca
K. planticola
ϕ – KX-32
ϕ – KX-32
ϕ – JL-1
ϕ – JL-1
E. coli F⁻
E. coli Hfr
ϕ – KX-32
ϕ – KX-32