NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 415

COURSE TITLE: VIROLOGY AND TISSUE CULTURE

COURSE GUIDE

BIO 415
VIROLOGY AND TISSUE CULTURE

Course Team Dr. O.H. Adekanmbi (Course Developer/ Writer) - University of Lagos
Prof. J.E. Okpuzor (Course Editor) - University of Lagos
Prof. A. Adebanjo (Programme Leader) - NOUN
Adams E. Abiodun (Course Coordinator) - NOUN

NATIONAL OPEN UNIVERSITY OF NIGERIA

National Open University of Nigeria
Headquarters
14/16 Ahmadu Bello Way
Victoria Island
Lagos

Abuja Office
No. 5 Dar es Salaam Street
Off Aminu Kano Crescent
Wuse II, Abuja

e-mail: centralinfo@nou.edu.ng
URL: www.nou.edu.ng

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INTRODUCTION

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The course guide tells you briefly what the course is all about, what course materials you will be using and how you can work your way through these materials. It also gives you some guidance on your Tutor-Marked Assignments (TMAs).

There are Self-Assessment Exercises (SAEs) at the end of each unit; these exercises are overview of the units which is designed to help you assess yourself at the end of every unit.

WHAT YOU WILL LEARN IN THIS COURSE

This course contains 15 study units which cover viruses, classification of viruses, structure of viruses, how to study viruses, case study of some viruses, viral tissue culture amongst others.

COURSE AIMS

This course aims at providing you with a broad knowledge of the concept of viruses, their replication, classification, identification, mode of transmission, diagnosis of viral infections, control and treatment of viral diseases, case study of some viruses and ethics in virology laboratory.

COURSE OBJECTIVES

In addition to the aim of this course, the course sets an overall objective which must be achieved. In addition to the course objectives, each of the units has its own specific objectives. You are advised to read properly the specific objectives for each unit at the beginning of that unit. This will help you to ensure that you achieve the objectives. As you go through each unit, you should from time to time go back to these objectives to ascertain the level at which you have progressed.

By the time you have finished going through this course, you should be able to:

- highlight and describe the general characteristics of viruses, structure of viruses, viral classification, viral taxonomy, viral replication
- explain the mode of transmission of viruses and diagnosis of

viral infections
- disuss the control and treatment of viral diseases
- identify viruses and molecular techniques used in virology
- explore the case studies of AIDS and rabies
- understand the ethics in a virology laboratory.

WORKING THROUGH THIS COURSE

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These are:

1) Course Guide
2) Study Units

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STUDY UNITS

This course is divided into three modules with a total of 15 study units which are as follows:

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Unit 1 Introduction to Viruses
Unit 2 Viral Taxonomy
Unit 3 Classification of DNA Viruses
Unit 4 Classification of RNA Viruses I
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Unit 4 Assessing the Purity of Virions and Identification of a Viral Particle
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ASSIGNMENT FILE

The assignment file will be given to you in due course. In this file, you will find all the details of the work you must submit to your tutor for marking. The marks you obtained for these assignments will count towards the final mark for the course.

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There are two components of the assessment for this course:

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TUTOR-MARKED ASSIGNMENTS (TMAS)

The TMA is the continuous assessment aspect of your course. It accounts for 30% of the total score you will obtain in this course.

FINAL EXAMINATION AND GRADING

This course is to be concluded by an examination. The final examination constitutes 70% of the whole course. You will be adequately informed of the time of the examination. The examination will consist of questions which reflect all the basic concepts you would have learnt through the duration of the course.

COURSE MARKING SCHEME

The table below clearly shows the actual marking is broken down.

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- diagnosis of viral infections
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Abuja Office
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We wish you success.

MODULE 1

UNIT 1 INTRODUCTION TO VIRUSES

CONTENTS

1.0 INTRODUCTION

Virology is the study of viruses. Viruses are small, acellular entities, inert in the extracellular environment and depend on the machinery of a living host to reproduce. In this unit, we shall be looking at the origin of viruses, some terms normally used in virology, the general characteristics of viruses and the chemical composition of viruses.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the origin of viruses
- explain some virology terms
- highlight the general characteristics of viruses
- explain the chemical composition of viruses.

3.0 MAIN CONTENT

3.1 Origin of Viruses

Virology is defined as the study of viruses, which are small, acellular entities that usually possess only a single type of nucleic acid and that must use the metabolic machinery of a living host in order to reproduce. Viruses are inert in the extracellular environment and only come alive when in contact with a living host cell. They replicate only in the living cells. The viral nucleic acid contains all information necessary for programming infected cells to synthesise a number of virus specific macromolecules required for the production of viral progeny. The viral genome takes control of the metabolism of the host cell. Viruses are known to infect a wide range of host cells ranging from mycoplasma, bacteria, algae, invertebrates, all higher plants and animals.

3.2 Terms in Virology

Capsid: This is the protein shell or coat that encloses the nucleic acid of the virus. Empty capsids may be by-products of replicative cycle of viruses with icosahedral symmetry.

Nucleocapsid: This refers to the capsid together with the enclosed nucleic acid.

Virion: This is the entire infectious unit or the complete viral particle. The virion serves to transfer the nucleic acid from cell to cell.

Viriod: This refers to some naked genetic materials that are air-borne but lacks capsid. They also cause infection on contact with a living host cell. They mostly cause plant infections.

Envelope: This is a lipid containing membrane that surrounds some viruses. It is acquired during virus maturation by budding process through the cellular membrane. Virus enclosed glycoproteins are exposed on the surface of the envelope.

Capsomers: This refers to morphologic unit seen in the electron microscope on the surface of the icosahedral virus particle. They represent clusters of polypeptides.

Capsomeres: They are the protein building blocks that constitute the capsomers.

Defective virus: This refers to a viral particle that is functionally deficient in some aspect of viral replication.

Replication: This is the mode of multiplication of the viral particles in the host cell for continuity and maintenance of the virus in the host.
Viroplasm: A virus factory or a modified region in an infected host cell where virus replication occurs.

3.3 General Characteristics of Viruses

- All viruses are acellular organisms and are the smallest known infective agents
- They posses no organelles and are unable to make their own proteins and essential enzymes
- They contain only one type of nucleic acid DNA or RNA as their genome but never the two at a time.
- They obligate on their host for energy and replication
- They are metabolically inactive outside their host
- They do not grow in ordinary laboratory or synthetic media used for bacterial cultivation, but they require a living host such as embryo or tissue for their cultivation
- Viruses can only be studied through the aid of an electron microscope that could magnify up to ×500,000
- Viruses cannot be gram stained or stained with common laboratory stains
- Viruses are not sensitive to antibiotics.

3.4 Chemical Composition of Viruses

Chemical constituents of viruses include the following: proteins, nucleic acid, lipids and carbohydrates.

They are discussed below.

3.4.1 Viral Protein

The major purpose of the viral protein is to aid transfer of viral genome. They protect the viral genome against the action of nucleases of the host cell and participate in the attachment of the virus particle to a susceptible cell during infection. The viral capsid is made up of protein sub-units known as capsomers which are made up of capsomeres. The viral protein determines the antigenic properties of the virus. The surface glycoproteins of some viruses exhibit specificity in their activities such as the heamaglutinin on influenza virus that agglutinates the red blood cells. Some viruses carry some enzymes inside the virion which are essential for the initiation of replication in the host cell such as RNA polymerase carried by rhabdoviruses (viruses containing RNA) and

reverse transcriptase carried by retroviruses that makes a DNA copy of the viral RNA.

3.4.1 Nucleic Acid

Viruses contain only a single type of nucleic acid, DNA or RNA that encodes all the genetic information necessary for the replication of the virus. The viral nucleic acid is also referred to as the *viral genome*. The viral genome can be single or double stranded, circular, linear or segmented. The type of nucleic acid, strandedness and the molecular weight of the viruses are used in viral classification into families. The molecular weight of viruses ranges from 1.5×10^6 to 200×10^6 Daltons.

The sequence and composition of the nucleotides of each viral nucleic acid are distinctive. The G+C content of the nucleic acid is one of the important properties used in characterising a viral genome. DNA viral genome can be analysed using restriction endonucleases and using molecularly clones CAN copies of RNA and restriction maps can be derived for analysing RNA viral genomes.

3.4.2 Viral Lipids

A number of different viruses contain lipid envelopes as part of their structure. The lipid is required when the virus nucleocapsid buds through a cellular membrane during maturation. The phospholipid composition is determined by the specific type of cell membrane in the budding process. The acquisition of a lipid containing membrane is an integral step in virion morphogenesis in some virus groups. Lipid containing viruses are sensitive to ether and organic solvent.

3.4.3 Viral Carbohydrate

Virus envelopes contain glycoproteins. In contrast to the lipids they are derived from the host cell. The envelope glycoproteins are virus-coded. However, the sugars added to the virus glycoprotein reflect the host cell in which the virus is grown. It is the surface glycoproteins of the enveloped viruses that attach the virus to a target cell by interacting with cellular receptors which are also important viral antigens.

4.0 CONCLUSION

Viruses contain lipids, carbohydrate, nucleic acids and a protein coat. They require a living host cell for replication and attack wide range of organism ranging from prokaryotic bacteria to higher eukaryotes like angiosperms and chordates.

5.0 SUMMARY

- Viruses are small acellular unit that require a lining host to become living a thing.
- They contain a nucleic acid and a protein coat, they could be enveloped or not.
- They attack a wide variety of organisms and they are infective agents.

6.0 TUTOR-MARKED ASSIGNMENT

i. Draw the structure of the DNA molecule.
ii. Draw and label a diagram showing the differences between naked and enveloped virus.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 2 VIRAL TAXONOMY

CONTENTS

1.0 INTRODUCTION

In this unit, we shall be looking into viral taxonomy, factors considered in classifying viruses and viral symmetry.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the history of viral taxonomy
- explain the factors considered in viral classification and viral symmetry
- describe symmetry in virus.

3.0 MAIN CONTENT

3.1 Viral Taxonomy

Several thousands of viruses have being classified and have been found in all major groups of organisms from prokaryotes to eukaryotes. There are over 2000 descriptions on virus infection bacteria alone. Taxonomy is the science of classification, very dynamic and substitution and subject to change on the basis of available data. Significant development in the classification of viruses is documented in the reports of the International Committee on Nomenclature of Viruses (ICNV). The reports of 1971, 1976 and 1979 dealt with human, low animal, insect and bacteria viruses. The organisation has however been renamed as

International Committee on Taxonomy of Viruses (ICTV). The viruses infecting humans and vertebrate animals of importance to man are most of the virus group recognised. Many of these have now being placed in families, genera and species.

In viral taxonomy, the family name ends with the suffix "VIRIDAE", the subfamily name with d suffix "VIRINAE" and the genus name with the suffix "VIRUS".

3.2 Factors Considered in Classification of Viruses

Viruses are classified into families on the basis of various factors stated below:

- The type of nucleic acid: a virus contains either RNA or DNA. The nature of the nucleic acid, single or double stranded is important as a strategy during replication.
- The size and morphology of viruses, type of virion symmetry, number of capsomers, presence or absence of an envelope.
- Presence of specific enzymes, particularly DNA and RNA polymerases concerned with genome replication.
- Susceptibility to physical and chemical agents such as ether.
- Immunologic properties.
- Natural methods of transmission.
- Host, tissue and cell tropism.
- Pathology and inclusion body formation.
- Symptomatology: This is the oldest form of viral replication and offers certain conveniences to clinicians but not satisfactory enough for virologist because the same virus may appear in several groups if it causes more than one disease.
- Sigla formation: Abbreviation from two or more names is joined together to form one name, part of the name could be derived from the type of genome and organ affected. Examples are as shown below:

a.	PAPOVA	PA meaning Papilloma PO meaning Polyoma VA meaning Vacuolating agent
b.	PICORNA	PICO meaning Small virus RNA meaning RNA-virus

3.3 Symmetry in Virus

The uses of electron microscope and x-ray diffraction techniques have made it possible to resolve the differences in the basic morphology of viruses. Heavy metal stains e.g. potassium phosphotungstate is used in emphasising the viral surface structure. The heavy metal permeates the virus particle like a cloud and brings out the surface structure of the viruses by virtue of negative staining. There are three basic symmetries in viral morphology, namely: cubic or icosahedral symmetry, helical symmetry and binal symmetry.

3.3.1 Cubic or Icosahedral Symmetry

All cubic symmetry observed with animal viruses to date is of the icosahedral structure. Icosahedron has 20 faces (each with an equilateral triangle), 12 vertices and 5-fold, 3-fold, 2-fold axes of rotational symmetry. The overall shape of viruses having icosahedral capsid symmetry is described as spherical.

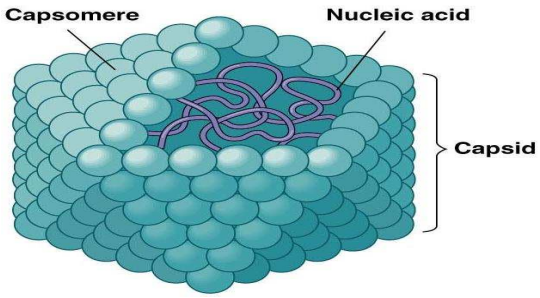

Fig. 1: Structure of an Icosahedral Virus

3.3.2 Helical Symmetry

The capsid is spiral in shape and surrounds a spiral shaped core of nucleic acid. All known examples of animal viruses with helical symmetry contain RNA genome with the exemption of rhabdoviruses. An example is the tobacco mosaic virus (TMV).

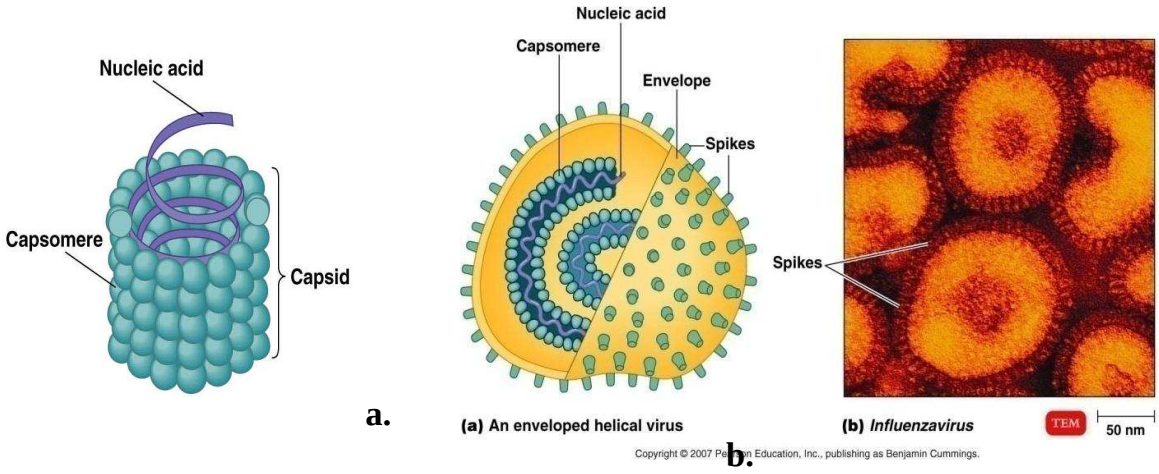

Fig. 2: Structure of a Helical Virus: a) A naked helical virus
b) An enveloped helical virus

3.3.3 Complex Structures

These are the symmetry that is neither icosahedral nor helical in nature. A good example is the pox virus whose symmetry is simply described as brick-shaped with ridges on the external surface and a core and lateral bodies.

3.3.4 Binal Symmetry

It consists of both cubical and helical symmetry e.g. T-even coliphage, a bacteriophage.

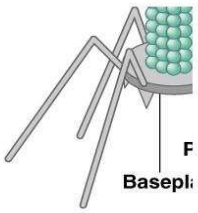

Fig. 3: Structure of T-Even Bacteriophage

4.0 CONCLUSION

Viruses are classified based on a number of factors such as type of nucleic acid, immunologic response, shape of viral genome, amongst a wide range of factors. Viral symmetry can either be icosahedral, helical, complex or binal.

5.0 SUMMARY

- Classification of viruses is documented in the reports of the International Committee on Nomenclature of Viruses (ICNV) now International Committee on Taxonomy of Viruses (ICTV).
- Several factors are considered in classifying viruses such as shape of viral genome, sensitivity of the virus to organic solvents, type of nucleic acid, symptomatology, amongst a host of others.
- Viral symmetry can either be icosahedral, helical, complex or binal.

6.0 TUTOR-MARKED ASSIGNMENT

i. An RNA virus contains the following sequence: A U C C C G A A U, What will be the sequence on the complementary strand in its DNA?
ii. What are the differences between DNA and RNA viral genomes?

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UNIT 3 CLASSIFICATION OF DNA VIRUSES

CONTENTS

1.0 INTRODUCTION

We shall be considering the classification of viruses based on the type of nucleic acids in their genome. In this unit we shall look into the various classes of DNA viruses.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the different classes of DNA viruses
- state the characteristics of DNA viruses.

3.0 MAIN CONTENT

3.1 Parvoviridae

They are very small DNA viruses with particle size of about 18-22nm in diameter with icosahedral symmetry of 32 capsomers. They are non-enveloped with single stranded DNA (ssDNA) of molecular weight (MW) of 1.5-2.0 $\times$ 10^6, G+C content of 41-53%. The physicochemical properties of the virion includes MW of 5.5 = 6.2 10^6; S_{20W} = 110-122; buoyant density of 1.39-1.42g/dm^3 in cesium chloride (CsCl). The mature virion/particle is stable in the presence of lipid solvent, pH of 3.9 and in most species at 56^0C for at least an hour.

The virion contains three polypeptides, MW of 60-90 $\times$ 10^3 and could be demonstrated in the genera parvovirus and dependovirus. Densoviruses

have four structural polypeptides. Viral replication takes place in only actively dividing cells while capsid assembly takes in the nucleus of the infected cells. Members of this group are parvovirus group (parvovirus), dependovirus (adeno-associated virus group) and insect parvovirus group (densovirus). Members of the dependovirus group require a "helper virus" (adenoviruses and herpesviruses) co-infection for efficient replication. Parvovirus replicates autonomously and preferentially encapsulates negatively sensed ssDNA. In the genera dependovirus and densovirus, the complementary positive (+ve) and negative (–ve) strands are encapsulated with about the same frequency.

3.2 Papoviridae

The name is derived from the sigla 'PA'= papilloma; PO = polyoma and VA = vacuolating agent (SV40). Members of this family are the genera polyomavirus and papillomavirus. Vrions are small in size of about 40-55nm in diameter, heat stable, ether and acid resistant. They have an icosahedral symmetry with 72 capsomers in skew arrangement, though filamentous forms occur.

The virus particle is non-enveloped containing a molecule of circular double stranded DNA (dsDNA) with molecular weight of 3-5 × 106, G+C content= 40-50%. About 6-9 polypeptides are found in the, MW of 3.82 × 102. Infections by these viruses are airborne but some forms of human papilloma virus are sexually transmitted. These agents have a slow cycle, stimulate cell DNA synthesis and replicate in them**.** They produce late and chronic infections in their natural host and all can induce tumors in some animal species.

3.3 Adenoviridae

Adenoviruses were first isolated from human adenoid tissue. Members of this family are the genera

- Mammalian Adenoviruses (Mastadenovirus), the prefix 'Mast' is derived from the Greek word 'Mastos', meaning 'breast'
- Avian Adenovirus.

Adenoviruses are non-enveloped isometric particles with icosahedral symmetry of about 70-90nm in diameter with 252 capsomers, 8-9nm in diameter. Twelve vertex capsomers (penton bases) carry one or two filamentous projections, 240 non-vertex (hexons) capsomers are different from penton bass and fibres. These fibres are glycoprotein nature. The physicochemical properties of adventure are: MW = 170 x 10^6 buoyant c1i density in CsCl = 1.32 - 1.35g/cm^3. The virion is stable on storage in frozen state, and not inactivated by lipid solvent. The Nucleic acid is a single linear molecule of DNA ct MW 20 - 25 x 106

for viruses isolated from mammalian (M) - species or 30 x 10^6 from avian (A) species. A viral coded terminal protein is covalently linked to each 5i end. The sequence of the human adenovirus 2 genome is 35,937 hp and contains an inverted terminal repetition (ITR) of 103bp. ITR's of 50-200bp's are found in all virus sequenced. G + C content varies from 48 - 61% (mastadenoviruses) and 54 -55% for aviadenoviruses.

At least 10 polypeptides with MW's of 5 - 121 x 10^3 are found in the M-Species. At least 41 types infect humans, especially in mucous membrane, and some types can persist in lymphoid tissue. Some adenoviruses cause acute respiratory diseases, phyaryngitis and conjunctivitis. Some human adenoviruses can induce tumors in new horn hamsters. There are many serotypes that infect animals. Characteristic Cytopathic Effect (CPE) without lysis occurs during multiplication in cell culture. After attachment of infectious particle to cell receptocytes by the glycoproteins, the virus gain entry into cell by endocytosis. Transcription, DNA replication, DNA replication, and virus assembly takes place in the cell nucleus.

Slow viruses are released after death of the cell. It has been observed that there is intranuclear inclusions, containing DNA, viral antigens and virions paracystailine array or otherwise. Transmission could be by direct, or in direct from throat, faeces, eye or urine, depending on the serotype.

3.4 Herpesviridae

The name was derived from the Greek word "Herpes", "herpetos" meaning creeping, or crawling creature, from the nature of herpes febrilis lesions in infected patients. There are three sub families in this family:

a) Herpes Simplex Virus group called Alpha Herpesvirinae
b) Cytomegalovirus group called Beta Herpesvirinae
c) Lymphnproliferative Virus group called Gamma Herpesvirinae.

Their host range in variable, from very wide to very narrow, e.g. warm and cold blooded vertebrates and invertebrates. The virion is 120 - 200mim in diameter and consists of 4 structural components. The capsid is 100 – 110nm in diameter, has 162capsomeres arranged as an icosahedron (150 hexameric and 12 pentameric Capsomeres). The capsomeres are hexagonal in cross section with a hole running half way down the long axis. The tegument surrounding the capsid consists of globular material which is frequently asymmetrically distributed and may be variable in amount.

They are enveloped viruses with the envelope being a bilayer membrane surrounding the tegument, and have surface projections. The core consists of a fibrillar spool on which the nucleic acid (DNA) is wrapped. The ends of the fibers are anchored to the underside of the capsid shell. Tie intact envelope is impermeable to negative stain.

The physicochemical properties of the viron are: MW is > 1000 x 10^6, buoyant density in CsCl = 1.20 – 1.29g/cm^3. Herpesviruses contain one molecule of ds DNA, 120 – 220kbp with G + C content of 35-75%. They contain more than 20 structural proteins, MW = 12000 to > 222,000. The lipid content is located in the viron envelope and probably variable. The carbohydrates are the glycoproteins in the envelope which also contain an FC-receptor. The viral DNA is transcribed in the nucleus with the mRNA being translated in the cytoplasm. The ability to infect cells building through the inner lamella of the nuclear membrane is particles are released by transport to the cell surface through modified endoplasmic reticulum (ER) in structures bounded by cytoplasmic membranes.

Herpesviridae are transmitted by contact between moist mucosal surfaces, trans-placentally, intrapartum, and breast milk, transfusions, airborne or waterborne. Herpesviruses may remain latent in their primary hosts for the lifetime of those hosts, usually in ganglial or lymphoblastoid cells. Human Herpesviruses include herpes simpler types 1 and 2 (oral and genital lesions), Varicella - Zooster Virus (Shingles and Chicken pox), cytomegalovirus (CMV), and Epstein-Barr virus (infectious mononucleosis and association with human neoplasm).

3.5 Poxviridae

The name was derived from the old English Poc, Pocc-, (Plural of Pock) meaning Pustule or Ulcer as one of the symptoms shown on infected individuals. They are large, somewhat pleomorphic, brick-shaped or ovoid virion, 230-450nm x 140-260nm, with external coat containing lipid and tubular or globular protein structures, enclosing one or two lateral bodies and a core, which contains the genome. They are enveloped viruses with some being ether resistant and some other member ether sensitive. The nucleic acid is a single molecule of ds DNA, 130 - 375 kbp with variable G+C contents. In vertebrate poxviruses, the G+C = 35-64% while it is 20% in the entmo poxviruses. More than 100 polypeptides are detected in the virion of poxviruses.

The virion core contains several enzymes concerned with transcription, and modifications of nucleic acids and proteins. The lipid content of the virion is about 4% by weight (e.g. in vaccinia virus) while the carbohydrate is about 3% by weight. They replicate in the cytoplasm

producing type B reactive occurs both with and between genera of vertebrate poxviruses. They are transmitted through airborne, contact, formites, and mechanically by arthropods. The family poxviridae is made up of 2 sub-families:

Poxviruses of vertebrates (Chordopox Virinae)

The word 'chordo' is from the word chordate. The genera in this subfamily are:

(a)	Orthopox virus	Ortho - front the Greek word 'Orthos' meaning straight, correct
(b)	Para Poxvirus	Para means 'by side of'
(c)	Avipox virus	Avi means Ave) front Latin word "AVIS"
(d)	Capripox virus	Capri from Latin word "Ceper' means Goat
(e)	Leporipoxvirus	Lepori from Latin word "Lepus, leporis" means Hare
(f)	Suipox virus	Sui from Latin word 'sus' means Swine
(g)	Molluscscipox virus	Mollusci from Latin word Molluscum means Cam or snail
(h)	Yatapox virus	'Yata' derived from the sigla Yaba poxvirus and Tanapox virus.

Entomopoxvirinae

Pox viruses infecting insects: "Entomo" was derived from Greek word 'Entomon meaning insect. This subfamily contains probably three (3) genera namely: Entomopoxvirus A, B and C.

3.6 Hepadnaviridae

The name was derived from the sigla 'HEPA' because of its affinity for liver cells i.e. heap tropism, DNA meaning the type of genome that is deoxyribonucleic acid (DNA). They are spherical in shape, 40-4nm in diameter, with no surface projections. They of which is 7nm in diameter and detergent sensitive. The nucleocapsid (icosahedral) is made up of 180 capsomeres arranged with T = 3 symmetry made up of one major, polypeptide species. The virion envelope is antigenically similar to the nucleic acid - free 22nm lipoprotein particles (HBs Ag) that occur naturally in the sera of infected patients.

The physicochemical properties are S = 280; buoyant density in CsCl = 1.24 - 1.26 g/crn^3, (surface antigen particles without core = 1.1.8g/cm.

The virus is unstable in acid pH; infectivity is retained for 6 months at 30-32°C or 1hour at 60°C.

The nucleic acid is a single circular molecule of partially ds and partially ssDNA with molecular weight of 1.6 - 106, S2 = 155; G+C content is 48%. One strand (negative sense, complementary to mRNA) is full length (3.02 - 3.32kb) and the other varies in length from 1.7 - 2.8kb. Length of clone DNA (fully double stranded) = 3.2 kbp. The virion coat is composed of following virus -coded proteins: S-proteins (P24, 0P27), M-proteins (GP33, GP36) L-proteins (P39, GP42). The virion core is composed of one major protein with molecular weight it of $22x\ 10^3$. Lipid has been demonstrated in the HBs Ag (22nm). The N-terminus of the L-proteins is myristoylated. The virions are probably derived from the ER. Carbohydrates have also been demonstrated in the 22nm HBs Ag particle and virions as N-linked glycans. HBsAg, HBcAg, HBeAg are the antigens. However, the S-proteins are sufficient to simulate protective immunity.

The two genera in this family are: Orthohepadnavirus (i.e. hepatitis-B virus group) and Avihepadnavirus (i.e. Duck hepatitis-B virus group).

4.0 CONCLUSION

DNA viruses contain DNA genome and infect mostly animal host. There are six major families as discussed in this unit.

5.0 SUMMARY

- DNA viruses contain double stranded helix in their genomes.
- They infect mostly animal cells.
- They are pathogenic to man and other vertebrates.

6.0 TUTOR-MARKED ASSIGNMENT

i. List five families of RNA viruses important to man.
ii. In a tabular form, list 10 medically important viruses and infection caused by each virus.

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UNIT 4 CLASSIFICATION OF RNA VIRUSES

CONTENTS

1.0 INTRODUCTION

In this unit, we shall continue our study on viral classification by looking into eight RNA viruses.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- highlight the different classes of RNA viruses
- state the characteristics of RNA viruses.

3.0 MAIN CONTENT

3.1 Picornaviridae

The name is derived as Time word 'PICO" meaning (micro or small). RNA is the sigla from ribonucleic acid. The family consists of five genera:

(a)	EnteroVirtis	-	Entero virus group (i.e. found in the intestine). For instance, poliovirus
(b)	Hepatovirims	-	Hepatitis-A virus group (affinity for liver)
(c)	Cardiovirus	-	EMC virus group (affecting the heart)

(d) Rhinovirus - Common cold virus group (affecting time nose)

(e) Aphthovirus - Foot and mouth disease virus group (causing vesicles in the mouth).

The virions in this family are icosahedral (T = 1) with no envelope. The core consists of RNA and a small protein 313VPg covalently linked to its 5-ends. The electron micrograph (EM) reveals almost a featureless surface with no projections. Hydrated native particles are 30nm it is from 22-30nm. The virion MW = 8-9 x 10^6: S_{20w} = 140 – 165: buoyant density in CsCl = 1.33-1.45g/cm^3 depending mainly on genus. Some species are unstable below pH6, many are less stable at low tonic strength than at high. They are insensitive to ether, chloroform, or non-ionic detergents. They are inactivated by tight when grown with or in the presence of dyes such as natural red and proflavin.

The nucleic acid is a molecule of infectious positively sensed ssRNA of MW: 2.4 – 2.7 x 10^6. A poly A tract, heterogeneous in length is transcribed onto the 3'-terminus. A protein VPq (MW=2,400) is linked covalently to the 5-terminus, capsid of 60 protein submits (protomers) each consisting of four polypeptides (three of MW=24-41x10 and one of MW=5.5 - 13.5x10) derived by cleavage of a single polyprotein. Protomers vary from 80kDa for aphthovirus to 97kDa for poliovirus and some may be incompletely cleared. The inner capsid polypeptide 1A (VP4) has a molecule of myristic acid covalently attached to the amino terminal end. Some strains of poliovirus may carry 60 of it to molecules each of a sphingosine – like molecules. Native virions are antigenically specific (designated 'N or 'D') but after gently heating, are converted to group specificity (designated 'H').

Replication of viral RNA occurs in complexes associated with cytoplasmic membranes apparently via two distinct replicative intermediates (RIs). One complex uses positive strand RNA and the other uses negative strand RNA as template. Functional proteins are mainly produced from a single large (MW=240-250x103) poly-protein by post translational cleavage. Coat protein is encoded by the 5'half; VPg, proteases and polymerases or polymerase factors are encoded downstream. Genetic recombination, complementation and phenotypic mixing occur.

Most species in this family are host-specific. The group infecting humans are rhinoviruses (more than, 100 serotypes causing common colds) and entervioruses (polio, coxsackie, and echoviruses). Those infecting animals include foot and mouth disease of cattle and encephalomy-o-carditis of rodents.

3.2 Calciviridae

These group of viruses are similar to Picornaviridae but slightly larger (about 35-39nm) in diameter. The genome is a single-stranded positively sensed RNA. They are non-enveloped viruses.

3.3 Reoviridae

The genera of this family are the reovirus group (i.e. the Orthoreovirus, Orbivirus, Coltivirus, Rotaviris, Aquareovirus); the cytoplasmic polyhedrosis virus group (cypovirus); plant reovirus subgroup 1 (phytoreovirus); plant reovirus subgroup 2 (Fijivirus) and plant reovirus subgroup 3.

The virus is an icosahedral particles with diameter range of 60-80nm. One or two outer protein coats and an inner protein coat are present. Transcriptase activity is associated with the core. The virion **molecular weight** is 120x10 buoyant density in CsCl = 1.36-1.39 g/cm^3. They are ether resistant because they are non-enveloped.

The nucleic acid is 10-12 segmented linear dsRNA with MW of 0.2-3.0 x 10^6. The total MW = 12-20x10^6 which is about 14-22% by weight of the virus particle. Each RNA segment has one open reading frame (ORE) encoding a protein requiring no further processing. About 6-10 proteins are found in the virus particle, M1vVs = 15-155x10^3 including transcriptase and messenger RNA-capping enzymes. Some of the proteins are glycosylated. Viral replication takes place in the cytoplasm with the presence of viroplasmas in the cytoplasm of infected cells. Genetic recombination occurs very efficiently by genome re-assortment.

Reoviruses of human include rotaviruses, which cause infantile gastroenteritis and have distinctive wheel-shaped appearance. Antigenically, similar reoviruses infect many animals. Orbiviruses constitute a distinct subgroup that includes Colorado tick fever virus of humans and other agents that infect plants, insects, and animals (blue-tongue of cattle and sheep).

3.4 Arboviridae

The sigla ARBO is derived from Arthropod (‘AR’) - Borne (‘BO’) viruses. An ecologic grouping of viruses with diverse physical and chemical properties. All these viruses (about 350 in number) have a complex cycle involving arthropods as vector that transmit the viruses to vertebrate hosts by their bite. Virus replication does not seem to harm the infected arthropod. Arboviruses infect humans, mammals, birds, and

snakes and use mosquitoes and tick as vectors. Human pathogens include dengue, yellow fever, encephalitis viruses, and others.

3.5 Toagaviridae

The name is derived from the Latin word 'toga' meaning "grown". Cloak"

Members of the genera in this family are:

(a) Alphavirus — Arbovirus group A
(b) Rubivirus — Rubella Virus (Rubi means Reddish)
(c) Arterivirus — Equine arteritris virus.

The virions are spherical, 60-70nrn in diameter, with an envelope tightly applied to a proven or presumed Icosahedral nucleocapsid 35-40nm in diameter. Surface projections are demonstrable in most togaviruses. The virion buoyant density in sucrose is $1.2g/cm^3$, S_{20w} = 280. The Nucleic acid is a single molecule of positively sensed ssRNA, MW= 4 x 10^6 which is 8-9% by weight of the virus. The genes for non structural proteins are located at the 5' end. The 5' terminus is capped, and the 3' end is poly adehylated, there are two or three envelope proteins one or more of which are glycosylated, and a small core protein. Members are antigenically related. The virus specific glycoproteins are inserted in the lipoprotein envelope whose lipids are cell-derived.

Replication is in the cytoplasm amid mature by budding. They infect arthropods as well as a wide range of vertebrates.

3.6 Flaviviridae

The name is derived from 'FLAVI' meaning yet low. The members of the genera of are:

(a) Flavivirus - Arhovirus group B e.g. Yellow fever virus
(b) Pestivirims - Mucosal disease virus group.
(c) Hepatitis - C virus group.

The virion is spherical in shape, 40-60nm in diameter. They are enveloped viruses, S_{20w} = 140 - 200. The nucleic acid is single molecule of infectious single stranded, positively sensed RNA. The structural and non-structural proteins are derived from the 51 - and 31- terminal sequences respectively. There are two to three membrane-associated problems and a core protein found in the virion. The membrane-associated proteins are inserted in the lipoprotein envelope whose lipids are cell derived.

Replication takes place in the cytoplasm and in association with membranes, and matures into cytoplasmic vesicles. Most members are transmitted by blood-sucking orthropods. Mature virions accumulate within cisternae of the endoplasmic reticulum.

3.7 Arenaviridae

The name 'Arena' is derived from Latin word 'arenosus' meaning 'Sandy' because of the appearance of the viral particles in EM sections. Members of this family are:

(a) Lymphocytic Chroriomenigtis (LCM) i.e. Lassa, Mobala, Mopeia and Ippy virus.
(b) Tacarbe Complex i.e. Tacaribe, Junin, Macupo, Amapari, Parana, Tamiami, Pichinde, Latino and Flexal virus.

They are enveloped viruses; spherical to pleomorphic particles, 50-300nm in diameter (usually 110-130nm). The dense lipid bi-layer envelope has surface projections 10nm long and club-shaped. Varying numbers of ribosome like particles (20-25nin diameter) that appear tree-like within the envelope. Isolated nucleocapsids vary in lengths from 450 1300nm.

The buoyant density in sucrose is 1.17 - 1.18g/cm^3 in CsCI = 1.19-1.20g/cm^3, in am idotrizoate compounds = 1.14g/cm^3 compounds = 114g, S_{20w} = 325-500. The virus is rapidly inactivated below pH5.5 and above pH 5.5, also rapidly inactivated at 56°C and by solvents. They are highly sensitive to UV and gamma radiation. The genome is two virus negative sensed specific ssRNA molecules, L and S (MWs = 2.2-2.8x10^5 and 1.1x10^6 respectively, and three RNAs of cell origin, = 28, l8 and 4-6). The nucleocapsid contain one glycosylated polypeptide (MW=63-72x10) associated with the RNA as part of RNP complex. One glycosylated polypeptide with MW=34-44x10^3 is found in all members of the family and a second glycosylated polypeptide of MW=44-72x10^3 noted II in some but not other members. At least three distinct antigenic molecules are known. Antigens on tile surface glycoprotein (MW=34-44x10) are involved in virus neutralisation. Most, if not all, arenaviruses probably have limited cell killing potential. Host range varies from animals, mammals and humans.

3.8 Coronaviridae

They have petal-shaped projections arranged in fringe like a solar corona. The name (prefix "corona") is derived from the Latin word "crown" from appearance of surface projections in negatively stained electron micrographs. The members of tile family are human

coronavirus, Mirune hepatitis virus, Porcine I Haemagglutinating encephalomyelitis virus, Porcine transmissible gastroenteritis virus, bovine coronavirus, canine coronavirus, Feline infectious peritonitis virus, Turkey, rat and rabbit coronavirus. The viral particle is spherical or pleomorphic enveloped particles, 60-220nm in diameter. There are club-shaped surface projections, 12- 24nm in length protruding from the envelope.

There is presence of ribonucleoprotein (RNA) structure seen by negative staining as helix of a 9-13nm or strands of 9nm in diameter. The buoyant density is 1.15-1.180g/cm^3 in sucrose. They are sensitive to ether, chloroform, and detergents. The envelope spikes (but not haemagglutiuin esterase protein of BCV) are removed by bromelain. The nucleic acid is a molecule of infectious unsegemented positively sensed ssRNA, MW of 9.0-11.0x 10^6. IBV genome is 27kb, MHV=33kb. The genome is adehylated at 3'-terminus but MHV genomic RNA is known to be capped at 5'-end. About three or four proteins are found in all coronaviruses:

(1) The spike (S) protein, MW = 170-220x10^3. This could be cleaved into two subunits: N-terminal (S1) and C-terminal (S2).

(2) The membrane (M) protein, MW of main species is 23-29x10^3.

(3) The Nucleocapsid (N) protein. MW 47-60x 10^3 and it is phosphorylated and associated with INA.

(4) The haemagglutinin-Esterase (HE) protein. Membrane fusion and esterase activity is associated with S and HE protein respectively. The enveloped of the viral particle is lipid in nature and s-protein is acylated. The S- and N- proteins are glycosylated. There are three or four major antigens corresponding to each virion protein. The S and HE proteins are predominant antigens involved in neutralisation.

The genomic RNA behaves as the mRNA for RNA polymerase responsible for amplification of genome and production of sub-genomic mRNAs. The virion matures in the cytoplasm by budding through the ER and Golgi membranes. There is no budding at plasmalemma.

The infection coronaviruses is restricted to natural vertebrate host and are often associated with respiratory or gastrointestinal organ. Transmission is by respiratory, facial-oral routes. Biological vectors and mechanical transmission is also possible.

4.0 CONCLUSION

RNA viruses are mostly pathogenic to plants and contain a single stranded RNA molecule in their genome.

5.0 SUMMARY

- RNA viruses are mostly pathogenic to plants.
- Some contain enzymes called reverse transcriptase, which enable them to synthesise a second strand while infecting their host.

6.0 TUTOR-MARKED ASSIGNMENT

In a tabular form, list 10 medically important viruses and their characteristics.

7.0 REFERENCES/FURTHER READING

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UNIT 5 CLASSIFICATION OF RNA VIRUSES II

CONTENTS

1.0 INTRODUCTION

We shall continue with the study of RNA viruses. The last eight groups of RNA viruses we shall be considering in this unit include the Retroviridae, a special group of RNA viruses that can make DNA from their RNA genome using the enzyme reverse transcriptase.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- state the different classes of RNA viruses
- highlight the characteristics of RNA viruses.

3.0 MAIN CONTENT

3.1 Retroviridae

They are enveloped viruses of about 90-120nm in diameter whose genome contains duplicate copies of high molecular weight, SSRNA of the same polarity as viral mRNA. The name RETRO is gotten from the presence of an enzyme **reverse transcriptase** associated with the virus genome. The enzyme is responsible for the reverse transcription of genomic RNA to DNA in infected host cells.

The virus is replicated from the integrated 'Provirus DNA' copy in infected cells. I-lost cells remain chronically infected. Leukaemia and sarcoma viruses of animal and human, loamy viruses of primates and some "slow viruses" called Lentiviruses (Visna, Maedi of Sheep) are included in this family. Retroviruses allow the identification of cellular oncogenesis. The human immunodeficiency virus (HIV) that causes AIDS in human is also a member of this viral family.

3.2 Bunyaviridae

The name was derived from "Bunyamwera" where it was first isolated. The viral genera in this family are:

1.	Bunyamwera Super Group	-	Btiniavirus
2.	Sandfly Fever & Uukuniemi Group	-	Phlehovirus
3.	Nairobi Sheep disease Group	-	Nairovirits
4.	Hantaan Group	-	Haittavirus
5.	Tomato Spotted Wilt Group	-	Tospovirus

The viral particles is spherical or pleomorphic. They are enveloped particles of about 80-100mm in diameter, with glycoprotein surface projections, ribonucleo capsids composed of three circular, helical strands, 2-2.5nm in diameter and sometimes super-coiled, 0.2-3m in length depending on the arrangement. The viral MW = 300 -400 X 10, S_{20w} =350-500, buoyant density in CsCl = 1.2g/cm^3. They are sensitive to lipid solvents and detergents.

The nucleic is made of 3 molecules; large (L), medium (M) and small (S), negatively or ambisensed SSRNA. The MW of L = 2.2 – 4.9 x 10^6 (6.5 - 14.4kb): M= 1.0 - 2.3 x 10^6 (3.2 - 6.3 kb): while 50.28 x 0.8 x 10^6 (0.8- 2.0kb). The constitute 1-2% by weight of a whole particle. Differences exist between terminal nucleotide sequences of gene segments of viruses of different genera. Ends are hydrogen bonded, RNA and nucleocapsids is circular. Usually, 4 - proteins consisting of two external glycoproteins (01 and 02), a nucleocapsid protein (N) and a Large (L) protein which is presumably a transcriptase are known.

Transcriptase activity is present in the virion. The lipid is 20-30% by weight with the lipoprotein envelope derived from host cell. The carbohydrate is 2-7% by weights of which are mainly glycoproteins and glycolipids. Haemaggatinin neutralising antigenic determinants are present on the viral glycoproteins. Complement fixing antigenic determinants is principally associated with N-protein. Cell fusion has been shown to be induced by these viruses at low PH. Most species cause CPE but Hantaviruses do not cause CPE. Some members have ion-dependent haemaglutinating activity. The virus replicate in the

cytoplasm. The host RNA sequences have been shown to prime viral mRNA synthesis. Genetic re-assortment is known for certain members. The virus mature by budding into smooth surface vesicles in or near the Golgi region but maturation at the plasma membrane has also been observed.

These viruses infect various arthropods and/or warm or cold blooded vertebrates. They are transmitted by mosquitoes, ticks, phlebotomine flies and other arthropod vectors. They have been demonstrated to be transmitted venereally. Aerosol infections also occur and could also be disseminated by avian host and, or vector movements. **No Arthropod Vector has been demonstrated in Hantavirus Transmission.** Hantaviruses are transmitted by rodents. They cause haemorrhagic fevers.

3.3 Orthomyxoviridae

The name is derived from the sigla; ORTHO from Greek 'Orthos meaning straight or correct; 'MYXO-" from Greek 'Myxa' relating to the activity of haemaglutinin and neuraminidase. Members of this family are influenza virus A, B, C. and D. The IV-D comprises the tick borne viruses, e.g. Dhori and Thogoto viruses which occasionally infect man. The Nucleocapids of helical symmetry and diameter of 9-15nm are enclosed within a lipoproteins of different sizes classes (50 - 130nm in length) with loop at each end, are extractable from virions or infected cells. The virion is pleomorphic, 20 - 120nm in diameter. Arrangement within virion uncertain, although coils of about 4 - 20 turns of a 7nm thick material are sometimes seen in partially disrupted virus. M - 1 protein is believed to form a layer inside the lipid bilayer, with HA and NA glycoproteins projecting about 10 - 14 nm from the surface.

About 500 spikes project from the surface of a spherical virion. Most are HA, with NA clusters interposed irregularly, but usually in the ratio 4 or 5 to 1 of HA and NA respectively. The HA spikes are rods, 13.5nm in length and 4nm in diameter. The NA glycoprotein has a box-shaped head, 10 x 10 x 6nm, attached to a slender stalk about l00nm long projecting from the membrane. Each NA subunit is composed of six (6) topologically identical beta sheets arranged in the formation of propeller. Cores containing MI, RNP and P-proteins may be generated by controlled chemical disruption of virions. Orthomyxoviruses are enveloped viruses.

3.4 Paramyxoviridae

The name "PARAMXYXO" is derived from the sigla PARA from Greek 'para' meaning 'by the side of' and 'myxo' from Greek 'myxa'

meaning mucus, relating to the activity of haemagglutini and neuraminidase.

There are two subfamilies:

1. Paramyxovirinae which consist of two genera:
   (a) Paratnyxovirus i.e the parainfluenza virus group
   (b) Morbillivirus i.e Mealses, Rinderpest. Canine distemper virus group.

2. Pneumorivinae which consist of a single genus i.e. the pneumovirus (The respiratory syncytial virus group). Other members of this genus are Bovine RSV, Rodent RVS and Avian RVS.

The virus particles are pleomorphic, usually roughly spherical, 150nm or more in diameter. Filamentous forms are common. They are enveloped, incorporating 2 or 3 virus glycoproteins and 1 or 2 unglycosilated proteins. There are surface projections of about 8-12nm in length, spaced 7 - 10nm apart according to genus, contain virus glycoproteins. Nucleocapsid is helical in symmetry, 13-l8nm in diameter and 5.5-7nm pitch according to genus; length up to 1µm in some genera.

The virus particle MW = > 500 x 10^6, much more for pleomorphic multiploid virions, S_{20w} at least 100; buoyant density in sucrose = 1.18 - l.20g/cm^3. They are sensitive to Lipid solvents, non-ionic detergents, formaldehyde and oxidising agents. The nucleic acid is a single molecule of non-segmented negatively sensed SSRNA of WM= 5-7 x 10^6 which is about 0.5% by weight of virus particle. The genomic size is fairly uniform 15.15kb for Newcastle disease virus, 15.222kb for human RVS, 15.285kb for sendai virus, 15.463kb for para influenza virus type 3 and 15.892 kb for measles virus. Some positive sensed template strands of RNA genome have been reported in some members thus, partial self - annealing of isolated RNA may occur.

3.5 Rhaboviridae

They are bullet shaped for those infecting vertebrates and invertebrates but are baciliform for those infecting plants. They are 100-430nm long and 45-100nm in diameter with surface projections (G-proteins), 5-10nm and long 3nm in diameter. A central axial channel is seen in thin section of the virion. There are characteristic cross-striations (spacing 4.5-5.0nm see in negatively stained and thin sectioned particles). Truncated particles of 0.1-0.5 of the length of the virus may be common except perhaps in members infecting plants. Abnormally long and double-length particles and tandem formations are sometimes observed.

The inner nucleocapsid is 50nm in diameter, with helical symmetry consisting of an RNA+N protein complex together with L- and NS-proteins, surrounded by an envelope containing M- protein. The nucleocapsid contains transcriptase activity and its infectious. It coils to a helical structure which is 20 x 700nm. The MW of virus particle is 300-1,000 x 10^6, S_{20w} = 550 - 1,000; buoyant density in CsCl = 1.19 - 1.20g/cm^3' and in sucrose, 1.17 – 1.19g/cm^3. The viral infectivity is stable at pH range 5 – 10 but rapidly X-irradiation. Since they are enveloped viruses, they are sensitive to lipid solvents.

The nucleic acid is a molecule of non-segmented, linear, negatively sensed SSRNA (non-infections). The MW of the genome is 3.5 - 4.6 x 10^6 which is about 1- 2% by weight of virus at id S_{20w} = 38 - 45. Five major polypeptides (proteins) designated as L, G, N, NS amid M for versicular stomatitis Idiana (VS-I) virus. The proteins are 65-75% by weight of the virus. Other polypeptides may be present in minor amounts. Transcriptase and other enzyme activities are present in virus. The lipid content is about 15-25% by weight of virus and the lipid composition is dependent on the host cell. The carbohydrate content is about 3% by weight of virus and is associated with surface projections, glycolipids, minor variation with the host cell type.

The genera or groups of this family rhabdoviridae are:

1. Vesiculovirus, i.e. vesicular stomatitis virus group
2. Lyssavirus, i.e. vabies virus group
3. Plant rhabdoviris group.

3.6 Toroviridae

The name 'Toro" is derived from the Latin word 'torus' meaning lowest convex melding in the base of a column. Other members are Breda virus (infecting cattle), Torovirus, infecting man and probably carnivores like mustellids. They are pleomorphic, biconcave disk, kidney, and rod-shaped viral particles of about 20-140nm diameter containing an elongated tubular capsid with helical symmetry. They are developed which hears some peplomers of spikes. The virus is table at PH 2.5 and 9.7. The buoyant density in sucrose is 1.16 - 1.17g/cm^3 and S20w = 380-400. The genome is a polyadenylated linear, non-segmented, positively segmented, positively sensed ssRNA that acts as an mRNA (infectious) of about >20kb.

Three major proteins are known in the virus particle. The nucleocapsid MW=180 x 10^3 while the envelope is 26 x 30^3 and the peplomer diner (derived from 200 x 10^3 precursor) is 80-100 x 10^3. Lipids are present in form of envelope with the glycosylated protein peplomer embedded in it

as the only carbohydrate. Replication take place in the cytoplasm with the 31-coterminal rested set of 5-subqenoinic mRNA detected. The polymerase gene contains two overlapping OREs; the downstream one expected by ribosomal frame–shifting during translation of genomic RNA.

Budding of preformed tubular capsids is through Golgi membranes and E.R. but host cell nuclear function is required. The transmission is probably via the faecal oral route.

3.7 Filoviridae

The name "FILO" is derived from Latin word 'filo' meaning 'like' relating to the morphology of the virus particle. Members are the Marburg Virus and Ebola Virus (Zaire and Sudan Biotypes). The natural reservoirs of these viruses or their source are unknown. However, monkey, mouse, Guinea Pig and hamster have been experimentally infected in the laboratory.

The virus particle is pleomorphic, appearing as a long filamentous forms (Sometimes with extensive branching) or as U-shaped, 6-shaped or circular forms. They vary greatly in length (up to 14,000nm), but of uniform diameter (80nm). Surface projections (about 7nm in length and spaced at 10nm intervals) are presented on the virion. Virions purified by rate-zonal-gradient centrifugation are infectious, uniform and bacilliform 3-shape; Ebola (970nm) and Marburg (790nm) long. They are enveloped viruses. Inside the envelope is a nucleocapsid with a dark central axis (20nm in diameter) surrounded by a helical tubular capsid (50nm in diameter) bearing cross - striations with a periodicity of 5nm.

The 20nm - central axis, also seen in infected cells appears to be the virion RNA. A structure with buoyant density of $1.32g/cm^3$ in CsCl is released from virions by detergent treatment and probably represented the viral RNP within the nucleocapsid which is an axial channel of 10 - l5nm with nucleocapsid proteins (N and VP 30) proteins-L and VP35. The whole virion MW = 300 600 x 60^6, S_{20w} of long particules very high but infectious bacilliform particles = 1,400S buoyant density is $1.14g/cm^3$ in Potassium tartarate. Infectivity is stable of room temperature but destroyed in 30nm at 600^0C. They are also sensitive to lipid solvents.

The genome is a molecule of linear, negatively sensed SSRNA (non-infectious), MW= 1.1% by weight of the virus, seven proteins designated as L.G.N VP40, VP35, VP30 and VP24 are known. The G-protein is very large and two are associated with RNA (N and VP30). Lipids are present in form of envelope while glycolipids and surface

projections (glycosylated) as the carbohydrates present. The virus cannot be neutralised *in vitro*.

3.8 Birnaviridae

The name is derived from the sigla 'Bi' meaning double and RNA meaning the type of genome (ribonucleic acid). The genome is a double stranded RNA and they are non-enveloped viruses. Not much is known about them. They mostly infect pets, e.g. Bovine Disease virus.

4.0 CONCLUSION

RNA viruses contain a single RNA molecule in their genome. There are 16 RNA virus families. The HIV virus is a member of the family Retroviridae.

5.0 SUMMARY

- There are 16 RNA virus families and the HIV virus is a member of the family Retroviridae.

6.0 TUTOR-MARKED ASSIGNMENT

i. Write short notes on viral replication.
ii. List three major information needed in estimating the size of a viral genome.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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MODULE 2

UNIT 1 OTHER CLASSES OF VIRUSES

CONTENTS

1.0 INTRODUCTION

We shall begin this module by looking into the special order of viruses known as mononegaviriales. Mononegaviriales contain a single negative sensed RNA in their genome. We shall also be looking at satellite structures in the genome of viruses and a quick look into the world of viriods.

2.0 OBJECTIVE

At the end of this unit, you should be able to:

- describe the order mononegavirales, viriods and satellites associated with plant viruses.

3.0 MAIN CONTENT

3. 1 The Order Mononegavirales

Derivation of the name is as follows:

'Mono' from Greek word 'Monos' meaning single.
'Nega' from negative strand or negatively sensed RNA (the genome) and
'Virales' from the viral orders which ends with the suffix - 'ales'.

This order encompasses the three viral families of eukaryotic viruses possessing linear, monosegemented negatively sensed RNA genome.
The three viral families are:

- The Filoviridae,
- Paramyxoviridae and
- Rhabdoviridae, all of which had been discussed earlier. Generally, their MW ranges from300 - 100 x 10^6; S_{20w} = 550 - > 1000. Buoyant density in sucrose = 1. 18 – 120g/cm^3 their genome MW = 3.5 – 7 × 10^6 and constitute about 0.5 - 20% of particle weight. Their Lipid content is about 15-25% by weight and the composition is dependent on the host cell. The carbohydrate is 3-6% by weight where known. The pathogenic potential in human tends to be characteristic of family:
  Haemorrhagic fever (Filoviridal);
  Respiratory and neurological disease (Paramyxoviridae);
  mild febrile to fatal neurological disease (Rhabdovirirdae).
  Replication occurs by synthesis of a complete positive sense RNA anti-genome. Maturation of the independently assembled helical nucleocapsids occurs by budding through the host membranes and investment by a host derived Lipid envelope containing trans-membrane virus proteins.

3.2 Viriods

These are small infectious agents that cause various plants diseases. Viroids are encapsidated low molecular weight, covalently closed circular, single stranded infectious positively sensed RNAs. The non-denatural viroid molecules adopt extensive internal base pairing to give rod-like structures that is 50nm long. They are denatured by co-operative melting to single - stranded circules of 100nm contour length.
The molecular weight = 80 - 122 x 10 S_{20w}8 – 10; Tm in 10mM Na^+ is 50 C; density in cesium sulphate is 1 .6g/cm^3They comprises about 246 to cover 370 nucleotides. They are rich in G+C content except few

members, with central conserved regions. They comprise highly base-paired rod like structure with unique properties. Each is arranged into 26 double-stranded regions separated by 25 regions of unpaired bases embodied in single-stranded internal loops. There is a loop at each end of the rod-like molecule.

3.2 Satellites

These are nucleic acid molecules that depend for their multiplication on co-infection of a host cell with a helper virus. Satellite nucleic acids have no approvable sequence homology with their helper virus genome and are not a part of its genome. The satellites are different and distinct nucleic acids from other types of dependent nucleic acid such as sub-genomic nucleic acids, e.g. defective interfering and messenger it molecules; genome parts and transmission - defective but independently replicating viruses. Some satellites may contribute advantageous character to their helper virus; the distinction between these and genome parts is sometimes no clear cut.

Most reported satellites are associated with plant viruses and these have been arbitrarily classified into four types according to physical and messenger properties of the satellite RNA. These are;

TYPE A: The RNA is large (>0.7kb) and encodes a capsid protein that fond in satellite specific particles.

TYPE B: The RNA is large (>0.7kb) and encodes a non structural protein.

TYPE C: The RNA is small (< 0.7kb), lacks significant mRNA properties

TYPE D: The RNA is small (<0.7kb), lacks mRNA activity and forms circular molecules during replication.

Most records satellites are of those associated with plant viruses. Satellites have also been found associated with viruses of other taxonomic groups, e.g. Bacteriophage p4, which is a dsDNA satellite virus dependent on Bacteriophage p2 adeno-associated viruses (Dependeovirus: Parroviridae). ssDNA satellite viruses dependent on adenoviruses or herpes viruses, hepatitis delta virus which is large, but circular, satellite RNA dependent on hepatitis B virus and a ssRNA satellite virus which is associated with chronic bee-paralysis virus.

4.0 CONCLUSION

Viriods are small infectious agents that cause various plants diseases. Satellites are nucleic acid molecules that depend on co-infection of a host cell with a helper virus for their multiplication on co-infection of a host cell with a helper virus.

5.0 SUMMARY

- Viriods are small infectious agents
- They infect plants causing disease
- Satellites require a helper virus for infection of a host cell.

6.0 TUTOR-MARKED ASSIGNMENT

i. List five diseases, hosts, sites of infection and agents of such diseases caused by viriods.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 2 VIRAL REPLICATION

CONTENTS

1.0 INTRODUCTION

Viruses have no metabolic activity of their own and thus replicate or multiply by taking over the biochemical machinery of the host cell and redirecting it to the manufacture of the virus components. Viruses multiply only in the living cells.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the concept of viral replication
- list and explain the seven stages involved in viral replication.

3.0 MAIN CONTENT

The unique feature of virus multiplications is that soon after interaction with host cell, the infecting virion is disrupted and its measurable infectivity is lost. The phase of this growth is called the ECLIPSE PERIOD. This duration depends on both the particular virus and the host cell, and ends with the formation of the first infectious progeny of virus particles. The eclipse period is actually one of intense synthetic activity as the cell is re-directed toward fulfilling the needs of the viral ‘pirate’. Viruses have evolved a variety of different strategies for accomplishing multiplication in their host cells. Although the details vary from one viral family to the other, but the general outline of the replication cycles

is similar. The replication process could be broken down into seven stages.

3.1 Absorption/Attachment

This is the first step in virus infection, during which there is interaction of a virion 'with a specific receptor site' on the surface of the cell to be infected. It is to be noted that receptor molecules differ for different viruses, e.g. poliovirus is able to attach only to cells in CNS and intestinal tract of primates, HIV binds to the CD4 receptor on cells of human immune system. Each susceptible cell probably contains at least 1,000,000 receptor sites for a given virus. Absorption is best achieved at 37°c but could also take place at 4°c but very slow. Adsorption is also enhanced by presence of Magnesium (Mg^{2+}) or Calcium (Ca^{2+}) ions.

3.2 Penetration/Entry

The mode of penetration of entry of viruses into the host cell is complex in nature. It is accomplished by receptor-mediated endocytosis with uptake of the ingested virus particles within endosomes. With syncytia producing viruses, it is by fusion of virus envelope with the cell membrane. Penetration in some other node is well-known.

3.3 Uncoating

Uncoating occurs concomitantly with or shortly after penetration. At this stage, there is physical separation of the viral nucleic acid (or in some cases, internal nucleocapsid) from the outer structural components of the virion. The infectivity in the parental virus is lost at this point. Viruses are only infectious agents for which dissolution of the infecting agent is an obligatory step in the replicative pathway. Uncoating is done enzymatically (from lysosome).

3.4 Transcription

At this stage, a specific mRNA must be transcribed from time viral nucleic acid for successful expression and duplication of genetic information contained in the viral genome. The mRNA produced serves as replicative intermediate from the viral genome. The process transcription could be carried out by either the host cell mechanism or a virus-specified enzyme. The patterns of transcription may differ before (early) at and after (late) virus nucleic acid replication. The primary transcripts are often spliced to remove intron sequences between expressed exons and transcription is sometimes overlapping with different starting and/or termination points within one gene to produce proteins from the same nucleic acid sequence.

Virus mRNA generally:

(i) Contains leader sequence
(ii) Capped at the 5-' end
(iii) Polyadenylated at time 3' terminus.

Some viruses carry RNA polymerases to synthesise mRNA.RNA viruses of this type are called Negative - sense since their single stranded RNA genome is complementary to mRNA which is designated positive strand (positiveness).

3.5 Synthesis of Viral Components

This is the stage where the virus makes use of the host cell component to translate the viral mRNA. All the virus specified macromolecules are synthesised in a highly organised sequences. The virus mRNA is translated on cell ribosome's to produce two types of virus-protein:

(a) The structural proteins which make up the virus particle and
(b) Non-structural protein, mainly enzymes for virus genome replications. This type of protein is not found in the viral particle.

In some virus infections, notably those involving double stranded, DNA-containing viruses, early viral proteins are synthesised soon after infection and late proteins are made only late in infecting viral DNA synthesis. Early genes may or may not be shut-off when late products are made. In contrast, most of the genetic information of RNA viruses is expressed at the same time. In addition to these temporal controls, quantitative controls also exist, since not all virus proteins are made in the same amounts. Virus specific protein may regulate the extent of transcription of the genome or translation of viral mRNA.

Small animal viruses and bacteriophage are good models for studies of gene expression. The widest variation in strategies of gene expression is found among RNA viruses. Some virions carry polymerases, some system utilise sub-genomic messages, sometimes generated by splicing (orthomyxoviruses, retroviruses) and some viruses synthesise large polyprotein precursors that are processed and cleaved to generate the final gene involved in these processes that varies from group to group. The larger viruses (herpesviruses, poxviruses) are more dependent of cellular functions than the smaller viruses. This is one reason the larger viruses are more susceptible to antiviral chemotherapy, because, more virus specific processes are available as targets for drug action.

The intercellular sites where the different events in virus replication take place vary from group to group. However, some general statements could be made as follows:

(a) Viral Proteins are synthesise in the cytoplasm of poly-ribosomes composed of virus-specific mRNA and host cell ribosomes
(b) Viral DNA is usually replicated in the nucleus
(c) Viral genomic RNA is generally duplicated in the cell cytoplasm, although there are exceptions.

3.6 Assemblage/Morphogenesis

The newly synthesised viral genomes and capsid polypeptides assemble together to form progeny viruses. Ichosahedral capsid can condense in the absence of nucleic acid, whereas nucleocapsids of viruses with helical symmetry cannot form without viral RNA. There are no special mechanisms for the release of non-enveloped viruses; the infected cells eventually lyses and release the virus particles. Assembly of viral proteins may take place in the cytoplasm or (as in most enveloped viruses) at the plasma membrane. After assemblage of viruses viral structural and essential proteins sub units, the viral becomes mature and ready for liberation or release.

3.7 Maturity and Release

On maturation, viral particles escape from the cell in several ways but most importantly done in two ways:

(a) bursting out in the cell thereby killing the host cells and
(b) budding out of the host cell which does not necessarily kill the cell.

Enveloped viruses mature by budding process, virus-specific envelope glycoproteins are inserted into cellular membranes, viral nucleocapsids then bud through the membrane at these modified sites, and in so doing, acquire an envelope. Budding frequently occur at the plasma membrane but may involve other membranes cell. It is important to note that enveloped viruses are not infectious until they acquired their envelopes. Therefore, infectious progeny virions typically do not accumulate within the infected cell.

Virus maturation is sometime a faculty process. Excess amounts of viral components may accumulate and be involved in the formation of inclusion bodies in the cell. As a result of the profound deleterious effects of virus replication, cellular cytopathic effects eventually develop and the cell dies. However, there are instances in which the cell is not

damaged by the virus and long-term persistent infections evolve. The basic fact is that each virus effectively utilise whichever cellular process is necessary to achieve its multiplication and morphogenesis.

4.0 CONCLUSION

Viral replication is essential for the successful integration and multiplication of the viral particle. It takes place in seven crucial stages.

5.0 SUMMARY

- Viral replication is essential for the survival and multiplication of viral particle.
- The viral particle must be able to effectively by-pass the host defence for effective replication.

6.0 TUTOR-MARKED ASSIGNMENT

i. Write short notes on reverse transcription.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 3 MODE OF TRANSMISSION VIRUSES AND DIAGNOSIS OF VIRAL INFECTIONS

CONTENTS

1.0 INTRODUCTION

We shall continue our study by looking into the mode of transmission of viruses which include direct transmission, animal-animal transmission and arthropod transmission. We shall also consider the methods of diagnosing viral infections.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe modes of viral transmission
- explain the various methods used in diagnosis of viral infections.

3.0 MAIN CONTENT

3.1 Mode of Transmission

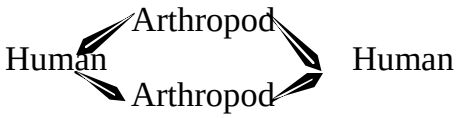
Different viruses have evolved ingenious and complicate mechanisms of survival in nature and transmission from one host to the other. The mode of transmission depends on the nature of the interaction between the virus and the host. Viruses may be transmitted in the following ways:

Direct Transmission: This is a person to person mode of virus transmission. The major means of transmission include droplet or earosol infection (influenza measles), faecal-oral route (enteroviruses), sexual contact (HIV, Hepatitis B), hand-hand, hand-mouth, mouth-mouth and exchange of contaminated blood.

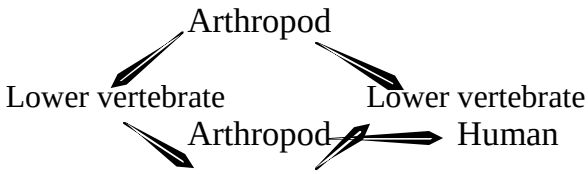
Animal-Animal: Humans are usually the accidental host. It spreads by bites (rabies from dogs), earosols infections from rodents contaminated quarters.

Arthropod Transmission: Viruses can be transmitted through insect vectors (e.g. Arboviruses). There are three different transmission patterns shown below:

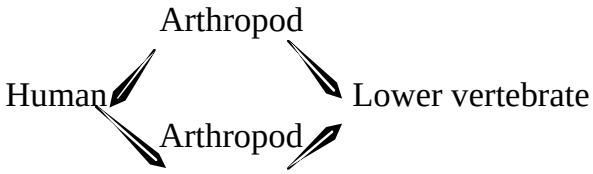
Human – Arthropod cycle, e.g. Urban yellow fever virus, Dengue virus.



Lower vertebrate - Arthropod cycle, e.g. Jungle yellow fever virus. The infected man is a dead end host.



Arthropod – Arthropod cycle, e.g. Colorado stick fever



3.2 Diagnosis of Viral Infection

It must be emphasised here that good diagnostic virology depends on rapid communication between the physician and the laboratory and also on the quality of specimens and information supplied to the laboratory. The choice of methods for laboratory-diagnosis and confirmation of viral infection depends upon the illness.

Antibody tests are more readily and inexpensively performed than virus isolations but they require samples taken at appropriate intervals, and the diagnosis often is not confirmed until convalescence stage. Antibody tests can be carried out only for those illness to which the causative viruses have been grown in the laboratory or for which the special sources of antigen become available (e.g. hepatitis B of HIV).

Virus isolation is required when:

- new epidemics occur as with influenza
- serologic tests overlap and do not allow differentiation between 2 viruses, as with small-pox and vaccinia
- it is necessary to confirm a presumptive diagnosis mode of direct microscopic observation e.g. detecting herpes virus in vesicle fluid and
- the same clinical illness may be caused by many different agents e.g. (non-bacterial) meningitis may be caused by many different viruses as well.

Similarly, respiratory diseases syndromes may be caused by many viruses as well as by mycoplasmas and other agents.

It is important to note that isolation of virus from a specimen is not necessarily equivalent to establishing the cause of a given diseases, e.g. the isolation of herpesviruses, polioviruses, echoviruses, or coxackieviruses from a patient with an undiagnosed illness does not prove that the virus is the cause of the disease. Thus, a consistent clinical and epidemiologic patter must be established with repeated studies before it can he determined that a particular agent is responsible for a specific clinical picture.

3.2.1 Specimen

All specimens must be safely contained for transport to the lab. Each specimen must be clearly labeled and should be accompanied by relevant information. Isolation of active virus requires proper collection of appropriate specimens, their preservation, both en-route to and in the laboratory, and inoculation in suitable cell cultures, susceptible animals or embryonated eggs.

The various specimens to be collected in a particular viral disease, the agent involved and the various or appropriate diagnostic test could be summarised in the control. A positive reaction is taken to indicate resistance to infection with some viruses.

3.2.1 Neutralisation Test (Nt-test)

Virus neutralising antibodies is determined or measured by adding serum containing these antibodies to a suspension of virus and then inoculating the mixture into specific cell cultures. The presence of neutralising antibodies is demonstrated if the cell culture fails to develop CPE while control cell cultures which have received virus plus a serum free of neutralising antibody develop CPE. To establish a diagnosis, one

looks for a significant rise in antibody titer - (4 fold or greater is desirable) during the course of the infection. Neutralising antibodies can persist for years and their presence may indicate an infection in a given individual. The Nt - tests are useful in serologic, epidemiology, in which it is important to know which viral agents have infected a given population in the past. Although Nt - tests principle is simple but are expensive in time and materials and must be standardised for each viral agent. Among the variables to be considered are:

1. Selection of cell culture, experimental animal or embryonated egg
2. Rate of inoculation of virus-serum mixture
3. Age of tested animals
4. Stability of the test virus
5. Reproducibility of the end point
6. Relative heat-stability of the specific antibody and possible, interfering substance in serum
7. Addition of an accessory factor found in fresh serum of the homologous species
8. Use of one concentration of virus and varying dilutions (and the relationship between varying concentrations of each)
9. Temperature of the neutralising mixtures
10. Incubation time for the mixture.

4.0 CONCLUSION

There are modes of transmitting viruses namely, direct transmission, animal-animal transmission and arthropod transmission. Good diagnostic virology depends on rapid communication between the physician and the laboratory and on the quality of specimens and information supplied to the laboratory. In diagnosing viral infections, antibody (neutralisation) test can be carried out or in cases of overlap, isolation of the virus is important for correct diagnosis.

5.0 SUMMARY

- There are modes of transmitting viruses namely, direct transmission, animal-animal transmission and arthropod transmission.
- Good diagnostic virology depends on rapid communication between the physician and the laboratory and on the quality of specimens and information supplied to the laboratory.
- Antibody testing or isolation of virus is used in diagnosing viral infections.

6.0 TUTOR-MARKED ASSIGNMENT

i. In a tabular form, describe five viral infections, specimen and the diagnostic tests.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 4 CONTROL AND TREATMENT OF VIRAL DISEASES

CONTENTS

1.0 INTRODUCTION

In this unit, we shall be looking into the roles and uses of interferons in managing viral diseases as well as anti-viral drugs.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the mode of actions of interferons and antiviral drugs
- state the various types of interferons.

3.0 MAIN CONTENT

3.1 Interferons (IFN)

Interferons are host-coded proteins (non-toxic antiviral agent) that inhibit viral replication. They are produced by intact animals or cultured cells in response to viral infection or other inducers. They are produced by all vertebrates' species but generally, normal cells do not produce or synthesise interferons unless they are induced to do so. They are believed to be the body's first line of defence against potent insult by viruses to the cells. IFN modulates humoral and cellular immunity and have broad cell growth regulatory activities. Interferons could also be induced by DS-RNA, bacteria endotoxin and small molecules such as Tilorone.

RNA-viruses are stronger inducers of IFN than DNA-viruses. All viruses are inhibited by IFN but RNA-viruses are more susceptible to it than DNA-viruses. IFN are active only in cells of the same animal species in which it was formed. There are three main types of IFN,

Alpha, Beta and Gamma interferons. They are similar in size but differ distinctly in antigenic nature. Alpha IFN and Beta-IFN are resistant to low pH. Beta-IFN and Gamma-IFN are glycosalated but the sugars are not necessary for biologic activity; so, cloned IFN produced in bacteria are biologically active. The different classes of IFN are produced by different cell types. Alpha-IFN is synthesized predominantly by leukocytes, Beta-IFN are produce mainly by fibroblasts while gamma IFN are produced only by lymphocytes.

Due to the fact that the amounts of IFN synthesised by induced cells are quite small, it has been difficult to purify and characterise the proteins. With recombinant-DNA techniques, cloned IFN genes are being expressed in large amount in bacteria and yeast, and the availability of genetically engineered IFN makes clinical studies feasible.

Mode of Action of IFN

IFN are always host species-specific in function. By contrast, IFN-activity is not specific for a given virus, but the replication of a wide variety of viruses can be inhibited. When IFN is added to cell prior to infection, there is marked inhibition of viral replication but nearly normal cell function. IFN are extremely potent, so that very small amount is required for function. It has been estimated that fewer than 50 molecules of IFN per cell are sufficient to induce the antiviral states.

The mechanism of action of IFN is still poorly understood. It is however established that IFN is not the antiviral agent; rather IFN induces an antiviral state by promoting the synthesis of other proteins that actually inhibit viral replication. IFN act by binding to cell surface receptors, with alpha-IFN and beta-IFN sharing a common receptor and gamma recognising a distinct receptor. This binding triggers the synthesis of several enzymes believed to be instrumental in the development of the antiviral state. These cellular enzymes subsequently block viral reproduction by inhibiting the translation of viral mRNA into viral protein. The mode of IFN action is from two points.

1. **Degradation of Viral mRNA:** This is basically done by an enzyme, endonuclease. The enzyme is activated by the presence of oligonuleotide synthetase, 2-5A synthetase, both of which are needed for oligoadenulic acid, 2, 5-oligoadenulic acid, a formation which in turn, degrades the viral mRNA.

2. **Inhibition of Protein Synthesis:** A protein kinase phosporylates and inactivates a cellular initiation factor (elF-2) and thus prevents the formation of the initiation complex needed for translation of viral proteins.

Interferons may also affect viral assembly, perhaps as a result of change at the plasma membrane.

It is noteworthy that IFN has been shown to have toxic side effects even when purified material is tested. Gastro-intestinal and nervous system side-effects proportionate of the dose given are common. Bone marrow suppression also may occur. Theoretically, IFN inducers could be administered therapeutically but every inducer that has been carefully studied has also been found to be toxic.

Uses of IFN

1. They have been shown to be effective against (prevents) Rhinovirus when administered intranasally
2. They inhibit vaccinia infection when administered intradermally
3. Chronic active hepatitis due to hepatitis B-Virus could also be prevented but not a dramatic effect and also herpes-keratitis Zoster and cytomegalovirus infections
4. IFN has been shown to have significant effect on several human tumors, e.g. Sarcoma, breast cancer, lymphoma, myeloma and
5. IFN is at present used for trials in cancer patients.

Table 1: Properties of Human Interferons

S/N	Property	Type ALPHA BETA GAMMA		
1	Current nomenclature	IFN α	IFN β	IFN γ
2	Former designation	Leukocytes	Fibroblast	Immune
3	Number of genes that codes for that family	14	>2	1
4	Principal cell source	Leukocytes	Fibroblast	Lymphocytes
5	Size of protein (MW)	17,000	17,000	17,000
6	Inducing agent	Viruses	Miltogens	
7	Stability at pH 2.0	Stable	Stable	Labile
8	Glycosylated	No	Yes	yes
9	Introns in genes	No	No	Yes

3.2 Anti-Viral Drugs

Since viruses are obligate intracellular parasites, a good antiviral agent must be capable of selectively inhibiting viral functions without damaging the host cells. Molecular virology studies have now succeeded in identifying virus specific functions that can serve as realistic targets for inhibition. Theoretically, any stage in viral replicative cycle could be a target for antiviral therapy. Recently, compounds have been found that are of value to treatment of viral diseases, while other compounds

appear promising. Seven antiviral drugs are currently licensed for use, i.e. Acyclovir, Amantadine, Doxuridine, Trifluriridine, Vidarabine, Ribavirin, and Azidothymidine. All these have one or more side-effects on the hosts; hence an ideal antiviral agent remains to be developed.

The majority of available antiviral agents are nucleoside analogues. Analogues inhibit nucleic acid replication by inhibition of enzymes of the metabolic pathways for purines and pyrimidines or by inhibition of polymerases for nucleic acid replication. In addition, some analogues can be incorporated into the nucleic acid and block further synthesis or alter its function. Analogues can inhibit cellular enzymes as well as virus-encoded enzymes. The new types of analogues are those able to inhibit specifically virus-encoded enzymes, with minimal inhibition of analogues of host cell enzymes.

4.0 CONCLUSION

Interferons are host-coded proteins (non-toxic antiviral agent) that inhibit viral replication, they are produced by intact animals or cultured cells in response to viral infection or other inducers. They act by degrading viral mRNA and inhibiting protein synthesis. Seven anti-viral drugs are licensed for use and they have one or more side- effects on the host cell. Viral infections cannot be cured but only be managed using drugs.

5.0 SUMMARY

- Interferons are host-coded proteins (non-toxic antiviral agent) that inhibit viral replication, they are produced by intact animals or cultured cells in response to viral infection or other inducers.
- They act by degrading viral mRNA and inhibiting protein synthesis.
- Seven antiviral drugs are currently licensed for use, i.e. Acyclovir, Amantadine, Doxuridine, Trifluriridine, Vidarabine, Ribavirin, and Azidothymidine.
- All these have one or more side-effects on the host; hence an ideal antiviral agent remains to be developed.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss host-cell response to viral infections.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 5 CONTROL AND TREATMENT OF VIRAL DISEASES I

CONTENTS

1.0 INTRODUCTION

In this unit, we shall be looking into the use of vaccines in preventing viral diseases and the future prospects of vaccination.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the concept of vaccination
- describe the prospects of further development of better vaccines especially through molecular and recombinant DNA technology.

3.0 MAIN CONTENT

3.1 Vaccination

Immunity to viral infection is based on the development of an immune response to specific antigens located on the surface of virus-particles or virus-infected cells. For enveloped viruses, the important antigens are the surface glycoproteins. Vaccines are available for the prevention of several significant human diseases but certain general principles apply to most virus vaccine for use in the prevention of human disease. Chart XI shows the principal vaccines used against most viral diseases of human. Viruses that have a viraemic mode of spread, e.g. polio, hepatitis and measles are controlled by serum antibodies. Cell mediate immunity also is involved in protection against systemic infection, e.g. Measles, herpes.

Neither vaccination nor recovery from natural infection always results in total protection against a later infection with the same virus. Control can

be achieved by limiting the multiplication of virulent virus upon subsequent exposure and preventing its spread to target organs where the pathologic damage is done, e.g. polio and measles viruses must be kept from the brain and spiral cord; Rubella virus must be kept from the embryo. There are various types of vaccine available for use against viral infections and diseases:

1. **Killed-Virus Vaccine:** These are made of purifying viral preparations to a (certain extent) and then inactivating viral infectivity in a way that does minimal damage to the viral structural proteins. This is done by treating the virus with mild formalin. Killed- virus vaccines prepared from a virion stimulates the development of circulating antibody against the viral protein coats; thus conferring some degree of resistor ace. For some diseases, only this type of vaccine is available for its prevention.
2. **Live-Attenuated Virus Vaccine:** Here, the viruses used are virus mutants that antigenically overlap with the wild-type but are restricted in some steps in the pathogenesis of disease. The development of virus strains suitable for live virus vaccines previously was done by selecting naturally attenuated strains or by cultivating the virus serially in various hosts and cultures with the hope of deriving an attenuated strain fortuitously. The search for such strains is now being approached by laboratory manipulations aimed at specific, planned genetic alterations in the virus (e.g. Rabies influenza, respiratory syncytial viruses).

Attenuated virus vaccines have the advantages of acting like the natural infection with regard to their effect on immunity. They multiply in the host and stimulate longer-lasting antibody production, to induce a good cell-mediated response, and to induce antibody production and resistance at the portal of entry.

The proper usage, strong and maintenance of vaccine is an important factor that determines its efficacy.

Table 2: Principal Vaccines Use in the Prevention of Viral Diseases of Human

S/N	Disease	Source of Vaccine	Condition of Virus	Route of Administration
1	Yellow fever	Tissue culture and eggs (17D strain)	Live attenuated	Subcutaneous
2	Hepatitis-B	HBsAg from recombinant DNA yeast	Sub-unit	Subcutaneous
3	Adenovirus-6	Human diploid cell cultures	Live attenuated	Oral by enteric coated capsule
4	Influenza	Highly purified or sub-units from chick embryo	Killed	Subcutaneous
5	Japanese B encephalitis	allantoic fluids Formalinsed mouse brain, tissue culture	Killed	Subcutaneous

3.2 Future Prospects in Vaccination

Molecular biology and modern technologies are combing to make novel approaches to vaccine development.

(a) **Attenuation of Viruses by Genetic Mapping:** This is where exploitation is on recombinant or mutants that can serve as live virus vaccines. The introduction on deletion mutations that damage virus do not completely inactivate; it should yield a vaccine candidate unlikely to revert to virulence.

(b) **Use of a Virulent Virus Vectors**: The concept here is to use recombinant DNA techniques to insert the gene coding for the protein of interest in the genome of a virulent virus that can be administered as the vaccine. The prototype vector under study is vaccinia virus.

(c) **Gene Cloning for Protein Production**: The idea is to clone the viral gene. The cloned DNA can then be expressed in prokaryotic and eukaryotic cells if appropriately engineered constructions are used. If the bacteria can be made to produce the antigen or sufficient quantity and immunogenicity, the production of a purified vaccine containing only the immunising antigen will be facilitated.

(d) Synthetic Peptides: Viral nucleic acids can be readily sequenced and the amino acid sequence of the gene products predicted. It is now possible to synthesise short peptides that correspond to antigenic determinants on viral protein. Although, this approach holds promise, there are several obstacles to overcome. The immune response induced by synthetic peptides is considerably weaker than that induced by intact protein or inactivated virus. It is not easy to identify peptide sequences able to induce a protective immune response. A single peptide representing a single epitope may not be able to induce resistance against a viral protein containing multiple antigenic determinants that are sequential; it may be very difficult to stimulate conformational determinants (i.e. those determined by the tertiary configuration of the protein, which juxtaposes amino acids that may be widely separated in the primary sequence).

(e) Subunit Vaccine: Sub-viral components are being obtained by breaking apart the virion to include in the vaccine only those viral components needed to stimulate protective antibody. Purified material can be administered in more concentrated form, containing greatly increased amounts of the specifically desired antigen.

(f) Local Administered Vaccines: Intranasally administered aerosol vaccines are being developed, particularly for respiratory disease viruses and also for measles virus. It is hoped that they will stimulate local antibody at the portal of entry of viruses.

(g) Conventional Vaccine: Considerable success has been claimed for a vacirella-Zoster vaccines developed in Japan and currently undergoing testing in the U.S.A. So also is Cytomegaloviruses, Epstein Barr virus and respiratory syncytial virus (RSV).

4.0 CONCLUSION

Viral infections cannot be cured but can be prevented by proper vaccination.

5.0 SUMMARY

- Vaccines are effective in preventing viral infections and
- Research is on going for the development of new vaccines in preventing existing viral diseases.

6.0 TUTOR-MARKED ASSIGNMENT

i. In a tabular form, list five viral diseases, source of vaccine, condition of virus and route of administration.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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MODULE 3

Unit 1 Case Study of Viral Diseases
Unit 2 Cultivation of Viruses
Unit 3 Purification of Viral Particles
Unit 4 Assessing the Purity of Virions and Identification of a Viral Particle
Unit 5 Preservation of Viruses and Ethics in a Virology Laboratory

UNIT 1 CASE STUDY OF VIRAL DISEASES

CONTENTS

1.0 INTRODUCTION

In this unit, we shall be looking at the case study of two human viral diseases namely AIDS and Rabies. We shall look into their epidemiology, history, management, diagnosis amongst others.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the virology
- highlight clinical presentation
- discuss diagnosis and treatment of representative viral diseases.

3.0 MAIN CONTENT

3.1 Acquired Immunodeficiency Syndrome (AIDS)

3.1.1 History

The first cases of AIDS were recognised in the U.S.A in 1981 while the virus that causes it was identified in 1983 at the Pasteur Institute in Paris and later re-discovered in U.S.A. at National Cancer Institute in Bethesda. Ever since, the AIDS pandemic was recognised.

3.1.2 Virology

The virus that causes AIDS is known as Human Immuno-deficiency Virus (HIV). It is a member of the family of Retroviridae and of sub-family Lentrivirinae. There are two variants of HIV (i.e. HIV-1 and HIV-2). The structure of the two variants is similar, like all retroviruses. HIV contains RNA in its core as the genome. The genome is a single stranded RNA, positive sense, of about 9-10kb. The virion is about 100 - l40nm in diameter with a cylindrical core. The genome contains at least five additional replication genes. The virus is enveloped with various glycoproteins. The virus is enveloped with various glycoproteins. The enveloped protein in HIV-l is GP16O and GP14 while those of HIV-2 are GP140 and GP36. The overall geographical distribution slows that 86% occurred in the US and Europe, 9% in Africa, 2% in the Caribbean region and 3% in other regions.

3.1.3 Clinical Presentation

After infection, a person may remain symptom-tree for years. An unknown proportion of infected people do experience fever, malaise and possible skin rashes between 21 weeks and 3 months after infection. From that point on, an average of S-9 years may pass before AIDS, once it has developed is very high and may reach 100%; perhaps, the provocative finding in AIDS is that vast majority of people infected with HIV, have no symptoms at all but may be spreading the disease.

AIDS victims may be divided into three tragic categories: the dead, dying and doomed. AIDS is characterised by severe cellular dysfunction, otherwise unexplained severe opportunistic infections (e.g. Pneumorytist carinii Pneumonia, PCP), and neurological disorders or selected malignancies including Kaposi sarcoma.

Provisional WHO Clinical Case Definitions for AIDS (WHO, 1986b)

Adults: AIDS in an adult is defined by the existence of at least two of the major signs in association with at least one minor sign in the absence of known causes of immunosuppression such as cancer or severe malnutrition with other recognised aetiologies.

Major Signs

- 10% weight loss of body weight
- Chronic diarrhoea for 1 month
- Prolong fever for 1 month (intermittent or constant)

Minor Signs

- Persistent cough for 1 month
- Generalised dermatitis
- Chronic progressive and disseminated herpes simplex infection
- Oropharygeal candidiasis and
- Generalised lymphadenipathy.

Note: The presence of generalised Kaposi's or Crytococcal meningitis is sufficient for AIDS diagnosis.

Children: Pediatric AIDS is suspected in an infant or child presenting with at least two major signs associated with at least two minor in the absence of known causes of immunosuppression.

Major Signs

- Weight loss or abnormally slow growth
- Chronic diarrhea for 1 month
- Prolonged fever for 1 month.

Minor Signs

- Generalised lymphadenopathy
- Orophargeal Candidiasis
- Repeated common infection (otitis, pharyngitis, etc.)
- Persistent cough
- Generalised dermatitis and
- Confirmed material HIV-infection.

3.1.4 Diagnosis

AIDS could be diagnosed in the Laboratory as follows:

1. Cellular test to evaluate the ratio of helper (T4) and suppressor (T8) lymphocyte subtypes
2. Antibody tests to detect HIV-antibodies
3. ELISA, of which most laboratory implored. The type of ELISA for HIV-antibody testing is called **COMPETITIVE ELISA.**

3.1.5 Treatment

No reliable anti-HIV therapy has been developed. The secondary opportunistic infections can be treated with drugs, surgery, and irradiation. AZT (an antiviral drug) developed against HIV that looks promising has been found to have a side effect of neurological disorders; same applies to Ribavirin, which also is another antiviral drug. Alpha-Interferon has been tried but no reliable result was achieved.

3.1.6 Vaccines

No anti-HIV vaccine is available till date. Various approaches toward development a vaccine are being investigated. Vaccine development against HIV is difficult because it mutates rapidly, undergoes latency, at id resists the immune responses that usually control viral infections. HIV also showed a marked variation, especially in the envelope antigens, yet parts of the envelope proteins and most core protein are served. **See Chart xii** for possible vaccines under study and prevention of AIDS.

Table 3: Candidate Vaccine against AIDS

S/N	General Vaccine Approaches	Possible Specific Approaches
1	Whole virus vaccine	Live attenuated virus, killed whole virus, defective virus
2	Subunit viruses	Based on gp 160 (gp120 plus gp41) gp 120 gp 41 As expressed in bacteria, animal cells. As part of vaccine envelope, adenovirus capsule protein, herpes virus outer membrane, bacterial outer membrane protein.
3	Target cell protection	As synthetic epilopes of the envelope glycoprotiens. Antibodies to the virus attachment protein. Antibodies to the CD4 receptor. Genetically engineered virus attachment proteins. Anti-idiotype antibody equivalent to the virus attachment protein. Anti-idiotype antibody equivalent to the CD4 receptor.
4	Antigen presentation options	Immunostimulatory complexes Attached to bacterial vectors Added with T-cell growth factors (IL-2) Non-specific immunostimulants.

3.2 Rabies

The disease is as old as the existence of man. The aetiological agent was first discovered by Lew Pasteur et al (1884) when he attenuated the virus. The virus rabies belongs to the family Rhandoviridae and Genus_Lyssavirus. The genome of the virus is a single stranded RNA, linear, non-segmented, and negative sense with molecular weight of four million. The virion is enveloped. The infection is zoonostic, and is transmitted to man via the bite of infected animal e.g. dogs. It causes a lethal form of encephalitis.

3.2.1 Epidemiology

It is a natural infection of dogs, cats, bats and wild animal like fox, wolf, skunk, etc. it could also be found in rodents and cattle. In Europe,

exposure to rabies is most common from cats than dogs except in Turkey where dog rabies is a particular problem. Infection by bite of vampire bats in Central and South America is also a problem rabies spread. Animal that remains healthy after l0 days of bite can be regarded as being free of the virus. Viruses are present in the saliva of infected animal usually 4 days before the onset of symptoms of the disease.

Usually, 15% of bitten individuals by a rabid animal developed the disease. Rabies is more common after bites on the head or neck rather than bites/wounds on limb. Rabies, as far back as 1921 was almost being eradicated in Britain due to quarantine law on imported animals. Rabies is present in wild animals in all continents of the world except in Australia (Antarctica). Case-to-case spread of human patients is not a source of infection. Rabies infection due to corneal transplantation had been reported. The spread of rabies virus is by the bite if infected vampire bats in the West Indies, Central and South America.

3.2.2 Pathogenesis and Pathology

Following an animal bite, the virus multiplies in the peripheral tissue of the wound and spread to the CNS via the nerves. The viruses attack the neuromuscular and neurotendonal spindle of the nerves and bud off to the CNS which is the seat of the virus. The disease is virtually always fatal, often leading to death. However, some rare cases of recovery had been reported.

Death Follows Convulsion: There is little or no lesions of the virus in the CNS with little evidence of destructive effects on cells but the main changes are the typical intra-cytoplasmic inclusions within the neurones called the **NEGRI BODIES.** They are specifically found in the perivascular mononuclear infiltration and are stained by sellers stain.

3.2.3 Clinical Features/Symptoms

Rabies infection begin with non-specific constitutional pro-dronatal including fever, fatigue, musculo-skeletal pains, occular pains, nervousness, hypersensitivity to stimulus by mainly excitement with tremor muscular contractions and convulsions: typically spasm of muscles of swallowing, hence the old name of the disease-HYDROPHOBIA. There is increase in sensitivity of the sensory nervous system. The infected animal especially dogs bark at anything and easily infuriated or excited hence, the classical syndrome of FURIOUS RABIES**.** There is presence of the virus in the saliva, skin, eyes as well as the brain. At this stage, there is hyper salivation, hyperpyrexia, excessive sweating, hypotension and tarchychadia which are all the autonomic disturbance of the infected animal or individuals.

This stage is followed by the dumb condition of the animal. Here, all the conditions in furious stage are aggravated and the animal now becomes dumb-like and paralysed. There is no hydrophobia in the dumb stage.

3.2.4 Incubation Period

The incubation period is usually long, taking about 4-12 weeks when the wound is on the limb but sometime take much more time. The incubation is however shorter if the wound is on the head or neck (about 10 days).

3.2.5 Isolation/Diagnosis

Rabies virus could be isolated from specimens of brain, tissue, CSE saliva and urine. The specimens are inoculated into healthy susceptible lab-mice intra-cerebrally. Observe for paralysis and convulsion. Post mortem demonstration of Negri bodies in the brain cells and immunoflouresence demonstration of rabies antibody with rabies antiserum is diagnostic.

Proper diagnosis of Rabies is as follows:

1. Direct demonstration of virus in smear or brain tissues by electron microscopy or immunoflourescence demonstration of rabies virus antigen.

2. Examination of brain smear by Selle's Stain to demonstrate inclusion bodies (NEGRI BODIES) which are stained RED.

Molecular analysis of viral genome by polymerase chain reaction (PCR) is possible. Complement fixation or neutralising antibodies, test on infected animal serum to detect rabies antibodies is diagnostic.

3.2.6 Treatment and Controls

Passive immunisation is done by injection of human anti-rabies immunoglobulin while active immunisation should be started after passive immunisation. Long incubation of rabies is a suitable disease for prophylactic immunisation after exposure.

Live attenuated vaccine made from human diploid cells is now the vaccine of choice and could be administered as a means of treatment. It is given subcutaneously in 6 doses of 0,3,7,14,30 and 90 days. This produces effective protection with high level of neutralising antibodies. Simple vaccine and Fermin vaccine were the early vaccine used but

produces side effects which include severe neuropamlytic effects due to allergic encephalomyelitis. This is due to repeated injection of nervous tissues. However, Duck-embryo-vaccine though less used now, produces reduced risks of neurological side effects.

The control of rabies could be by:

(1) Government introduction of quarantine on the influx of dogs and other domestic animals into the country

(2) Campaign on vaccination of domestic animals as done in some countries like Latin America

(3) Pre-exposure vaccination which is desirable for all persons who are at high risk of contact with rabid animals within the country and about to travel to other countries and

(4) A booster vaccine dose of 1mg to be administered every two years to have their serum tested for rabies neutralising antibodies every two years before booster does is administered (if serum titre is found inadequate) if needed.

4.0 CONCLUSION

AIDS is a sexually transmitted disease that is becoming an epidemic all over the world. A good and monogamous sexual lifestyle will help in reducing its spread while rabies is spread through animal bites and can be treated via vaccination.

5.0 SUMMARY

- There is no known cure for AIDS, as vaccines are under development. But recent development and research reports indicated that drugs to manage them are now available to give hope to the infected people.
- Vaccination is possible against rabies.
- Both diseases are caused by RNA viruses.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss the prevention and control of AIDS.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 2 CULTIVATION OF VIRUSES

CONTENTS

1.0 INTRODUCTION

We shall be looking into various means of detecting infected viral cells and cultivation of virus.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the methods of cultivating viruses
- identify virus-infected cells.

3.0 MAIN CONTENT

3.1 Detection of Virus- Infected Cells

Multiplication of viruses can be monitored in a variety of ways, some of which are:

1. Cytopathic Effects (CPE): The growth of viruses in cells causes morphological changes in the cells. These types of virus in cells may be necrosis, cytoplasmic vacuolisation. Most viruses produce some obvious CPE which includes lysis or necrosis, cytoplasmic vacuolisation. Most virus produce obvious CPE in infected cells that is generally characteristic of the virus group.

2. Appearance of a virus-coded protein, such as the haemagglutinin of influenza virus. Specific antisera can he used to detect the synthesis of viral proteins in infected cells.

3. Adsorption of erythrocytes to infected cells called haemasorption, due to the presence of virus-encoded haemagglutinin (parainfluenza and influenza) in cellular membranes. This reaction becomes positive before cytopathic changes are visible and in some cases occur in the absence of CPE.

4. Interference by a non-cytopathogenic virus (e.g. rubella) with the replication and induction of CPE by a second challenge virus (e.g. echovirus) added as an indicator.

5. Morphologic transformation by an oncogenic virus (e.g. Raus Sarcoma virus), usually accompanied by the loss of contact inhibition and the piling up of cells into discrete foci.

6. Virus growth in an embryonated chick egg result in death of the embryo (e.g. encephalitis virus), production of pocks or plaques on the CAM. (e.g. herpes, small pox vaccina), development of haemagglutinin in the embryonic fluids or tissues (e.g. influenza) or development of infective virus (e.g. Poliovirus types 2).

7. In the course of virus multiplication within cells, virus-structured called inclusion bodies may be produced. In many viral infections, the inclusion bodies are the site of development of the virions (referred to as the virus factory). Variations in the appearance of inclusion materials depend largely upon the tissue fixative used. The presence of inclusion bodies may be of considerable diagnostic acid. The intracytoplasmic inclusion in nerve cells, the Negri bodies is pathognomonic for rabies.

8. One of the consequences of infection of cells by certain viruses is derangement of the karyotype (chromosome). Breakage, fragmentation, re-arrangement of the chromosomes, abnormal chromosomes and changes in chromosome number may occur. To date, no pathognomonic chromosomes alterations have been identified in virus infected cells in humans. Cells transformed by viruses also exhibit random chromosomal abnormalities. Particular chromosomal alterations, including translocations, inversions and deletions are frequently observed in human cancer cells especially specific types of Leukaemia. More than 20 cellular oncogenes have been localised to specific human chromosomes many that are located at bands are involved in translocations or deletions.

3.2 Cultivation

Many viruses can be grown in cell cultures or in fertile eggs (embryonated eggs) under strictly controlled conditions or inoculation into suitable host animals.

3.2.1 Embryonated Eggs

These are more practical for cultivation of viruses, ethical and economic and/or ease of handling and relative freedom from contaminants. A developing chick-embryos are immune-deficient thus favours the growth of viruses. Most viruses will grow or can be adapted to grow in fertile eggs, and some may kill the embryo or may produce visible evidence of specific infection on the choricallantoic membrane (C.A.M). Haemagglutinnating viruses in allantoic and amniotic fluids will cause haemagglutinnation when incubated with appropriate species of erythrocytes (red blood cells).

There are four routes of inoculating eggs for viral cultivation:

- C.A.M used for many pox and some herpesviruses
- Allantoic cavity used for ortho-paramyxo and rhabdo viruses
- Amniotic cavity used for ortho- and paramyxo-viruses
- Yolk sac used for many togaviruses.

3.2.1 Animal Host

Ideally, the natural host of a viruses or closely related species should be used for animal inoculation. This mode of viral cultivation is not always practical on ethical or economic ground, while there is also the possibility of latent infection with the virus under consideration.

The route of inoculation of the animal is an important factor due to the specific affinity some viruses have for certain tissues, e.g. intracerebral-inoculation of mice with rabies virus; subcutaneous inoculation of swine vesicular disease virus into pigs. Animal may show clinical signs of infection and these must be observed, or biopsy material taken for examination. Necrosis must be conducted thoroughly and any microscopic abnormalities and histological changes noted. Serology may be necessary for the presence of specially acquired antibodies.

Isolation or demonstration of the virus may be attempted by egg inoculation or tissue culture, and by electron microscopy. Neutralisation of virus with a specific antiserum before inoculation of animals will, of course prevent the occurrences of infection.

The growth of virus in animal hosts is still used for primary isolation of certain viruses and for studies of the pathogenesis of viral disease and of viral oncogenesis.

3.2.3 Cell Cultures

The availability of cell grown in-vitro has facilitated the identification and cultivation of newly isolated viruses and the characterisation of the previously known ones. Cell cultures have been on the success since the advent of antibiotics and fungicides which have made it possible to prevent contamination of cultures. The introduction of trypsin facilitated monolayer growth of cells. Chemically defined growth media have been produced to satisfy the nutritional requirements of many different types of cells.

There are three basic types of cell culture:

(a) Primary cultures are made dispersing cells (usually with trypsin) from freshly removed host tissues. In general, they are unable to grow for more than a few passages in culture, e.g. monkey, kidney cells and human-amnion cells.

(b) Secondary cultures (semi-continuous cells) are also known as diploid cells. They have undergone a change that allow their limited cultures (up to 50 passages) but which retain their normal chromosome pattern, e.g. Human embryo lung.

(c) Continuous cell lines are cultures capable of more prolonged, perhaps indefinite growth that have been derived from diploid cell lines or form malignant tissues. They invariably have altered an irregular numbers of chromosomes. The types of cell culture used for virus cultivation depend on the sensitivity of the cells to a particular virus. Continuous cell lines are also referred to as Heteroploids cells lines, e.g. Hela cells derived from human cervical cancer.

4.0 CONCLUSION

Viruses can be cultivated via several means including embryonated eggs, animal host and cell cultures.

5.0 SUMMARY

- The best method for culturing viruses is cell cultures as even the most difficult virus can grow in cell lines suitable as host.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss viral pathogenesis.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 3 Purification of Viral Particles

CONTENTS

1.0. INTRODUCTION

In this unit, we shall consider methods of purifying viral particles. Special attention will be paid to centrifugation as a method.

2.0. OBJECTIVES

At the end of this unit, you should be able to:

- discuss the methods of purifying viral nucleic acids
- explain factors to be considered in identifying a viral particle.

3.0 MAIN CONTENT

3.1 Purification

For purification, the starting material is usually large volumes of tissue culture medium, body fluids or infected cells. Pure virus is important so as to have meaningful studies on the properties and molecular biology of the virion. The first frequently involved concentration of the virus particles by precipitation with ammonium sulphate, ethanol or polyethylene glycol or by ultra filtration. Haemagglutination and elution can be used to concentrate orthomyxoviruses. Once concentrated, virus can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.

More than one step is usually necessary to achieve adequate concentration. A preliminary purification will remove non-virus material: the first step may include centrifugation while the final purification step almost always involves density gradient centrifugation. The band of purified virus may be detected by optical methods, by

following radioactivity if the virus is radiolabelled, or by assaying infectivity.

Viruses can also be purified by high speed centrifugation in density gradients of cesium chloride (CsCl), potassium tartarate, potassium citrate of sucrose. The gradient material of choice is the one that is least toxic to the virus. The virus particles migrate to the equilibrium position where the density of the solution is equal to their buoyant density and form a visible band. Virus bands are harvested by puncture through the bottom of the plastic centrifuge tube and assayed for infectivity.

In column chromatography, virus is bound to substance such as DEAE or phosphocellulose and then eluted by changes in pH or salt concentration. Zone electrophoresis permits the separation of virus particles from contaminant on the basis of charge. Specific antisera also can be used to remove virus particles from host materials.

Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contains variable amounts of envelope per particle. The virus population is heterogeneous in both size amid density. It is very difficult to achieve complete purity of viruses. Small amounts of cellular material tend to adsorb to particles and this purity with the virion. The minimal criteria for purity are a homogenous appearance in electron micrographs and the failure of additional purification procedure to remove contaminants without reducing infectivity.

3.1.1 Centrifugation

Centrifugation as a purification and characterisation procedure:

Ultracentrifuge: A centrifuge is capable of generating large centrifugal fields by rotating samples at 20,000-100,000 rpm. Centrifugal forces of greater than 100,000 x gravity can be generated.

Sedimentation Coefficient

- Rate at which a macromolecule sediments under a defined gravitational force.
- This parameter is influenced by both the molecular weight and the shape of a macromolecule (larger and more spherical sed. faster).
- The basic unit is the Svedberg (S) which is 10^{-13} sec.
- This value can be used to estimate molecular weights in conjunction with other values.

Buoyant density - density at which a virus or other macromolecule neither sinks nor floats when suspended in a density gradient (e.g., $CsCl_2$ or sucrose).

The Svedberg equation: $s = \frac{v}{\omega^2 r} = \frac{m(1 - \bar{v}\rho)}{f} = \frac{\varphi(\rho_p - \rho_m)}{f}$

Where:
S = Sedimentation co-efficient

v = velocity
r = radius, i.e. distance from center of rotation
m = mass (grams)
v= partial specific volume of particle (in nm)
r = density of solvent (g/cm^3)
f = frictional co-efficient between particle and solvent. For a globular protein, $f \approx 1$ (f_p = frictional co-efficient of the particle; f_m = frict. coeff. of solvent).

Types of Sedimentation Medium

1. **Aqueous Buffer (Water based)** – This can be used to separate molecules with widely different S-values (e.g. Nuclei from ribosomes).

2. **Sucrose or glycerol gradients or cushions (isokenetic or rate-zonal)** - A fixed concentration or a linear gradient of these agents in buffer is used. The compounds increase the density and viscosity of the medium therefore, decreasing the rate at which macromolecule sediment through them and preventing the sedimentation molecules with densities less than the medium. General approach is to pour a "cushion" of material at the bottom of the centrifuge tube and centrifuge the virion onto the cushion (cushion need not always be used). By controlling the time and speed of centrifugation, a significant purification can be obtained. Since most macromolecules have greater densities than these mediums, separation is based on S-values. This can be used to separate molecules with relatively close S-values.

3. **CsCl gradient centrifugation (isopycnic or buoyant density) -** A linear gradient of these compounds in buffer is prepared in the centrifuge tube. As the concentration of the compound is increased, the density of the medium increases in the tube. Density is low at the top and high at the bottom. Macromolecule centrifuged through will form a band at a position equal to their buoyant density. Useful for separating molecules of different

densities even when the densities are very close. Drawback is that CsCl can permanently inactivate some viruses.

4.0 CONCLUSION

Virus can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis. Ultracentrifuge is a centrifuge that is capable of generating large centrifugal fields by rotating samples at 20,000-100,000 rpm.

5.0 SUMMARY

- Viruses can be purified from tissue culture mediums.
- Virus can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.
- Viruses can also be purified by high speed centrifugation in density gradients of cesium chloride (CsCl), potassium tartarate, potassium citrate of sucrose.
- Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contains variable amounts of envelope per particle, the virus population is heterogeneous in both size amid density.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss the parthenogenesis of two named virus.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 4 ASSESSING THE PURITY OF VIRIONS AND IDENTIFICATION OF A VIRAL PARTICLE

CONTENTS

1.0 INTRODUCTION

This unit covers assessing the purity of virions and how to identify a viral particle. You will also learn the factors to be considered when identifying a viral particle.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

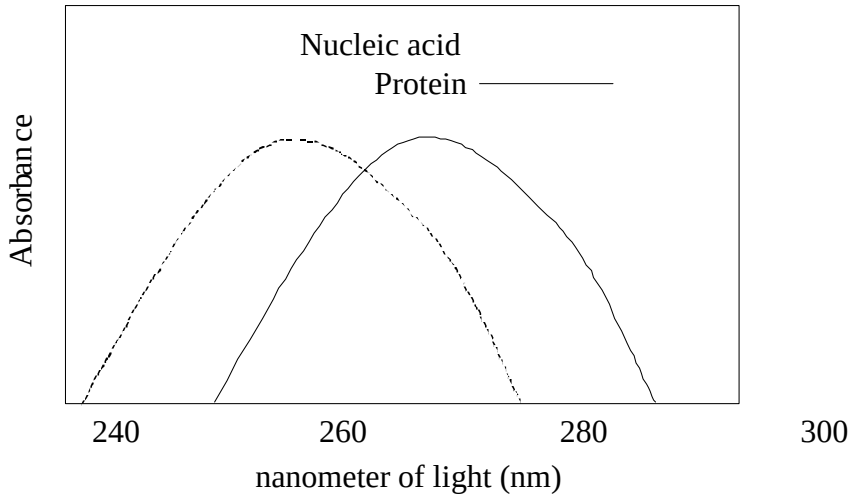
- discuss the different methods used in assessing the purity of viral particles
- explain how to identify viral particles
- state factors to be considered in identifying a viral particle.

3.0 MAIN CONTENT

3.1 Methods for Assessing the Purity of Virions

3.1.1 Spectrophotometric Analysis

UV absorption range is at 260 and 280 nm. This ratio (260/280) is a characteristic of a pure virus and is dependent on the amount of nucleic acid and protein in the virus. The number can be used to estimate the amount in the preparation. Nucleic acid absorbs light about twice as well at 260 *vs.* 280 and vice versa for protein.



3.1.2 Serological Methods

Antibodies for viral proteins are used to characterise, detect, or quantify virions. Antibodies can be made in several ways. Whole virus possibly attenuated (modified so can't cause disease), can be injected into animals (rabbit or mouse) and monoclonal (single type of antibody generally recognises a single epitope) or polyclonal (several different antibodies that may recognise several epitopes).

A second approach is to purify or clone individual viral proteins and inject these directly. Methods available for using antibodies include ELISA (Enzyme-linked immunosorbent assay), RIA (radioimmune assay), RIPA (radioimmune precipitation assay), western blotting, direct precipitation of virus with antibody, neutralisation of viral infectivity, complement fixation by the virus-antibody complex, and others.

3.1.3 Electron Microscopy Method

It allows the visualisation of single virus particles. It is based on the principle of electron scattering. A beam of electrons is focused on the sample. Electrons within the specimen will scatter the electron beam. The scattering effect is enhanced by the presence of heavy, electron-rich metal ions (i.e. gold, platinum) within the sample. This is why the sample is coated with a solution containing a heavy metal. Resolution in the nm range (10^{-9} meters) is possible. *Negative staining (sodium phosphotungstate or uranyl acetate that will stain background but not the virus particles) or shadowing techniques (place specimen on support and direct a vaporised heavy metal across the sample at an angle). This creates a region where relatively little metal deposits just behind the viral particle (resulting in a shadow).*

3.1.4 X-Ray Crystallography

This involves the analysis of crystallised virus. Virus crystals are symmetrical structures composed of many isometric viruses. The atoms of the crystal will diffract x-rays in a structure dependent manner. This approach has been used to analyse the structure of the viruses at the molecular level. Resolution at the Armstrong level (10^{-10} meters, in the bond length range) is possible.

3.2 Identification of a Viral Particle

A purified physical particle should fulfill the following criteria before it is identified as a virus particle:

1) The particle can be obtained only from infected cells or tissues.
2) Particles obtained from various sources are identical, regardless of the cellular species in which virus is grown.
3) The degree of infective activity of the preparation varies directly with the number of particles present.
4) The degree of destruction of the physical particle by chemical or physical means is associated with a corresponding loss of virus activity.
5) Certain properties of the particles and infectivity must be shown to be identical, such as their sedimentation behavior in the ultracentrifuge and their pH stability curves.
6) The absorption spectrum of the purified physical particle in the ultraviolet range should coincide with ultraviolet inactivation spectrum of the virus.
7) Antisera prepared against the infective virus should react with the characteristic particles and vice versa. Direct observation of an unknown virus can be accomplished by electron microscopic examination of aggregate formate in a mixture of antisera and crude virus suspension.
8) The particles should be able to induce the characteristic disease in-vivo (if such experiments are feasible).
9) Passage of the particles in tissue culture should result in the production of progeny with biologic and serologic properties of the virus.

4.0 CONCLUSION

Spectrophotometery, serological assays, electron microscopy and x-ray crystallography are methods of assessing the purity of a viral particle. There are nine important criteria a purified particle must satisfy to be identified as a viral particle.

5.0 SUMMARY

- A purified physical particle should fulfill nine important criteria before it is identified as a virus particle as listed in the unit content.
- Spectrophotometery, serological assays, electron microscopy and x-ray crystallography are methods of assessing the purity of a viral particle.

6.0 TUTOR-MARKED ASSIGNMENT

i. List methods of detecting viral nucleic acids.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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UNIT 5 PRESERVATION OF VIRUSES AND ETHICS IN A VIROLOGY LABORATORY

CONTENTS

1.0 INTRODUCTION

In rounding up this course, we shall look into methods of preserving viruses such as freezing and lyophilisation and ethics in a virology laboratory.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe the methods of preserving viruses
- familiar with the “do and don’ts” in a virology laboratory.

3.0 MAIN CONTENT

3.1 Preservation of Viruses

The preservation of virus is an important sensitive area in virology. It is necessary to preserve viruses after being purified for research purposes and in the development of vaccines. Viruses cannot be preserved on ordinary laboratory media as can be done for most bacteria or fungi.

They are preserved as follows:

3.1.1 Freezing

A large wide-mouthed thermo jar or insulated carton, half-filled with pieces of solid Co_2 (dry ice), serves for transport and storage of material

containing viruses. If dry ice is unavailable, the specimens should be kept cold and transported ordinarily. The temperature in a dry ice storage cabinet is close to -76°C. Electric deep freezer can maintain temperatures of -50°C to -105°C.

3.1.2 Lyophilisation

This procedure consists of rapid freezing at low temperature (in a bath containing Alcohol and dry ice) and dehydration from the frozen state at high vacuum; 1.0-50% of normal plasma or serum in the fluid menstruum protects the virus to be frozen and dried. The plasma or serum must not contain neutralising antibodies. Skimmed milk is also another "protective" menstruum in which virus-containing material may be suspended.

The use of dinitrogen oxide (N_2O) which maintains a temperature of 160°C is also a better way of preserving viruses for many years.

3.1.3 Ethics in a Virology Laboratory

The virology laboratory is a place where the scientist needs to take special caution in addition to normal laboratory practices:

- you must wear a sterile laboratory coat every time
- you must wear a shoe cover
- you must not eat in the laboratory
- you must not wear make ups, jewelry or wear your hair down in the laboratory
- the work benches must be free of unnecessary items such as bags
- nose mask and sterile gloves must be available at all times
- you must not talk when working with RNA viruses as RNAases are everywhere and may degrade your RNA genome
- safety signs must be in appropriate places and on chemicals
- proper storage of chemicals before and after use must be maintained
- proper labelling of samples and chemicals must be done always
- the laboratory must be clean at all times.

4.0 CONCLUSION

Freezing is best for preserving samples during active research and lyophilisation is used in preserving viruses for a long time. Good laboratory practices are essential for obtaining excellent results from research.

5.0 SUMMARY

- Lyophilisation and freezing are methods of preserving viruses.
- Good laboratory practice is important in getting good result from virus studies.

6.0 TUTOR-MARKED ASSIGNMENT

i. List methods used in the study of viruses.

7.0 REFERENCES/FURTHER READING

Flint, S.J. *et al.* (2004). *Principles of Virology*. ASM Press.

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