

MICR1010 Microbes and Man

Extended Lecture Synopses

Diseases of Plants Caused by Fungi and Bacteria

Current significance of plants diseases - world population expected to be close to 7 billion by year 2,000 - must increase world food supply e.g. by countering microbial pathogens which account for £billions worth of losses per annum

4 major groups of microbial plant pathogens: fungi, bacteria, mycoplasmas, viruses

Dispersal of pathogens: facultative parasites; importance of spores; mycelial strands and rhizomorphs; vectors

Infection and colonization: seed-borne pathogens; direct penetration of plant tissues - the importance of the fungal hypha; penetration through wounds

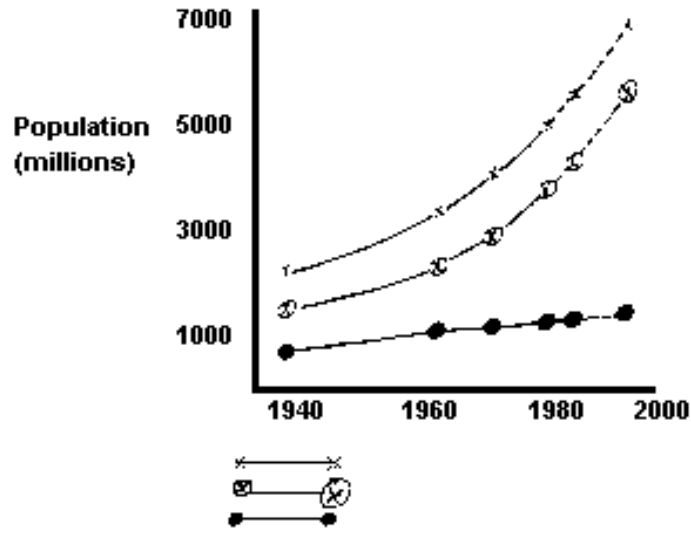
Host-pathogen interface: growth of pathogen between, partially within (haustoria) and entirely within host cells

Control of plant diseases caused by fungi and bacteria: surface and systemic fungicides; biological control - *Agrobacterium tumefaciens* antagonised by *A. radiobacter*, *Heterobasidion annosum* antagonised by *Peniophora gigantea*.

...further details

Current significance of plant diseases; dispersal of pathogens; infection and colonisation; host-pathogen interface; control of plant diseases caused by fungi and bacteria.

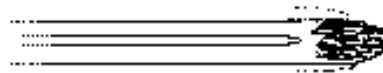
Real and projected population changes to the year 2000



DISPERSAL OF FUNGAL HYPHAE



mycelial strand



Rhizomorph

Biological Control

Pathogen: *Agrobacterium tumerifaciens*

- -causes 'crown gall'
- -antagonist: *A. radiobacter* produces a bacteriocin

Pathogen: *Heterobasidion annosum*

- -causes death of various tree species especially conifers
- -antagonist: *Peniophora gigantea* (saprophyte)

Plants

Anatomical Features

Rigid cell wall

No circulatory system

Internal environment

Acid pH

High C/N ratio

No temperature regulation

Animals

Anatomical Features

No cell wall

Circulatory system

Internal environment

Alkaline pH

Low C/N ratio

Temperature regulation (some)

Host-Pathogen Interface

Pathogen grows between host cells

e.g. Necrotrophic bacteria and fungi

Pathogen grows partially or entirely within host cells

Intracellular hyphae produce intracellular haustoria

e.g. biotrophic fungi

Pathogen contained within host cell

Plasmodial fungi,

mycoplasmas,

viruses

Diseases of Plants Caused by Viruses

**This information is for general interest
and will not be examined as part of
MICR1010 Microbes and Man**

These notes cover an introduction to the history of plant viruses, families and genera of plant viruses, infection of plants by viruses, plant virus genomes, viroids, satellite agents, the economic importance of plant viruses and control measures used to limit plant virus infection.

In 17th century in Holland, colour variegation or striping of tulip petals was much prized by Dutch tulip growers. Although the cause of the symptoms on the petals was unknown, some growers were aware that the condition could be grafted to a normal bulb. It was not until 1926 however that **tulip breaking** was associated with a **virus** and shown to be transmitted by infected sap or by aphids.

In 1886, Mayer in Holland found that a mosaic disease of tobacco could be transmitted to healthy tobacco plants in juice extracts taken from infected plants. A few years later (1892), Ivanowski confirmed this and showed that the sap was still infectious after it had been passed through a Chamberlain filter which was known to retain bacteria. Meyer suggested that the mosaic disease might be caused by a toxin produced by bacteria but in 1898 Beijerinck independently repeated these experiments and called the plant pathogen **tobacco mosaic virus**.

With the advent of the **electron microscope** in the 1930s, the existence of viruses was confirmed. There are now over 650 identified plant viruses which cause significant losses in agriculture, pasture, horticulture and ornamental crops.

Families and Genera of Plant Viruses

No official names for plant viruses have so far been approved. Viruses are therefore still called by their common names which often include the name of the first described or most common host plant together with a major feature of the disease caused *e.g.* tobacco mosaic virus. Pathological changes in the plant that can be recognised without a microscope have often been adopted as names for the diseases. Thus, in addition to mosaics, there are mottling, crinkling, stripes, streaks, curling, bushy tops, stunted growth, necrosis, leaf roll etc.

Infection of Plants by Viruses

Infection occurs by quite different means to those for bacteria and animals. Most plants have rigid cell walls of **cellulose** and therefore viruses must be introduced into the host cytoplasm by some traumatic process such as by feeding animals, by invading fungi or by mechanical damage. The first of these is the most common *e.g.* by leafhoppers, aphids, thrips, whiteflies, mealy bugs, mites, beetles and nematodes. Once inside the cell, **uncoating** probably occurs in a similar fashion to animal viruses. Understanding of the regulation and genome expression in plant viruses is still limited.

Plant Virus Genomes

Relatively speaking, there are only a small number of identified plant viruses whose genomes are composed of single stranded DNA, double stranded DNA or of double stranded RNA although examples of each do exist. The vast majority of plant viruses have single stranded RNA as their genetic material. Most such viruses have a single piece of RNA inside the capsid and are said to be **monopartite** *e.g.* tobacco mosaic virus. Others are **bipartite** and their genomes consist of two molecular species of RNA *e.g.* cowpea mosaic virus. Still others are **tripartite** with three RNA species *e.g.* cucumber mosaic virus.

Viroids

A variety of virus-like diseases in plants are in fact caused by something even smaller than viruses called **viroids**. Viroids are small, circular, single stranded RNA molecules a few hundred nucleotides long (250-575) with a high degree of secondary structure. They do **not** contain any coat protein and are therefore not viruses. They **do not code for** any polypeptides and replicate quite independently of any associated plant virus. They are transmitted as coat-free nucleic acid and are replicated totally by cellular enzymes but they **cause disease in plants**. Palm trees, tomatoes and potatoes are examples of crops that have proved vulnerable to serious damage from viroids *e.g.* potato spindle tuber viroid. There is nothing that distinguishes disease symptoms produced by viroids from those caused by viruses - *e.g.* symptoms include stunting, mottling, leaf distortion, necrosis etc. Viroids could either be **precursors** of viruses or, recent, sophisticated parasites that have **evolved from** viruses.

Satellite Agents

Some isolates of certain plant viruses contain **satellite agents**. There are two classes of these:

- 1) in **satellite viruses**, the satellite RNA codes for its own coat protein and
- 2) in **satellite RNA's**, the RNA becomes packaged in protein shells made by the helper virus.

These satellite agents can influence the outcome of infection with the virus.

Economic Importance of Plant Viruses

Accurate global figures for crop losses due to viruses are not available but figures of the order of $\tilde{\text{A}}\text{£}40$ billion/year have been suggested. It is generally accepted that of the various plant pathogens, viruses rank second only to fungi with respect to the disease losses they cause. Most, if not all economically important crop plants may become infected with viruses. In most cases, the virus infection will cause a reduction in **yield** or **quality** of the infected crop but the extent of the economic loss can vary greatly.

Control Measures

The main approaches adopted are:

1. Removal of the source of the infection *e.g.* plant remains, contaminated equipment.
2. Control of vectors which can be either airborne or soil-borne, both difficult tasks.
3. Protect the plant, either with anti-viral chemicals, by cross protection or by genetic protection.

The last example is providing some unexpected success. It involves introducing a piece of DNA from a plant virus genome into a plant cell in such a way as to **integrate** it stably into the cell genome where it can be expressed as though it was a cell gene. Plants that have received a new gene in this way are called **transgenic plants** and can often exhibit increased resistance to virus disease. The transgene may act on initiation of infection, on replication of the virus or on spread of the infection through the plant or on the development of symptoms. It provides an exciting new approach to potential control of at least some plant virus infections.

Diseases of Humans and Animals Caused by Fungi

Allergies, mycetisms (consumption of poisonous mushrooms), **mycotoxicoses** (consumption of mouldy foodstuffs which contain toxic fungal secondary metabolites).

Infectious mycoses: - three broad groups: superficial, subcutaneous and systemic mycoses

Superficial mycoses: - Dermatophytoses: three closely-related genera of dermatophyte fungi: *Epidermophyton*, *Microsporum* and *Trichophyton*; dermatophytes grow only in the keratinous tissues of the skin, hair and nails; 'ringworm' and 'athlete's foot' etc. are contagious mycoses

- **Superficial candidosis: - 'thrush'** (mucosal infections of the mouth and vagina); oropharyngeal candidosis and AIDS patients; other factors contributing to oral and vaginal candidoses including sexual transmission of latter; - **skin and nail infections**

- ***Candida* spp. as opportunists and the importance of dimorphic *C. albicans***

- **Pityriasis versicolor:** patchy discolouration of skin caused by *Malassezia furfur* which interferes with melanin production.

Subcutaneous mycoses: involve the skin, subcutaneous tissues and bone and show slow, localized spread; affected individuals accidentally inoculated with fungi growing as saprophytes in soil and decaying vegetation e.g. 'Madura foot' caused by *Madurella mycetomatis*

Systemic mycoses: - primary disease caused by 'true' pathogens e.g. Paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* - this disease endemic in Central and South America; *P. brasiliensis* is dimorphic - inhaled mycelial elements/spores convert to budding yeasts in human host; untreated disease becomes disseminated and is fatal.

- **opportunistic infections:** factors contributing to the increased prevalence of disease caused by opportunistic fungal pathogens

Antifungal drugs: targets for amphotericin B, azoles (imidazoles and triazoles), allylamines, 5-fluorocytosine and griseofulvin; fungi as eukaryotes/host toxicity of antifungals; urgent requirement for new, broad-spectrum fungicidal agents with low host toxicity

Superficial mycoses

Dermatophytosis

- *Epidermophyton* spp.
- *Microsporum* spp.
- *Trichophyton* spp.

Superficial Candidosis

- *Candida* spp.
- *Candida albicans*
- - dimorphic

Pityriasis versicolor

- *Malassezia furfur*

Subcutaneous Mycoses

e.g 'Madura Foot' (*Madurella mycetomatis*)

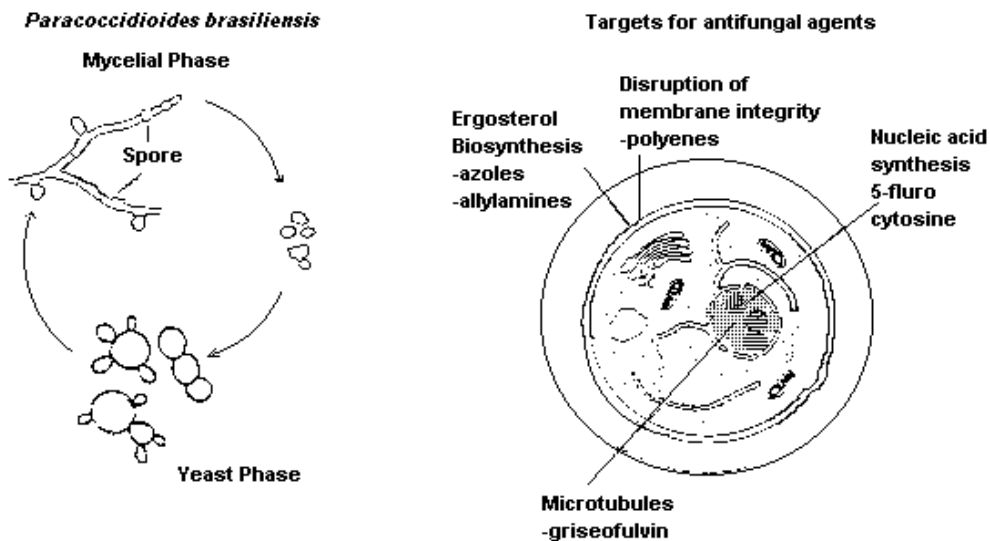
Systemic Mycoses

Primary Infections e.g.

- Coccidiomycosis (*Coccidioides immitis*)
- Cryptococcosis (*Cryptococcus neoformans*)
- Paracoccidiomycosis (*Paracoccidiomycoides brasiliensis*)

Opportunistic infections e.g.

- Aspergillosis
- Candidosis
- Associated with
 - a) Naturally receptive states e.g. very young or old people, pregnancy
 - b) Broad-spectrum antibacterial antibiotics
 - c) Immunocompromised hosts
 - -underlying diseases e.g leukaemia or AIDS
 - -transplant surgery
 - - during chemo- or radio-therapies



Diseases of Plants caused by Fungi and Bacteria

Current significance of plant diseases

The world population is in excess of 6 billion. It is estimated that today well over 2 billion people suffer from hunger, malnutrition or both. To feed these people and the additional millions to come in the next few years, all possible methods of increasing the world food supply are currently being pursued. Important amongst these is improved crop protection. Microbial pathogens account for £billions worth of losses per annum. Microbial plant pathogens fall into the following groups

Fungi

- Fungi are the most important plant pathogens - around 70% of crop losses are attributable to these organisms (the figure of 70% is based on a survey in the USA after control measures had been applied)
- Some 8,000 species are known to cause disease in plants excellent example is Irish potato famine of 19th century - caused by fungus *Phytophthora infestans* - resulted in deaths of one million by starvation and migration of at least same number to other parts of Europe and the USA - as we will see next lecture only some 60-150 species of fungi are 'true' pathogens of animals including humans.
- Fungi are capable of causing disease in any cultivated plant

Plasmodial fungi (wall-less) e.g. 'Club Root' disease of brassica (cabbage, turnip etc) crops - the whole root system may be converted to a swollen, distorted mass which ceases to function. This disease develops following the germination of a spore in close proximity to a plant root. (NOTE that unlike mycoplasmas (wall-less bacteria, see later), plasmodial fungi are dispersed by spores.)

Bacteria

- Only some 200 species of bacteria are recognised as plant pathogens - all of these walled bacteria are facultative parasites.
- Most species of plant pathogenic bacteria are rod shaped e.g. *Erwinia carotovora*, the organism that causes bacterial soft rot of potato tubers. This pathogen gains access to the tuber at sites of damage. Two factors that contribute to the spread of the bacteria through the tuber are: (i) the production of extracellular enzymes (although most plant pathogenic bacteria are not able to degrade complex substances such as cellulose or lignin *c.f.* fungal pathogens of plants); and (ii) the fact that *Erwinia*, like many other plant pathogenic bacteria, is flagellate and is therefore motile.
- Some actinomycetes are plant pathogens - actinomycetes are prokaryotes/bacteria that form narrow, branching, aseptate filaments that resemble the fungal mycelium. Form spores.
- Neither the numerous single-celled bacteria nor the filamentous forms are able to produce modified cells to facilitate penetration into, or spread within, host plants (*c.f.* fungi later).

Mycoplasmas (wall-less)

It seems probable that these organisms cannot survive away from their hosts or vectors* i.e. they are entirely dependent on vectors for transmission from host to host e.g. dodder (a 'dodder' is a plant that is a parasite of a plant of another species) or insect vectors. Mycoplasmas can cause serious economic losses and were responsible for the virtual destruction of the coconut industry in the Caribbean.

* A 'vector' is defined as any organism which transmits or disperses a pathogen.

Why are fungi so successful as pathogens of plants?

I. Dispersal

- Spores Both fungi and bacteria produce spores - but importance of spores for bacterial plant pathogens is confined to actinomycetes which comprise a minority of bacterial plant pathogens. Spores are extremely important for dispersal of fungal plant pathogens.
- Mycelial strands and rhizomorphs These are produced only by fungi and are aggregate structures consisting of a number of hyphae grouped together. At their simplest, these form 'mycelial strands' [NOTE in earlier lecture in this series described mycelial strand of *S. lacrymans*]; some of the hyphae form thick walls while others become hollow water/nutrient-conducting vessels. In more massive and elaborate structures known as 'rhizomorphs' the outermost hyphae, which have thickened and pigmented walls, form a resistant rind while the internal hyphae are differentiated into a highly efficient transporting system. Both mycelial strands and rhizomorphs are capable of transporting nutrients over very considerable distances. In addition, rhizomorphs grow much more rapidly than normal vegetative hyphae. Thus mycelial strands and rhizomorphs enable fungi to pass across hostile conditions ensuring efficient dispersal.

Other dispersal mechanisms (these may also apply for other pathogens besides fungi)

- Many fungal pathogens are saprophytes As indicated above, all bacterial pathogens of plants are facultative parasites *i.e.* they also grow as saprophytes - so too do many fungal pathogens of plants so that they are readily dispersed in the environment.
- Vectors A 'vector' is defined as an organism which transmits or disperses a pathogen. Vectors are of particular importance in the dispersal of viruses and bacteria/mycoplasmas pathogenic for plants. In addition, they often play a role in the dispersal of fungal pathogens - excellent example is dispersal of Dutch Elm disease pathogen *Ophiostoma ulmi*: bark beetles are insect vectors that enable dispersal and penetration of pathogen through bark of tree; in addition humans have acted as supremely efficient vectors of this disease - the aggressive strain of Dutch Elm disease pathogen responsible for the recent epidemic in Europe was almost certainly introduced in elm logs shipped from North America.
- Seed-borne pathogens This mechanism avoids the need to penetrate host tissues, the pathogen being dispersed/transmitted in embryonic tissues.

II. Infection and colonization

Both fungal and bacterial pathogens will readily gain access to the host plant at wounds/sites of damage. Fungi have a number of additional infection/colonisation mechanisms:

- Thigmotropism The most important natural openings on plants that microorganisms can enter are stomata. Both bacterial and fungal pathogens may enter the plant by these pores. When a rust spore germinates on a cereal leaf the germ tube grows at right angles to the long axis of the leaf. This precise orientation of growth is an example of 'thigmotropism' = contact guidance - it is probably organized through the recognition by the hyphae of the regular pattern formed by the crystals of epicuticular wax. The orientation of hyphae across the long axis of the epidermal cells ensures that they will sooner or later encounter a stoma pore, as these occur in longitudinal rows. Once the germ tube has penetrated a stoma pore, colonization of the inner tissues can occur more readily: the substomatal cuticle appears to be substantially thinner than that on exposed leaf

surfaces and growth of the pathogen is probably aided further by the high water content in the substomatal cavity).

- Specialised infection/penetration structures *e.g.* appressorium Direct penetration of herbaceous tissues requires that a pathogen must enter through layers of wax, cutin, pectin and a network of cellulose fibrils impregnated with wall polymers before making contact with living protoplasm. Only fungal hyphae are capable of penetrating these layers. Direct penetration of the host is frequently associated with the production of specialized structures *e.g.* appressoria. When the spore of *e.g.* the powdery mildew fungus, *Erysiphe graminis*, germinates on the surface of a leaf of barley it produces a short germ tube which appears to 'search' for a favourable site for penetration. Eventually the tip of the hypha swells to form an 'appressorium'. This spherical or ovoid structure increases the area of contact and attachment between the fungus and the host surface. Penetration then takes place by the downward growth of a thin hyphal thread formed on the lower surface of the appressorium where it adheres to the host. In some species, penetration appears to depend largely on the build-up of very high turgor pressure in the appressorium/hyphal thread, while in others the release of enzymes that degrade cellulose, cutin and pectin is of greater importance. In many species both mechanisms are likely to have important roles during penetration.
- Haustoria In the majority of biotrophic parasites, haustoria develop from intercellular hyphae - see earlier lecture: 'Modes of Life of Fungi'. Typically haustoria are produced by biotrophic fungi such as rusts and powdery mildews.

Control of plant diseases caused by fungi and bacteria

Those fungicides that are currently available to treat plant disease are not ideal:

'Surface' fungicides - include inorganic and sulphur compounds - they have been used to protect crops from pathogens for over a century - they do not penetrate plants, cannot cure disease and are subject to weathering.

'Systemic' fungicides - these became available in the mid 1960s - they enter plants, are translocated and can cure disease - however, these compounds are not ideal and, in particular, resistance of pathogens to systemic fungicides is occurring for many classes of these compounds - in addition, many of these compounds are potentially toxic to humans.

'Biocontrol' - this term has been used to describe control measures in which one organism is used as an antagonist of another - an excellent example of the successful biological control of plant disease is the following:

The fungus, *Heterobasidion annosum*, causes rotting and death of various tree species, particularly conifers. It causes serious losses in pine plantations in various parts of Britain. Pine stumps are liable to infection by *H. annosum* which may then use the stumps as a food/inoculum base for attacking adjacent trees. Various saprophytic fungi grow on pine stumps and of these, *Peniophora gigantea* (which is not an active pathogen of pines), invades rapidly and is highly antagonistic to *H. annosum*. Therefore, an effective and persistent control programme was developed which involves spraying each pine stump immediately after felling with a commercially prepared suspension of *P. gigantea* spores. This regime has proved highly effective in controlling the disease in the UK.

Diseases of Humans and Animals caused by Fungi

Introduction

A mycosis (pl. mycoses) is a disease of humans or animals caused by a fungus - for reasons that should become apparent, mycoses are less of a problem for the vet. than for the clinician and for most of this lecture we will consider human mycoses.

- **Allergies** Fungi cause serious allergic diseases which usually affect the lungs and nasal passages following inhalation of fungal spores e.g. Farmer's lung (which can be acute or chronic, sometimes fatal) is caused principally by sensitisation to spores of fungi (Farmer's lung may also be caused by sensitisation to spores of actinomycetes = bacteria).
- **Mycetismus and mycotoxicoses** The ingestion of poisonous mushrooms can be fatal (described as a 'mycetismus') as can the consumption of mouldy foodstuffs which contain toxic, fungal secondary metabolites (mycotoxicosis).

However, in this lecture we shall focus on infectious diseases caused by fungi.

- **Infectious mycoses** As indicated in previous lecture, some 8,000 species of fungi are capable of causing disease in healthy plants. In contrast only some 150 are capable of causing disease (mycosis) in man and animals by tissue invasion. Such organisms may be described as 'true' pathogens causing 'primary' disease. However, a very large no. of fungi - potentially almost any fungus - can act as an opportunistic pathogen in a debilitated/compromised host and it is as opportunistic pathogens that fungi are becoming of great importance in the clinic - more of this later. Will consider the infectious mycoses under three headings: *Superficial*, *Subcutaneous* and *Systemic* mycoses.

Infectious mycoses

The infectious mycoses, which may occur in otherwise healthy individuals, can be divided into three broad groups: Superficial, Subcutaneous and Systemic mycoses.

Superficial mycoses

If asked to name a human mycosis, most people would suggest 'athlete's foot', or 'thrush' (which is a yeast infection of the mouth or vagina) - these conditions occur almost as frequently as the common cold and can lead to very considerable morbidity. Tissues affected are skin, hair and nails, and mucous membranes. We shall consider the following superficial mycoses in more detail: Dermatophytosis, Superficial Candidosis and Pityriasis versicolor.

Dermatophytosis

- **Genera** - three closely-related genera of dermatophyte fungi: *Epidermophyton*, *Microsporum* and *Trichophyton*
- **Affected tissues** - dermatophytes grow only in keratinous tissues of *skin, nails, hair*. For infections of skin, 'dermatophytosis' is synonymous with 'ringworm' - the 'worm' part of the name comes from the Romans who associated the disease with insect larvae. In the smooth skin, growth spreads outwards to produce approximately circular, red, scaly, itchy lesions - the livid, red circumference of the lesion represents the region of active growth of the fungus; in the toe webs ('athlete's foot') the lesions are red, itchy and may be macerated or cracked; finger and toe nails

become discoloured, thickened and either very hard or flaky; scalp lesions vary, according to the causal fungus, from diffuse loss of hair to very severe and painful lesions secondarily infected by bacteria].

- **Contagious** - most mycoses are not contagious (i.e. communicated by contact) - ringworm is an exception - it is a truly contagious mycosis: the majority of dermatophytes do not occur as saprophytes in nature and are known only as agents of disease - infection results from the transfer of arthrospores or keratinous material containing fungus from the infected to the uninfected. (In addition to exposure to the fungus, some abnormality of the epidermis, such as slight peeling or minor trauma, is probably necessary for the establishment of infection.)
 - Examples of transmission of disease in this way are:
 - Athlete's foot* (with associated infections of the nail and groin) commonly occurs in e.g. competitive swimmers and industrial workers because of regular use of communal bathing facilities
 - Animal ringworm*, an occupational hazard for farmers, vets and others closely associated with animals (transfer occurring e.g. via. animal grooming implements).

Superficial candidosis

- **Candida species are opportunistic pathogens** Infections due to *Candida* spp are normally endogenous in origin i.e. the causal fungi are common commensals ['**Commensal**' is an organism that lives in close association with another organism of a different species without either harming or benefitting it' (e.g. some microorganisms living in the gut obtain both food and a suitable habitat but neither harm nor benefit man)]. Thus the fungus is already present in the affected person and is kept in check by the normal defence systems of the body and by competition from the bacterial flora - disease occurs only when there is some localized or general abnormality of the host, or in natural receptive states e.g. infancy, old age or pregnancy. Under these circumstances, *Candida* species can be described as 'opportunistic pathogens' - again, will return to this later when discussing more serious diseases caused by *Candida* and other species.
- **Affected tissues**
 - **Mucous membranes (thrush)** Infections of the mouth and vagina are commonly referred to as 'thrush' and are characterised by the development of discrete white patches on the mucosal surfaces. This will usually be cleared easily in a patient with a normal immune system but oropharyngeal thrush (an infection of the mouth and other linings) can be a major problem for AIDS patients. Vaginal candidosis is common, particularly during pregnancy.
 - **Skin and nail infections** Skin infections normally occur on sites that become abnormally moist - in infants, candidosis is a frequent complication of napkin dermatitis = 'nappie rash'. People who frequently immerse their hands in water e.g. bar staff suffer from candidosis of the hands and nails.
- **Sexual transmission** Vaginal candidosis provides a further exception to the non-contagious 'rule' (cf. dermatophytes, above) in that this condition may be transmitted sexually.
- ***Candida albicans*** A normal commensal of the human digestive tract, including the mouth, and of the vagina: thought that more than 30% of the population carries *C. albicans* as a commensal i.e. the organism is causing no harm. Most frequently encountered and most virulent *Candida* pathogen of humans. The organism is dimorphic with the capacity to grow both as a budding yeast and a mycelium.

Pityriasis versicolor

This is a mild, chronic infection which (as the name indicates) causes a patchy discolouration of the skin. It is caused by the fungus *Malassezia furfur* which interferes with melanin production. The lesions vary in

appearance according to the degree of pigmentation of the surrounding skin. In light-skinned individuals, lesions appear as pale brown, hyperpigmented patches and these are hardly noticeable until exposure to sunlight renders them more obvious by their failure to tan. In dark-skinned races, the lesions are always lighter in colour than the adjacent normal skin. *M. furfur* is a member of the normal microflora of the skin of many individuals and contagious spread of the disease, although it occasionally occurs, is not thought to play a significant role in its epidemiology. There is a much higher incidence of infection in warm climates than in temperate zones and also in individuals with illnesses causing high temperatures or necessitating long periods confined to bed, indicating that excessive sweating is one of the predisposing factors.

Subcutaneous mycoses

For example, 'Madura foot' caused by *Madurella mycetomatis*. Subcutaneous mycoses involve the skin, subcutaneous tissues and bone and show slow, localised spread. These diseases frequently involve inoculation of individuals with fungi growing as saprophytes in soil and decaying vegetation. Field workers in warm climates, particularly those who wear little protective clothing in regions where thorny vegetation is common, frequently contract subcutaneous infections following minor injuries.

Systemic mycoses

'True' pathogens

Systemic mycoses are usually initiated in the lung and sometimes become widely disseminated. The reason for the frequent initiation of these diseases in the lung is that systemic mycoses are usually caused by soil fungi, which produce large nos. of airborne spores, the size of which, if inhaled, allows them to penetrate deep into the respiratory system and initiate the primary pulmonary disease. Therefore, such systemic mycoses are caused by 'true' fungal pathogens i.e. highly pathogenic species capable of causing an infection in all exposed individuals. They occur most frequently in agricultural workers, or workers in the construction industries, following disturbance of soils containing the causal fungi.

For a number of fungal pathogens that cause systemic mycoses the capacity to grow in two major growth forms, 'dimorphism', is of key importance. An example is *Paracoccidioides brasiliensis* which causes paracoccidioidomycosis, a disease endemic in Central/South America. Inhaled mycelial elements/spores give rise to the pathogenic budding yeast growth phase in the human host. Untreated, the disease may become disseminated and fatal.

Opportunistic pathogens

Examples are the diseases caused by *Aspergillus* species (e.g. *A. fumigatus*) and *Candida* species (e.g. *C. albicans*).

Opportunistic mycoses are associated with:

- Naturally receptive states e.g. premature babies, during pregnancy, old age
- The use of broad spectrum antibacterial antibiotics
- Immunocompromised hosts e.g.
 - underlying disease e.g. leukaemia, AIDS
 - during transplant surgery (i.e. deliberate immunosuppression)
 - during chemo- and radio-therapies

Opportunists like *Aspergillus fumigatus* and *Candida albicans* can give rise to serious systemic disease and are among the most frequent causes of failure of liver transplants due to infection. As indicated earlier, *C. albicans* is frequently a member of the commensal flora and is an important example of a fungus that can give rise to a systemic disease that is not initiated in the lung.

Antifungal drugs

None of the currently available antifungal drugs is ideal and there is a need for novel, broad-spectrum antifungal agents. The problem stems, at least in part, from the fact that fungi are eukaryotes. This means that targets for antifungal agents in fungi are also present in human cells, so that toxic side-effects are frequently associated with antifungal chemotherapy. Furthermore, the problem of drug resistance which already causes great concern following the widespread use of many antibacterial agents, is becoming increasingly significant during antifungal chemotherapy.

SYPHILIS

Syphilis has been called "The Great Mimicker" because of the protean symptoms and signs associated with the disease.

Although there has been a continuing decline in the incidence of syphilis over the past two decades, it is still an important disease in this country today. Total number of new cases reported in 1983 was over 3,000.

It is endemic in most countries of the world. Controlling factors; poverty and promiscuity, However, this disease is also of historical interest.

LANDMARKS IN HISTORY

1. SYPHILIS "THE RISE AND FALL"

Known since biblical times?

Postulated that Job's affliction was in fact pustular lesions of secondary syphilis. (Job 2:7) In Deuteronomy (28:27-29) we have what is probably a description of the various stages of syphilis; "The Lord will smite thee with the botch of Egypt, and with the emerods, and with the scab, and with the itch whereof thou can'st not be healed. The Lord shall smite thee with madness, and blindness and astonishment of the heart".

Isaiah (3:16-26) refers to genital lesions and baldness resulting from the 'infected daughters of Israel'.

Exodus (20:5) and Jeremiah (3:29) appear to describe congenital syphilis; "Visiting the iniquity of the fathers upon the children unto the third and fourth generation" - "In those days they shall say no more, The fathers have eaten a sour grape, and the children's teeth are set on edge".

Certainly known by the early Romans and Greeks. Celsus (25BC - 50AD) described hard and soft lesions of mouth, genitalia, tonsils and nostrils. Pliny (23-79AD) described painless ulcers that were self-limiting, disappearing after several weeks without treatment, and which were associated with sexual activity. Enlargement of the great blood vessels and general paralysis of the insane were common among the Romans, Nero, Caligula, Augustus, Tiberius, were probably afflicted. However there are no other obvious references to its existence until centuries later.

Recently, excavations at a monastery in Hull have revealed bones with lesions of syphilis. These pre-date the Columbian voyages of discovery and raise the possibility that syphilis never really disappeared from Europe; its eclipse was because Europeans were dying too young for bone lesions to develop.

During the last decade of the fifteenth century this disease became disseminated throughout Europe. Examination of bones of pre-Columbian Native Americans has shown evidence of late syphilis, such evidence is rare among pre-Columbian European bones. Therefore Columbus and his crew have been implicated in bringing or returning this disease to Europe. They visited the West Indies during their great voyage of discovery. Yaws or syphilis was endemic among the natives of these islands The ship's pilot and members of the crew were 'treated' for a 'new exotic' disease upon their return to Barcelona in 1493.

Within a short time these sailors were spreading the disease among the water-front prostitutes, who in their turn passed it on to other unsuspecting individuals.

The disease was particularly active over the next 60 years, giving the appearance of a true pandemic! It became known as the 'Great Pox'. Its rapid spread lent support to the idea of Isla (1500-) and Oviedo (1478-1557) that it was indeed a new disease from the 'New World'. It was spread from the ports of Spain and Portugal to Italy and the rest of Europe by French and Spanish mercenaries.

1495 - Reported in Germany, Switzerland, and Greece.

1496 " " in Paris Netherlands.

1498 " " in England; spread via sailors to India and the Philippines.

1505 - Appeared in China.

1646 - 'Returned' to America.

1800s- Moved west to the Pacific islands.

Whether this represented a real pandemic is difficult to ascertain. The printing press developed in the fifteenth century, therefore these reports may indicate an actual increased incidence or may merely reflect more widespread reporting and hence awareness of a long-standing problem.

2. EFFECTS ON THE COURSE OF HISTORY?

Henry VIII (1491-1547) was infected before his first marriage. He searched for a non-infected wife - Only one of his six wives appears to have been disease free. Of his progeny, the following are thought to have manifested signs of congenital syphilis, Mary Tudor, Elizabeth I, and Edward VI.

Others - Francis I of France.

Many painters and composers - link between spirochaete and genius!

It has been suggested that Wagner's *Parsifal* is an allegory of syphilis. The never-healing wound suffered by Amfortas may be a syphilitic lesion, acquired through liaison with Kundry...

3. JOURNEY TOWARDS UNDERSTANDING AND CURE.

1496 - Theodore Ulsensius of Nuremberg attributed its emergence to the conjunction of Jupiter and Saturn in 1484. A more jovial reference to the association of syphilis with the stars - "A night with Venus leads to a lifetime of Mercury"! In the same year, mercury was first used to treat patients with the disease. Although its use proved as dangerous to the recipient as the disease itself, it continued to be used for four centuries.

1530 - Girolamo Fracastoro, poet, physician and astronomer named the disease Syphilis after a mythical shepherd.

From the earliest times its sexual mode of transmission was clearly recognised, throughout the sixteenth and seventeenth centuries there was a period of rampant confusion during which gonorrhoea, chancroid, and syphilis were all thought to be manifestations of the same disease.

1767 - John Hunter helped to fuel this confusion. He inoculated himself with pus from a patient with symptoms of gonorrhoea. However this patient also had syphilis, so when he himself contracted syphilis following this experiment it seemed to corroborate this long-standing misconception.

1879 - Albert Neisser discovered the organism causing gonorrhoea.

1889 - Augusto Ducrey discovered the organism associated with chancroid.

1903 - Metchnikoff and Rowe inoculated primates with syphilitic material and induced syphilitic lesions in the animals.

1905 - Shaudinn and Hoffman described an organism observed in chancres and inguinal glands of syphilitic patients: *Treponema pallidum*.

1906 - Landsteiner confirmed *Treponema pallidum* as the causative agent.

1906-1910 Wassermann, Neisser and Bruck introduced a complement-fixation method for detecting antibodies in patient's serum.

1906-1907 Ehrlich and Hata used Compound 606, Salversan as a therapeutic agent (arsenic derivative, arsphenamine)

1917 - Wagner von Jauregg Introduced fever therapy for paresis.

1921 - Sazerac and Levaditi used bismuth in therapy.

1922 - Khan introduced tube flocculation method using cardiolipin as the antigen

1941 Dr John Mahoney of the United States Public Health Service reported four cases of syphilis successfully treated with penicillin. The age of the cure had arrived!

1941 Pangborn modified the cardiolipin test by adding lecithin and cholesterol to improve the sensitivity and specificity of the test.

1950's- VDRL test introduced.

An increased awareness of the extent of the problem, the introduction of compulsory reporting of all diagnosed cases and contact tracing, the initiation of serological testing during pregnancy and finally the advent of the antibiotic era, all contributed to the decline of the disease to its present level.

SYPHILIS THE DISEASE

Causative organism: *Treponema pallidum*

An extremely thin, delicate organism which contains a number of spirals (4-14) that appear uniform near the centre of the cell but which tend to increase in periodicity and decrease in amplitude towards its tapered ends. It has a low refractive index therefore it is difficult to see except by dark ground or phase contrast microscopy.

Motile, it spins around on its longitudinal axis, but has no external flagella. There are three axial fibrils inserted at each end of the protoplasmic cylinder and which overlap in the mid-region which act as 'internal flagella' and which are also responsible for the spirals. The bacterium cannot be cultured on ordinary laboratory media. *Treponema pallidum* can only be propagated in laboratory animals, namely rabbit testes and eyes.

Mode of transmission

Horizontal Sexual contact.
Vertical. Congenital syphilis.
Blood. Blood transfusions.

Incubation period: 9-90 days. Average three weeks.

Incidence: Peak incidence between 20-40 years.

U.K. M>F 6:1

High incidence among men who have sex with men. Although numbers declined sharply in the 1980's, as a benefit of the sex education campaigns associated with the appearance of HIV and AIDS, in the first years of the 21st Century, numbers of cases of syphilis have shown an alarming exponential increase.

Natural course of disease.

Organism penetrates intact skin/mucous membranes. Spreads via lymphatics to blood within a few hours, to produce metastatic foci. (Blood from a patient incubating syphilis is infectious).

At the end of the incubation period the primary lesion 'chancre' appears at the site of the inoculation. Lasts 2-3 weeks then heals. Inguinal lymph nodes become enlarged. (Painless).

After another 6-8 weeks, secondary stage occurs. Generalised symptoms of infection, malaise, etc., and the appearance of a characteristic non-itchy rash. Lymph nodes throughout the body become enlarged. Some patients develop snail-track ulcers in the mouth, or patchy baldness or eye problems (iritis). This stage can last months - years. Patients remain infectious until the last lesion of secondary syphilis heals.

Then the disease enters a latent stage. Patient appears well. This period can last 3-30 years. However pregnant women with latent disease may still infect foetus as intermittently seed the blood stream during this stage.

Untreated, one third of patients will go on to develop tertiary syphilis with gummatous lesions in skin, mucous membranes, bones, and joints. More serious manifestations include lesions involving the cardiovascular (valvular lesions, aortic aneurysms) and central nervous system (meningovascular syphilis, tabes dorsalis, general paralysis of the insane). Tertiary lesions contain very few organisms. Tissue damage probably involves a delayed type hypersensitivity response.

Remaining 50-70% of patients probably remain seropositive for life.

Congenital Syphilis:

The organism readily crosses the placental barrier transmitting the disease to the foetus. The lesions of congenital syphilis resemble those of acquired syphilis of comparable duration. Early manifestations include rhinitis rashes enlarged liver and spleen and bone lesions. Late manifestations include interstitial keratitis, neurosyphilis, gummatous lesions in bone (5-25 years). Stigmata associated with congenital syphilis; Hutchinson' s teeth, saddle nose, saber shins.

LABORATORY DIAGNOSIS

Dark ground microscopy & Direct fluorescent antibody test (Abs).

Exudate aspirated from chancre and examined immediately, or sealed into capillary tubes. Saline aspiration of lymph node material. Negative results do not exclude syphilis. Need 1,000,000 orgs/ml to see at least one per field. Very sensitive to penicillin, cannot be found within 4 hours of commencing therapy.

Tissues:

Direct fluorescent antibody test.

Silver stain. Often see artefacts.

Serology:

Three distinct antibodies appear in serum

1. Lipoidophil or reagin antibody. Antigen is cardiolipin, prepared from alcoholic extract of beef heart muscle; lecithin and cholesterol are added to give greater sensitivity. Non-specific test but sensitive. Becomes positive 1-3 weeks after primary lesion appears Flocculation tests - VDRL; Rapid plasma reagin test.
Can be used to monitor therapy.
Biological false positives - pregnancy, malaria, thyroid disease, etc.
Theory of non-specific reaction? Cardiolipin present in normal tissues plus a carrier (microbial cell) gives rise to an immune response. Microbial cell incorporating host lipid?
2. Antibody to Reiter protein. Reiter protein is common to pathogenic and non-pathogenic treponemes alike.
Complement-fixation tests. Obsolete.
3. Antibodies to specific *Treponema pallidum* antigens.

Treponema pallidum immobilisation test. - Need live organisms which are incubated with test sera and fresh complement anaerobically at 35 degrees C for 18 hours. Becomes Positive 3-4 months after onset of disease, i.e. end of primary stage. (Reference Laboratory Only). Only becomes negative if successfully treated in primary stage, once secondary stage established remains positive indefinitely.

FTA (Abs) - Fluorescent antibody test which uses an absorbing agent to remove any antibodies reactive against the group antigen. Allows demonstration of different antibody subclasses in serum, e.g. IgG, IgM. Useful in diagnosing early and congenital syphilis. Very sensitive.

Treponema pallidum haemagglutination test (TPHA). - again very sensitive and specific test Sonicate of *Treponema pallidum* attached to tanned turkey or sheep red blood cells. Presence of specific antibodies in serum causes these red cells to agglutinate. Remains positive despite therapy - marker of past infection.

TREATMENT

Mainstay of treatment remains penicillin. In patients who are allergic to penicillin, erythromycin can be used.

Mycobacterial infections

Leprosy is a disease that has been known since biblical times, with over 20 references to the disease made in the King James translation of the Bible. In the book of Leviticus, there are laws for the control of the disease involving segregation of those infected and disinfection of the material that they contacted. Chapter 13 in this book details a description of various skin conditions, indicating the nature of leprosy as diagnosed by the Priests; declaring 'raw flesh' to be 'unclean'. Two examinations are required, seven days apart for a diagnosis of leprosy to be made.

Any clothing belonging to someone declared unclean from leprosy was also considered unclean, no matter of what material or skin it was made. Clothing was burned by fire to purify it.

Priests were also the people who could declare lepers cleansed. In the ritual of cleansing, two birds were taken: one was sacrificed in an earthen vessel over running water. The second bird was dipped in the blood of the first bird, together with cedar wood, scarlet and hyssop. The blood was sprinkled over the person to be declared cleansed who had to shave off all his hair and to bathe in running water, then wait seven days before returning to the society of others. The second bird was released. The reference to hyssop in cleansing is of note since *Penicillium notatum* was first isolated from a hyssop plant: In Psalm 52 it says 'Purge me with hyssop and I shall be whiter than snow'. Given the very widespread dispersion of this fungus, this must be regarded as nothing more than a very interesting coincidence.

There are also references in the Book of Deuteronomy, again indicating the role of the Priesthood in regulating this disease and its sufferers. Further references appear in the second Book of Samuel, the Second Book of Kings, where King Azariah was smitten with the disease after usurping the duties of the High Priest, handing his reign to his son Jotham. In the Second Book of Chronicles the king is named Uzziah. His picture can be found as part of the ceiling of the Sistine Chapel. His afflictions were also the subject of a painting by Rembrandt, owned by the Duke of Devonshire and housed in Chatsworth. Despite his royal personage, Uzziah is still forced to withdraw from the people. The Gospel of Matthew has Christ curing a leper and commanding the cured man to tell no one but to show himself to the Priest and offer the gift that Moses commanded. In the version in Mark and Luke's Gospels, the cured leper blazed the matter abroad. This was why Jesus withdrew into the wilderness, to escape from the crowds that came to be healed. It was in the House of Simon the Leper that Christ's feet were anointed, just before Judas betrayed him.

It is thought that the Egyptians were aware of the disease with records dating to 4,000 BC. In Asia, there are reports of a disease like leprosy from both Japan and from India dating to about 1,000 BC. Leprosy was referred to as 'Kushtha' in the sacred Vedic scriptures.

The spread of leprosy was aided when large numbers of European soldiers joined the Crusades, bringing back this disease on their return home. By the 13th Century, 'leprosy' reached epidemic proportions in Europe and specialised Leper hospitals were set up, often associated with religious houses. These were often called 'Lazarets', after Lazarus, said to have been raised from the dead (John 11:1-44) and people with leprosy were referred to as the living dead. Funeral services were held to mark the entry of a victim into a leper colony. In England in the 13th Century there were over 200 leper hospitals. These were not confined to mediaeval Europe; Leper hospitals were and are found in Asia and Africa and were found in the Americas as well. The disease was greatly feared: Shakespeare has the ghost of Hamlet's father told the following of his death:

'... Upon my secure hour thy uncle stole
 With juice of cursed hebona in a vial,
 And in the porches of my ears did pour
 The leperous distilment...!'

These days, leprosy is still found in tropical and sub-tropical regions Africa, Asia and Latin America. It is associated with unsanitary living conditions and overcrowding. In the developed world, it occurs sporadically, particularly among emigrants from endemic areas. Leprosy is one of the major health problems of developing countries including India, Brazil, Africa, Nepal and Bangladesh. The figures below are from the WHO (2001):

WHO Region	New Cases Reported
Africa	39,612
Americas	42,830
Middle East	4,758
S.E. Asia	668,658
Western Pacific	4,786
Europe	53
Total	763,317

Leprosy is caused by a rod-shaped bacterium, *Mycobacterium leprae*. The Norwegian physician G. Armauer Hansen first described this in 1874. Leprosy is still also referred to as Hansen's disease in his honour. Of all infections, leprosy is one of the least contagious, with only ~5% of those exposed to infection succumbing to clinical disease. Children exposed to leprosy seem to be at more risk than are adults. Prolonged close contact with an infected person is necessary for infection to occur. It is not known how the disease spreads with any certainty. Infected nasal droplets have been suggested, as has direct contact with leprosy lesions.

The disease has a long incubation period, measured in years rather than months or weeks and the appearance of symptoms is gradual. It may take up to twenty years for the disease to become manifest after contact. Leprosy is rarely fatal but it does damage the peripheral nerves. This leads to a loss of sensation in the affected areas of skin and this can lead to inadvertent injury, which may be serious. Victims frequently suffer damage from burns because they have no sensation of getting too hot.

There is a continuous spectrum of clinical manifestations at the opposite ends of which are tuberculoid and lepromatous leprosy. Whether an individual develops lepromatous or tuberculoid leprosy is dependent on the nature of the immune response mounted against the bacterium, with the tuberculoid form of the disease being associated with a strong cellular immune response.

Tuberculoid leprosy is characterised by one or a few blotchy red lesions on the face and on the extremities. These have a very dry appearance and sensation is absent from the affected areas. Enlargement or destruction of the cutaneous nerves is common in tuberculoid leprosy but it very rarely affects the larger peripheral nerves. There are comparatively few bacteria found in lesions associated with tuberculoid leprosy. Because this form of the disease occurs in individuals with a strong cell-mediated immunity, the Lepromin test is strongly positive. This is a skin test to diagnose leprosy and involves sub-dermal injection of inactivated *M. leprae*, usually on the forearm. The prognosis for people suffering from tuberculoid leprosy is good and this form of the disease is the least easily transmissible.

In contrast, people with a weak cell-mediated immunity develop the lepromatous form of leprosy, for which the prognosis is very poor and for which transmission is the most likely. Patients with lepromatous leprosy fail to respond to the Lepromin test. This form of the disease is characterised by numerous shiny skin lesions and enlargement of the larger peripheral nerves. The lesions of lepromatous leprosy contain very many bacilli. In lepromatous leprosy there is extensive damage involving numerous organs. The skin becomes thickened and nodular and there is enlargement of the skin of the nostrils and cheeks. Patients are said to have a leonine appearance - looking like a lion.

M. leprae has a lower growth temperature than *M. tuberculosis* and cannot be cultured *in vitro*. It can, however, be grown in the footpad of the nine-banded armadillo (*Dasypus novemcinctus* [Linnaeus]). This animal has the lowest core body temperature of the mammals. Diagnosis, therefore, does not rely upon artificial culture. Rather, it depends upon the observation of acid-alcohol-fast bacilli in tissues or skin scrapings removed from an infected individual.

The mycobacteria have unusual cell walls; covalently attached to peptidoglycan are polymers whose subunits are composed of arabinose and galactose. The arabinogalactan structure is esterified to mycolic acids - complex fatty acids that give the cell wall a waxy nature. Approximately 60% of the cell wall of a mycobacterium comprises lipid. These bacteria are thus difficult to stain using conventional methods.

Mycobacteria are visualised using the Ziehl-Neelsen method. The microscopic preparation is flooded with strong carbol fuchsin and the slide is heated until the dye steams. It is kept hot for ten minutes. During this time the dye is able to penetrate the waxy cell walls. The carbol fuchsin is then washed off from the slide and the preparation is flooded with a mixture of mineral acid in alcohol. Either hydrochloric or sulphuric acid is used. This treatment decolourises all the material on the slide apart from mycobacteria, which retain the red dye. To visualise the preparation, a counterstain is used. This is methylene blue, unless the microscopist is red-blue colour blind, in which case malachite green makes a good alternative. The bacillary load is much greater in lepromatous leprosy than it is in tuberculoid leprosy.

Treatment for the lepromatous form is prolonged, lasting at least one year. The WHO has recently reduced this recommendation from two years. For the tuberculoid form of the disease therapy is still for at least six months. The drug of choice used to be dapsone. Resistance to this drug has made single therapy unreliable and these days multiple drug therapy is the rule. The long course of antimicrobial therapy is required because the pathogenic mycobacteria grow extremely slowly; hence, they require a very long treatment. A consequence of this is that bacteria are exposed to antimicrobial drugs for a very long period. Consequently, point mutations that confer resistance are selected much more commonly than is the case with many other combinations of bacteria and antimicrobial agent. Multiple drug therapy delays the emergence of resistance. Daily oral dapsone plus one dose of rifampicin each month for 6 months is a standard therapy for tuberculoid leprosy, while daily dapsone and clofazimine plus monthly doses of rifampicin for 12 months, and with clofazimine pulses each month are required to treat lepromatous leprosy. If a patient has a single-lesion tuberculoid form of the disease, a single dose of rifampicin plus ofloxacin plus minocycline may be all that is required to treat the disease. Ideally patients should be given DOT treatment: Daily Observed Therapy. The drugs do have unpleasant side effects and patients do not always comply. By observing patients taking their drugs, the course of therapy may be monitored and this reduces the risk of selecting a resistant strain.

There are other mycobacterial infections that are associated with lesions of the skin. Scrofula is a tuberculoid infection of the skin of the neck and the cervical lymph nodes. It is caused by a number of different mycobacteria including *Mycobacterium scrofulaceum*, bacteria of the *Mycobacterium avium-intracellulare* complex and *Mycobacterium tuberculosis*. The disfigurement it caused started a fashion for

high collars and neck ruffs that became prominent in Regency England. Aquarium keepers and swimmers are also prone to skin ulcerations, fish-tank granuloma or swimmer's granuloma, caused by *Mycobacterium marinum*. This bacterium has a low optimal growth temperature compared with other pathogenic bacteria and this is one reason that the lesions it causes are confined to the skin. This bacterium also causes infections in fish.

Like the other diseases discussed in the Infections Ancient And Modern lectures, tuberculosis has been known since the Dawn of Civilisation. Egyptian mummies dating to 2400 BC have been found where the skeletons show clear evidence of the disease. As with many infections, tuberculosis flourishes in conditions of overcrowding and poor nutrition. During the Stewart Dynasty, Tuberculosis was said to be cured by the King's Touch and as early as 1692, Thomas Sydenham advocated the use of fresh air for the treatment of 'the white death': a practice that was still used in the 1950s in the TB sanatoria in the UK.

In Victorian Europe it reached epidemic proportions. Indeed, it was almost fashionable to die of consumption. The disease claimed the lives of a number of famous people. The poet Keats probably contracted tuberculosis while a medical student at Guy's hospital and he died from the disease at the age of 26. Chopin died from TB, the disease that claimed the lives of Anne, Branwell Charlotte and Emily Bronte. Scarborough became a popular resort for Victorians suffering from TB. George Orwell and DH Lawrence also died from the disease. It became the focus of much art and literature. Alexandre Dumas killed the heroine of *La Dame aux Camélias* of consumption: she became the heroine Violetta in Verdi's *La Traviata*. Other operatic heroines dying of TB included the seamstress Mimi in *La Bohème* and Antonia in *The Tales of Hoffman* by Jacques Offenbach. Antonia died after Dr Miracle the quack doctor had treated her.

It was the German bacteriologist Robert Koch, who first described the isolation of *Mycobacterium tuberculosis* in 1882. TB was also one of the first diseases to be diagnosed by X-ray, still a cornerstone in the diagnostic arsenal for this disease. Each year worldwide, over 2 million people die of TB. The wit who wrote the following advice on a lavatory wall had obviously learned how to break a cycle of infection: '*... half the girls in this college have TB, the others have VD. Sleep with the ones who cough*'.

A third of the world's population is estimated to be infected with the bacterium that causes TB, and across the world about 8 million people develop the disease every year. The World Health Organization estimate that between 2002 and 2020, approximately 1000 million people will be newly infected, over 150 million people will get sick, and 36 million will die of TB - if control is not further strengthened. The incidence of TB is increasing in both the developing and developed world. In Europe ten years ago there were 5 cases per 100,000 head of population. Last year the figure was 11 per 100,000 head of population. In the UK, there are currently about 7, 000 new cases annually. Ten years ago, the figure was about 2,000 cases annually.

Primary pulmonary TB occurs following inhalation of 'respiratory droplets'. The causative bacterium, *Mycobacterium tuberculosis* is an obligate aerobic bacterium and the lesions of TB are typically found in the upper part of the lung. The infection may, however, affect almost any part of the body. The symptoms of Pulmonary TB are a persistent cough lasting for more than three weeks, coupled with night sweats, weight loss and a high temperature. In 90-95% of all cases of primary TB the condition resolves spontaneously. Spontaneously resolved TB may, however, reactivate. Factors that are associated with reactivation include: old age; excess alcohol consumption; immunosuppression (particularly suppression of the cellular immune response) and co-existing lung disease (*e.g.* pneumoconiosis). This causes a particular problem for visitors to the South Coast of England. Unvaccinated grandchildren visiting grandparents with a cough are vulnerable to infection with TB.

Genetic factors undoubtedly predispose people to infection. A twin study showed that an identical, monozygotic, twin had an 87% probability of contracting TB if the other twin had the disease, whereas a non-identical twin had only a 26% chance of succumbing to tuberculosis. Further evidence for a genetic component in susceptibility to tuberculosis comes from the Lubeck disaster, which occurred in Germany in 1929-30. Two hundred and forty-nine babies were vaccinated with a virulent strain of *Mycobacterium tuberculosis* instead of the attenuated BCG strain. In consequence, 76 children died of tuberculosis. The remainder suffered only minor lesions, and all were alive and well twelve years later.

During the opening of the North American landmass during the 19th Century, European settlers introduced TB to populations who had never previously been exposed to this infection. However bad TB seemed to Europeans it was as nothing compared with the plight of The Plains Indians of the Qu'Appelle Valley Reservation on Saskatchewan. In 1886, this settlement suffered an epidemic of miliary tuberculosis. The mortality was 9,000/100,000. Although in many cases, tuberculosis is considered a pulmonary disease, it may spread to other parts of the body. In its most serious form, it becomes disseminated in a condition known as miliary TB. It may also cause renal infection and chronic osteomyelitis. This is an infection of the bone and has a gradual onset. *Mycobacterium tuberculosis* often infects the bones of the pelvis and of the spinal column. The bacterium is also causes a rare and insidious form of meningitis. On tapping a CSF, initial results are more suggestive of meningitis caused by a virus rather than a bacterium. Instead of the CSF being markedly turbid, it appears only slightly cloudy and microscopic examination reveals that the cells causing turbidity are lymphocytes, associated with virus meningitis, rather than the neutrophils that are typically associated with acute bacterial meningitides.

Mycobacterium tuberculosis causes an intracellular infection. Although the bacteria are phagocytosed by macrophages, the bacteria can survive within the phagosomal vacuole. Ultimately, this failure to eliminate the bacterium leads to the formation of a granuloma, which then undergoes caseation and, subsequently, calcification.

Unlike *Mycobacterium leprae*, *Mycobacterium tuberculosis* can be grown in artificial culture. It grows well on egg-based media such as Lowenstein-Jensen medium. It does, however, take a very long time to grow: four to six weeks is the average time for the characteristic breadcrumb like colonies to appear and a negative culture cannot be declared until slopes have been incubated for eight weeks. Because of the delay in reporting culture, provisional diagnosis is often made on the basis of ZN staining. An alternative to the Ziehl-Neelsen method has been developed. The auramine-rhodamine stain works by the same principle but the dyes are fluorescent, making the screening of large numbers of slides much easier.

Care must be taken with microscopy. The water used to prepare the stains must be passed through a bacteriological filter: there have been instances where environmental mycobacteria have contaminated stains and have given rise to false-positive microscopy results. Similarly, on examination of urine samples, the commensal bacterium *Mycobacterium smegmatis* has been confused with *Mycobacterium tuberculosis*. Culture is not without its problems. If sputum is to be tested for the presence of *Mycobacterium tuberculosis*, it must be decontaminated to remove the commensal flora of the upper respiratory tract and mouth. This is done with alkali treatment, since the waxy wall protects the mycobacterium from extremes of pH. Following alkali treatment, sputum samples are neutralised before inoculating onto slopes in screw-topped bottles. These are necessary to retain sufficient moisture in the culture that must be maintained for eight weeks. A culture in a Petri dish would resemble a crispy pancake after such a prolonged incubation. Because of the prolonged incubation necessary to detect *Mycobacterium tuberculosis*, more rapid, methods of diagnosis that rely on DNA hybridisation or PCR-based technology have been introduced. These techniques are also being applied to the identification of mycobacteria and also for antimicrobial susceptibility testing.

The first successful antimicrobial drug effective against *Mycobacterium tuberculosis* was streptomycin. Its discovery was attributed to Selman Waksman but his PhD student, Albert Schatz, was the person who made the first observations on this drug and to whom the discovery ought properly to be attributed. These days because of the emergence of multiply drug resistant strains of *Mycobacterium tuberculosis* (MDR-TB) combination therapy is used to ensure effective treatment and to help reduce the risk of selecting new resistant strains. Precise therapy depends on susceptibility of the causative strain. Combination therapy that is often used includes combinations of isoniazid, rifampicin, pyrazinamide and/or ethambutol/streptomycin. Therapy must be administered regularly for at least six months. There have been many cases of failure of 'compliance' *i.e.* the likelihood of the patient taking all of the tablets at the correct times and in the correct doses. This has undoubtedly contributed to the emergence of MDR-TB. As with leprosy, use of DOTS is encouraged to prevent this from spreading.

Each year in the UK, about 40 people succumb to bovine tuberculosis, caused by *Mycobacterium bovis*. This is acquired by drinking unpasteurised milk. Early pasteurisation measures, the Low Temperature Holding system, where milk was held at 62.8°C for at least 30 minutes failed to control this route of transmission. High Temperature Short Time pasteurisation, with milk is held at a temperature of 71.7°C for at least 15 seconds was introduced to prevent milk being the vector for tuberculosis. Although cattle are the human pool of this bacterium, controversy rages over badgers as the source of infection for dairy cows. The argument has continued from the 1970's with farmers arguing that badgers are the source of *Mycobacterium bovis* and conservationists arguing that badgers catch the infection from cows!

Infections with the atypical mycobacteria are increasing - mainly due to an expanding population of immunocompromised individuals. These infections are difficult to treat; not only because of the immunological problems of the patient, but also because these bacteria are often resistant to many of the currently available antimycobacterial antibiotics. The most important atypical mycobacteria include those of the *Mycobacterium avium-intracellulare* complex (MAI or MAC). For people who do not have AIDS, these bacteria cause pulmonary lesions; in AIDS patients, they may cause generalised infections. In children, these bacteria result in infections of the cervical lymph nodes. Treatment may involve ethambutol, amikacin, quinolones or macrolides. As with other mycobacterial infections, drugs are given in combination to prevent the further selection of resistant strains. Currently, the macrolides azithromycin or clarithromycin are used as the first-line drug in combination with others.

Virus Infections of Humans and Animals

The importance of viruses as pathogens: the broad ranges of diseases caused by viruses and the number of visits to GP's (by far the most common reason for visiting a family doctor is respiratory infection caused by a virus).

Epidemiology and spread: skin, respiratory, gastro-intestinal, venereally (horizontal): placental, breast feeding, during birth (vertical). Examples and means of spread given for each. Anecdotal case histories and approaches to controlling cross-infection by education are discussed.

The outcome of virus infection: inapparent infection, disease and recovery, fatality, latency (herpes), carrier status, oncogenesis. Examples of each.

Chemotherapy - very brief concepts.

Vaccines (live, dead, sub-unit) very brief concepts.

HIV and AIDS

THE HUMAN IMMUNE DEFICIENCY VIRUS

The Human Immune Deficiency Virus (HIV) is probably the most studied virus in the world. Almost 3/4 million articles have been written on or about HIV/AIDS. Millions of pounds have been ploughed into it. We understand much of its molecular biology. Recent 'dual-approach' drug trials appear to be useful (even if they will never cure the disease).

Some vaccine trials are in hand, but we do not have a vaccine - many scientists think we never will, and some companies are pulling out of the market (Nature 378, 23 November 1995).

Some children appear to have eradicated the virus - some prostitutes have not become HIV positive even though probably infected. Many HIV positive individuals have not progressed to AIDS, even 20 years on from their infection. These individuals are being studied to give us an insight into HIV and its progression to the clinical syndrome of AIDS.

Basically HIV/AIDS research is about what causes HIV to win in the battle between it and the immune system. One minute it is in check, the next it is out of control, killing T-cells and causing immune suppression. I think if we understand these key issues we may start to solve other questions.

Brief notes on HIV/AIDS

- 1) At present predicted to be 4.5 m cases world-wide (70% in Africa). 14-15 m HIV positive individuals predicted to exist (8.5 m in Africa, 3.5 m in S.E. Asia, 1.5 m in Latin America).
- 2) Virus probably arose from Central Africa in 1950's - mutated from monkey virus, taken to Haiti by workers from Zaire. Transmitted to gay men on holiday in Haiti. These returned to W. coast of USA and promiscuity allowed virus to spread (some men had more than 200 partners per year). In May 1981 5 cases of severe immunosuppression in promiscuous gay men were reported in LA & San Francisco. These patients had Kaposi's Sarcoma (a tumour) and *Pneumocystis carinii* (a fungal parasite); the latter causes fatal pneumonia.
The disease was later shown to be transmitted by blood products when haemophiliacs presented with identical syndrome.
- 3) Infectious agent isolated in 1983 (Montagnier, Paris) and shown to be a **RETROVIRUS**, later called **human immunodeficiency virus (HIV)**. HIV-1 & HIV-2 now known to exist - little cross reaction between them. Many strains of HIV-1 exist as the antigens are capable of rapid mutation, therefore, many specific epitopes exist which will make vaccination difficult.
- 4) Virus not a typical RETROVIRUS it is a **LENTIVIRUS** and kills, not transforms infected cells. Important surface antigens are gp120 and gp45 - glycoproteins required for attachment to CD4+ receptor of cells and fusion in the cell. Internal enzymes carried by particle are integrase (integrates DNA copy of RNA into cell chromosome) protease (processes translation product of virus (mRNA) and Reverse Transcriptase (essential for making initial DNA copy of virus RNA genome). The glycoproteins are centre of recombinant DNA technology approach to making a vaccine (i.e. sub unit approach, as in HBsAg - the hepatitis B surface antigen). The enzymes are targets for chemotherapeutic agents e.g. **AZT (RETROVIR)** which inhibits Rev. Transcriptase. Dual approach to chemotherapy will be the best one.

QUESTIONS

Will chemotherapy stop HIV positive individuals progressing to AIDS?

Will vaccines stop progression to AIDS?

Can HIV be eradicated from HIV+ individuals?

Will vaccination of HIV - individuals prevent virus infection and 'latency'?

Progress to date with vaccination not good - animal models for HIV not plentiful, therefore, SIV model used. Responses can be induced - what about PROTECTION?

Virus transmitted in blood and body fluids (semen, vaginal fluids (low) saliva (low) tears (very low).

NOT in swimming pools, on cups, knives, forks. If you can, avoid practices which increase transmission.

The virus is quite labile and falls apart easily (not like hepatitis B). If celibacy is out of the question sex should be accompanied by **CONDOMS**?

Groups considered at risk were

- GAY MEN,
- BISEXUAL MEN,
- HAEMOPHILIACS,
- RECIPIENTS OF INFECTED BLOOD/PRODUCTS,
- I.V. DRUG ABUSERS
- NEONATES BORN TO HIV POSITIVE MOTHERS.
- OTHERS AT RISK ARE SEXUAL PARTNERS OF ABOVE (IF SAFE SEX NOT PRACTISED).

At present it is unsafe practices or exposure to untested blood that will lead to virus transmission. As a group educated gay men have got transmission under control.

Attempts to curb transmission by needles has had some but disappointing impact. Blood products in most parts of the world (not all) are tested. Vertical transmission remains a sad increasing statistic.

In the UK, the predominant spread appears to be in H.M. Prisons.

PLEASE BE AWARE OF THIS VIRUS
&
ACT SENSIBLY
