UNIT:II
VIRUSES

Definition: Viruses are simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein (and sometimes, in addition,substances such as lipids and carbohydrates). They can reproduce only within living cells and are obligately intracellular parasites. Viruses are cultured by inoculating living hosts or cell cultures with a virion preparation. Purification depends mainly on their large size relative to cell components, high protein content, and great stability. The virus concentration may be determined from the virion count or from the number of infectious units.

Early Development of Virology:

Although the ancients did not understand the nature of their illnesses,they were acquainted with diseases, such as rabies, that are now known to be viral in origin. In fact, there is some evidence that the great epidemics of A.D. 165 to 180 and A.D. 251 to 266, which severely weakened the Roman Empire and aided its decline, may have been caused by measles and smallpox viruses. Smallpox had an equally profound impact on the New World. Hernán Cortés's conquest of the Aztec Empire in Mexico was made possible by an epidemic that ravaged Mexico City. The virus was probably brought to Mexico in 1520 by the relief expedition sent to join Cortés. Before the smallpox epidemic subsided, it had killed the Aztec King Cuitlahuac (the nephew and son-in-law of the slain emperor, Montezuma II) and possibly 1/3 of the population. Since the Spaniards were not similarly afflicted, it appeared that God's wrath was reserved for the Native Americans, and this disaster was viewed as divine support for the Spanish conquest.

The first progress in preventing viral diseases came years before the discovery of viruses. Early in the eighteenth century, Lady Wortley Montagu, wife of the English ambassador to Turkey, observed that Turkish women inoculated their children against smallpox. The children came down with a mild case and subsequently were immune. Lady Montagu tried to educate the English public about the procedure but without great success.

Later in the century an English country doctor, Edward Jenner, stimulated by a girl's claim that she could not catch smallpox because she had had cowpox, began inoculating humans with material from cowpox lesions. He published the results of 23 successful vaccinations in 1798. Although Jenner did not understand the nature of smallpox, he did manage to successfully protect his patients from the dread disease through exposure to the cowpox virus. Until well into the nineteenth century, harmful agents were often grouped together and sometimes called viruses [Latin virus, poison or venom]. Even Louis Pasteur used the term virus for any living infectious disease agent. The development in 1884 of the porcelain bacterial filter by Charles Chamberland, one of Pasteur's collaborators and inventor of the autoclave, made possible the discovery of what are now called viruses. Tobacco mosaic disease was the first to be studied with Chamberland's filter. In 1892 Dimitri Ivanowski published studies showing that leaf extracts from infected plants would induce tobacco mosaic disease even after filtration to remove bacteria. He attributed this to the presence of a toxin. Martinus W. Beijerinck, working independently of Ivanowski, published the results of extensive studies on tobacco mosaic disease in 1898 and 1900. Because the filtered sap of diseased plants was still infectious, he proposed that the disease was caused by an entity different from bacteria, a filterable virus. He observed that the virus would multiply only in living plant cells, but could survive for long periods in a dried state.

At the same time Friedrich Loeffler and Paul Frosch in Germany found that the hoof-and-mouth disease of cattle was also caused by a filterable virus rather than by a toxin. In 1900 Walter Reed began his study of the yellow fever disease whose incidence had been increasing in Cuba. Reed showed that this human disease was due to a filterable virus that was transmitted by mosquitoes. Mosquito control shortly reduced the severity of the yellow fever problem. Thus by the beginning of this century, it had been established that filterable viruses were different from bacteria and could cause diseases in plants, livestock, and humans. Shortly after the turn of the century, Vilhelm Ellermann and Oluf Bang in Copenhagen reported that leukemia could be transmitted between chickens by cell-free filtrates and was probably caused by a virus. Three years later in 1911, Peyton Rous from the Rockefeller Institute in New York City reported that a virus was responsible for a malignant muscle tumor in chickens. These studies established that at least some malignancies were caused by viruses. It was soon discovered that bacteria themselves also could be attacked by viruses. The first published observation suggesting that this might be the case was made in 1915 by Frederick W. Twort. Twort isolated bacterial viruses that could attack and destroy micrococci and intestinal bacilli. Although he speculated that his preparations might contain viruses, Twort did not follow up on these observations. It remained for Felix d'Herelle to establish decisively the existence of bacterial viruses. D'Herelle isolated bacterial viruses from patients with dysentery, probably caused by *Shigella dysenteriae*. He noted that when a virus suspension was spread on a layer of bacteria growing on agar, clear circular areas containing viruses and lysed cells developed. A count of these clear zones allowed d'Herelle to estimate the number of viruses present (plaque assay, p. 368). D'Herelle demonstrated that these viruses could reproduce only in live bacteria; therefore he named them bacteriophages because they could eat holes in bacterial "lawns." The chemical nature of viruses was established when Wendell M. Stanley announced in 1935 that he had crystallized the tobacco mosaic virus (TMV) and found it to be largely or completely protein. A short time later Frederick C. Bawden and Norman W. Pirie managed to separate the TMV virus particles into protein and nucleic acid. Thus by the late 1930s it was becoming clear that viruses were complexes of nucleic acids and proteins able to reproduce only in living cells.

General Properties of Viruses

- **Viruses** are a unique group of infectious agents whose distinctiveness resides in their simple, acellular organization and pattern of reproduction.
- A complete virus particle or **virion** consists of one or more molecules of DNA or RNA enclosed in a coat of protein, and sometimes also in other layers. These additional layers may be very complex and contain carbohydrates,lipids, and additional proteins.
- Viruses can exist in two phases:extracellular and intracellular. Virions, the extracellular phase, possess few if any enzymes and cannot reproduce independent of living cells.
- In the intracellular phase, viruses exist primarily as replicating nucleic acids that induce host metabolism to synthesize virion components; eventually complete virus particles or virions are released.
- viruses differ from living cells in at least three ways: (1) their simple, acellular organization; (2) the presence of either DNA or RNA, but not both, in almost all virions (human cytomegalovirus has a DNA genome and four mRNAs); and (3) their inability to reproduce independent of cells and carry out cell division as procaryotes and eucaryotes do. Although bacteria such as chlamydia and rickettsia are obligately intracellular parasites like viruses, they do not meet the first two criteria.

Virus Purification and Assays

Virologists must be able to purify viruses and accurately determine their concentrations in order to study virus structure, reproduction,and other aspects of their biology. These methods are so important that the growth of virology as a modern discipline depended on their development.

Virus Purification

Purification makes use of several virus properties. Virions are very large relative to proteins, are often more stable than normal cell components, and have surface proteins. Because of these characteristics, many techniques useful for the isolation of proteins and organelles can be employed in virus isolation. Four of the most widely used approaches are (1) differential and density gradient centrifugation, (2) precipitation of viruses, (3) denaturation of contaminants, and (4) enzymatic digestion of cell constituents.

1. Host cells in later stages of infection that contain mature virions are used as the source of material. Infected cells are first disrupted in a buffer to produce an aqueous suspension or homogenate consisting of cell components and viruses.Viruses can then be isolated by **differential centrifugation,** the centrifugation of a suspension at various speeds to separate particles of different sizes . Usually the homogenate is first centrifuged at high speed to sediment viruses and other large cellular particles, and the supernatant, which contains the homogenate's soluble molecules, is discarded. The pellet is next resuspended and centrifuged at a low speed to remove substances heavier than viruses. Higher speed centrifugation then sediments the viruses. This process may be repeated to purify the virus particles further. Viruses also can be purified based on their size and density by use of **gradient centrifugation**. A sucrose solution is poured into a centrifuge tube so that its concentration smoothly and linearly increases between the top and the bottom of the tube. The virus preparation, often after purification by differential centrifugation, is layered on top of the gradient and centrifuged. The particles settle under centrifugal force until they come to rest at the level where the gradient's density equals theirs (isopycnic gradient centrifugation). Viruses can be separated from other particles only slightly different in density. Gradients also can separate viruses based on differences in their sedimentation rate (rate zonal gradient centrifugation). When this is done, particles are separated on the basis of both size and density; usually the largest virus will move most rapidly down the gradient. Viruses differ from one another and cell components with respect to either density (grams per milliliter) or sedimentation coefficient(s). Thus these two types of gradient centrifugation are very effective in virus purification.

2. Viruses, like many proteins, can be purified through precipitation with concentrated ammonium sulfate. Initially,sufficient ammonium sulfate is added to raise its concentration to a level just below that which will precipitate the virus. After any precipitated contaminants are removed, more ammonium sulfate is added and the precipitated viruses are collected by centrifugation. Viruses sensitive to ammonium sulfate often are purified by precipitation with polyethylene glycol.

3. Viruses frequently are less easily denatured than many normal cell constituents. Contaminants may be denatured and precipitated with heat or a change in pH to purify viruses. Because some viruses also tolerate treatment with organic solvents like butanol and chloroform, solvent treatment can be used to both denature protein contaminants and extract any lipids in the preparation. The solvent is thoroughly mixed with the virus preparation, then allowed to stand and separate into organic and aqueous layers. The unaltered virus remains suspended in the

aqueous phase while lipids dissolve in the organic phase. Substances denatured by organic solvents collect at the interface between the aqueous and organic phases.

4. Cellular proteins and nucleic acids can be removed from many virus preparations through enzymatic degradation because viruses usually are more resistant to attack by nucleases and proteases than are free nucleic acids and proteins. For example, ribonuclease and trypsin often degrade cellular ribonucleic acids and proteins while leaving virions unaltered.
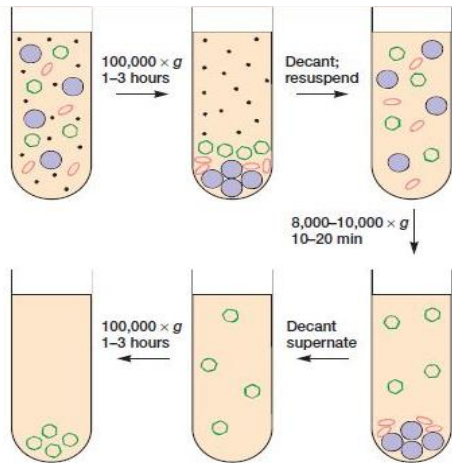
The Use of Differential Centrifugation to Purify a Virus. At the beginning the centrifuge tube contains homogenate and icosahedral viruses (in green). First, the viruses and heavier cell organelles are removed from smaller molecules. After resuspension, the mixture is centrifuged just fast enough to sediment cell organelles while leaving the smaller virus particles in suspension; the purified viruses are then collected. This process can be repeated several times to further purify the virions.
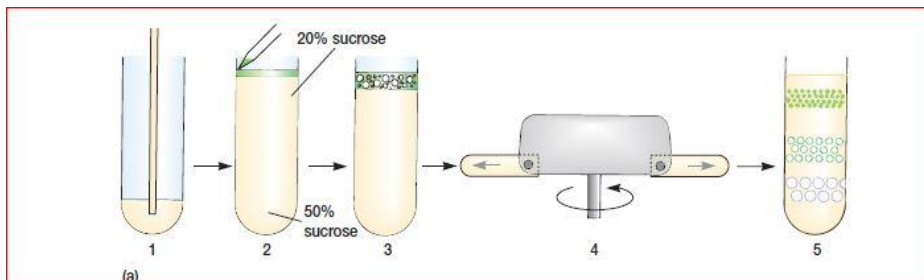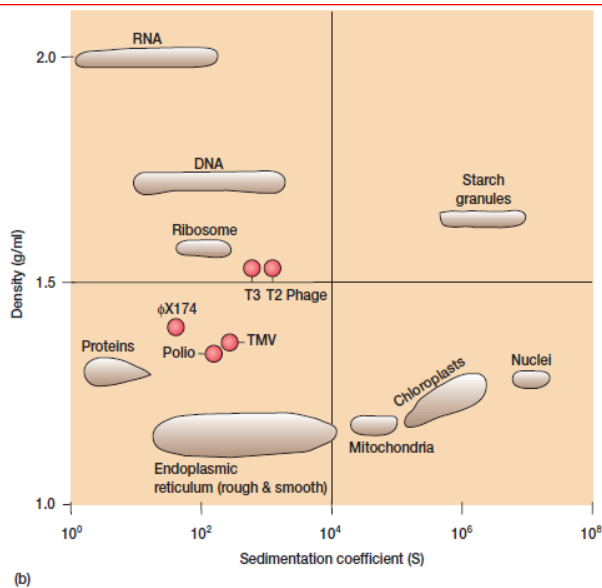
Fig: Gradient Centrifugation. (**a**) A linear sucrose gradient is prepared, *1*, and the particle mixture is layered on top, *2* and *3*. Centrifugation, *4,* separates the particles on the basis of their density and sedimentation coefficient, (the arrows in the centrifuge tubes indicate the direction of centrifugal force). *5.* In isopycnic gradient centrifugation, the bottom of the gradient is denser than any particle, and each particle comes to rest at a point in the gradient equal to its density. Rate zonal centrifugation separates particles based on their sedimentation coefficient, a function of both size and density, because the bottom of the gradient is less dense than the densest particles and centrifugation is carried out for a shorter time so that particles do not come to rest. The largest, most dense particles travel fastest. (**b**) The densities and sedimentation coefficients of representative viruses (shown in color) and other biological substances.

Virus Assays

The quantity of viruses in a sample can be determined either by counting particle numbers or by measurement of the infectious unit concentration. Although most normal virions are probably potentially infective, many will not infect host cells because they do not contact the proper surface site. Thus the total particle count may be from 2 to 1 million times the infectious unit number depending on the nature of the virion and the experimental conditions. Despite this, both approaches are of value. Virus particles can be counted directly with the electron microscope.In one procedure the virus sample is mixed with a known concentration of small latex beads and sprayed on a coated specimen grid. The beads and virions are counted; the virus concentration is calculated from these counts and from the bead concentration .This technique often works well with concentrated preparations of viruses of known morphology. Viruses can be concentrated by centrifugation before counting if the preparation is too dilute. However, if the beads and viruses are not evenly distributed (as sometimes happens), the final count will be inaccurate.

The most popular indirect method of counting virus particles is the **hemagglutination assay.** Many viruses can bind to the surface of red blood cells. If the ratio of viruses to cells is large enough, virus particles will join the red blood cells together, forming a network that settles out of suspension or agglutinates.In practice, red blood cells are mixed with a series of virus preparation dilutions and each mixture is examined. The hemagglutination titer is the highest dilution of virus (or the reciprocal of the dilution) that still causes hemagglutination. This assay

is an accurate, rapid method for determining the relative quantity of viruses such as the influenza virus. If the actual number of viruses needed to cause hemagglutination is determined by another technique, the assay can be used to ascertain the number of virus particles present in a sample.A variety of assays analyze virus numbers in terms of infectivity,and many of these are based on the same techniques used for virus cultivation. For example, in the **plaque assay** several dilutions of bacterial or animal viruses are plated out with appropriate host cells. When the number of viruses plated out are much fewer than the number of host cells available for infection and when the viruses are distributed evenly, each plaque in a layer of bacterial or animal cells is assumed to have arisen from the reproduction of a single virus particle. Therefore a count of the plaques produced at a particular dilution will give the number of infectious virions or **plaque-forming units (PFU),** and the concentration of infectious units in the original sample can be easily calculated. Suppose that 0.10 ml of a 10–6 dilution of the virus preparation yields 75 plaques. The original concentration of plaque-forming units is PFU/ml - (75 PFU/0.10 ml)(10^6) - 7.5- 10^8.Viruses producing different plaque morphology types on the same plate may be counted separately. Although the number of PFU does not equal the number of virus particles, their ratios are proportional: a preparation with twice as many viruses will have twice the plaque-forming units. The same approach employed in the plaque assay may be used with embryos and plants. Chicken embryos can be inoculated with a diluted preparation or plant leaves rubbed with a mixture of diluted virus and abrasive. The number of pocks on embryonic membranes or necrotic lesions on leaves is multiplied by the dilution factor and divided by the inoculum volume to obtain the concentration of infectious units.

Virion Size

Virions range in size from about 10 to 300 or 400 nm in diameter. The smallest viruses are a little larger than ribosomes, whereas the poxviruses, like vaccinia, are about the same size as the smallest bacteria and can be seen in the light microscope. Most viruses, however, are too small to be visible in the light microscope and must be viewed with the scanning and transmission electron microscopes.

General Structural Properties

All virions, even if they possess other constituents, are constructed around a **nucleocapsid** core (indeed, some viruses consist only of a nucleocapsid). The nucleocapsid is composed of a nucleic acid, either DNA or RNA, held within a protein coat called the **capsid,** which protects viral genetic material and aids in its transfer between host cells.

There are four general morphological types of capsids and virion structure.

1. Some capsids are **icosahedral** in shape. An icosahedron is a regular polyhedron with 20 equilateral triangular faces and 12 vertices. These capsids appear spherical when viewed at low power in the electron microscope.

2. Other capsids are **helical** and shaped like hollow protein cylinders, which may be either rigid or flexible .

3. Many viruses have an **envelope,** an outer membranous layer surrounding the nucleocapsid. Enveloped viruses have a roughly spherical but somewhat variable shape even though their nucleocapsid can be either icosahedral or

helical.

4. **Complex viruses** have capsid symmetry that is neither purely icosahedral nor helical. They may

possess tails and other structures (e.g., many bacteriophages) or have complex, multilayered walls surrounding the nucleic acid (e.g., poxviruses such as vaccinia).

Both helical and icosahedral capsids are large macromolecular structures constructed from many copies of one or a few types of protein subunits or **protomers.** Probably the most important advantage of this design strategy is that the information stored in viral genetic material is used with maximum efficiency. For example, the tobacco mosaic virus (TMV) capsid contains a single type of small subunit possessing 158 amino acids. Only about 474 nucleotides out of 6,000 in the virus RNA are required to code for coat protein amino acids. Unless the same protein is used many times in capsid construction, a large nucleic acid, such as the TMV RNA, cannot be enclosed in a protein coat without using much or all of the available genetic material to code for capsid proteins. If the TMV capsid were composed of six different protomers of the same size as the TMV subunit, about 2,900 of the 6,000 nucleotides would be required for its construction, and much less genetic material would be available for other purposes.

Nucleic Acids

Viruses are exceptionally flexible with respect to the nature of their genetic material. They employ all four possible nucleic acid types:single-stranded DNA, double-stranded DNA, single-stranded RNA, and double-stranded RNA. All four types are found in animal viruses. Plant viruses most often have single-stranded RNA genomes. Although phages may have single-stranded DNA or single-stranded RNA, bacterial viruses usually contain double-stranded DNA.

Viral Envelopes

Many animal viruses, some plant viruses, and at least one bacterial virus are bounded by an outer membranous layer called an envelope . Animal virus envelopes usually arise from host cell nuclear or plasma membranes; their lipids and carbohydrates are normal host constituents. In contrast, envelope proteins are coded for by virus genes and may even project from the envelope surface as **spikes** or **peplomers ,**These spikes may be involved in virus attachment to the host cell surface. Since they differ among viruses, they also can be used to identify some viruses. Because the envelope is a flexible, membranous structure, enveloped viruses frequently have a somewhat variable shape and are called pleomorphic. However, the envelopes of viruses like the bullet-shaped rabies virus are firmly attached to the underlying nucleocapsid and endow the virion with a constant, characteristic shape. In some viruses the envelope is disrupted by solvents like ether to such an extent that lipid-mediated activities are blocked or envelope proteins are denatured and rendered inactive. The virus is then said to be "ether sensitive."

Viral classification

The classification of viruses is in a much less satisfactory state than that of either bacteria or eucaryotic microorganisms. In part, this is due to a lack of knowledge of their origin and evolutionary history.Usually viruses are separated into several large groups based on their host preferences: animal viruses, plant viruses, bacterial viruses, bacteriophages, and so forth. In the past virologists working with these groups were unable to agree on a uniform system of classification and nomenclature. Beginning with its 1971 report, the International Committee for Taxonomy of Viruses has developed a uniform classification system and now divides viruses into three orders, 56 families, 9 subfamilies, 233 genera, and 1,550 virus species.The committee

places greatest weight on a few properties to define families: nucleic acid type, nucleic acid strandedness, the sense (positive or negative) of ssRNA genomes, presence or absence of an envelope, and the host. Virus family names end in *viridae;* subfamily names, in *virinae;* and genus (and species) names, in *virus.* For example, the poxviruses are in the family *Poxviridae;* the subfamily *Chorodopoxvirinae* contains poxviruses of vertebrates. Within the subfamily are several genera that are distinguished on the basis of immunologic characteristics and host specificity. The genus *Orthopoxvirus* contains several species, among them variola major (the cause of smallpox), vaccinia, and cowpox. Viruses are divided into different taxonomic groups based on characteristics that are related to the type of host used, virion structure and composition, mode of reproduction, and the nature of any diseases caused. Some of the more important characteristics are:

1. Nature of the host—animal, plant, bacterial, insect, fungal
2. Nucleic acid characteristics—DNA or RNA, single or
double stranded, molecular weight, segmentation and
number of pieces of nucleic acid (RNA viruses), the sense
of the strand in ssRNA viruses
3. Capsid symmetry—icosahedral, helical, binal
4. Presence of an envelope and ether sensitivity
5. Diameter of the virion or nucleocapsid
6. Number of capsomers in icosahedral viruses
7. Immunologic properties
8. Gene number and genomic map
9. Intracellular location of viral replication
10. The presence or absence of a DNA intermediate
(ssRNA viruses), and the presence of reverse
transcriptase
11. Type of virus release
12. Disease caused and/or special clinical features, method of transmission

REFERENCE:

Prescott, Harley, and Klein's Microbiology. Seventh Edition, 2008

R. P. Singh, Microbiology, 2016