

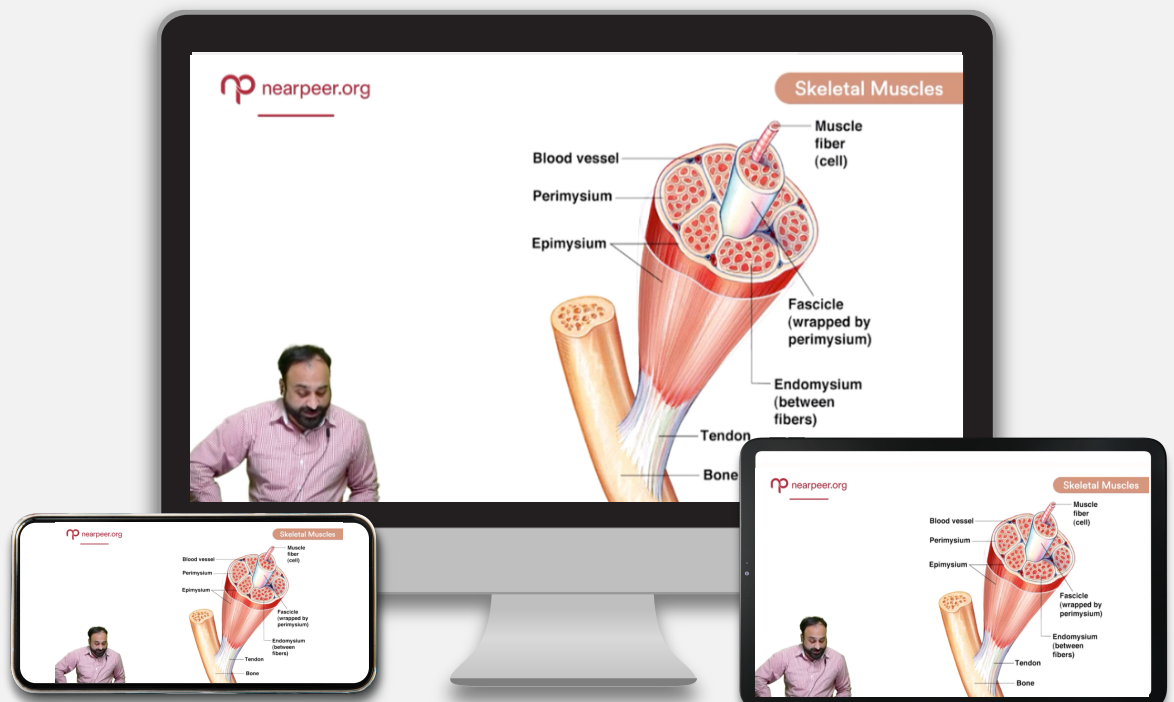
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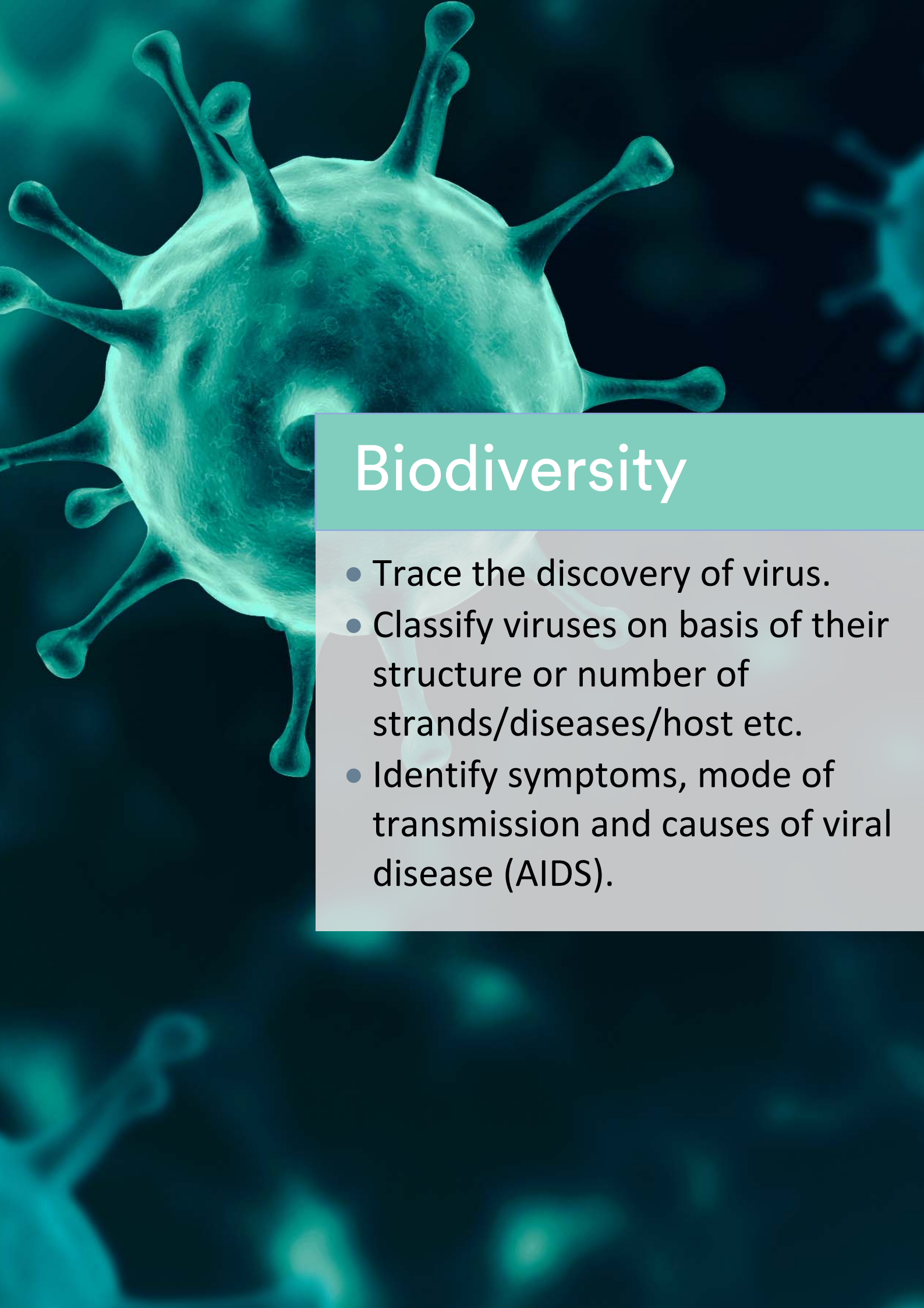
Biology

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Biodiversity

- Trace the discovery of virus.
- Classify viruses on basis of their structure or number of strands/diseases/host etc.
- Identify symptoms, mode of transmission and causes of viral disease (AIDS).

Viruses

The **word** virus is derived from Latin word 'venome' which means poisonous fluid. Viruses are: Non-cellular, submicroscopic infectious agents which contain either RNA or DNA, enclosed by proteinaceous coat, and reproduce only in living cells (obligate intracellular: parasites) Viruses use biosynthetic machinery of the host to make their materials and then transfer to their cells. Study of virus is known as **virology**.

Historical Background of Virus

Some viral diseases have been known from centuries.

The first infectious disease against which presentation was developed was a viral disease.

1. Work of Edward Jenner:

In 1796, Edward Jenner discovered an effective method for the prevention of a viral disease small pox. He removed material from cowpox lesion on the hand of milkmaid and injected into an 8 years old boy (James Phipps). After six weeks the boy was injected with pus from a small pox victim. He did not develop the disease. Jenner used material for vaccination from cowpox lesions and successfully vaccinated 23 persons. As the material was obtained from cow (called vacca in Latin), this method was named as vaccination by Louis Pasteur.

2. Work of Charles Chamberland:

Charles Chamberland (1884) found that bacteria cannot pass through porcelain filters. However, agents responsible for rabies can pass through these filters.

Any toxic substance that caused disease was called virus. These unseen filterable agents of rabies were called as filterable viruses.

Rabies is a disease which is transferred to human by bites of rabid dogs, foxes, cats, bats and other animals.

3. Work of Ivanowski:

In, 1892, Ivanowski discovered that the agent which caused tobacco mosaic disease was filterable.

He obtained bacteria free filtrate from infected plants and placed it on healthy leaves of tobacco.

The filtrate caused the disease in healthy plants.

Later these ultramicroscopic agents were also observed in victims of many diseases including foot and mouth disease (1898) and yellow fever (1901).

In 1898 the Dutchman Beijerinck formed the name 'virus' (Latin for poison) to describe the infectious nature of certain filtered plant fluids.

Although progress was made in isolating highly purified samples of viruses and in identifying them chemically as nucleoproteins (nucleic acids combined with proteins). the particles still proved mysterious because they were too small to be seen with the light microscope. They were among the first

biological structures to be studied when the electron microscope was developed in the 1930s.

Stanley (1935) crystallized the tobacco mosaic virus.

Characteristics of Viruses

Viruses are small infectious agents and can be seen under electron microscope. They have following characteristics:

1. **Size:**
They range in size from 250 nm of Pox viruses to 20 nm of Parvoviruses.
2. **Filterable:**
They are 10 to 1000 times smaller than bacteria. So they can pass through the pores of filter from which bacteria cannot pass.
3. **Obligate Intracellular Parasites:**
Viruses cannot grow on artificial media. They can reproduce in animal cells, plant cells or in microorganisms.
Here they reproduce by replication (a process by which many copies or replicas of virus are formed).
Therefore, the viruses are obligate intracellular parasites.
4. **No Metabolic Machinery:**
Viruses have no metabolic machinery for the synthesis of their nucleic acid and protein. They depend on the host cell to complete vital functions.
5. **Disease Production:**
They can cause disease in the host during reproduction.
6. **Resistant to Antibiotics:**
They are generally resistant to many antibiotics such as penicillin, streptomycin and others. Each type of virus will recognize and infect only certain types of cell. In other words, viruses are highly specific to their hosts.

Structure of Virus

Virion:

The complete, mature and infectious particle is known as virion. It has following parts:

1. **Genome:** The virions are composed of a central core of nucleic acid (DNA or RNA) which is also called genome.
2. **Capsid:** The genome is surrounded by a protein coat, the capsid.
Nucleic acid and capsid is collectively called nucleocapsid.
Capsid gives definite shape to virion.
Capsid is made up of protein subunits known as capsomeres.
The number of capsomeres varies in a particular virus.

Examples:

- 162 capsomeres in the capsid of herpes virus.
- 252 capsomeres in the capsid of adenovirus which cause some common colds.

Enveloped or Naked Virions:

- (i) In some animal viruses the Nucleocapsid is covered by a membrane called envelope. This membrane is derived from the host cell.
- (ii) The viruses which are not enveloped are known as naked virions.

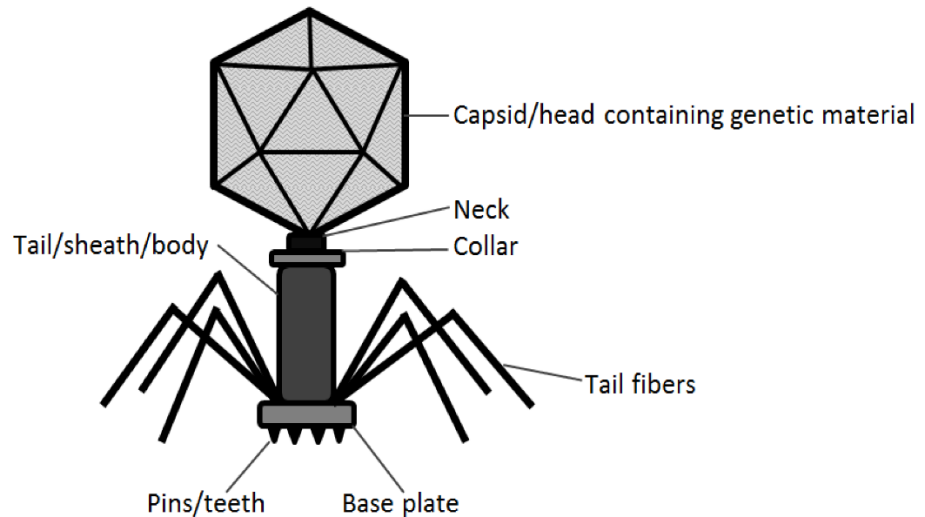


Figure 1. A bacteriophage

Shapes of Animal and Plant Viruses

Animal and plant viruses may be:

- polyhedron (having many sides)
- helical (spiral)
- enveloped or
- complex

Shapes of Bacteriophages:

These have two shapes (structural forms):

1. Cubical: These are regular solid or icosahedral (having 20 triangular faces).
2. Helical Symmetry: They are rod shaped. Many phages consist of head and tail. The heads are polyhedral but tails are rod shaped.

Structure of Bacteriophage

Bacteriophage meaning bacteria eater, earlier workers studied few phages that infect *Escherichia coli*. Of these the best phages are T phages (T for type). Two main types of T phages are T₂ and T₄.

Structure of T₄:

The structure under electron microscope looks like a tadpole and consists of head and tail.

1. Head: The head is elongated having Pyramidal (having two triangular structures with common. base), hexagonal or prism shaped structure to it straight tail is attached. Head contains double stranded DNA.
2. Tail: The structure of tail is more complex than head. Tail has a core of protein which is surrounded by a sheath of another protein. On one side of the sheath is collar and on the other side is end plate (Base plate). Six tail fibers are attached to the end plate. These fibers are for attachment.

Volume of the Phage:

It is about 1/1000 of the host.

Classification of Viruses

Different types of plant, animal and. bacterial viruses are classified on the basis of morphology, cover and nucleic acid properties.

1. Genetic Material: The genetic material may be DNA or RNA
2. Cover: The genetic material may be naked, enveloped or complex.
3. Morphology: On the basis of morphology viruses are classified into rod shaped (T.M.V), spherical (poliovirus), tadpole like (bacteriophage) and many more figure.

Retroviruses (Oncoviruses)

RNA tumor viruses are widely distributed in nature: These produces tumor in the fowls, rodents, cats and many other animals. The most familiar virus is the human immune deficiency virus (HIV) which causes acquired immune deficiency syndrome (AIDS).

1. Structure: They are single stranded RNA viruses which are spherical in shape and about 100 nm in diameter. They are enveloped by host plasma membrane.
2. Characteristics:

Retroviruses have following characteristics:

- a. Host Specificity:
 - i. A few retroviruses are non-specific and can infect any cell. Most of them infect host cell that have required receptors.
 - ii. In the case of AIDS virus, the host cell has a receptor that allows viral adsorption and penetration of many types of leukocytes (white blood cells) and tissue cells.
- b. Reverse Transcriptase:
 - i. The retroviruses have a special enzyme called reverse transcriptase. It can convert a single stranded RNA genome into double stranded viral DNA.
- c. Mode of Action:
 - i. The DNA of the retrovirus infects the host cells. They also unite with host genome as a provirus that can be passed to progeny cells. In this way some of the retroviruses can convert normal cells into cancer cells.

Diseases Caused by Viruses

1. Small Pox:

- a. Virus: It is caused by pox viruses: This virus is DNA enveloped.
- b. History: This disease occurred as epidemic in China in 12th century B.C. Until the early 20th century, it was common.
- c. Symptoms/Effects: This disease results in the formation of raised fluid-filled vesicles on the body. These become pustules later on and form pitted scars, the pocks.
- d. Prevention: By 1950's immunization and other control measures had decreased the disease. However, it is still present in the third world countries where many people are affected.

2. Herpes Simplex:

- a. Virus: It is caused by Herpes virus (DNA virus). It is naturally occurring disease of mankind.
- b. Symptoms / Effects: This disease most occurs in the mouth. on the lips, and at other skin sites. In this disease vascular lesions are formed in the epithelial layers of ectodermal tissues.

3. Influenza:

- a. Virus: Influenza viruses are enveloped RNA viruses.
- b. Epidemic Disease: It is very common in man and occurs in epidemic form.

4. Mumps and Measles:

- a. Virus: Mumps and Measles viruses belong to group paramyxoviruses. They are large, enveloped, RNA viruses.
- b. Occurrence: It is highly contagious (communicable) wide spread, but rarely fatal. About 60 of adults are immune to it.
- c. Measles is a common disease among children and adults all over the world. This disease develops immunity in its victim.

5. Polio:

- a. Virus: Poliomyelitis is caused by polio virus. It is the smallest known virus. It contains RNA in spherical capsid.
- b. Occurrence: It is present all over the world. It occurs mostly in children. The age at which primary infection occurs depends upon social and economic factors.

6. Acquired Immunodeficiency Syndrome (AIDS):

- a. Discovery: The AIDS was reported by some physicians in early 1980's in the young males. All these young patients were homosexuals.
 - i. This disease was also discovered in non-homosexual patients who were given blood (blood transfusion) or blood products.
 - ii. In 1984 the agent causing the disease was identified by research teams from Pasteur Institute in France and National Institute of health in USA.
 - iii. In 1986 the virus was named as human immunodeficiency virus (HIV).

- b. Symptoms: The patient has complex symptoms such as severe pneumonia, a rare vascular cancer, sudden weight loss, swollen lymph nodes and general loss of immune system.
- c. Pathogenicity: The major cell infected by HIV is the helper T -lymphocyte. It is the major part of the immune system.
 - i. The decrease of helper T-lymphocytes results in failure of the immune system. Now the infected person is susceptible to other diseases. Cells of central nervous system can also be infected by HIV.
- d. Host Specificity: It has been found recently that HIV infect and multiply in monkey but do not cause disease in them. It means that HIV is host specific.

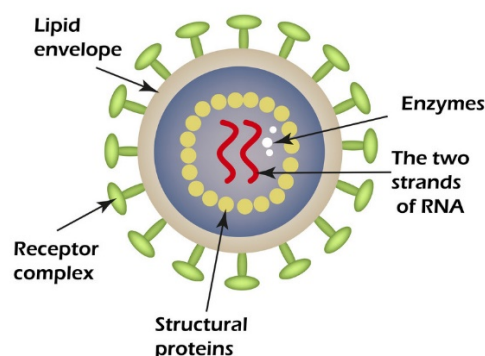


Fig. Human immunodeficiency virus (HIV)

- e. Transmission: The HIV is transmitted by:
 - (a) Sexual contact
 - (b) Contact with blood
 - (c) Breast feeding and
 - (d) Healthcare workers can also acquire HIV during professional activities.
- h. Prevention: Important preventive measure is avoiding the direct contact with HIV. Intravenous injection if drugs with common syringes must be prevented. Sterile needles/syringes and utensils must be used.
- i. Vaccination: Now the vaccine against HIV has been formed and its experimental supply to humans started in early 2001 in South Africa.

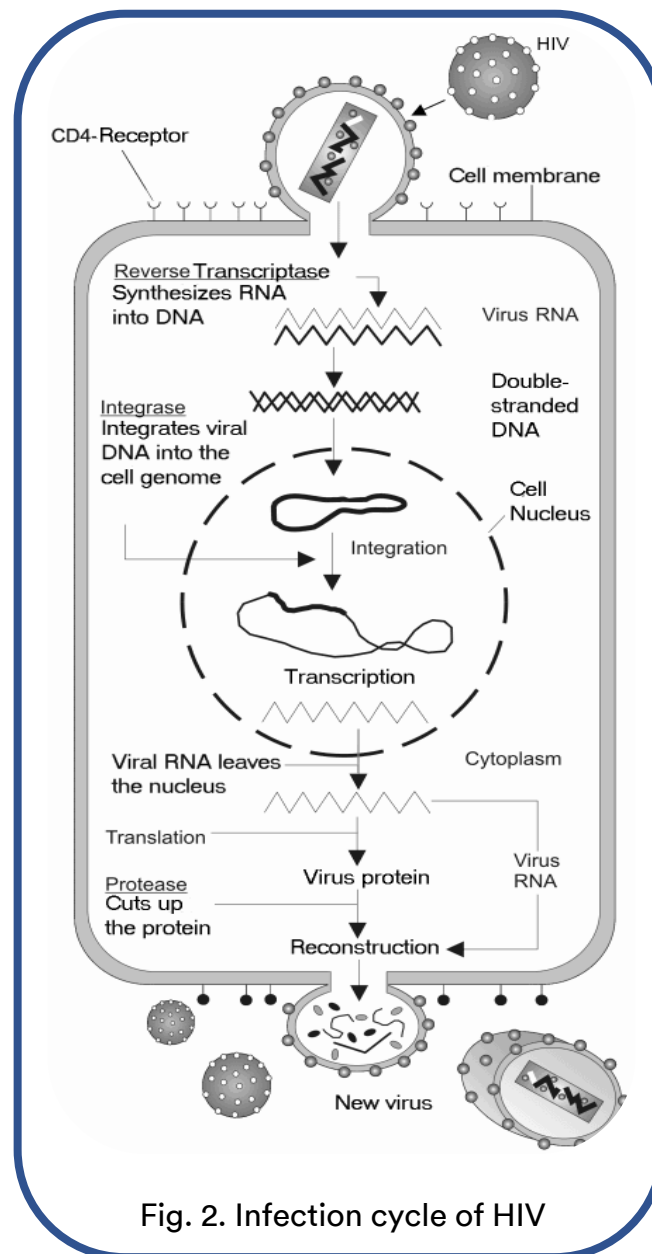


Fig. 2. Infection cycle of HIV

Hepatitis:

(i) Pathogenicity:

It is an inflammation of the liver. It is usually caused by viral infection, toxic agents or drugs.

(ii) Following are symptoms:

- | | |
|-----------------|---------------------|
| (a) Jaundice | (b) Abdominal pain |
| (d) Fatigue and | (e) Sometimes fever |

It may be mild or can be sharp and can lead to liver cancer.

(iii) Liver enlargement

Types of Hepatitis:

Following are different types of hepatitis:

Hepatitis A:

It was formerly called infectious hepatitis.

Hepatitis A virus (HAV) is an RNA virus (non-enveloped).
The disease is mild, short term and less virulent.
It is transmitted by contact with feces from infected individuals.
Vaccine is available for HAV.

Hepatitis B:

It is also called serum hepatitis. Hepatitis B (HBV) is the second major form of hepatitis.

(i) Virus:

Hepatitis B virus (HBV) is a DNA virus.

(ii) Occurrence:

It is very common in Asia, China, Philippines, Africa and the Middle east.

(iii) Transmission:

It is transmitted by the exchange of body fluids, for example blood serum, breast milk and saliva.

During birth it is transmitted from mother to child.

It is also transmitted by sexual contact.

(iv) Symptoms:

During acute attacks of Hepatitis B different symptoms appear like fatigue, loss appetite and jaundice.

Infected persons can recover completely and become immune to the virus.

People with chronic hepatitis infection are at the risk of liver damage.

Prevention:

Hepatitis can be controlled by:

Taking hygienic measures, Vaccination. (Genetically engineered vaccine is available for HBV) and screening of blood/organ/tissue of the donor.

Hepatitis C:

It was formerly called non-A. non-B hepatitis.

(i) Virus:

Hepatitis C virus (HCV) is also RNA virus (enveloped)

(ii) Symptoms / Effects:

It causes infusion hepatitis, which is less severe than hepatitis A or hepatitis B.

However, hepatitis C often leads to chronic liver disease.

(iii) Transmission:

Through blood from mother to child during pregnancy and afterward and by sexual contact.

Note: Vaccine is not available for HCV.

Hepatitis D:

It is also called as delta hepatitis.

Hepatitis E:

HEV is transmitted through the feces of an infected person.

Halbur and coworker (2001) said that hepatitis E could be transmitted through pig.

3. Hepatitis F and G:
Hepatitis F & G are caused by unidentified virus.

Prions

These are infectious proteins discovered in 1983. They contain the information that codes for their own replication. All other organisms contain their genetic information in nucleic acid (DNA or RNA).

Effects

Prions are responsible for: mad cow infection and mysterious brain infection in man.

Life Cycle of Bacteriophages

The bacteriophage replicates only inside the bacterial cell.

There are many steps in replication.

Attachment (Adsorption) of Phage to the Host Cell:

First of all, the bacteriophage attaches to the bacterial cell at receptor site. The receptor sites are present on the cell wall of bacterium.

During attachment weak chemical union occurs between virion and the receptor site.

Penetration:

- In this step the tail releases the enzyme lysozyme. This enzyme dissolves a portion of the bacterial cell wall.
- The tail sheath contracts and tail core is forced into the cell through cell wall and cell membrane.
- The virus injects its DNA into the cell (just as the syringe is used to inject the vaccine).
- The protein coat, consisting of head and tail, remains outside the cell.
- Many animal viruses enter the host cell as a whole.

After penetration one of the following cycles takes place:

- (i) Lytic cycle (ii) Lysogenic cycle

1. Lytic Cycle:

During lytic cycle following steps occur:

- (a) Multiplication: Soon after entering the bacterium, the viral DNA takes the control of the biosynthetic machinery of the host.
The host is forced to synthesize viral DNA and proteins. As a result, viruses begin to multiply.
Within 25 minutes about 200 new bacteriophages are formed.
- (b) Lysis: After the formation of bacteriophages, the bacterial cell bursts (lysis occurs).
Newly formed bacteriophages are released to infect other bacteria. A new lytic cycle may start.
The phage that causes lysis of the host cell is called lytic or virulent phage.

2. Lysogenic Cycle:

In some cases, instead of lytic cycle, lysogenic cycle takes place. It occurs as follows:

- (a) **Formation of Prophage:** The viral DNA does not take over the control of host's machinery.
The DNA is incorporated into the bacterial chromosome. Phage at this state is called prophage and this process is known as lysogeny.
The phage which causes lysogeny is called temperate (lysogenic) phage.
Lysogenic bacteria are resistant to infection by the same or related phages.
- (b) **Replication:** During lysogeny the bacterium lives and reproduces normally.
Viral DNA is the part of bacterial chromosome and passes to each daughter cell generation after generation.
- (c) **Induction:** Sometimes the viral DNA detaches from the chromosome of the host and lytic cycle starts. This process is called induction. Induction is spontaneous or environmentally induced excision of the prophage from the bacterial chromosome.

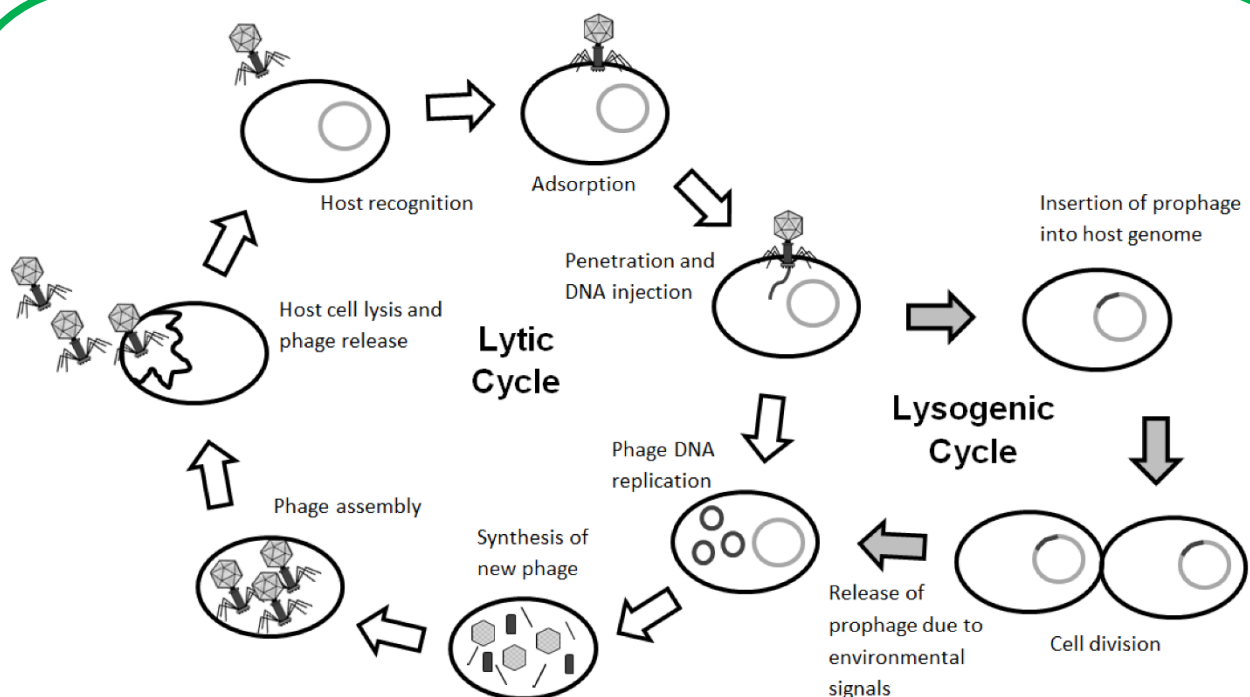


Fig. 3. Replication of a bacteriophage



Additional Notes

1. Taxonomy is the branch of biology which deals with the naming and classification of individuals.
2. The arrangement of organisms into systematic groups is called as classification.
3. More than 1.5 million species of animals and more than 0.5 million species of plants are known.
4. All organisms are related to one another at some point in their, evolutionary histories.
5. Classification is based on homologies, comparative biochemistry, cytology and genetics. However, the major base of classification is homologies.
6. A species is a group of natural population which can interbreed freely among them and produce fertile off springs, but are reproductively isolated from another such groups in nature.
7. Each species has its own structure, ecology and behavior.
8. Species is the basic unit of classification. The taxonomic groups from species to kingdom form a classification ladder.
9. During 18th century, Carious Linnaeus (1707-1778), a Swedish botanist, provided a system for naming and classifying the organisms.
10. Linnaeus published the list of names of plants in 1753. His system became popular and in 1758 he published the list of names of animals. Many of his names are in use today.
11. Linnaeus's system of giving each species a scientific name comprising two words is known as binomial nomenclature.
12. The scientific names are mostly taken from Latin word. The scientific name is Latinized or Italicized.
13. Examples of scientific names:

(i)	Onion	<i>Allium cepa</i>
(ii)	Amaltas	<i>Cassia fistula</i>
(iii)	Man	<i>Homo sapiens</i>
(iv)	Potato	<i>Solanum tuberosum</i>
(v)	Tomato	<i>Solanum esculentum</i>
14. E-Chatton (1937) described two terms:
Procariotique to describe bacteria and blue-green algae.
EU-CARITIQUE to describe animal and plant cells
15. Robert Whittekar (1969) proposed five kingdom system of classification. It is based on three levels of cellular organization (prokaryotes, Single cell Eukaryotes and Multicellular Eukaryotes) and three types of nutrition (photosynthesis, absorption and ingestion).
16. KINGDOM MONERA includes prokaryotic unicellular organisms. (bacteria and cyanobacteria).



Additional Notes

17. KINGDOM PROTISTA includes eukaryotic unicellular organisms such as Euglena and Amoeba. This kingdom also includes simple multi-cellular organisms that were directly evolved from unicellular protists. However, most protists are unicellular.
18. KINGDOM PLANTAE includes eukaryotic multi-cellular autotrophs. They prepare their food by photosynthesis. Examples are mosses, ferns, gymnosperms and angiosperms.
19. KINGDOM FUNGI are heterotrophic having absorptive method of nutrition. Most fungi are decomposers that live on organic material, secrete digestive enzymes and absorb small organic molecules which are produced by digestion. These are also called eukaryotic multi-cellular reducers (e.g. mushrooms).
20. KINGDOM ANIMALIA consists of eukaryotic, multi-cellular consumers. Animals live by ingesting and digesting food within special cavities. They can move and lack cellulose. Examples are birds and reptiles.
21. Lynn Margulis and Karlene Schwartz (1988) modified five kingdom classification of Whittaker by considering: cellular organization, mode of nutrition, cytology, genetics and organelles of symbiotic origin (mitochondria, chloroplast).
22. Binomial Nomenclature: The assignment of names to organisms using two Latin words. the first denoting the genus and the second descriptive name, the two. together constitute the name of species e.g., Homo sapiens.
23. Carcinoma: A tumor arising in one of the epithelial sheets that cover the outer and inner surfaces of the body.
24. Kingdom: The most inclusive taxonomic grouping, such as the classification of all plants into the Kingdom Plantae.
25. Lytic pathway: The serial events in which viral genes within a host cell begin to replicate independently, mature virus particles assemble and the host cell bursts, releasing the particles, which may then infect other host cells.
26. Viroid: A minute particle of RNA that lacks a protein coat and is capable of causing disease in both plants and animals.

Assessment 1

1. Most of the scientific names are:

- (a) Greek
- (b) Latin
- (c) English
- (d) Greek and Latin

2. A virion is:

- (a) Virus
- (b) Capsid
- (c) Viroid
- (d) Genome

3. Smallest virus is:

- (a) Picorna virus
- (b) Influenza virus
- (c) TMV
- (d) Wound tumour virus

4. Viral genetic material is:

- (a) RNA only
- (b) DNA or RNA
- (c) DNA only
- (d) DNA and RNA

5. A virus is obligate parasite of:

- (a) Metabolic machinery
- (b) Nucleus
- (c) Cell
- (d) Metabolic machinery and nucleus

6. RNA particles producing symptoms similar to viral disease are known as:

- (a) Viroids
- (b) Virion
- (d) All of these
- (c) Prions

7. Enzymes are present in:

- (a) Mycoplasma
- (b) Spirochaetes and slime molds
- (c) Virus
- (d) All of these

8. A phage that infects algae is:

- (a) Phycophage
- (b) Virulent phage
- (c) Temperate phage
- (d) Viroids

9. Influenza virus contains:

- (a) RNA
- (b) Proteins
- (c) DNA
- (d) Both DNA and RNA

10. Hydrophobia is caused by:

- (a) Diffugia
- (b) Virus
- (c) Mycoplasma
- (d) Bacteria

Assessment 2

1. How does a T phage enters its DNA in its host?
(a) Tail piece has a TP (b) Injection like process
(c) Contraction of protein (d) All of these
2. Virus that infects Escherichia coli is called:
(a) Viroid (b) Prions
(c) Bacteriophage (d) Phycophage
3. The virus responsible for AIDS is a kind of:
(a) Mosaic virus (b) Retrovirus
(c) Immuno virus (d) Viroid
4. Capsomeres in TMV are made up of:
(a) RNA only (b) RNA and proteins
(c) Amino acids (d) Proteins and DNA
5. A process absent in virus is:
(a) Mutation (b) Replication
(c) Energy production (d) All of these
6. Genetic transfer through virus is called:
(a) Transduction (b) Conjugation
(c) Transformation (d) Transposition
7. What is the most effective protection against viral infections?
(a) Disinfectants (b) Natural immunity of the host
(c) Antibiotic (d) Microbistatics
8. Bacteriophages were discovered first time in 1915 by:
(a) Twort (b) Ivanowsky
(c) D. Herelle (d) Stanley
9. Smallest disease causing agent in plants:
(a) Virion (b) Prions
(c) Viroids (d) Mycoplasma
10. Who made modification in five kingdom classification system?
(a) Margulis and Schwartz (b) Linnaeus
(c) Robert Whittacker (d) Ernst Hackel

Assessment 3

1. The shape of bacteriophages:

- (a) Cubical
- (b) Cubical and helical
- (c) Helical and polyhedron
- (d) Cubical and polyhedron

2. Halbur and Coworker (2001) said that hepatitis _____ could be transmitted through pig.

- (a) E
- (b) C
- (c) B
- (d) D

3. The filterable agents were first purified in 1935, when Stanley was successful in crystallizing the viruses of:

- (a) AIDS
- (b) Tobacco mosaic
- (c) Polio
- (d) Hepatitis

4. The major cell infected by HIV is the _____ lymphocyte

- (a) Helper- T
- (b) Killer – T
- (c) B
- (d) Both T and B

5. First of all, the bacteriophage attaches to the bacterial cell at _____ site.

- (a) Receptor
- (b) Landing
- (c) Attacking
- (d) Inducing

6. The enzyme which dissolves a portion of the bacterial cell wall and is released by the tail of bacteriophages is the:

- (a) Arginase
- (b) Protease
- (c) Lysozyme
- (d) Amylase

7. The capsomeres in the capsid of herpes virus are:

- (a) 162
- (b) 262
- (c) 362
- (d) 462

8. Transfer of lysogenic into lytic is:

- (a) Isolation
- (b) Stimulation
- (c) Induction
- (d) Coordination

9. Which of the following illness is not caused by a retrovirus?

- (a) smallpox
- (b) Hepatitis B
- (c) herpes simplex
- (d) all of these

10. Who discovered in 1796 an effective method for the prevention of a viral disease small pox?

- (a) Ivanowski
- (b) Edward Jenner
- (c) Chamberland
- (d) Louis Pasteur

Assessment 4

1. Foot and mouth disease and yellow fever is caused by:

- (a) Fungus
- (b) Bacteria
- (c) Virus
- (d) Protozoan

2. Most commonly herpes simplex occurs in the mouth, on the lips and at other _____ sites.

- (a) Receptor
- (b) Binding
- (c) Active
- (d) Skin

3. Which statement is true about viruses?

- (a) Each virus has both DNA and RNA in it
- (b) Most virus are obligate parasite
- (c) Viruses exhibit all of the characteristic associated with living organisms
- (d) A protein capsid surrounds the nucleic acid in each virus

4. Serum hepatitis is hepatitis:

- (a) A
- (b) B
- (c) C
- (d) D

5. Hepatitis can be controlled by:

- (a) Adopting hygienic measures
- (b) With routine vaccination
- (c) Screening of the blood/organ/tissue of the donor
- (d) All of these

6. In small pox, raised fluid filled vesicles are formed on the body, which become pustules later on and form pitted scars which are the:

- (a) Pox
- (b) Cow pox
- (c) Small pox
- (d) Pocks

7. Temperate phage may exist as:

- (a) Prophage
- (b) Viroid
- (c) Capsid
- (d) Retrovirus

8. Virus tail has:

- (a) ATP
- (b) Tail sheath and tail core
- (c) Tail fibers
- (d) All of these

9. Which hepatitis is by RNA virus which is enveloped?

- (a) E
- (b) B
- (c) C
- (d) D

10. Icosahedral means:

- (a) Less than 10 faces
- (b) 5 faces
- (c) 20 faces
- (d) More than 20 face

Key

Assessment 1

1. d
2. a
3. a
4. d
5. d
6. a
7. d
8. a
9. a
10. b

Assessment 2

1. b
2. c
3. b
4. c
5. c
6. a
7. b
8. a
9. c
10. a

Key

Assessment 3

1. b
2. a
3. b
4. a
5. a
6. c
7. a
8. c
9. d
10. b

Assessment 4

1. c
2. d
3. b
4. b
5. d
6. d
7. a
8. d
9. c
10. c