

## Lab-1

Virus:- particles composed of core which contains either DNA or RNA (Not Both), covered by protein coat called capsid, some viruses have outer lip protein membrane called an envelope.

### \* properties of virus:-

- ① Don't have cytoplasm, nucleus, and mitochondria and ribosomes.
- ② Obligate intracellular parasites, must replicate within cell, because they can not generate energy or synthesize proteins.
- ③ viruses don't undergo binary fission or mitosis but one virus can replicate and produce hundreds of progeny viruses.

### Viral Nucleic acid:-

All the viruses contain one type of nucleic acid

DNA: single strand (SS)  
double strand (ds) → or

RNA: single strand (SS) ↙  
double strand (ds)

the nucleic acid may be linear or circular, divide or non divide.

### Capsid:- protein coat covered of nucleic acid

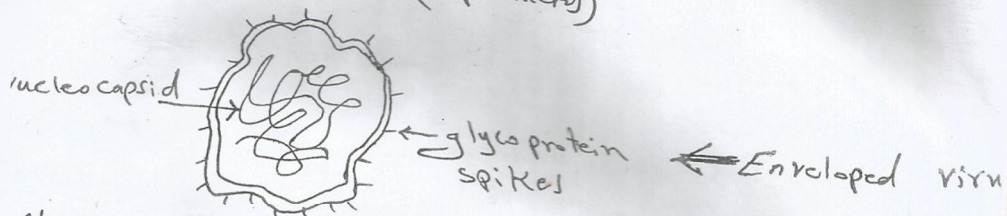
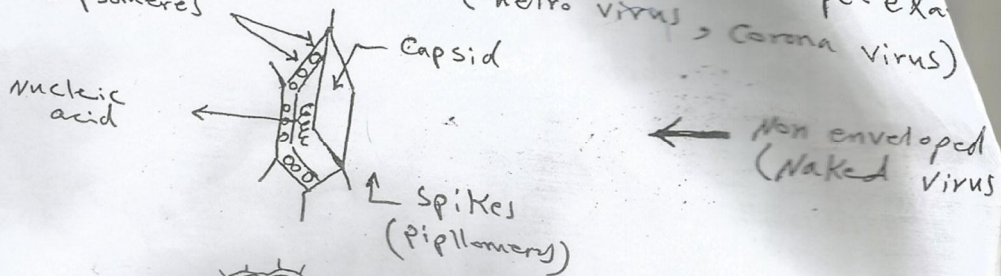
Composed of subunits which is capsomeres.

Function of capsid:-

- ① protective of nucleic acid from nucleases enzyme.
- ② play role in attachment of particle virus with the cell.
- ③ play role in transport of viral nucleic acid from one cell to another.

Nucleo Capsid:- this term called on capsid and the nucleic acid (RNA or DNA)

many viruses have a lipid component, generally the surface of the virion forming an envelope. enveloped viruses are (Retro virus, Corona virus)



### Atypical agents :-

#### \* prions :-

infection particles composed of protein coat only without presence of nucleic acid. prions resistant to inhibition by heat, u.v. ray, ion's ray, formaldehyde and nucleic acid enzymes.

- ① Transmissible spongiform encephalopathies.
- ② Mad Cow disease.
- ③ Creutzfeldt Jakob disease in human.

#### \* viriods :-

infection agent composed of nucleic acid (RNA) without protein coat. viriods considered as obligate intracellular agent and caused many of diseases in the plants.

Virion - the pathogen complete virus unit - extracell. phase.

Spikes - glycoprotein particles present in envelope ~~of some~~ surface of some particle virus such as animal bacterial virus (Bacteriophage) play role in entry of nucleic acid to infected cell because carry of lysozyme enzyme.

Capsid symmetry is the arrangement of capsomers give virus its geometric symmetry.

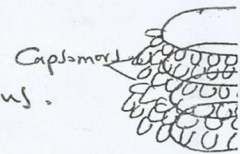
① Cubic (Icosahedral) symmetry -

The virus showed under electron microscope circular particles - such as Adeno virus, Herpes virus.



② Helical symmetry -

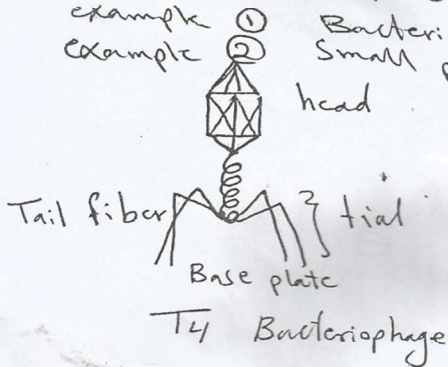
The virus showed linear such as Tobacco mosaic virus & Influenza virus.



③ Complex symmetry (Binary) -

The viruses infect bacteria such as Bacteriophage in this virus, the head cubic symmetry while the tail helical symmetry.

example ① Bacteriophage T4  
example ② Small pox virus



Small pox virus

## Method of classification the viruses

- ① Nature of host
  - ① plants viruses.
  - ② Animals viruses.
  - ③ Bacteriophage.
  - ④ Insect viruses.
  - ⑤ Fungal viruses.
- ② Type of nucleic acid.
  - ① DNA viruses (ds or ss)
  - ② RNA viruses (ds or ss)
- ③ Diseases caused or special clinical Feature.
  - ① liver viruses (example: Hepatitis B virus, enterovirus)
  - ② Salivary gland viruses (example: Mumps v., CMV)
  - ③ Gastrointestinal viruses (example: Rota virus, Adenovirus)
  - ④ sexually transmitted viruses  
(example: HSV, retro virus, papilloma virus)
- ④ Diseases of skin or mucous membranes  
(example: HSV type 1).
- ⑤ Diseases of respiratory tract.  
(example: Adeno virus, orthomyxo virus, paramyxo v.)
- ⑥ Diseases of the nervous system  
(example: polio virus, rabid virus).
- ⑦ Diseases of the eye.  
(example: Adeno virus, HSV).

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(-Lab-2-) (Lab 3)

\* Classification of viruses according to type of Nucleic acid

① ⑤

| The virus family        | Capsid      | envelope              | Nucleic acid | spike   | Genus   | the disease   |
|-------------------------|-------------|-----------------------|--------------|---------|---|---|
| 1- Adenoviridae         | Icosahedral | Non enveloped (Naked) | DS DNA       | present | Adeno virus                                     | pharyngitis, pneumonia<br>Acute respiratory   |
| 2- papillomaviridae     | Icosahedral | Non enveloped (Naked) | DS DNA       | Absent  | ① papilloma virus<br>② polyoma virus            | warts<br>warts  |
| 3) Herpes viridae       | Icosahedral | enveloped             | DS DNA       | present | ① HSV-1, V.<br>② HSV-2, V.<br>③ CMV V.<br>④ EBV | Fever blister<br>genital herpes<br>Cytomegalo virus<br>inclusion D<br>Kissing D or<br>mononucleosis |
| 1) pox viridae          | Complex     | enveloped             | ds DNA       | present | ① HAV<br>② HBV<br>③ HCV                         | Small pox (Variola)<br>liver Cancer   |
| 2) Hepadeno-<br>viridae | Icosahedral | enveloped             | ds DNA       | present | ① HAV<br>② HBV<br>③ HCV                         | liver infection<br>liver Cancer<br>liver Cancer   |

\* Classification of viruses according to type of Nucleic acid

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| The virus family   | Capsid                   | Envelope               | Multiplicity                                 | Spike  | Genus   | The disease                                      |
|--|--------------------------|------------------------|--|--|---|--|
| 1. Retroviridae  | helical                  | enveloped              | RNA → DNA<br>SS RNA<br>Reverse Transcription | present  | Retro virus<br>lentivirus   | maligant tumours and AIDS                        |
| 2. Rhabdoviridae   | helical                  | enveloped              | SS RNA                                       | present  | Rabies virus  | Rabies   |
| 3. Paramyxoviridae                                       | helical and polymorphism | envelope               | SS RNA (1 segment)                           | present  | mumps v.<br>measles v.<br>Rubella v.  | mumps<br>measles<br>Rubella<br>chickenpox        |
| 4. Antigenic shift<br>Antigenic drift<br>or<br>virulence | helical and polymorphism | envelope               | SS RNA (7-9 segments)                        | <p>① Neuraminidase (NA) :-<br/>Damaged of cell membrane + release of virus.</p> <p>② Hemagglutinin (HA) :- play role in attachment of virus with host + RBC agglutination.</p> | <p>G1 = influenza virus A<br/>G2 = influenza virus B<br/>G3 = influenza virus C</p> | <p>influenza in birds<br/>influenza in human</p> |
| 5. Reo viridae   | Icosahedral              | Non enveloped (Vaccet) | DS RNA (10 segments)                         | Absent   | G1 = Reo virus  | infant gastroenteritis<br>diarrhoea              |
| 6. Corona viridae  | helical                  | enveloped              | SS RNA (1 segment)                           | present  | G1 = Corona virus<br>G2 = SARS V  | Acute gastroenteritis<br>Respiratory infection   |

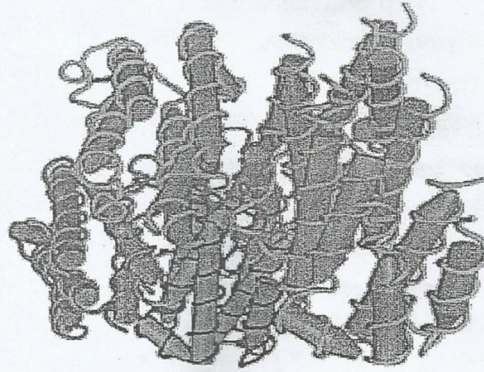
\* Classification of viruses according to type of nucleic acid

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| The virus family                                  | Capsid      | envelope                 | Nucleic acid              | Spike   | Genus  | The disease  |
|---|-------------|--------------------------|---------------------------|---------|--|--|
| 1. Bunya viridae                                  | helical     | enveloped                | SS RNA<br>(3 segments)    | Present | Bunya virus  | Chikungunya fever<br>hemorrhagic fever<br>(LCMV)       |
| 2. Picorna viridae                                | Icosahedral | Non enveloped            | SS RNA                    | Absent  | <sup>G<sup>1</sup></sup> Rhino virus<br><sup>G<sup>2</sup></sup> Polio virus<br>Coxsackie v. | encephalitis<br>poliomyelitis<br>Rhinitis              |
| 3. plant viruses<br>Tobacco mosaic virus (T.M.V.) | helical     | enveloped                | SS RNA                    | Absent  | T.M.V. virus   | Tobacco mosaic   |
| 2. Caliciviridae                                  | helical     | Non enveloped<br>(Naked) | SS RNA                    | Absent  | Calici virus   | Gastroenteritis  |
| 3. Arona viridae                                  | helical     | enveloped                | SS RNA<br>(many segments) | Absent  | G <sup>1</sup> = Arona v.<br>G <sup>2</sup> = Lassa v.                                       | Lassa fever<br>lymphocytic choriomeningitis<br>of mice |

(lab- 4)

## Interferons



The molecular structure of human interferon-alpha

(IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors.

IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes; and they increase the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection.

About ten distinct IFNs have been identified in mammals; seven of these have been described for humans. They are typically divided among three IFN classes: Type I IFN, Type II IFN, and Type III IFN. IFNs belonging to all IFN classes are very important for fighting viral infections.

### Types of interferon

Based on the type of receptor through which they signal, human interferons have been classified into three major types.

#### IFN- $\alpha$

The IFN- $\alpha$  proteins are produced by leukocytes. They are mainly involved in innate immune response against viral infection.

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## IFN- $\beta$

The IFN- $\beta$  proteins are produced in large quantities by fibroblasts. They have antiviral activity that is involved mainly in innate immune response.

### • Function

- All interferons share several common effects; they are antiviral agents and can fight tumors.
- As an infected cell dies from a cytolytic virus, viral particles are released that can infect nearby cells. However, the infected cell can warn neighboring cells of a viral presence by releasing interferon. The neighboring cells, in response to interferon, produce large amounts of an enzyme known as protein kinase R (PKR), and infected host cells.
- Another function of interferons is to upregulate major histocompatibility complex molecules, MHC I and MHC II,
- Interferons, such as interferon gamma, directly activate other immune cells, such as macrophages and natural killer cells.
- Interferons can inflame the tongue and cause dysfunction in taste bud cells, restructuring or killing taste buds entirely.

### • Induction of interferons

- Production of interferons predominantly occurs in response to microbes, such as viruses and bacteria, and their products. Binding of molecules uniquely found in microbes—viral glycoproteins, viral RNA, bacterial endotoxin (lipopolysaccharide), bacterial flagella

### • Interferon therapy

Interferon therapy is used (in combination with chemotherapy and radiation) as a treatment for many cancers. This treatment is most effective for treating hematological malignancy; leukemia and lymphomas including hairy cell leukemia, nodular lymphoma, Both hepatitis B and hepatitis C are treated with IFN- $\alpha$ , often in combination with other antiviral drugs. IFNs are mostly administered by an intramuscular injection. The injection of IFNs in the muscle, in the vein, or under skin is generally well tolerated. The most frequent adverse effects are flu-like symptoms: increased body temperature, feeling ill, fatigue, headache, muscle pain, convulsion, dizziness, hair thinning, and depression. Erythema, pain and hardness on the spot of injection are also frequently observed. IFN therapy causes immunosuppression.

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# Electron Microscopy

The electron microscope is a type of microscope that uses a beam of electrons to create an image of the specimen. It is capable of much higher magnifications and has a greater resolving power than a light microscope, allowing it to see much smaller objects in finer detail.

## Types of Electron Microscopes

All electron microscopes use electromagnetic and/or electrostatic lenses to control the path of electrons. The electron beam passes through the centre of such solenoids on its way down the column of the electron microscope towards the sample. Electrons are very sensitive to magnetic fields.

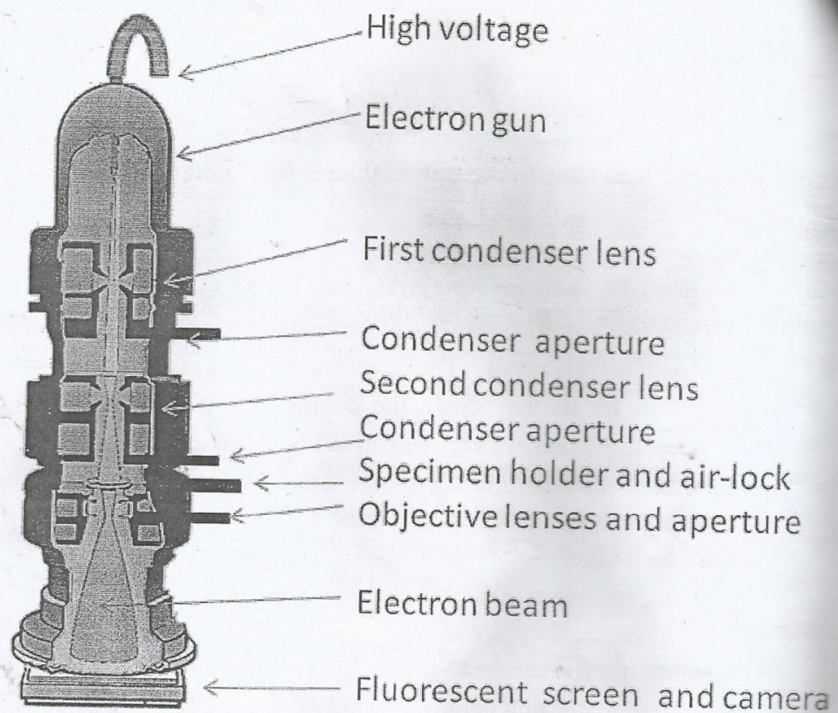
The faster the electrons travel, the shorter their wavelength. The resolving power of a microscope is directly related to the wavelength of the irradiation used to form an image.

Although, modern electron microscopes can magnify objects up to about two million times, they are still based upon the correlation between wavelength and resolution.. Researchers can use it to examine biological materials (such as microorganisms and cells), a variety of large molecules, medical biopsy samples.

## Transmission Electron Microscope (TEM)

(TEM) involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses. The electron beam that has been partially transmitted through the very thin (and so semitransparent for electrons) specimen carries information about the structure of the specimen. The spatial variation in this information (the "image") is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor such as a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer. Transmission electron microscopes produce two-dimensional, black and white images.

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Transmission Electron Microscope

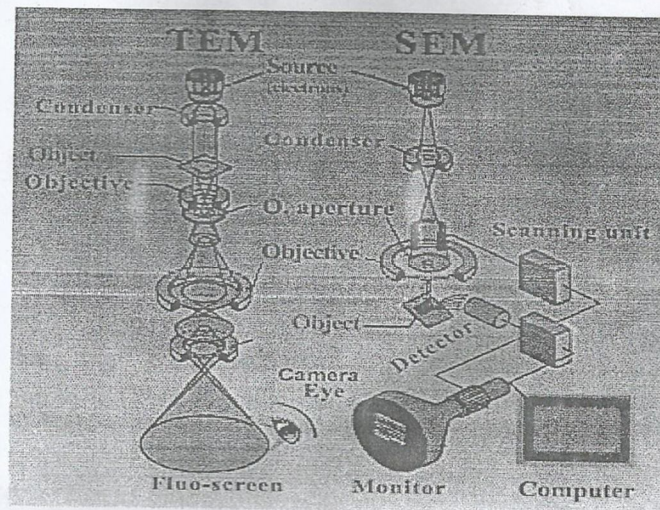
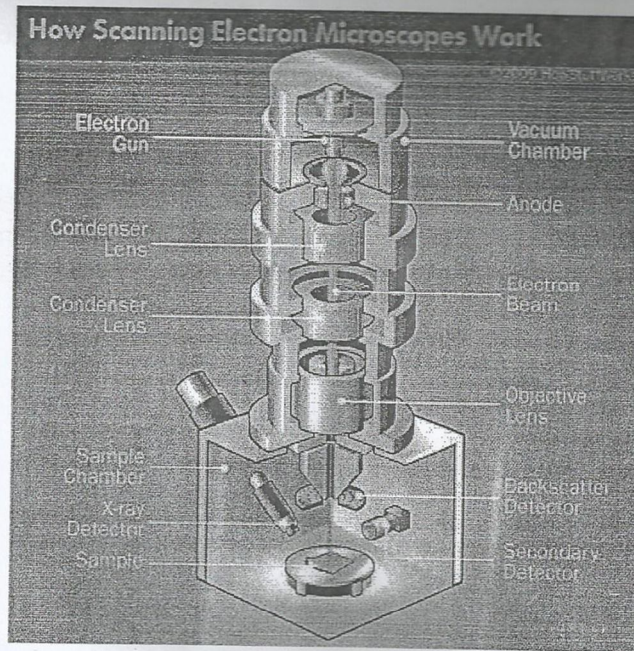
**Scanning Electron Microscope (SEM)**

Unlike the TEM, where the electrons in the primary beam are transmitted through the sample, the Scanning Electron Microscope (SEM) produces images *reflected* detecting secondary electrons which are emitted from *vel* the surface due to excitation by the primary electron beam.

TEM resolution is about an order of magnitude better than the SEM resolution. Our TEM can easily resolve details of 0.2nm. Our two SEMs at both relatively recent are high-resolution instruments capable of about 2 μm resolution on biological samples. Because the SEM image relies on electron interactions at the surface rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample. .

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### Sample Preparation

Materials to be viewed in an electron microscope generally require processing to produce a suitable sample. This is mainly because the whole of the inside of an electron microscope is under high vacuum in order to enable the electron beam to travel in straight lines. The technique required varies depending on the specimen, the analysis required and the type of microscope:

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**Cryofixation** - freezing a specimen rapidly, typically to liquid nitrogen temperatures or below, An entire field called cryo-electron microscopy has branched from this technique.

**Fixation** - a general term used to describe the process of preserving a sample at a moment in time and to prevent further deterioration so that it appears as close as possible to what it would be like in the living state, although it is now dead. In chemical fixation for electron microscopy, glutaraldehyde is often used to crosslink protein molecules and osmium tetroxide to preserve lipids.

**Dehydration** - removing water from the samples. The water is generally replaced with organic solvents such as ethanol or acetone as a stepping stone towards total drying for SEM specimens or infiltration with resin and subsequent embedding for TEM specimens.

**Embedding** - a resin (for electron microscopy) which can then be polymerised into a hardened block for subsequent sectioning.

**Sectioning** - the production of thin slices of the specimen so that they are semitransparent to electrons, typically around 90nm. These ultra-thin sections for electron microscopy are cut on an ultramicrotome with a glass or diamond knife.

**Staining** - uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to the electron beam.

**Freeze-fracture and freeze-etch** - a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. For the SEM, the sample is now ready for imaging. For the TEM, it can then be rotary-shadowed with evaporated platinum at low angle in a high vacuum evaporator. A second coat of carbon, The specimen is returned to room temperature and pressure.

**Sputter Coating** - an ultra-thin coating of electrically-conducting material, deposited by low vacuum coating of the sample. This is done to prevent charging of the specimen which would occur because of the accumulation of static electric fields due to the electron irradiation required during imaging. It also increases the amount of secondary electrons that can be detected from the surface of the sample in the SEM and therefore increases the signal to noise ratio. Such coatings include gold, gold/palladium, platinum, chromium etc.

### **Disadvantages of Electron Microscopy**

Electron microscopes are very expensive to buy and maintain. They are dynamic rather than static in their operation: requiring extremely stable high voltage

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supplies, a cooling water supply circulation through the lenses and pumps. As they are very sensitive to vibration and external magnetic fields,

The samples have to be viewed in a vacuum, as the molecules that make up air would scatter the electrons. This means that the samples need to be specially prepared by sometimes lengthy and difficult techniques to withstand the environment inside an electron microscope.

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