BACTERIA

FUNGI

VIRUS

MICROBIOLOGY-INTRODUCTION

- Microbiology is the study of living organisms of microscopic size, which include Bacteria, Fungi, Algae, Protozoa, Viruses about their form, structure, reproduction, physiology, metabolism and classification
- It also include the study of their distribution in nature, their relation ships to each other and to other living orgnisms, their effects on human beings and on other animals and plants, their ability to make physical and chemical changes in our environment.

HISTORY :

Microbiology has had a long, rich history, initially centered in the causes of infectious diseases but now including practical applications of the science. Many individuals have made significant contributions to the development of microbiology.

Early history of microbiology.

Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named **Antony van Leeuwenhoek** made careful observations of microscopic organisms, which he called **animalcules.** Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

Louis Pasteur and the germ theory.

Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would

not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease.

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism.

Robert Koch's postulates are the four criteria designed to assess whether a microorganism causes a disease. As originally stated, the four criteria are: (1) The microorganism must be found in diseased but not healthy individuals; (2) The microorganism must be cultured from the diseased individual; (3) Inoculation of a healthy individual with the cultured microorganism must recapitulated the disease; and finally (4) The microorganism must be re-isolated from the inoculated, diseased individual and matched to the original microorganism. Koch's postulates have been critically important in establishing the criteria whereby the scientific community agrees that a microorganism causes a disease.

The development of microbiology.

In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified.

The Spectrum of Microbiology

Like all other living things, microorganisms are placed into a system of **classification.** Classification highlights characteristics that are common among certain groups while providing order to the variety of living things. The science of classification is known as **taxonomy**, and **taxon** is an alternative expression for a classification category. Taxonomy displays the unity and diversity among living things, including microorganisms. Among the first taxonomists was **Carolus Linnaeus.** In the 1750s and 1760s, Linnaeus classified all known plants and animals of that period and set down the rules for nomenclature.

In the classification scheme, various species are grouped together to form a **genus.**Among the bacteria, for example, the species *Shigella boydii* and *Shigella flexneri*are

in the genus *Shigella* because the organisms are at least 70 percent similar. Various genera are then grouped as a **family** because of similarities, and various families are placed together in an **order.** Continuing the classification scheme, a number of orders are grouped as a **class**, and several classes are categorized in a single **phylum** or **division.** The various phyla or divisions are placed in the broadest classification entry, the **kingdom.**

Prokaryotes and eukaryotes.

Because of their characteristics, microorganisms join all other living organisms in two major groups of organisms: prokaryotes and eukaryotes. Bacteria are **prokaryotes** (simple organisms having no nucleus or organelles) because of their cellular properties, while other microorganisms such as fungi, protozoa, and unicellular algae are **eukaryotes** (more complex organisms whose cells have a nucleus and organelles). Viruses are neither prokaryotes nor eukaryotes because of their simplicity and unique characteristics.

The five kingdoms.

The generally accepted classification of living things was devised by **Robert Whittaker** of Cornell University in 1969. Whittaker suggested a five-kingdom classification.

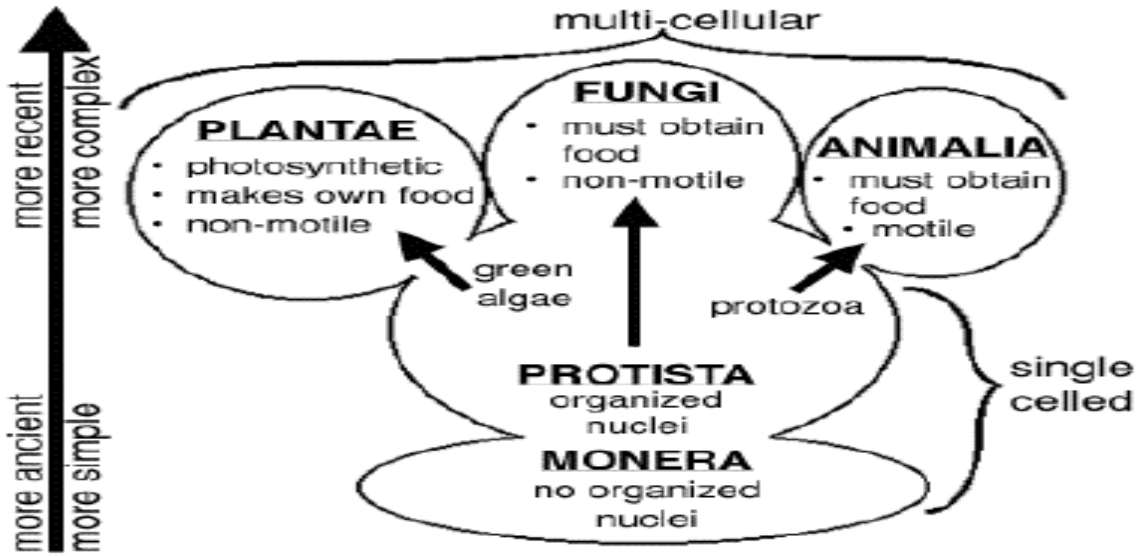


Figure 2-A: Five Kingdoms, their Characteristics & Relationships

The first of the five kingdoms is **Monera** (in some books, Prokaryotae). Prokaryotes, such as bacteria and cyanobacteria (formerly, blue-green algae), are in this kingdom; the second kingdom, **Protista**, includes protozoa, unicellular algae, and slime molds, all of which are eukaryotes and single-celled; in the third kingdom,**Fungi**, are the molds, mushrooms, and yeasts. These organisms are eukaryotes that absorb simple nutrients from the soil (See Figure). The remaining two kingdoms are **Plantae** (plants) and **Animalia** (animals).

BRIEF DESCRIPTIONS OF MICROORGANISMS.

Bacteria

are relatively simple, prokaryotic organisms whose cells lack a nucleus or nuclear membrane. Size range: 0.5 to 1.5 micro m in dia. and 3 to 5 micro m in length.The bacteria may appear as rods (bacilli), spheres (cocci), or spirals (spirilla or spirochetes). Bacteria reproduce by binary fission, have unique constituents in their cell walls, and exist in most environments on earth. For instance, they live at temperatures ranging from 0° to 100°C and in conditions that are oxygen rich or oxygen free. A microscope is necessary to see and study them.

Fungi

are eukaryotic microorganisms. Size range: 2 to 10 micron. that include multicellular molds and unicellular (single-celled) yeasts. The **yeasts** are slightly larger than bacteria and are used in alcoholic fermentations and bread making. Certain yeasts such as *Candida albicans*are pathogenic (disease causing). **Molds** are filamentous, branched fungi that use spores for reproduction. The fungi prefer acidic environments, and most live at room temperature under oxygen-rich conditions. The common mushroom is a fungus.

Algae

Algae implies a variety of plantlike organisms. Size range: 1 micro m to many feets. In microbiology, several types of single-celled algae are important. Examples are the diatoms and dinoflagellates that inhabit the oceans and are found at the bases of marine food chains. Most algae capture sunlight and transform it to the chemical energy of carbohydrates in the process of photosynthesis.

Protozoa

are eukaryotic, unicellular organisms. Size range: 2 to 200 micro m. Motion is a characteristic associated with many species, and the protozoa can be classified according to how they move: Some protozoa use flagella, others use cilia, and others use pseudopodia. Certain species are nonmotile. Protozoa exist in an infinite variety of shapes because they have no cell walls. Many species cause such human diseases as malaria, sleeping sickness, dysentery, and toxoplasmosis.

Viruses

are ultramicroscopic bits of genetic material (DNA or RNA) enclosed in a protein shell and, sometimes, a membranous envelope. Size range: 0.015 – 0.2 micron. Viruses have no metabolism; therefore, it is difficult to use drugs to interfere with their structures or

activities. Viruses multiply in living cells and use the chemical machinery of the cells for their own purpose. Often, they destroy the cells in the process of replicating.

Bacterial Classification [Bergey's Manual of Systematic Bacteriology]

Microorganisms represent an exceptionally large conglomerate of minute living body with enormous diversity having a procaryotic cellular organization. Several sincere intensive and extensive studies were duly made with particular reference to their broad spectrum physical, structural, and functional characteristic qualities, but none of them could ever produce and evolve an overall satisfactory generally acceptable classification.

Chester (1899 and 1901) initiated and took active interest in the classification of bacteria, and subsequently published for the first time—**'The Manual of Determinative Bacteriology'.** The said manual was painstakingly and meticulously revised, substantiated, and modified by David Hendrick's Bergey (1923) and entitled as—**'Bergey's Manual of Systematic Bacteriology'**, later on commonly termed as **'Bergey's Manual'**. In fact, **Bergey's Manual** is being recognized as the *'official compendium* of all identified and classified bacteria, and serves as an indispensable and valuable guide to the **microbiologists** across the globe.

TAXONOMY

Taxonomy (**Greek** : *taxis* = arrangement or order), and *nomos* = law, or *nemein* = to distribute or govern) refers to the science or discipline that essentially deals with the logical arrangement of living things into categories. It may also be defined as *'the laws and principles of classification of living organisms'.*

Aristotle—in fact, was the first ever taxonomist in the fourth century BC who painstakingly and meticulously categorized the so-called *'living objects'* in the universe into almost 500 well defined species of plant and animal kingdoms.

Robert H Whittaker (1969) — duly put forward a most scientific, plausible, and logical system of classification of the living organisms which was widely accepted by the modern microbiologistsacross the world. However, Robert's system articulately recognizes the **five kingdoms** applicable to all living things, namely: *Monera, Protista, Fungi, Animalia,* and *Plantae*.

Monera — predominantly includes **bacteria** and **cyanobacteria.**

Protista — essentially comprises of **eukaryotes** and **protozoa.**

Fungi — specifically belongs to the organisms attached to the kingdom of fungi.

Animalia and Plantae — particularly include the traditional animals and plants.

It is, however, pertinent to mention here some of the main terminologies, one may frequently come across in the proper and elaborated description of the **taxonomy** of microorganisms, such as:

(*a*) **Species** – *i.e.,* the fundamental rank in the classification system;

(*b*) **genus** – *i.e.,* clubbing together

of two or more *species* ;

(*c*) **family –** *i.e.,* the collection of genera;
(*d*) **order –** *i.e.,* the collection of *families* with identical characteristic features ;
(*e*) **class –** *i.e.,* the arranging together of *order* ;
(*f*) **phylum** (or **division**) – *i.e.,* grouping together of *classes*; and
(*g*) **kingdom** – *i.e.,* collection of two or more *phyla*.

Taxon, also known as the *basic taxonomic group* represents the **species** *i.e.,* a collection of strains with almost similar characteristic features. In usual practice, the **microbial species** invariably comprise of a specialized typical strain termed as the **type strain,** along with all other strains which are regarded very much identical to the **type strain** so as to justify their logical inclusion in the species. In other words, the **type strain** is symbolized and designated to be the *permanent reference specimen* for the species. however, it may be stressed that it is not necessarily always the particular strain which happens to be most characterwise typical of all the strains strategically included in the **species,** whereas it is essentially the specific strain to which all the rest of the strains should be critically compared to ascertain, whether they do have a close resemblance sufficient enough to belong to the same **species.** The above glaring statement of facts pertaining to the **type strains** are extremely vital and important; and, therefore, specialized and particular attention need to be given to their genuine and regular maintenance as well as preservation. The following are *two* world famous reference collection centres located in USA and UK, namely:
(*a*) **American Type Culture Collection (ATCC),** Rockville, Maryland, USA, and
(*b*) National Collection of Type Cultures (NCTC), UK.

APPLIED AREAS OF MICROBIOLOGY

- MEDICAL MICROBIOLOGY- diseases, diagnoses
- AQUATIC MICROBIOLOGY- water purification, ecology, waste degradation…
- AERO MICROBIOLOGY- contamination & spoilage
- FOOD MICROBIOLOGY- preservation, food borne diseases, preparation etc..
- AGRICULTURAL MICROBIOLOGY- soil fertility, diseases
- INDUSTRIAL MICROBIOLOGY- antibiotics, beverages..
- EXO MICROBIOLOGY- life in outer space.
- GEO CHEMICAL MICROBIOLOGY- coal, oil, gas.

Applications of microbiology

- In food and diary
  - Yeast in wine, beer, vinegar production
  - Cheese production
- In production of industrial products
  - Antibiotics, enzymes, aminoacids, vitamins
- In genetic engineering and Biotechnology
  - Diagnostic kits, HGH, insulin
- In medical microbiology
  - Vaccines, culture and sensitivity test
- In agriculture
  - Compost and organic manures
  - Bio gas plant
- In BIO-TERRORISM
  - Anthrax, cholera, small-pox

INTRODUCTION TO MYCOLOGY

The term "mycology" is derived from Greek word "mykes" meaning mushroom. Therefore mycology is the study of fungi.
The ability of fungi to invade plant and animal tissue was observed in early 19th century but the first documented animal infection by any fungus was made by Bassi, who in 1835 studied the muscardine disease of silkworm and proved the that the infection was caused by a fungus *Beauveria bassiana*.
In 1910 Raymond Sabouraud published his book *Les Teignes*, which was a comprehensive study of dermatophytic fungi. He is also regarded as father of medical mycology.

Importance of fungi: Fungi inhabit almost every niche in the environment and humans are exposed to these organisms in various fields of life.

Beneficial Effects of Fungi:

1. Decomposition - nutrient and carbon recycling.
2. Biosynthetic factories. The fermentation property is used for the industrial production of alcohols, fats, citric, oxalic and gluconic acids.
3. Important sources of antibiotics, such as Penicillin.
4. Model organisms for biochemical and genetic studies. Eg: *Neurospora crassa*
5. *Saccharomyces cerviciae* is extensively used in recombinant DNA technology, which includes the Hepatitis B Vaccine.
6. Some fungi are edible (mushrooms).
7. Yeasts provide nutritional supplements such as vitamins and cofactors.
8. Penicillium is used to flavour Roquefort and Camembert cheeses.
9. Ergot produced by Claviceps purpurea contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
10. Fungi *(Leptolegnia caudate* and *Aphanomyces laevis*) are used to trap mosquito larvae in paddy fields and thus help in malaria control.

Harmful Effects of Fungi:

1. Destruction of food, lumber, paper, and cloth.
2. Animal and human diseases, including allergies.
3. Toxins produced by poisonous mushrooms and within food (Mycetism and Mycotoxicosis).
4. Plant diseases.
5. Spoilage of agriculture produce such as vegetables and cereals in the godown.
6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues, bones and wax.

General properties of fungi:

1. They are eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, golgi apparatus, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.
2. Have ergosterols in their membranes and possesses 80S ribosomes.
3. Have a rigid cell wall and are therefore non-motile, a feature that separates them from animals. All fungi possess cell wall made of chitin.
4. Are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.
5. Fungi are osmiotrophic; they obtain their nutrients by absorption.
6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).
7. All fungi require water and oxygen and there are no obligate anaerobes.
8. Typically reproduce asexually and/or sexually by producing spores.
9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.
10. Food storage is generally in the form of lipids and glycogen.

Classification of fungi:

Fungi were initially classified with plants and were a subject of interest for botanists; hence the influence of botany can be seen on their classification. In 1969 R.H Whittaker classified all living organisms into five kingdoms namely Monera, Protista, Fungi, Plantae and Animalia.
Traditionally the classification proceeds in this fashion:
Kingdom - Subkingdom - Phyla/phylum - Subphyla - Class - Order - Family - Genus-
Species This classification is too complicated to be dealt here.
There are alternate and more practical approaches, one based on sexual reproduction and the other based on morphology of the thallus (vegetative structure).

Based on Sexual reproduction:

1. Zygomycetes: which produce through production of zygospores.
2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.
3. Basidiomycetes: which produce exogenous spores called basidiospores in cells called basidia.
4. Deuteromycetes (Fungi imperfecti): fungi that are not known to produce any sexual spores (ascospores or basidiospores). This is a heterogeneous group of fungi where no sexual reproduction has yet been demonstrated.

Based on Morphology:

1. Moulds (Molds): Filamentous fungi Eg: *Aspergillus sps*, *Trichophyton rubrum*
2. Yeasts: Single celled cells that buds Eg: *Cryptococcus neoformans, Saccharomyces cerviciae*
3. Yeast like: Similar to yeasts but produce pseudohyphae Eg: *Candida albicans*
4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions. They exist as yeasts in tissue and in vitro at 37^{o}C and as moulds in their natural habitat and in vitro at room temperature. Eg: *Histoplasma capsulatum*, *Blastomyces dermatidis*, *Paracoccidiodes brasiliensis*, *Coccidioides immitis*

Some 200 "human pathogens" have been recognized from among an estimated 1.5 million species of fungi.

Morphology of fungi:

Fungi exist in two fundamental forms; the filamentous (hyphal) and single celled budding forms (yeast). But, for the classification sake they are studied as moulds, yeasts, yeast like and dimorphic fungi.

All fungi have typical eukaryotic morphology. They have rigid cell wall composed of chitin, which may be layered with mannans, glucans and other polysaccharides in association with polypeptides. Some lower fungi possess cellulose in their cell wall. Some fungi such as Cryptococcus and yeast form of *Histoplasma capsulatum* possess polysaccharide capsules that help them to evade phagocytosis.

Inner to the cell wall is the plasma membrane that is a typical bi-layered membrane in addition to the presence of sterols. Fungal membranes possess ergosterol in contrast to cholesterol found in mammalian cells. The cytoplasm consists of various organelles such as mitochondria, golgi apparatus, ribosomes, endoplasmic reticulum, lysosomes, microtubules and a membrane enclosed nucleus. A unique property of nuclear membrane is that it persists throughout the metaphase of mitosis unlike in plant and animal cells where it dissolves and re-forms. The nucleus possesses paired chromosomes.

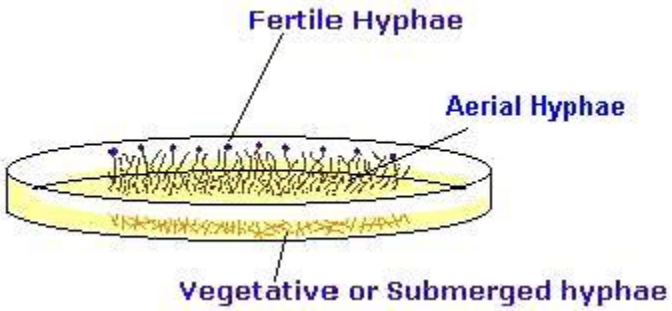
Moulds:

The thallus of mould is made of hyphae, which are cylindrical tube like structures that elongates by growth at tips. A mass of hyphae is known as mycelium. It is the hypha that is responsible for the filamentous nature of mould. The hyphae may be branched or unbranched. They may be septate or aseptate. Hyphae usually have cross walls that divide them into numerous cells. These cross walls, called septa have small pores through which cytoplasm is continuous throughout the hyphae. Therefore all hyphal fungi tend to be coenocytic (multinucleate). With exception of zygomycetes (Rhizopus, Mucor), all moulds are septate. Non-septate hyphae are considered to be more primitive because if a hyphal strand is damaged the entire strand dies. When a septate hyphal strand is damaged, the pores between adjacent compartments can be plugged, thus preventing death of the whole hyphal strand.

Mycelium are of three kinds:

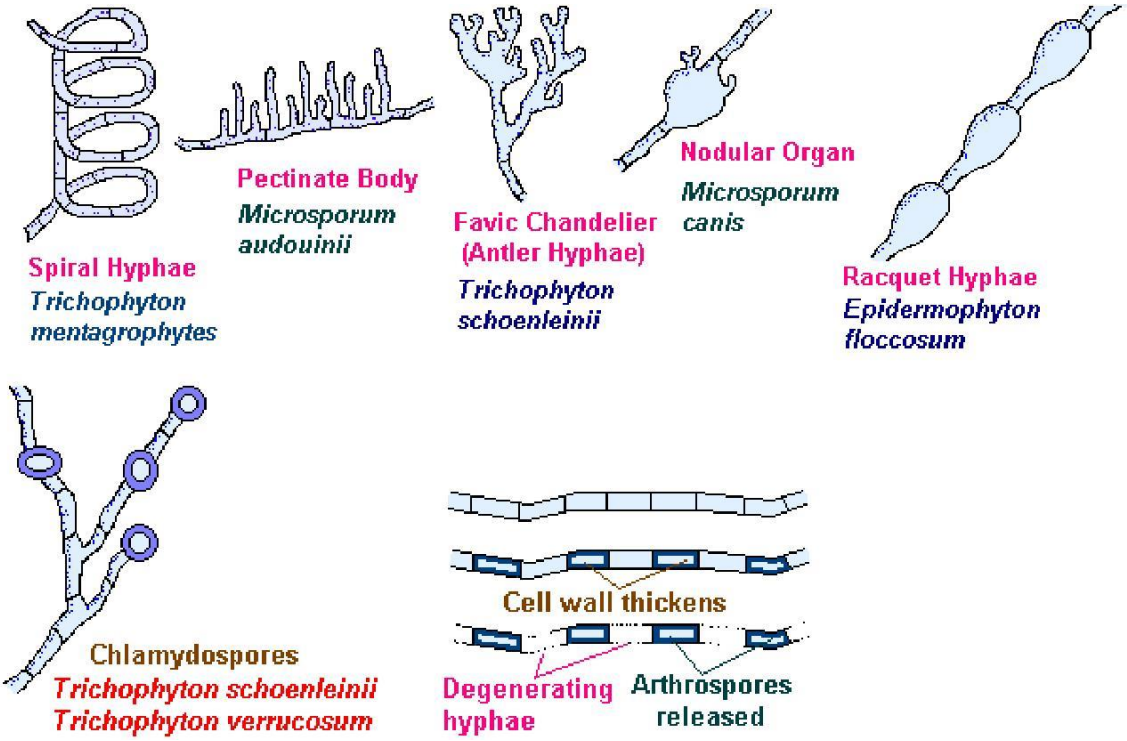
1. **Vegetative mycelium** are those that penetrates the surface of the medium and absorbs nutrients.

2. **Aerial mycelium** are those that grow above the agar surface
3. **Fertile mycelium** are aerial hyphae that bear reproductive structures such as conidia or sporangia.



Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are species of Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella Those hyphae that don't possess any pigment in their cell wall are called hyaline. Hyphae may have some specialised structure or appearance that aid in identification.
Some of these are:

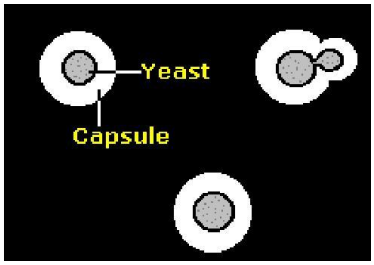
a) **Spiral hyphae**: These are spirally coiled hyphae commonly seen in *Trichophyton mentagrophytes*.
b) **Pectinate body**: These are short, unilateral projections from the hyphae that resemble a broken comb. Commonly seen in *Microsporum audouinii*.
c) **Favic chandelier**: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (antler hyphae). They occur in *Trichophyton schoenleinii* and *Trichophyton violaceum*.
d) **Nodular organ**: This is an enlargement in the mycelium that consists of closely twisted hyphae. Often seen in *Trichophyton mentagrophytes* and *Microsporum canis*.
e) **Racquet hyphae**: There is regular enlargement of one end of each segment with the opposing end remaining thin. Seen in *Epidermophyton floccosum*, *Trichophyton mentagrophytes.*
f) **Rhizoides**: These are the root like structures seen in portions of vegetative hyphae in some members of zygomycetes.
g) There are structures in the hyphae, which arise out of modification of a single cell and transform into thick walled resting cells. Chlamydospore (or chlamydoconidia), which are produced by *Trichophyton schoenleinii* and *Trichophyton verrucosum* are thick walled cells that are larger than other cells and arranged singly or in groups. In some fungi such as *Trichosporon beigeilli* and *Coccidioides immitis* some alternating cells become thick walled and subsequently the intervening cells disintegrate leaving behind arthrospores (or arthroconidia).



Yeasts:

Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which result in blastospore (blastoconidia) formation. In some cases, as the cells buds the buds fail to detach and elongate thus forming a

chain of elongated hyphae like filament called pseudohyphae. This property is seen in *Candia albicans*. The same species also have the ability to produce true hypha, which is seen as germ tube. The difference between the two is that there is a constriction in psueudohyphae at the point of budding, while the germ tube has no constriction.



Some yeast such as Cryptococcus and the yeast form of *Blastomyces dermatatidis* produce polysaccharide capsule. Capsules can be demonstrated by negative staining methods using India ink or Nigrosin. The capsule itself can be stained by Meyer Mucicarmine stain.
Some yeasts are pigmented. *Rhodotorula sps* produces pink colonies due to carotenoid pigments while some yeasts such as *Phaeoannellomyces werneckii* and *Piedraia hortae* are dematiaceous, producing brown to olivaceous colonies.
True yeasts such as *Saccharomyces cerviciae* don't produce pseudohyphae. Yeast-like fungi may be basidiomycetes, such as *Cryptococcus neoformans* or ascomycetes such as *Candida albicans.*

Reproduction in fungi:

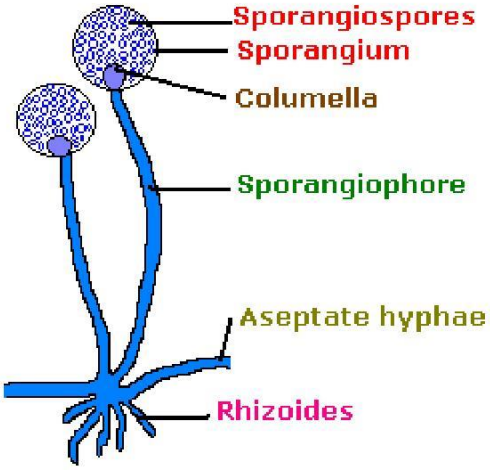
Fungi reproduce by asexual, sexual and parasexual means.

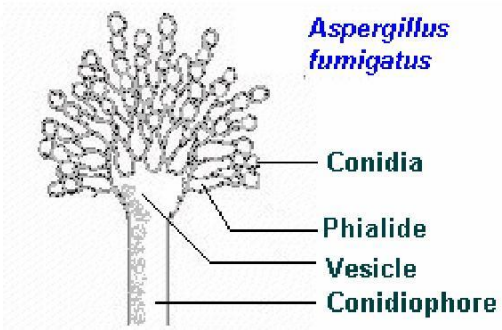
Asexual reproduction is the commonest mode in most fungi with fungi participating in sexual mode only under certain circumstances. The form of fungus undergoing asexual reproduction is known as anamorph (or imperfect stage) and when the same fungus is undergoing sexual reproduction, the form is said to be teleomorph (or perfect stage). The whole fungus, including both the forms is referred as holomorph. *(Taxonomically, the teleomorph or the holomorph is used, but practically it is more convenient to use the anamorph.)*

Asexual reproduction:

> Asexual propagules are termed either spores or conidia depending on their mode of production. Asexual spores are produced following mitosis where as sexual spores are produced following meiosis. *(Cells divide and reproduce in two ways: mitosis and meiosis. **Mitosis** is a process of cell division that results in two genetically identical daughter cells developing from a single parent cell. **Meiosis**, on the other hand, is the division of a germ cell involving two fissions of the nucleus and giving rise to four gametes, or sex cells, each possessing half the number of chromosomes of the original cell.)*

The asexual spores of zygomycetes, which are known as sporangiospores form within sac like structure known as sporangia. The sporangiospores result from the mitotic cleavage of cytoplasm in the sporangium. The sporangia are borne on special hyphae called sporangiophore. This endogenous process of spore formation within a sac is known as sporogenesis.





Conidia arise either by budding off conidiogenous hyphae or by differentiation of preformed hyphae. These develop

following mitosis of a parent nucleus and are formed in any manner except involving cytoplasmic cleavage. This exogenous process is known as conidiogenesis, a process that occurs both in yeasts and moulds. Conidia are borne on specialised structures called conidiophore.
Conidia production may be blastic or thallic. In blastic development the conidium begins to enlarge and a septum is formed. Here the conidium originates from part of parent. In thallic mode of development the conidium is differentiated by a septum before its differentiation. Thus the conidium results from the conversion of entire parent cell into the conidium.
The cell that gives rise to a conidium is called a conidiogenous cell. Conidiophores are specialised hyphae that bear conidia or conidiogenous cells. In many cases conidiogenous cells are referred as phialides.

Sexual Reproduction:
Sexual propagules are produced by the fusion of two nuclei that then generally undergo meiosis.
The first step in sexual methods of reproduction involves plasmogamy (cytoplasmic fusion of two cells). The second step is karyogamy (fusion of two compatible nuclei), resulting in production of diploid or zygote nucleus. This is followed by genetic recombination and meiosis. The resulting four haploid spores are said to be sexual spores, e.g. zygospores, ascospores and basidiospores.
If a sexual spore is produced only by fusion of a nucleus of one mating type with a nucleus of another mating type (+ and - strains), the fungus is said to be heterothallic. In contrast, homothallic moulds produce sexual spores following the fusion of two nuclei from the same strain. For sexual reproduction to occur, two compatible isolates are required.
Zygospores, which are the sexual spores of zygomycetes are round, thick walled reproductive structures that result from the union of two gamentagia. Ascomycetes produce sexual spores called ascospores in a special sac like cell known as ascus. In basidiomycetes the basidiospores are released from basidium, which is the terminal cell of a hyphae.

Parasexual reproduction:
Parasexual reproduction, first seen in Aspergillus is known to occur in basidiomycetes, ascomycetes and deuteromycetes. The process involves genetic recombination without the requirement of specific sexual structures.

Importance of Spores:
A. Biological
1) Allows for dissemination
2) Allows for reproduction
3) Allows the fungus to move to new food source.
4) Allows fungus to survive periods of adversity.
5) Means of introducing new genetic combinations into a population

B. Practical
1) Rapid identification (also helps with classification)
2) Source of inocula for human infection
3) Source of inocula for contamination

ZYGOMYCETES
Commonly known as bread moulds, these are fast growing, terrestrial, largely saprophytic fungi. Hyphae are coenocytic and mostly aseptate. Asexual spores include chlamydoconidia, conidia and sporangiospores. Sporangiophores may be simple or branched. Sexual reproduction involves producing a thick-walled sexual resting spore called a zygospore.
Medically important orders and genera include:
1. Entomophthorales: Conidiobolus and Basidiobolus are involved in subcutaneous zygomycosis
2. Mucorales: Rhizopus, Mucor, Rhizomucor, Absidia and Cunninghamella are involved in subcutaneous and systemic zygomycosis (formerly called Mucormycosis)

BASIDIOMYCETES
They exist as saprobes and parasites of plants. Hyphae are dikaryotic and can often be distinguished by the presence of clamp connections over the septa. Sexual reproduction is by the formation of exogenous basidiospores, typically four, on a basidium. Occasional species produce conidia but most are sterile.

Genera of medical importance include:
1. Teleomorph of *Cryptococcus neoformans*, which is *Filobasidiella neoformans*
2. Agents of basidiomycosis such as Coprinus and Schizophyllium
3. Mushroom poisoning by Aminita, Lepiota, Coprinus and Psilocybe etc.

ASCOMYCETES

They exist as saprophytes and parasites of plants. Hyphae are septate with simple septal pores. Asexual reproduction is by conidia. Sexual reproduction is by the formation of endogenous ascospores, typically eight, in an ascus.

Medically important genera include the:

1. Teleomorphs of known pathogenic fungi e.g. Arthroderma (of Trichophyton and Microsporum), *Ajellomyces dermatitidis* (of *Blastomyces dermatitidis*), *Pseudallescheria boydii* (of *Scedosporium apiospermum*)
2. Agents of mycetoma, like Leptosphaeria
3. Agents of black piedra, like *Piedraia hortae*.

DEUTEROMYCETES

Deuteromycetes are also known as Fungi Imperfecti because of absence of sexually reproducing forms (teleomorph or perfect stage). As their teleomorph continue to be discovered, they would be classified among the previous categories, until then this remains an artificial and heterogeneous group.

There are three classes of Fungi Imperfecti.

1. **Blastomycetes:** These include asexual budding forms of Cryptococcus, Candida, Torulopsis and Rhodotorula. Depending on the presence of melanin in their cell walls, they may be non-dematiaceous or dematiaceous.
2. **Hyphomycetes:** A class of mycelial moulds which reproduce asexually by conidia on hyphae. Hyphae are septate. This class contains the majority of medically important fungi. Dematiaceous hyphomycetes are those conidial fungi that produce dark brown, green-black, or black colonies and are the causative agents of phaeohyphomycosis. Hyaline hyphomycetes include those conidial fungi, which are not darkly pigmented; colonies may be colourless or brightly coloured. These include the agents of hyalohyphomycosis, aspergillosis, dermatophytosis and the dimorphic pathogens, like *Histoplasma capsulatum*.
3. **Coelomycetes:** These produce acervuli, which are tightly bound mats of hyphae on which conidia are produced.

Pathogenesis of fungal diseases (Mycoses):

Most fungi are saprophytic or parasitic to plants and are adapted to their natural environment. Infection in humans is a chance event, occurring only when conditions are favourable. Except for few fungi such as the dimorphic fungi that cause systemic mycoses and dermatophytes, which are primary pathogens, the rest are only opportunistic pathogens.

Human body is a hostile environment and offers great resistance to fungal invasion. Most fungi are saprophytic and their enzymatic pathways function more efficiently at the redox potential of non-living substrates than at the relatively more reduced state of living metabolizing tissue. Some fungi such as Candida and Malasezzia have adapted to human environment and exist as commensals.

The complex interplay between fungal virulence factors and host defence factors will determine if a fungal infection will cause a disease. Infection depends on inoculum size and the general immunity of the host.

Fungal Pathogenicity (virulence factors):

- Ability to adhere to host cells by way of cell wall glycoproteins
- Production capsules allowing them to resist phagocytosis
- Production of a cytokine called GM-CSF by *Candida albicans* that suppress the production of complement.
- Ability to acquire iron from red blood cells as in *Candida albicans*
- Ability to damage host by secreting enzymes such as keratinase, elastase, collagenase
- Ability to resist killing by phagocytes as in dimorphic fungi
- Ability to secrete mycotoxins
- Having a unique enzymatic capacity
- Exhibiting thermal dimorphism
- Ability to block the cell-mediated immune defences of the host.
- Surface hydrophobicity

Host defence factors:

- Physical barriers, such as skin and mucus membranes
- The fatty acid content of the skin
- The pH of the skin, mucosal surfaces and body fluids
- Epithelial cell turnover

- Normal flora
- Chemical barriers, such as secretions, serum factors
- Most fungi are mesophilic and cannot grow at $37^{O}C$.
- Natural Effector Cells (polymorphonuclear leucocytes) and the Professional Phagocytes (monocytes and macrophages)

Factors predisposing to fungal infections:

- Prolonged antibiotic therapy
- Underlying disease (HIV infection, cancer, diabetes, etc.)
- Age
- Surgical procedures
- Immunosuppressive drugs
- Irradiation therapy
- Indwelling catheters
- Obesity
- Drug addiction
- Transplants
- Occupation

Immunity to fungal infections:

Mechanism of immunity to fungal infections can be innate or acquired. The non -specific immunity includes the physical barriers offered by skin and mucus membranes along with their secretions and normal flora. The pH, body temperature and serum factors along with phagocytic cells play an important part in providing non-specific immunity. Even though body mounts both humoral and cell mediated immunity, it is the latter that is the mainstay of host defence.

Cell mediated immunity:

Immunity is provided non-specifically be effector cells (polymorphonuclear leucocytes) and professional phagocytes (monocytes and macrophages) and specifically by T lymphocytes. The phagocytes are very important in defence against Candia, Aspergillus and Zygomycetes as is evidenced by their severity in granulomatous diseases, myeloperoxidase deficiency and cytotoxic chemotherapy.

Expression of T-cell-mediated immunity to fungi includes:

- delayed-type hypersensitivity
- contact allergy
- chronic granulomatous reactions

Humoral immunity:

Even though antibodies are produced against many fungi, their role in protection is not very clear. However, antibodies help in clearing fungal pathogens through opsonisation, which is important against Candida and Cryptococcus. Another component of humoral immunity is the complement, which can act as opsonins and may even cause damage to their cells through complement activation. Antibodies are important to fungal serodiagnosis.

Hypersensitivity:

As a result of dermatophyte infection some fungus-free skin lesions of variable morphology occur elsewhere on the body, which are thought to result from hypersensitivity to the fungus. These reactions are called "id reaction". These reactions are also seen n Candida infections. An inflamed boggy lesion of the scalp called the kerion may result from a strong immune reaction to the dermatophyte. Granulomas due to intracellular fungi represent delayed hypersensivities. Many fungi are significant allergens to humans, the allergens being spores, conidia, hyphae and other fungal products. On inhalation they may produce allergic pulmonary diseases such as allergic bronchopulmonary aspergillosis, farmer's lung, maple bark stripper's lung, bronchial asthma etc, which may be Type I or III hypersensitivity.

Fungal Diseases (Mycoses):

Mycoses can be conveniently studied as:

1. Superficial mycoses
    I. Superficial phaeohyphomycosis
    II. Tinea versicolor
    III. Black piedra IV.
    White piedra

2. Cutaneous mycoses
    I. Dermatophytosis
    II. Dermatomycosis
3. Subcutaneous mycoses
    I. Chromoblastomycosis
    II. Rhinosporidiasis
    III. Mycetoma
    IV. Sporotrichosis
    V. Subcutaneous phaeohyphomycosis VI. Lobomycosis
4. Systemic (deep) mycoses
    I. Blastomycosis
    II. Histoplasmosis
    III. Coccidioidomycosis
    IV. Paracoccidioidomycosis
5. Opportunistic mycoses
    I. Candidiasis
    II. Cryptococcosis
    III. Aspergillosis
6. Other mycoses
    I. Otomycosis
    II. Occulomycosis
7. Fungal allergies
8. Mycetism and mycotoxicosis

Laboratory diagnosis of mycoses:

Specimen collection: specimen collection depends on the site affected. Different specimens include hair, skin scrapings, nail clippings, sputum, blood, CSF, urine, corneal scraping, discharge or pus from lesions and biopsy.

- All specimens must be transported to the laboratory without any delay to prevent bacterial overgrowth. In case of delay specimens except skin specimen, blood and CSF may be refrigerated for a short period.
- Infected hairs may be plucked using forceps. Those hairs that fluoresce under Wood's lamp may be selectively plucked. Hairs may be collected in sterilized paper envelopes.
- Surface of the skin must be disinfected with spirit before specimen collection. The advancing edge of the lesion is scraped with the help of a blunt forceps and collected in sterilized paper envelopes.
- Discoloured or hyperkeratotic areas of nail may be scraped or diseased nail clipping may be collected in sterilized paper envelopes.
- Specimens from mucus membranes (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs may be collected from vagina.
- Corneal scrapings may be collected using a fine needle and inoculated at bedside.
- Pus may be collected by aspiration; use of cotton swabs may give false positive microscopic results.
- Clean catch urine may be collected in a sterile wide-mouthed container.
- Biopsy specimens must be transported in saline.

In certain cases, pus or exudates must be looked for presence of granules.

Microscopy: Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. In the latter case, lactophenol cotton blue is stain of choice, which stains the fungal elements blue. Direct examination of clinical specimens could be stained or unstained.

- Wet mount: Candida may be observed in urine wet mounts
- 10-20% KOH mount: Several specimens are subjected to KOH mount for direct examination. The material is mixed with 20% KOH on a slide and a cover slip is placed. The slide is then gently heated by passing through the flame 2-3 times. The slide is observed on cooling. KOH serves to digest the protein debris and clears keratinised tissue and increases the visibility. Addition of Dimethyl sulphoxide (DMSO) permits rapid clearing in the absence of heat.
- Calcofluor white: This is a fluorescent dye, which binds selectively to chitin of the fungal cell wall. The specimen then can be observed under fluorescent microscope.
- India Ink: Capsules of *Cryptococcus neoformans* can be demonstrated by this negative staining technique.
- Periodic Acid-Schiff (PAS) stain: On staining by this stain, fungal elements appear bright magenta coloured while the background stains green. It is useful in staining tissue specimens.

- Giemsa's stain: It is particularly useful in the detection of *Histoplamsa capsulatum* in the bone marrow smears.
- Haematoxylin and Eosin (H&E) stain: Useful for staining tissue sections.
- Gomori's methenamine silver nitrate (GMS) stain: Outlines of the fungi are black, internal parts stain pink-black while the background stains light green. Candida and Aspergillus may be missed in H&E stained sections, therefore GMS stained sections are essential for tissue pathology.
- Gridley's stain: It stains hyphae and yeasts dark blue-pink, tissues deep blue and background yellow.
- Meyer mucicarmine stain: Capsules of *C. neoformans* and inner walls of *Rhinosporidium seeberi*'s sporangium are stained pink.
- Gram stain: Candida is best demonstrated in clinical specimen by Gram stain.
- Masson-Fontana stain is helpful in staining phaeoid (dematiaceous) fungi in tissue.
- Immunofluorescence: Monoclonal antibody labelled with fluorescent dyes can be used to detect several fungi in the clinical specimens.

Culture: One of the most common media used to culture fungi in laboratory is Sabouraud's Dextrose Agar (SDA). It consists of peptone, dextrose and agar. High concentration of sugar and a low pH (4.5-5.5) prevents growth of most bacteria and makes it selective for fungi. Emmon's modification of SDA contains 2% dextrose and has pH of 6.8.

Other basal media to grow fungi include Potato Dextrose Agar, Malt Extract Agar etc. Most fungi are able to grow at room temperature while few pathogenic fungi (e.g, Cryptococcus, dimorphic fungi) can grow at 37^{o}C. Saprophytic fungi grow much quickly than pathogenic fungi (e.g, dermatophytes). In such situations the saprophytic fungi can be inhibited by the addition of cycloheximide (actidione) to the SDA. Addition of antibiotics such as Chloramphenicol, Gentamicin or Streptomycin to SDA serves to inhibit bacterial multiplication. An example of SDA with cycloheximide and Chloramphenicol is Mycosel agar.
Other specialized media used for different fungi include:

- Brain Heart Infusion Agar general isolation of fungi and conversion of dimorphic fungi.
- Inhibitory Mould Agar, an isolation medium with Chloramphenicol to suppress most bacteria.
- Caffeic Acid Agar and Birdseed Agar for isolation of *Cryptococcus neoformans*.
- Corn Meal Agar: Enhances production of chlamydospores in *Candida albicans* and formation of conidia in fungi.
- Trichophyton Agars: Used for selective identification of Trichophyton species.
- Dermatophyte Test Medium: Used for recovery of dermatophytes from clinical specimens.
- Sabhi Medium: Isolation of *Histoplasma capsulatum*.
- 'CHROM agar Candida' is useful in identification of Candida species.

Conversion of mould to yeast phase must be demonstrated in vitro for identification of dimorphic fungi. Since some fungi grow slowly cultures should not be discarded for 4-6 weeks. Fungi are identified on the basis of colony morphology (including pigmentation) and microscopic observation by tease-mount preparation or slide culture technique.

Serology: Detection of anti-fungal antibody is helpful in diagnosis of sub-cutaneous and systemic mycoses, prognosis and response to anti-fungal drugs. Different serologic techniques that are used include agglutination, immunodiffusion, counter-immunoelectrophoresis, complement fixation test, immunofluorescence, RIA and ELISA.

Antigen detection: It is particularly useful in the diagnosis of cryptococcal meningitis from CSF specimens. The test is performed by Latex Agglutination or immunodiffusion tests. It is also helpful in the detection of Aspergillus and Candida antigens in systemic infections.

Skin tests: Delayed hypersensitivity reactions to fungal antigens can be demonstrated by skin tests. A positive skin does not necessarily indicate an active infection; it only indicates sensitization of the individual. Hence, its value is in epidemiological studies than diagnosis. These tests may be performed in Histoplasmosis, Candidiasis, Sporotrichosis, Coccidioidomycosis, Blastomycosis, Paracoccidiodomycosis and dermatophytosis.

Molecular techniques: Newer techniques such as DNA hybridization, PCR are useful in diagnosis of mycoses in a shorter period as well as detect those fungi that are difficult or dangerous to cultivate in vitro.

VIRUS

General properties of viruses

Three main properties distinguish viruses from their various host cells: size, nucleic acid content and metabolic capabilities.

Size

Whereas a bacterial cell like a staphylococcus might be lOOOnm in diameter, the largest of the human pathogenic viruses, the poxviruses, measure only 250 nm along their longest axis, and the smallest, the poliovirus, is only 28 nm in diameter.

Nucleic acid content

Viruses contain only a single type of nucleic acid, either DNA or RNA.

Metabolic capabilities

Virus particles have no metabolic machinery of their own. They cannot synthesize their own protein and nucleic acid from inanimate laboratory media and thus fail to grow on even nutritious media. They are obligatory intracellular parasites, only growing within other living cells whose energy and protein-producing systems they redirect for the purpose of manufacturing new viral components.

Classification

Baltimore classification

The Nobel Prize-winning biologist David Baltimore devised the Baltimore classification system.

The Baltimore classification of viruses is based on the mechanism of mRNA production.

Viruses must generate mRNAs from their genomes to produce proteins and replicate themselves, but different mechanisms are used to achieve this in each virus family. Viral genomes may be single-stranded (ss) or double-stranded (ds), RNA or DNA, and may or may not use reverse transcriptase (RT). In addition, ssRNA viruses may be either sense (+) or antisense (−).

This classification places viruses into seven groups:

I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)

II: ssDNA viruses (+ strand or "sense") DNA (e.g. Parvoviruses)

III: dsRNA viruses (e.g. Reoviruses)

IV: (+)ssRNA viruses (+ strand or sense) RNA (e.g. Coronaviruses, Picornaviruses, Togaviruses)

V: (−)ssRNA viruses (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)

VI: ssRNA-RT viruses (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)

VII: dsDNA-RT viruses DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)

Holmes classification

Holmes (1948) used Carl Linnaeus's system of binomial nomenclature to classify viruses into 3 groups under one order, Virales. They are placed as follows:

- **Group I:** *Phaginae* (attacks bacteria)
- **Group II:** *Phytophaginae* (attacks plants)
- **Group III:** *Zoophaginae* (attacks animals)

LHT System of Virus Classification

The LHT System of Virus Classification is based on chemical and physical characters like nucleic acid (DNA or RNA), symmetry (helical or icosahedral or complex), presence of envelope, diameter of capsid, number of capsomers.

•**Phylum Vira** (divided into 2 subphyla)

- **Subphylum Deoxyvira** (DNA viruses)
  - **Class Deoxybinala** (dual symmetry)
    - **Order *Urovirales***
      - **Family *Phagoviridae***
  - **Class Deoxyhelica** (helical symmetry)
    - **Order *Chitovirales***
      - **Family *Poxviridae***
  - **Class Deoxycubica** (cubical symmetry)
    - **Order *Peplovirales***
      - **Family *Herpesviridae*** (162 capsomeres)
    - **Order *Haplovirales*** (no envelope)
      - **Family *Iridoviridae*** (812 capsomeres)
      - **Family *Adenoviridae*** (252 capsomeres)
- **Subphylum Ribovira** (RNA viruses)
  - **Class Ribocubica**
    - **Order *Togovirales***
      - **Family *Arboviridae***
    - **Order *Tymovirales***
      - **Family *Napoviridae***
      - **Family *Reoviridae***
  - **Class Ribohelica**
    - **Order *Sagovirales***
      - **Family *Stomataviridae***
      - **Family *Paramyxoviridae***
    - **Order *Rhabdovirales***
      - **Suborder *Flexiviridales***
        - **Family *Mesoviridae***
        - **Family *Peptoviridae***
      - **Suborder *Rigidovirales***
        - **Family *Pachyviridae***

<u>Structure of virus</u>

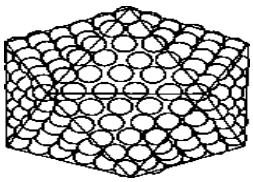
In essence, virus particles are composed of a core of genetic material, either DNA or RNA, surrounded by a coat of protein. The function of the coat is to protect the viral genes from inactivation by adverse environmental factors, such as tissue nuclease enzymes which would otherwise digest a naked viral chromosome during its passage from cell to cell within a host. In a number of viruses the coat also plays an important part in the attachment of the virus to receptors on susceptible cells, and in many bacterial viruses the coat is further modified to facilitate the insertion of the viral genome through the tough structural barrier of the bacterial cell wall. The viral protein coat, or *capsid,* is composed of a large number of subunits, the *capsomeres.* Viruses can have a lipid "envelope" derived from the host cell membrane.

Virions are either enveloped or non enveloped (Naked).

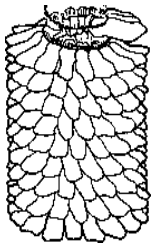
The envelope surface have projecting spikes of protein subunits, called PEPLOMERS.

Peplomers are of 2 types:

1. Haemagglutinin – a triangular spike
2. Neuraminidase – a mushroom shaped.



An icosahedral virus particle composed of 252 capsomeres 240 being hexons and 12 being pentons



A helical virus partially disrupted to show the helical coil of viral nucleic acid embedded in the capsomeres

Helical symmetry

These viruses are composed of a single type of capsomere stacked around a central axis to form a helical structure, which may have a central cavity, or tube. This arrangement results in rod-shaped or filamentous virions which can be short and highly rigid, or long and very flexible.

The genetic material (typically single-stranded RNA, but ssDNA in some cases) is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. S The tobacco mosaic virus (TMV) is the classic example. X-ray diffraction data and electron micrographs have revealed that 16 subunits per turn of the helix project from a central axial hole that runs the length of the particle. The nucleic acid does not lie in this hole, but is embedded into ridges on the inside of each subunit and describes its own helix from one end of the particle to the other. Helical symmetry was thought at one time to exist only in plant viruses. It is now known, however, to occur in a number of animal virus particles

Icosahedral symmetry

The viruses in this architectural group have their capsomeres arranged in the form of regular icosahedra, i.e. polygons having 12 vertices, 20 faces and 30 sides. At each of the 12 vertices or corners of these icosahedral particles is a capsomere, called *apenton,* which is surrounded by five neighbouring units. Each of the 20 triangular faces contains an identical number of capsomeres which are surrounded by six neighbours and called

hexons. In plant and bacterial viruses exhibiting this type of symmetry, the hexons and pentons are composed of the same polypeptide chains; in animal viruses, however, they may be distinct proteins. The number of hexons per capsid varies considerably in different viruses. Adenovirus, for example, is constructed from 240 hexons and 12 pentons, while the much smaller poliovirus is composed of 20 hexons and 12 pentons.

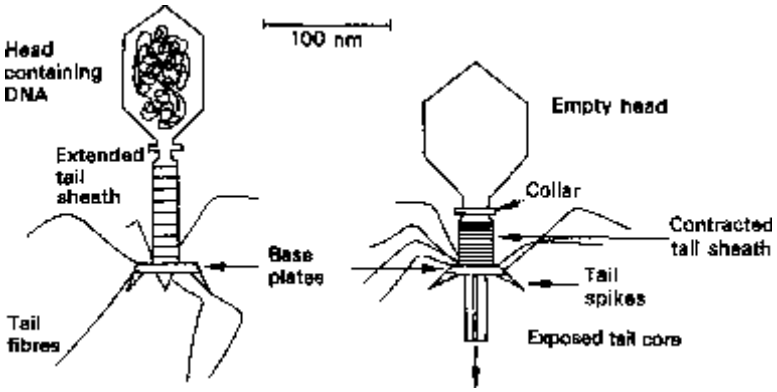
Complex or polyhedral

These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall.

Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres.

This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell

Bacteriophages



T-even phage structure before and after tail contraction

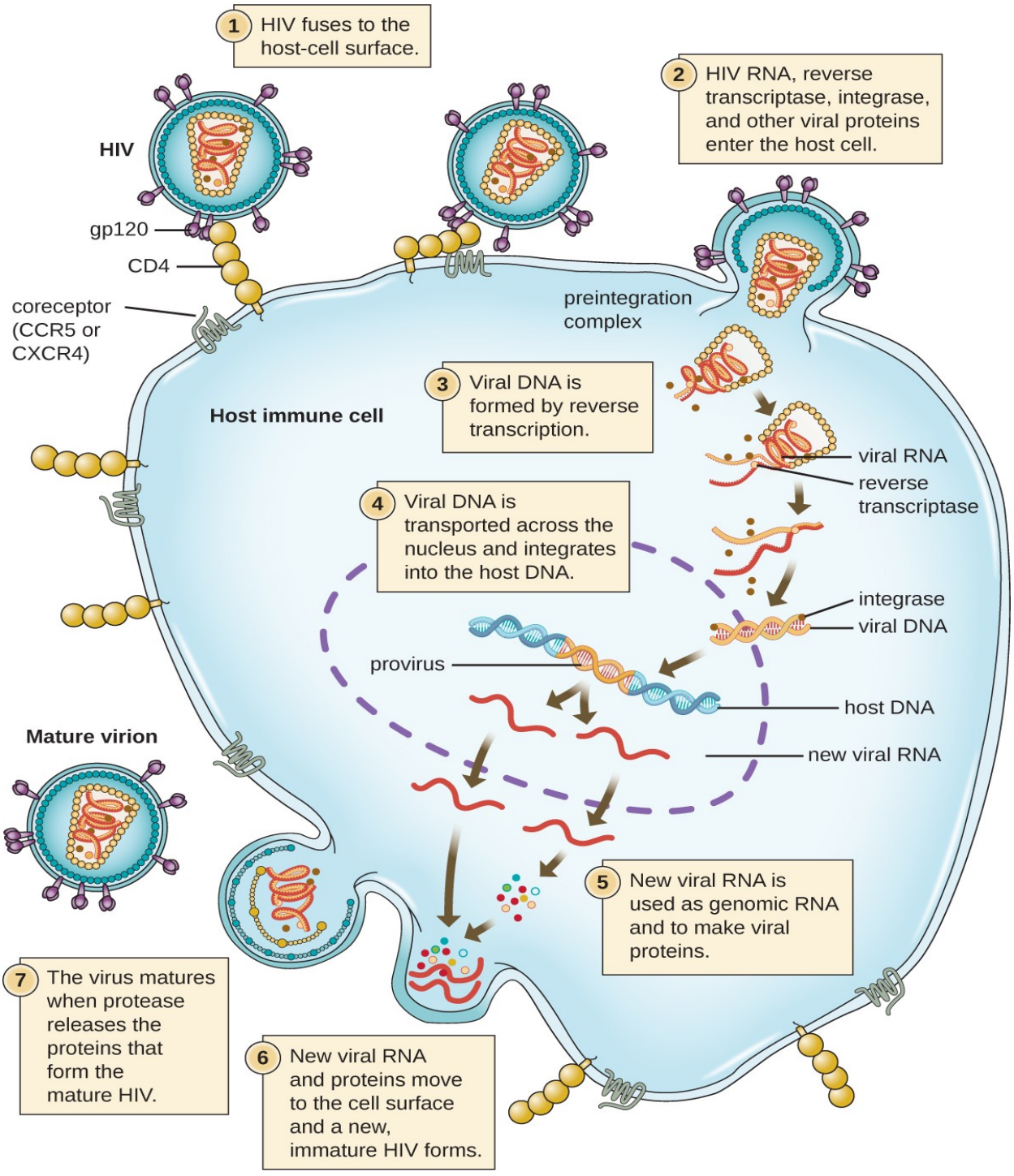
Bacteriophages, or as they are more simply termed, phages, are viruses that have bacteria as their host cells. The name was first given by D'Herelle to an agent which he found could produce lysis of the dysentery bacillus *Shigella shiga.* It is probable that all species of bacteria are susceptible to phages. Any particular phage will exhibit a marked specificity in selecting host cells, attacking only organisms belonging to a single species. A *Staphylococcus aureus* phage, for example, will not infect *Staph, epidermidis* cells. In most cases, phages are in fact strain-specific, only being active on certain characteristic strains of a given species. Most phages are tadpole-shaped structures with heads which function as containers for the nucleic acid and tails which are used to attach the virus to its host cell. There are, however, some simple icosahedral phages and others that are helically symmetrical cylinders. The dimensions of the phage heads vary from the large T-even group *of Escherichia coli* phages (60 x 90 nm) to the much smaller ones (30 x 30nm) of certain *Bacillus* phages. The tails vary in length from 15 to 200 nm and can be quite complex structures.

While the majority of phages have double-stranded DNA as their genetic material, some of the very small icosahedral and the helical phages have single-stranded DNA or RNA. On the basis of the response they produce in their host cells, phages can be classified as *virulent* or *temperate.* Infection of a sensitive bacterium with a virulent phage results in the replication of the virus, lysis of the cell and release of new infectious progeny phage particles. Temperate phages can produce this lytic response, but they are also capable of a symbiotic response in which the invading viral genome does not take over the direction of cellular activity, the cell survives the infection and the viral nucleic acid becomes incorporated into the bacterial chromosome, where it is termed *prophage.* Cells carrying viral genes in this way are referred to as *lysogenic.*

Replication of virus :

The virus replication occurs in seven stages, namely;

1.Adsorption
2.Entry,
3.Uncoating,
4.Transcription / mRNA production,
5.Synthesis of virus components,
6.Virion assembly and
7.Release (Liberation Stage).



Adsorption

It is the first step of viral replication. The virus attaches to the cell membrane of the host cell. It then injects its DNA or RNA into the host to initiate infection. In animal cells these viruses get into the cell through the process of endocytosis which works through fusing of the virus and fusing of the viral envelope with the cell membrane of the animal cell and in plant cell it enters through the process of pinocytosis which works on pinching of the viruses.

Entry

The cell membrane of the host cell invaginates the virus particle, enclosing it in a pinocytotic vacuole. This protects the cell from antibodies like in the case of the HIV virus.

Uncoating

Cell enzymes (from lysosomes) strip off the virus protein coat. This releases or renders accessible the virus nucleic acid or genome.

Transcription / mRNA production

For some RNA viruses, the infecting RNA produces messenger RNA (mRNA). This is translation of the genome into protein products. For others with negative stranded RNA and DNA, viruses are produced by transcription then translation.The mRNA is used to instruct the host cell to make virus components. The virus takes advantage of the existing cell structures to replicate itself.

Synthesis of virus components

The following components are manufactured by the virus through the host's existing organelles:

•Viral protein synthesis: virus mRNA is translated on cell ribosomes into two types of virus protein.

•Structural: the proteins which make up the virus particle are manufactured and assembled.

•Non – structural: not found in particle, mainly enzymes for virus genome replication.

•Viral nucleic acid synthesis (genome replication) new virus genome is synthesized, templates are either the parental genome or with single stranded nucleic acid genomes, newly formed complementary strands. By a virus called polymerate or replicate in some DNA viruses by a cell enzyme. This is done in rapidly dividing cells.

Virion assembly

A virion is simply an active or intact virus particle. In this stage, newly synthesized genome (nucleic acid), and proteins are assembled to form new virus particles. This may take place in the cell's nucleus, cytoplasm, or at plasma membrane for most developed viruses.

Release (liberation stage)

The viruses, now being mature are released by either sudden rupture of the cell, or gradual extrusion (force out) of enveloped viruses through the cell membrane.

The new viruses may invade or attack other cells, or remain dormant in the cell. In the case of bacterial viruses, the release of progeny virions takes place by lysis of the infected bacterium. However, in the case of animal viruses, release usually occurs without cell lysis.

The lytic growth cycle (of bacteriophage)

The replication of virulent phage was initially studied using the T-even-numbered (T2, T4 and T6) phages of *E. Coll.* These phages adsorb, by their long tail fibres, on to specific receptors on the surface of the bacterial cell wall. The base plate of the tail sheath and its pins then lock the phage into position on the outside of the cell. At

this stage, the tail sheath contracts towards the head, while the base plate remains in contact with the cell wall and, as a result, the hollow tail core is exposed and driven through to the cytoplasmic membrane (Fig. 3.3). Simultaneously, the DNA passes from the head, through the hollow tail core and is deposited on the outer surface of the cytoplasmic membrane, from where it finds its own way into the cytoplasm. The phage protein coat remains on the outside of the cell and plays no further part in the replication cycle.

Within the first few minutes after infection, transcription of part of the viral genome produces 'early' mRNA molecules, which are translated into a set of 'early' proteins. These serve to switch off host-cell macromolecular synthesis, degrade the host DNA and start to make components for viral DNA. Many of the early proteins duplicate enzymes already present in the host, concerned in the manufacture of nucleotides for cell DNA. However, the requirement for the production of 5-hydroxymethylcytosinecontaining nucleotides, which replace the normal cytosine derivatives in T-even phage DNA, means that some of the early enzymes are entirely new to the cell. With the build-up of its components, the viral DNA replicates and also starts to produce a batch

of 'late' mRNA molecules, transcribed from genes which specify the proteins of the phage coat. These late messages are translated into the subunits of the capsid structures, which condense to form phage heads, tails and tail fibres, and then together with viral DNA are assembled into complete infectious particles. The enzyme digesting the cell wall, lysozyme, is also produced in the cell at this stage and it eventually brings about the

lysis of the cell and liberation of about 100 progeny viruses, some 25 minutes after infection.

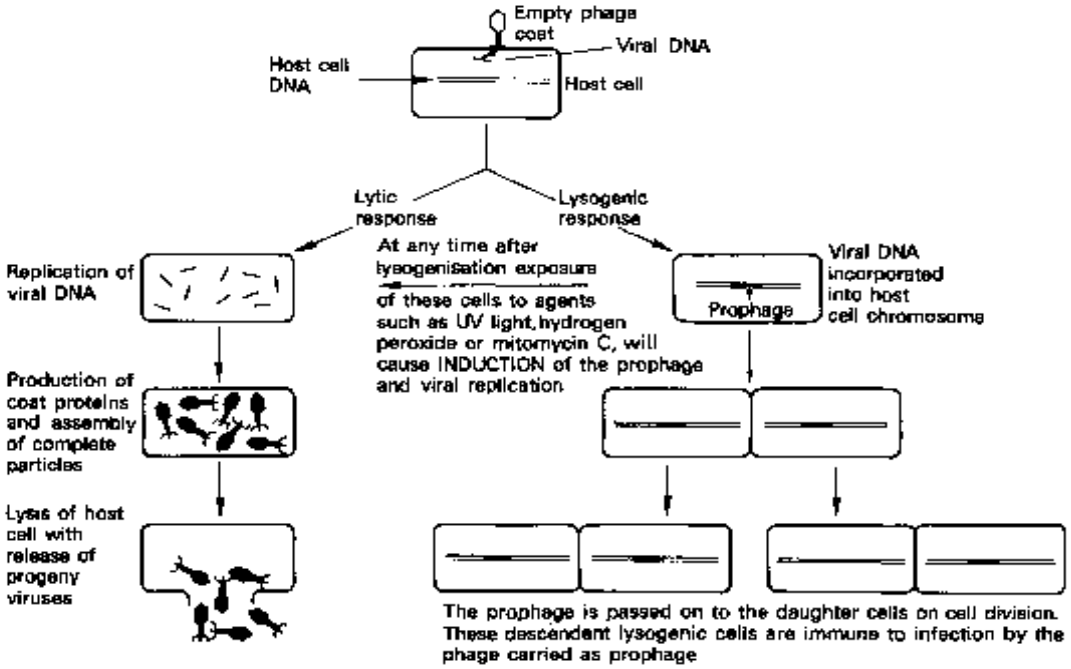


Fig. 3.5 Scheme to illustrate the lytic and lysogenic responses of bacteriophages.

Lysogeny (of bacteriophage)

The essential features of lysogenic cells and the phenomenon of lysogeny are listed below and summarized in Fig. 3.5.

1 Integration of the prophage into the bacterial chromosome ensures that, on cell division, each daughter cell will acquire the set of viral genes.

2 In a normally growing culture of lysogenic bacteria, the majority of bacteria manage to keep their prophages in a dormant state. In a very small minority of cells, however, the prophage genes express themselves. This results in the multiplication of the virus, lysis of the cells and liberation of infectious particles into the medium.

3 Exposure of lysogenic cultures to certain chemical and physical agents, e.g. hydrogen peroxide, mitomycin C and ultraviolet light, results in mass lysis and the production of high titres of phage. This process is called *induction.*

4 When a lysogenic cell is infected by the same type of phage as it carries as prophage, the infection is aborted, the activity of the invading viral genes being repressed by the same mechanism that normally keeps the prophage in a dormant state.

5 Lysogeny is generally a very stable state, but occasionally a cell will lose its prophage and these 'cured' cells are once more susceptible to infection by that particular phage type.

Lysogeny is an extremely common phenomenon and it seems that most natural isolates of bacteria carry one or more prophages; some strains of *Staph, aureus* have been shown to carry four or five different prophages.

Cultivation of human viruses

The cultivation of viruses from material taken from lesions is an important step in the diagnosis of many viral diseases. Studies of the basic biology and multiplication processes of human viruses also require that they are grown in the laboratory under experimental conditions. Human pathogenic vpiruses can be propagated in three types of cell systems.

1 Cell culture

Cells from human or other primate sources are obtained from an intact tissue, e.g. human embryo kidney or monkey kidney. The cells are dispersed by digestion with trypsin and the resulting suspension of single cells is generally allowed to settle in a

vessel containing a nutrient medium. The cells will metabolize and grow and after a few days of incubation at 37°C will form a continuous film or monolayer one cell thick. These cells are then capable of supporting viral replication. Cell cultures may be divided into three types according to their history.

a) Primary cell cultures, which are prepared directly from tissues.

b) Secondary cell cultures, which can be prepared by taking cells from some types of primary culture, usually those derived from embryonic tissue, dispersing them by treatment with trypsin and inoculating some into a fresh batch of medium. A limited number of subcultures can be performed with these sorts of cells, up to a maximum of about 50 before the cells degenerate.

Media:

- A balanced salt solution, to provide the optimum pH and osmotic pressure.
- Nutrients: approximately 60 ingredients. Eg: essential amino acids, growth factors, dextrose, purines pyrimidines& inorganic salts.
- A pH indicator to show the state of cell metabolism. Eg: often **Phenol red** is used.
- Antibiotics, both antibacterial and antifungal to prevent growth of contaminants.

2 The chick embryo

Fertile chicken eggs, 10-12 days old, have been used as a convenient cell system in which to grow a number of human pathogenic viruses.

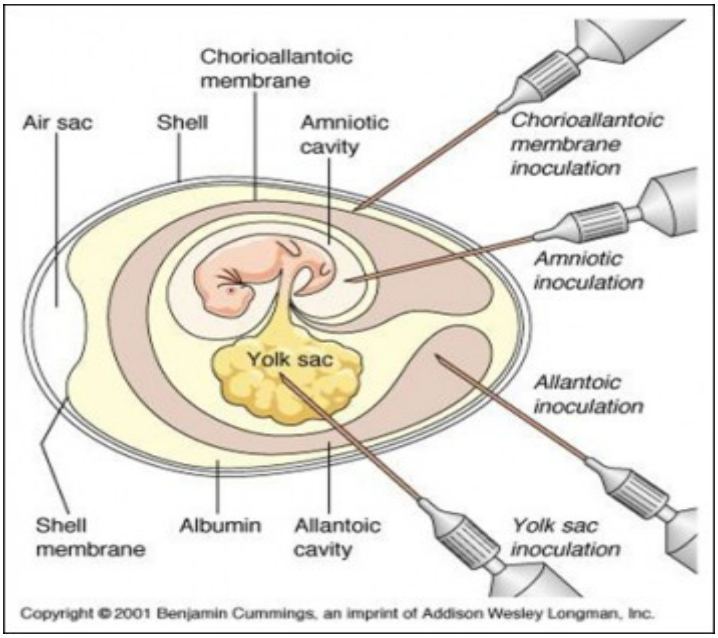


Figure shows that viruses generally have preferences for particular tissues within the embryo. Influenza viruses, for example, can be grown in the cells of the membrane bounding the amniotic cavity, while smallpox virus will grow in the chorioallantoic membrane. The growth of smallpox virus in the embryo is recognized by the formation of characteristic pock marks on the membrane. Influenza virus replication is detected by exploiting the ability of these particles to cause erythrocytes to clump together. Fluid from the amniotic cavity of the infected embryo is titrated for its haemagglutinating activity.

It is much easier to keep the product free from contaminating organisms.

Embryo cannot produce antiviral antibodies. But ....

- ✓ Strict aseptic conditions must be maintained throughout.
- ✓ Repeated passage from egg to egg must be avoided because the virus may become adapted to embryo and less virulent to its natural host.
- ✓ Traces of egg protein get into the vaccine may cause reactions in the recipient.
- ✓ The eggs must be candled to to confirm that the embryos are alive.

3 Animal inoculation

Experimental animals such as mice and ferrets have to be used for the cultivation of some viruses. Growth of the virus is indicated by signs of disease or death of the inoculated animal. Nowadays very few vaccines are made from free living animals. They pproduce good antigens but the method is inconvenient and costly and contamination is difficult to prevent.

Vrus Host Interaction

Interferon

Although it is difficult to obtain drugs capable of interrupting viral replication, it had been known for many years that infection of a host with one virus could sometimes prevent infections with a second, quite unrelated virus. This phenomenon was called *interference* and in many cases it proved to be due to the production of a substance called *interferon,* Interferons are low molecular weight proteins produced by virus-infected cells. They have no direct antiviral activity. They bind to the cell membranes and induce the synthesis of secondary proteins. If interferon-treated cells are then infected with a virus, although adsorption, penetration and uncoating can take place, the interferon-induced proteins inhibit viral nucleic acid and protein synthesis and the infection is aborted. Interferons have major roles to play in protecting the host against natural virus infections. They are produced more rapidly than antibodies and the outcome of many natural viral infections is probably determined by the relative early titres of interferon and virus, protection being most effective when the infecting dose of virus is low. Potentially, interferon is an ideal antiviral agent in that it acts on many different viruses and is not toxic to host cells. However, the exploitation of this agent in the treatment of viral infections has been delayed by a number of factors. For example, it has proved to be species-specific and interferons raised in animal sources offered little protection to human cells. Human interferon is thus needed for the treatment of human infections and the production and purification of human interferon on a large scale has proved difficult. The insertion of human genes for interferon into *E. coli* has resolved the production problems. Clinical trials have demonstrated that interferon prevents rhinovirus infection and has a beneficial effect in herpes, cytomegalovirus and hepatitis B virus infections. Interferon does not only inhibit virus replication, it also has multiple effects on cell metabolism and slows down the growth and multiplication of treated cells. This is probably responsible for its widely reported antitumour effect. Encouraging results have been reported from clinical trials of interferon against several human tumours such as osteogenic sarcoma, myeloma, lymphoma and breast cancer.