Introduction.

Microbiology is the study of microorganisms, which are unicellular or multi-cellular organisms. This includes eukaryotes such as fungi, and prokaryotes such as bacteria and certain algae. Viruses, though not strictly classed as living organisms, are also studied. Microbiology is a broad term which includes virology, mycology, parasitology and other branches. In reality it is likely that only about 1% of all of the microbial species have been studied, and although microbes were first observed over three hundred years ago, the field of microbiology can be said to be in its infancy relative to older biological disciplines.

The existence of microorganisms was hypothesised for many centuries before their actual discovery in the 17th century. The first theories on microorganisms was made by Roman scholar Marcus Terentius Varro in a book titled 'On Agriculture' in which he warns against locating a homestead in the vicinity of swamps:

> " *...and because there are bred certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases.* "

Even though he accepted that he could not see the organisms, he was aware that disease may well be spread this way.

In The Canon of Medicine (1020), Abū Alī ibn Sīnā (Avicenna) stated that bodily secretions were contaminated by 'foul foreign earthly bodies' before being infected. He also hypothesised on the contagious nature of tuberculosis and other infectious diseases, and used quarantine as a means of limiting the spread of contagious diseases.

When bubonic plague reached al-Andalus in the 14th century, Ibn Khatima suggested that infectious diseases are caused by "minute bodies" which enter the human body and cause disease.

In 1546 Girolamo Fracastoro proposed that epidemic diseases were caused by transferable seed-like entities that could transmit infection by direct or indirect contact or even without contact over long distances.

All these early claims about the existence of microorganisms were purely speculative in nature as there did not exist the equipment to provide evidence of their existence. Microorganisms were neither proven, observed, nor correctly and accurately described until the 17th century. The reason for this was that all these early inquiries lacked the most fundamental tool in order for microbiology and bacteriology to exist as a science - the microscope.

Discovery and origins of microbiology

Bacteria and micro-organisms were first observed by Antonie van Leeuwenhoek in 1676 using a single-lens microscope of his own design. In doing so Leeuwenhoek made one of the most important discoveries in biology and initiated the scientific fields of bacteriology and microbiology. The name "bacterium" was introduced much later, by Ehrenberg in 1828, derived from the Greek, and meaning "small stick". While Van Leeuwenhoek is often cited as the first microbiologist, the first recorded microbiological observation, that of the fruiting bodies of molds, was actually made earlier in 1665 by Robert Hooke.

The field of bacteriology (later a subdiscipline of microbiology) is generally considered to have been founded by Ferdinand Cohn (1828–1898), a botanist whose studies on algae and photosynthetic bacteria led him to describe several bacteria including Bacillus and Beggiatoa. Cohn was also the first to formulate a scheme for the taxonomic classification of bacteria. Louis Pasteur (1822–1895) and Robert Koch (1843–1910) were contemporaries of Cohn’s and are often considered to be the founders of medical microbiology. Pasteur is most famous for his series of experiments designed to disprove the then widely-held theory of spontaneous generation, as well as designing methods for food preservation (pasteurisation) and vaccines against several diseases such as anthrax, fowl cholera and rabies. Koch is best known for his contributions to the germ theory of disease, proving that specific diseases were caused by specific pathogenic microorganisms. He developed

a series of criteria that have become known as the Koch's Postulates. Koch was one of the first scientists to focus on the isolation of bacteria in pure culture resulting in his description of several bacteria including Mycobacterium tuberculosis, the causative agent of tuberculosis.

While Pasteur and Koch are often considered the founders of microbiology, their work did not accurately reflect the true diversity of the microbial world because of their exclusive focus on microorganisms having direct medical relevance. It was not until the work of Martinus Beijerinck (1851–1931) and Sergei Winogradsky (1856–1953), the founders of general microbiology (an older term encompassing aspects of microbial physiology, diversity and ecology), that the true breadth of microbiology was revealed. Beijerinck made two major contributions to microbiology: the discovery of viruses and the development of enrichment culture techniques. Whilst his work on the Tobacco Mosaic Virus established the basic principles of virology, it was his development of enrichment culturing that had the most immediate impact on microbiology by allowing for the cultivation of a wide range of microbes with wildly different physiologies. Winogradsky was the first to develop the concept of chemolithotrophy (organisms which use inorganic materials to help produce energy), and to thereby reveal the essential role played by microorganisms in geochemical processes. He was responsible for the first isolation and description of both nitrifying and nitrogen-fixing bacteria.

A modern perspective.

Worldwide more than 20% of deaths are caused by infectious disease, and this number is increasing in both wealthy and poor economies. The picture in Europe is similar to that in the USA :

- deaths from HIV peaked at around 50,000 in 1995, but still exceed 12,000 each year;
- influenza and pneumonia kill 61,000 people each year and affect many millions;
- some 4 million people carry hepatitis C virus, and 15% develop life-

threatening cirrhosis as a result;
- drug-resistant tuberculosis (TB) is a major cause of concern, as are food-borne infections and hospital-acquired infections.

The burden of infectious disease in the resource-poor world continues to increase rapidly, particularly in sub-Saharan Africa and south-east Asia. Although sub-Saharan Africa has only about 10% of the world's population, it has 60% of AIDS infections and a majority of all AIDS-related deaths, the highest HN-TB co-infection rates and most of the global malaria burden. TB and HIV-AIDS are of increasing importance in south-east Asia and the Pacific, where drug resistant malaria is also common.

Children younger than 5 years are most at risk from infectious diseases. Of the 10.6 million deaths in this age group recorded by WHO for the year 2002, more than half were due to infection, acute respiratory infection and diarrhoeal diseases accounting for 36% of all deaths. Almost all of these infection-related deaths occurred in Africa, south-east Asia and the Western Pacific. It is obvious that the prevalence and importance of infectious diseases in the resource-poor world are directly linked to poverty.

In the last 35 years familiar diseases such as TB, malaria, hepatitis, and cholera have re-emerged as major infections. Since 1980, some 25 new infectious agents have been identified, of which HIV is the most important. For many new diseases, there is no effective treatment. The economic cost of these diseases is enormous: Around £270 billion was the estimated cumulative cost of the AIDS epidemics by 2000 and the cost of malaria in Africa was estimated at £7 billion in 2000. Successful eradication can therefore save very large sums, for example an estimated £11 billion from eradicating smallpox.

The reasons for the resurgence of infectious diseases are multiple. They include:

- New patterns of travel and trade (especially food commodities), new agricultural practices, altered sexual behaviour, medical interventions and overuse of antibiotics.

- The evolution of multi-drug resistant bacteria, such as MRSA, and their frequency in hospital patients, has become a major problem.
- Breakdown of economic, social and political systems in the resource-poor world and the countries of the former Soviet Union has weakened medical services and increased the effects of poverty and malnutrition.
- The dramatic increase in air travel over the last few decades has facilitated the spread of infection and increased the threat of new pandemics. The Spanish influenza pandemic in 1918 spread along railway and sea links. Modern air travel moves larger numbers of people more rapidly and more extensively and makes it possible for microbes to cross geographical barriers, a recent example of this being the potential for spread of the SARS virus from Asia to Europe and North America, as well as the avian flu virus.

Predictions based on data from the United Nations and the World Health Organization give a choice of optimistic, stable or pessimistic scenarios. Optimistically, the aging population, coupled with socioeconomic and medical advances, should see a fall in the problems posed by infectious disease, and a decrease in deaths from these causes from 34% of the global total in 1990 to 15% in 2020.

The pessimistic view is that population growth in resource-poor countries, especially in urban populations, the increasing gap between rich and poor countries and continuing changes in lifestyle will result in surges of infectious disease. Even in resource-rich countries, increasing drug resistance and a slowing of developments in new antimicrobials and vaccines will create problems in control. Added to these are three additional factors. These are:

- the emergence of new human infections such as a novel strain of influenza virus, or a new infection of wildlife origin
- climate change, with increased temperatures and altered rainfall adding to the incidence of vector-borne infection
- the threat of bioterrorism, with the possible deliberate spread of viral and bacterial infections.

Whatever the view, it is without doubt microbiology and the continuing study of micro-organisms remains essential to the future of medicine and disease management, and its importance in every-day health care should never be under-estimated.

Characteristics of Bacteria.

All bacteria have two names; firstly their generic (genus) name, e.g. *Staphylococcus,* then their specific (species) name, e.g. *aureus*

Special additions to this universal scheme may include:

- a third name to distinguish several varieties within one species, e.g. *Acinetobaeter calcoaceticus* var *anitratus*
- a common, non-scientific, historical name, e.g. pneumococcus for *Streptococcus pneumoniae,* gonococcus for *Neisseria gonorrhoeae,* meningococcus for *Neisseria meningitidis*
- a serological group name, e.g. *Streptococcus pyogenes* is also called 'the group A streptococcus'
- a toxin profile name, e.g. Clostridium *perfringens* type A.

Increasing knowledge of the properties of bacteria, fungi, protozoa and algae has necessitated revision of the simple division of living things into two kingdoms: plants and animals. The animal group have two sub-groups - procaryotes (bacteria and blue-green algae) and eucaryotes.

Eucaryotes include Protista (Protozoa and Algae), Fungi, Animalia and Plantae. Viruses are not included because they do not have the essential characteristics of living organisms, i.e. not capable of independent replication or survival.

Bacteria are fundamentally different from all other living things in being procaryotes, distinguished by:

- DNA in a double-stranded loop, not within a nuclear membrane
- small ribosomes free in the cytoplasm, no endoplasmic reticulum
- the absence of mitochondria or other membrane-enclosed organelles
- a complex peptidoglycan-protein cell wall

Bacteria are classified by several criteria :

- shape
- stain
- ability to grow with or without oxygen
- size
- growth characteristics
- DNA content

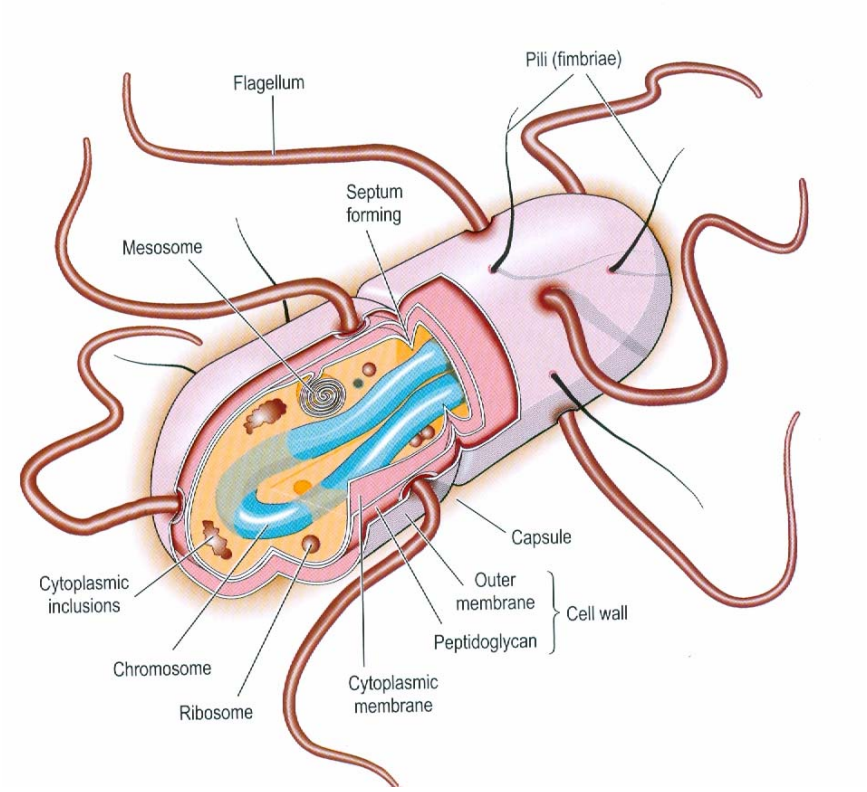
The Gram stain is the most important staining procedure in medical microbiology. Gram-positive organisms retain the purple of crystal violet after iodine fixation and alcohol washing, whereas Gram-negative organisms lose the colour with alcohol and need counterstaining with a pink dye. Special stains are needed for organisms with unusual cell walls, e.g. acid-fast stains for mycobacteria.

Although staining and growth characteristics have formed the basis of diagnostic microbiology, the availability of techniques such as DNA probes and amplification, and polyclonal and monoclonal antibodies have greatly increased the speed, range and sensitivity of diagnostic testing.

Size and Structure.

Pathogenic bacteria vary widely in size: from *Mycoplasma* spp. *(0.2-0.8μm* diameter) to enteric Gramnegative rods *(O.5- 6.0 μm* diameter*).* Gram-positive and Gram-negative bacteria differ in their cell wall composition but have typical procaryote internal structure.

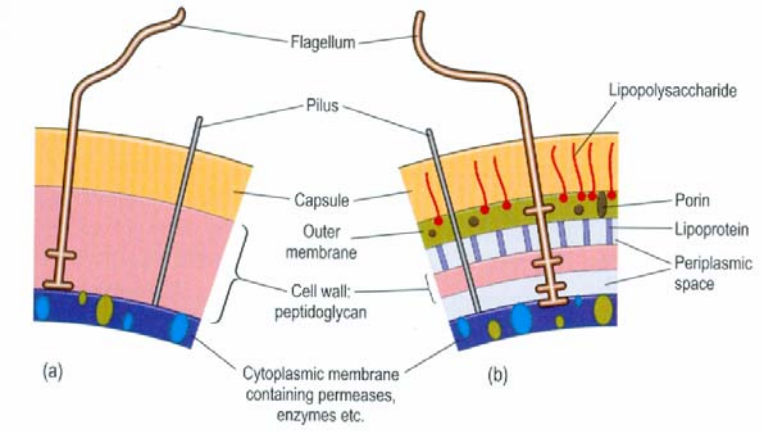
Cell membrane - The cell (cytoplasmic) membrane is made of protein and phospholipids, but not the sterols that are found in eucaryotes. It is the osmotic barrier between cell and environment, and its essential functions include electron transport, enzyme systems (as in eucaryotic mitochondria), solute transport and cell product transport.



Cell wall - The cell wall has numerous functions reflected in its structure:

- protects the cell membrane from osmotic or mechanical rupture
- contains numerous characteristic antigens, important both in bacterial virulence and endotoxins, and in host antibody formation
- provides a firm base for pili (fimbriae) and flagella.

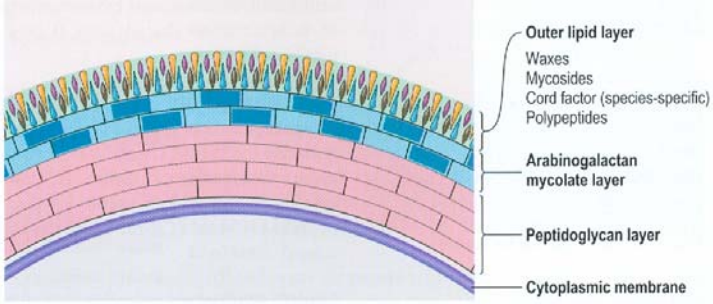
Gram-positive cells (figure a) The cell wall of Gram-positive organisms consists mainly of many layers of peptidoglycan (murein), a complex polymer of long glycan (sugar) chains of alternating N-acetylglucosamine and N-acetyl muramic acid with short pentapeptide side chains cross-linked to each other by peptide bonds between the lysine of one and the D-alanine of the other, giving a rigid polar wall. Other polymers in the wall include teichoic acids and chains of glycerol or ribitol linked by phosphodiester bonds.



Gram-negative cells (figure b) The cell wall of Gram-negative organisms consists of a thinner layer of the same peptidoglycan but the crosslinking is between D-amino pimelic acid and D-alanine. This peptidoglycan layer is in a periplasmic space between the inner cytoplasmic membrane and a unique bi-layered phospholipid outer membrane, with lipoprotein on the inner surface binding to the peptidoglycan, and a special lipopolysaccharide (LPS) on the outer. The three components of LPS are the lipid (lipid A, the active component of endotoxin, very important in causing septic shock), a core polysaccharide and a variable carbohydrate chain. Its hydrophobicity gives some antibiotic and bile salt resistance.

Mycobacteria

The cell wall of mycobacteria and other acid-fast organisms contains characteristic waxes, complex long-chain hydrocarbons with sugars. This almost impervious coat prevents stains being removed by acid and gives resistance to desiccation and many disinfectants and antibiotics. It also slows the entry of nutrients.



Capsule

A capsule protects the cell wall of many bacteria, particularly in adverse conditions; this mucoid polysaccharide layer may be lost in laboratory cultures. In infections, it resists phagocytosis by white blood cells and aids adherence to tissues, catheters and prostheses.

Pili

Pili (fimbriae) are hair-like in appearance and are of at least two types:

- Sex pili are specialised structures that enable DNA transfer by conjugation (literally, 'joined with')
- Common pili are shorter and aid attachment to host cells, are antiphagocytic and can change their antigenic protein to avoid host antibody response.

Flagella

Flagella are much longer than pili and give motility to bacteria, which may be monotrichous (one flagellum at one or both ends), lophotrichous (many flagella at one or both ends), or peritrichous ('covered with hair').

Spores

Spores, formed especially by *Clostridium* spp. and *Bacillus* spp., are concentrated bacterial DNA surrounded by an extremely tough protective coat. The cell is metabolically inert and survives drying, heat and most chemical agents for months, years or more.

KEY LEARNING POINTS.

1. **The first name of a bacteria is its genus, the second is its species**
2. **Bacteria are procaryocytes with free circular DNA, ribosomes, no mitochondria, and a peptidoglycan cell wall.**
3. **Spores are metabolically inert, and surrounded by a protein coat.**

Energy, nutrition, and growth.

Energy sources and processes

Bacteria use three sources for their energy requirements: chemical reactions (chemotrophy), light (phototrophy) or the host cell (paratrophy). If the energy-yielding reactions use organic compounds they are termed organotrophy those using inorganic compounds are termed lithotrophy. So energy derived from light using organic hydrogen donors would be described as photo-organotrophy (in some anaerobic bacteria).

Nutrition

Bacteria show a wide variety of nutritional requirements. Autotrophs can live in an entirely inorganic environment - they are free living and rarely are of medical importance. Heterotrophs need an exogenous supply of one or more essential metabolites. Most bacteria of medical importance come into this group and they vary from those with great synthetic capacity (e.g. Escherichia coli) to those pathogens that require exogenous supplies of growth factors such as vitamins to grow (e.g. some streptococci). Finally, some of the parasitic and pathogenic species can only survive intracellularly (paratrophy or auxotrophy); they possess DNA and RNA, but only a limited range of independent metabolic activity.

Growth

The doubling time or the generation time is the time between bacterial divisions (by binary fission) and ranges from about 20 minutes for E. coli and similar bacteria provided with rich nutrients like laboratory media, to 24 hours for tubercle bacilli. Balanced growth occurs when all necessary nutrients are supplied, while unbalanced growth is more usual in nature where changing environments and unbalanced nutritional supplies will occur.

Characteristics of Fungi

Fungi, like bacteria, are named by the binomial Linnaean system with a generic name (capitalised and in italics) and a specific name (not capitalised, in italics). However all fungi reproduce asexually, giving the anamorphic state, and most also reproduce sexually giving the teliomorphic state. Unfortunately many pairs of these were described and named before it was realised that they were different forms of the one fungus, e.g. *Cryptococcus neoformans* (anamorph) = *Filobasidiella neoformans* (teliomorph). The name of the tissue form (anamorph) is used by clinicians.

Fungi can be normal inhabitants of the mouth and intestinal tract. In predisposing conditions, e.g. pregnancy, diabetes, immunodeficiency, therapy with broad-spectrum antibiotics, the fungi can proliferate and cause disease, e.g. thrush or even endocarditis.

Fungi are divided into two phyla (groups):

- *Zygomycota,* which quickly produce a diploid zygote sexually and sporangiophores asexually.
- *Dikaryomycota,* in which the haploid nuclei do not fuse quickly, giving a prolonged dikaryotic sexual cycle.

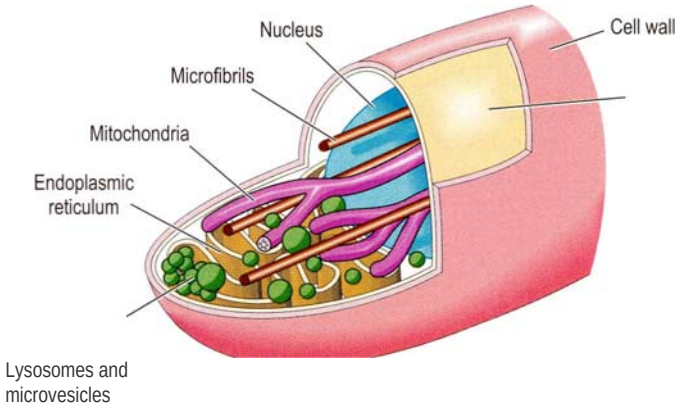
Fungi for which a sexual form is not yet recognised and which, therefore, cannot be fully classified are called Fungi imperfecti. These include many pathogens: e.g. *Candida, Epidermophyton* spp.

Structure.

Fungi, being eucaryotes, have a structure (Fig. 2) differing considerably from procaryotic bacteria (see p. 2-3). Fungi have *a nucleus* containing their chromosomal DNA and an RNA-rich nucleolus within a nuclear membrane The cytoplasm contains not only ribosomes but also *mitochondria, lysosomes and microvesicles,* microtubules, *Golgi apparatus and a double-membraned endoplasmic* reticulum.

Surrounding the above structures (the cytosol) is the cell membrane or *plasmalemma,* which contains not only lipids and glycoproteins but also ergosterol. Bacteria (except for *Mycoplasma)* do not contain sterols, and mammalian cells contain cholesterol rather than ergosterol, which is, therefore, a site of attack by antifungal drugs.

Outside the plasmalemma is a rigid cell wall containing a polymer of N-acetylglucosamine, *chitin,* on which are layers of polypeptides with complex polysaccharides including mannans and glucans. Some fungi, e.g. *Cryptococcus* spp., have a further layer, a polysaccharide *capsule.* The cell wall and capsule have multiple functions, including protection, transport and virulence, and are involved in invoking the host response.



Morphology

There are two major morphological forms of fungi : small round yeasts, and long filaments called hyphae. Both have sexual and asexual forms.

Yeasts - Yeasts are round, unicellular and multiply by budding or by fission. Some yeasts form long buds called pseudo-hyphae ('germ tubes', used to identify *Candida albicans)* but not true hyphae.

Filamentous fungi - Filamentous fungi form hyphae, long tubes which may have cross-walls called septa, or simply be multinucleate. A collection of hyphae is called a mycelium, which may be vegetative, growing on a nutrient surface, or extending upwards as an aerial mycelium producing conidia ('spores') which

spread very easily, contaminating a laboratory if culture plates are carelessly opened! The morphology of conidia is important in classifying fungi.

Dimorphic fungi - Dimorphic fungi exist in both forms. Many pathogenic fungi are dimorphic, usually the yeast form occurring in tissues and the filamentous (mould) form in the environment or on culture at 25°C. *Candida albicans* is an exception, forming mycelium in tissues.

Diagnosis of fungal infection

Yeasts and fungi grow on ordinary media but are mostly slow growing, and cultures need to be examined over 2-3 weeks. A glucose or blood agar is often used at acid pH to inhibit bacterial growth. Identification of fungi is mainly made from morphology, and yeasts may be detected in stained films during routine examination of swabs etc. Special stains are usually needed for filamentous fungi.

Clinical classification

Fungi are sometimes grouped by the clinical syndromes they cause:

- superficial and cutaneous mycoses , e.g. athlete's foot caused by *Trichophyton* spp.
- subcutaneous mycoses,e.g. sporotrichosis, ulcerative lesions caused by *Sporothrix* spp.
- systemic/deep mycoses, e.g. histoplasmosis, a pulmonary or generalised disease caused by *Histoplasma capsulatum.*

Reproduction

Asexual reproduction is most commonly seen when haploid cells divide by mitosis to form spores, the chromosome number being unchanged.

Sexual reproduction produces spores by mating when two haploid cells fuse to become diploid and then divide by meiosis. Many fungi need two colonies of the

opposite mating type for sexual mating to occur, while others need only one colony. Spore formation occurs either within a sac called an ascus or partly on the surface of a bag called a basidium. The characteristic appearance of the spore-bearing structure and the spore is used in classification and hence identification of pathogens.

Pathogenicity

Fungal pathogenicity describes the pathogen's attack mechanisms, whereas resistance describes the host's defence mechanisms. It is essential to distinguish between:

- primary pathogens, i.e. fungi such as Cryptococcus spp. that can infect normal hosts
- opportunistic fungi, i.e. those only able to infect abnormal hosts with impaired defences resulting from, for example, antibiotics, cytotoxics, x-ray therapy, steroids and other immunosuppressant drugs, endocrine disease such as diabetes mellitus, or AIDS.

Although less is known about fungal than bacterial pathogenicity, the following mechanisms are recognised:

- mycotoxins
- hypersensitivity
- invasive infection.

Mycotoxins

Unlike bacteria, fungi are not known to produce any endotoxins. Some make exotoxins, i.e. made outside the fungus. These are also only made outside the human body.

Hypersensitivity

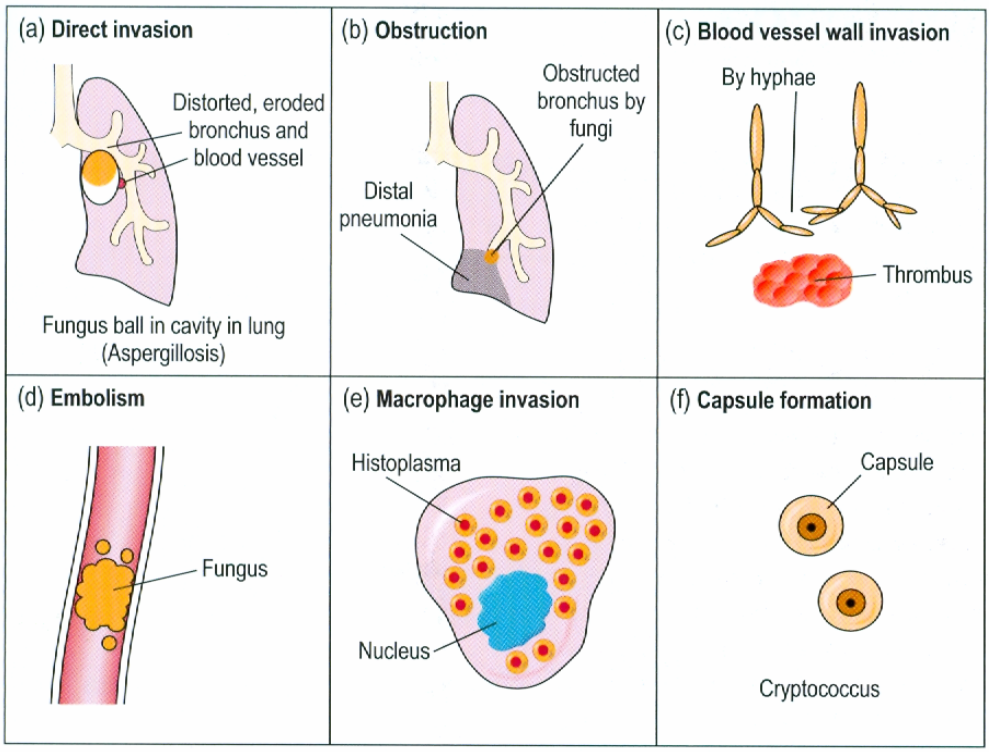
Hypersensitivity results from repeated exposure to fungal spores and consequent immunoglobulin or sensitised lymphocyte production. There is no toxin production

or tissue invasion. Inhalation of spores (e.g. of *Aspergillus)* causes allergic rhinitis, asthma and alveolitis, i.e. hypersensitivity pneumonitis.

Invasive infection

Colonisation is the continuing presence of the organism without disease, whereas infection means tissue invasion and damage. Anatomically, fungal infection causes superficial, cutaneous, subcutaneous or systemic mycoses. Tissue damage in infection can occur by at least six mechanisms:

1. direct invasion leads to distortion (e.g. fungus balls formed from *Aspergillus*) hence tissue destruction and ill-understood toxic effects
2. obstruction leads to secondary bacterial infection and further tissue damage
3. blood vessel wall invasion causes thrombosis, obstruction, ischaemia and infarction of tissues.
4. embolism to distant vessels occurs, e.g. in endocarditis
5. intracellular persistence and even multiplication in macrophages and neutrophils by some fungi.
6. capsule formation can occur in species such as *Cryptococcus;* this protects the fungus and may damage tissues.



Host resistance

All four lines of defence are of some but variable importance against fungi:

- Intact skin and mucous membranes plus the associated chemical and bacterial factors are primary barriers. Normal bacterial flora compete with fungi for nutrients, the balance being upset by antibiotics.
- Non-specific inflammatory reactions occur, though neutrophil phagocytosis and macrophage activity is often less against fungal than bacterial infection.
- Antibodies and complement can kill *Aspergillus* and *Candida* spp., though many antibodies are not protective.
- Cell-mediated immunity is the most important defence, and its loss, in diseases such as AIDS, causes a multitude of serious and often fatal fungal infections.

Fungal Ecology

The ecology of fungi include their reservoirs in the environment, animals and humans, hence the sources of infection.

Environmental reservoirs - These are the natural habitat of many fungi, e.g. free living in soil or air. These reservoirs are the usual source of most human pathogenic fungi, infection occurring after inhalation or implantation. Geophilic ('soil loving') dermatophytes live in the soil.

Animals - Zoophilic ('animal-loving') dermatophytes obviously live on (and infect) animals, such as cats, dogs and horses, and, at times, humans.

Humans - Two yeasts are commonly found as part of our normal flora: *Candida albicans* on skin and mucous membranes, and *Pityrosporum ovale* on skin rich in nutrient lipids from sebaceous glands. Thirdly, dermatophytes are sometimes found in the absence of symptoms, probably as colonisation.

KEY LEARNING POINTS.

1. **Fungi are eukaryotic, having a nucleus with a nuclear membrane, and mitochondria, golgi apparatus, lysosomes, and endoplasmic reticulum.**
2. **All fungi reproduce asexually.**
3. **Primary pathogens are fungi able to infect normal hosts**
4. **Opportunistic pathogens are fungi which can infect abnormal hosts with impaired defences**
5. **Fungi cause disease via mycotoxins, hypersensitivity, or invasive infection**
6. **Reservoirs and sources of infection are usually environmental, occasionally animals, and more commonly from other humans.**

Viruses.

Basic properties

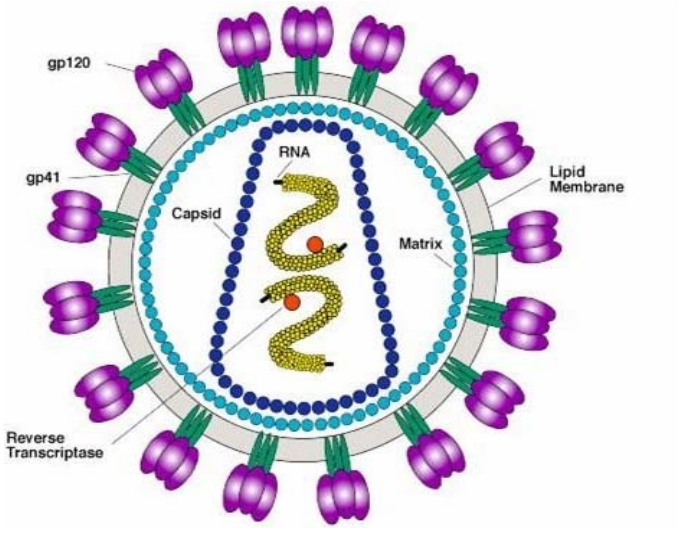
Viruses are not living, as they lack two essential properties of life - they cannot replicate independently, and they cannot survive long-term independently, needing a bigger living organism for both functions, as they contain no ribosomes, so unaided cannot synthesise protein.

They are much smaller than bacteria: human viral pathogens are usually 20 to 300 nanometres (nm) in diameter, compared to about 1000nm for coccal bacteria like staphylococci. Fungi and protozoa are much bigger again.

Their structure is simple: a nucleocapsid consisting of a central genome of either DNA or RNA, and a protein shell called the capsid. Many viruses also have an outer envelope.

Structure and composition

The virus particle is called a virion, and consists (Fig. 1) of the nucleocapsid with two components - the single- or double-stranded, linear, circular or segmented D A or RNA genome, with a surrounding protein shell called the capsid. Viruses contain either DNA or RNA, never both like living organisms and cells.



The capsid's repeating protein units form structural units called capsomeres: these are usually arranged with either helical or icosahedral (20-sided) symmetry, the exceptions including pox viruses with complex symmetry. Small icosahedral viruses appear spherical on electron microscopy.

The nucleocapsid of some human viruses is 'naked', but all helical and many icosahedral are sheathed by a large envelope with an inner structural protein layer, an outer lipid layer, and projecting spikes of glycoprotein.

Viral nucleic acids are usually double-stranded (DS) DNA or single-stranded (SS) RNA. The RNA can be either positive-strand (+) which functions as messenger RNA (mRNA), or negative strand (-) RNA which functions as a template for the production of mRNA.

Viral proteins are either structural or non-structural. Structural proteins are essential components, either of the capsid, or basic core proteins to 'package' the nucleic acid, or envelope glycoproteins. Non-structural proteins are usually enzymes to produce virus components, for example RNA-dependent transcriptase in negative-strand RNA viruses to produce mRNA.

Viral envelopes are lipoprotein, composed of an inner structural virus-derived protein and an outer host-cell-derived lipid layer. In addition there are often projecting spikes of glycoprotein, which are important as viral attachment proteins (VAPs) to host cells or erythrocytes (haemagglutinins), as neuraminidases (influenza virus), as receptors, or as antigens which stimulate protective immunity.

Atypical viral-like agents

There are four types:

- **Defective viruses** such as Hepatitis D have viral protein but defective nucleic acid (by mutation or deletion), so cannot replicate without a helper virus, in this case Hepatitis B.
- **Pseudovirions** have the viral DNA replaced by host-cell DNA which has fragmented and been incorporated into the viral capsid. They can

infect but cannot replicate.

- **Viroids** are only a single small molecule of RNA, with no capsid or envelope. How they replicate is unclear. They can infect plants, but apparently not humans.
- **Prions** are made of a single glycoprotein with no detectable nucleic acid, yet they can replicate. The protein is encoded by a host cell gene, not a viral gene. The increase in numbers of prions in infected nervous tissue is apparently due to a post-translational modification in the conformation of the normal form to the abnormal form. These abnormal forms then recruit further normal forms and change their configuration. They cause the transmissible spongiform encephalopathies including scrapie in sheep and Creutzfeldt-Jakob disease in humans.
-

The virus and the host cell

The infected cell

Three major types of viral infection occur:

1. **Abortive**, with no replication, no visible host cell effects, no disease.
2. **Cytolytic**, with cell death and virus dissemination, then disease, then death or recovery of the host.
3. **Persistent**, which is of three further sub-types:
    - *Latent,* with no apparent effect on the cell, but may re-activate, e.g. herpes.
    - *Productive,* which may give chronic carriage or disease e.g. hepatitis B.
    - *Transforming,* producing tumours e.g. EBV lymphomas.

Host cell changes due to viral infection can be:

1. No morphological change, in abortive or latent infection.
2. Multi-nucleated cells by fusion due to herpes or para myxovirus

infection.

3. Cytopathic effects (CPE) including rounding or darkening of the cell, or inclusion bodies in the nucleus, or in the cytoplasm, or actual cell death from inhibition of cellular (but not viral) protein synthesis.
4. Haemagglutination, by viral surface haemagglutinins adhering to red blood cells.
5. Transformation to malignant cells e.g. by papilloma viruses, EBV, Hepatitis B or C viruses.

Host cell pathologic mechanisms(pathogenesis) are:

1. Virus-induced inhibition of macromolecular synthesis causing cell death. This is the commonest and most important mechanism.
2. Virus-induced immunologic attack i.e. immunopathogenesis. This can be by:
    - Immune attack by cytotoxic T cells on viral antigens in the cell membrane, e.g. on hepatocytes in hepatitis B, and in hepatitis C, and on vascular endothelium in the rash of measles.
    - Immune-complexes of virus-antibody-complement depositing in tissues, e.g. causing arthritis in hepatitis B, rubella, and parvovirus B19 infections.
3. Virus-induced cytokines from infected cells stimulating other cells, e.g. Rotavirus infected enterocytes produce cytokines which stimulate enteric neurons, causing fluid and electrolyte loss into the bowel, with consequent diarrhoea.
4. Virus envelope glycoprotein damage to other cells, e.g. vascular endothelial cells by Ebola virus.

KEY LEARNING POINTS.

1. **Viruses can only replicate inside a host cell. and cannot survive long-term outside a host cell.**
2. **Viruses consist of a central genome of either RNA or DNA, and a protein capsid shell (together forming the nucleocapsid). Many viruses also have an outer** *envelope.*
3. **The genome is usually double-stranded DNA. or single-stranded RNA, which may be positive or negative polarity, linear or circular, one piece or segmented.**
4. **Viral proteins are either structural, or non-structural (usually enzymes)**
5. **Viral envelopes are lipoprotein, often with glycoprotein spikes.**
6. **Viral infection of the host cell may be abortive, cytolytic or persistent (latent, productive or transforming to tumours).**
7. **Cell morphologic changes include giant-cell formation, cytopathic effects, inclusion bodies, haemagglutination or malignant transformation.**
8. **Cell pathologic mechanisms include arrest of macro-molecular synthesis causing cell death, immunologic attack, cytokine production and envelope damage to other cells**

Replication

Growth curve - This describes the number of virus particles in a host cell from the time of infection. Often one virion infects, and after a period of about 10 hours progeny are released. Once a virus enters a cell it seems to disappear for up to five hours (eclipse period), and then during the 'rise period' the nucleic acid content and then the number of new virus particles rise exponentially.

Growth cycle - Events during the growth cycle may be understood in a number of stages.

a. Recognition and attachment are due to interaction between each type of virus and a specific receptor on the human cell The virus may have an identifiable structure such as the spikes on adeno-viruses, and the haemagglutinin of influenza virus, while numerous receptors are known on human cells, such as the CD4 molecule on T cells for HIV (see diagram), and sialic acid on glycoproteins for influenza virus.

b. Penetration or entry is either by uptake into a phagosome, or by fusion of viral and host cell membranes.

c. Uncoating in the cell cytoplasm is by cell enzymes from lysosomes, which remove the virus protein coat and so make the viral genome accessible for the next stage.

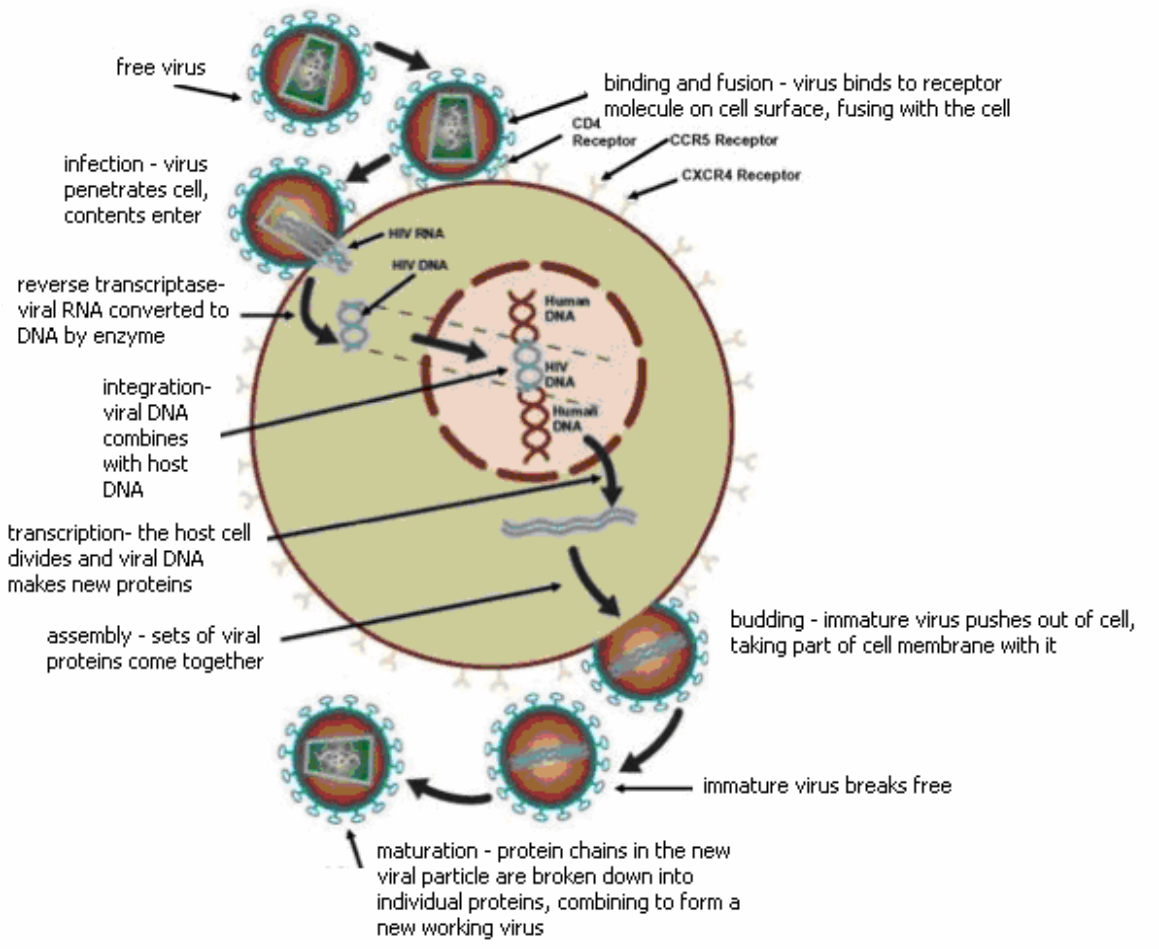
d. mRNA synthesis, protein synthesis and genome replication –

1. RNA synthesis, depending on the type of genome.
2. Early protein synthesis is by translation of the above mRNA using host cell ribosomes in the cytoplasm to make viral protein. If the viral genome is a single nucleic acid molecule, one large polyprotein is produced and then cleaved by enzymes into a number of smaller proteins. If the viral genome consists of several nucleic acid molecules, several mRNAs are made, each translated into one

protein. These early proteins are usually enzymes and regulatory molecules for the next stage.

3. Genome replication - like mRNA synthesis, this depends on the type of genome.
4. Late protein synthesis by translation of viral mRNA produces the capsid structural proteins.

e. Assembly of new virus particles occurs in the cytoplasm, the nucleus, or at the cell membrane of the host cell. The viral genome is assembled with the capsid proteins and viral enzymes into new viral progeny. Release of unenveloped virus usually occurs through the host cell wall by lysis, causing cell death. Enveloped viruses undergo a further step, by incorporating host cell nuclear or plasma membrane components and inserted viral proteins and glycoproteins to form the envelope prior to release. Release is usually by budding, and does not necessarily cause cell death, so infectious enveloped virus can be shed for a long time.



KEY LEARNING POINTS.

1. **Viruses can only replicate inside a host cell, and cannot survive for long without one**
2. **Viruses have either RNA or DNA, and a protein shell (capsid). Some also have an outer envelope.**
3. **The usual growth cycle of viruses involves recognition, attachment, penetration and uncoating of the cytoplasm.**
4. **Protein synthesis and genome replication then occurs**
5. **New viruses are assembled, and released.**
6. **Mutations can occur, producing a defective virus.**
7. **Interactions may also occur with either host cells or other viruses.**