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Viruses, fungi and parasites

There is, of course, little in common between these groups of organisms and they deserve individual attention. In the previous chapter I discussed the general properties of bacteria, the nature of bacterial infections and the laboratory methods used in their diagnosis. In this chapter I propose to follow a similar plan for the other main groups of medically important micro-organisms, which are brought together here for convenience.

The viruses

> So, naturalists observe, a flea
> Hath smaller fleas that on him prey;
> And these have smaller fleas to bite 'em,
> And so proceed ad infinitum.
>
> *On Poetry*, Jonathan Swift (1667—1745)

The viruses form a large group of infectious agents parasitizing other microbes, plants, animals and man. There is a wide variation in particle size, but the medically important viruses range from 20 to 300 nm in diameter. The genetic material of a virus is composed of RNA or DNA but, unlike that of a bacterium, never both. Organisms such as the Chlamydiae which, because of virus-like properties, used to be regarded as honorary viruses but which contain both nucleic acids, are

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now recognized to be bacteria. Surrounding the central nucleic acid core there may be a layer of protein sub-units arranged either spirally (in which case the virus is said to show 'helical symmetry') or in an icosahedron ('cubic symmetry'). Some viruses are surrounded by an outer membrane containing lipids. Most such enveloped viruses can be inactivated by exposure to lipid solvents such as ether. Just as Table 1 set out a simple classification of the bacteria using only shape, Gram stain response and aerobic or anaerobic growth, so Table 2 offers a simple classification of the main groups of medically important viruses using only nucleic acid type, nature of symmetry and presence or absence of an outer membrane.

Table 2   Provisional classification of viruses pathogenic to man (after Andrewes and Pereira, 1972)

Nucleic acid	Symmetry	Presence of outer membrane	Viruses
RNA	cubical	−	Picornaviruses, Reoviruses
		+	Togaviruses
	helical	+	Orthomyxoviruses, Paramyxoviruses, Rhabdoviruses
	uncertain	+	Arenaviruses, Coronaviruses
DNA	cubical	−	Papovaviruses, Adenoviruses
		+	Herpesviruses
	uncertain	+	Poxviruses

Advances in our knowledge of the properties of viruses have followed closely upon the development of new technology. Two major characteristics of viruses are their inability to propagate themselves outside their living host cells, and their small size. Research on viruses was therefore severely hampered until the introduction of tissue culture and electron microscopy. Most of the work prior to this, was conducted using bacterial filtrates (containing 'filter passing viruses') which provided a means of assessing the size of the viral particles by grading the pore size of the filter used. Such filtrates could then only be examined by inoculation into living animals. Subsequently, it became possible to propagate viruses in fertile hens' eggs, and later still, in tissue cultures. The application of light microscopy to virology produced only modest results due to the limited resolution of optical microscopes. However, the technique was of some value in the

recognition of individual particles (elementary bodies) of some of the larger viruses and of clumps (inclusion bodies) of others in infected cells. The introduction of electron microscopy made it possible for the detailed structure of virus particles to be seen for the first time.

I have avoided using the word 'organism' in relation to viruses because it is difficult to describe as a living creature a structure which is incapable of replicating itself without the agency of the genetic material of alien cells, and which is capable of renewed activity after crystallization of its nucleic acid. The role of viral nucleic acid in relation to host cells is analogous to the plastic instruction cards inserted into some modern washing machines which select the sequence of tasks to be fulfilled by the machine. The process is initiated by the attachment and absorption of a virus to its host cell. The specificity of the relationship between host and virus is determined by the outer layers of the virus, and a 'good fit' is required for successful host—virus attachment. Naked viral nucleic acid may have a much wider range of possible hosts than the intact virus, the outer layers of which are antigenic and provoke the immune response of the host. Once attached, the virus is either ingested by, or injects itself into, the host cell. A quiescent (eclipse) stage follows, during which infective virus cannot be retrieved from the host cell. At this stage the viral nucleic acid is subverting the host cell's enzymatic armoury, by use of modifications controlled by the host's DNA, to forge new viral components and to assemble them into new virus particles. When a sufficient number of such virions has been generated, they are released from the host cell either by extrusion or by lysis, and are free to find new host cells and to repeat the process.

Commonly, cells can be invaded successfully by only one type of virus at a time, the multiplication of the second being prevented either by 'interference' (mediated by 'interferon') or by direct blocking by the first. Upon occasion, however, two dissimilar viruses can co-exist in the same cell and, under specialized conditions, the host cell may generate 'recombinant' virions with some genetic characteristics of each invading virus.

As has already been noted (Chapter 2), some viruses may remain quiescent for lengthy periods. This is the case with those temperate bacteriophages which do not lyse their host cells. These 'phages reproduce at the same time as their host and are passed down from

generation to generation of host cells. Such lysogenized viruses may be released spontaneously or be 'cured' from their host cells by exposure to chemicals such as dyes or to ultraviolet light. In some cases, virus nucleic acid may integrate with the host cell's genetic material and alter the characteristics of the host cell (lysogenic conversion). One example of this is the production, under the influence of a 'phage, of diphtheria toxin by strains of otherwise non-toxigenic *Corynebacterium diphtheriae*. Another example is the virulence factor for the intestinal tract of young animals which is conferred by other 'phages on some serotypes of *Escherichia coli*. The occasional role of 'phages in accidentally carrying plasmids mediating antibiotic resistance or other characteristics from one bacterial host to another (transduction) has been mentioned in Chapter 2.

Laboratory methods in clinical virology, as in clinical bacteriology, are based upon microscopy, culture, and serological techniques.

Direct light microscopy of clinical specimens has little to offer except in a limited range of circumstances (e.g. the presence of inclusion bodies, of elementary bodies, or of atypical mononuclear cells or 'virocytes' seen in the blood films of patients with some viral infections). However, the use of the light microscope in the examination of tissue cultures inoculated with material containing certain viruses is an important diagnostic technique (see below). The value of microscopy as a diagnostic procedure has been transformed by the advent of the electron microscope. Some electron photomicrographs of viruses from clinical specimens are shown in Figure 1. Electron microscopy of suitably collected material has permitted the rapid identification (within 2 hours) of viruses (e.g. varicella zoster, herpes, variola and its variants, and orf) collected from vesicular lesions. Electron microscopy for rotaviruses from stools takes only a little longer. This rapid identification of pathogens from clinical material is much faster than anything so far available in bacteriological diagnosis. Using the technique of immunofluorescence, similarly rapid viral diagnostic methods have been developed for the demonstration of infection with respiratory syncytial virus (RSV), a troublesome respiratory infection in children, sometimes giving rise to respiratory obstruction. Methods of immuno-electronmicroscopy capable of giving rapid diagnostic information are now being developed.

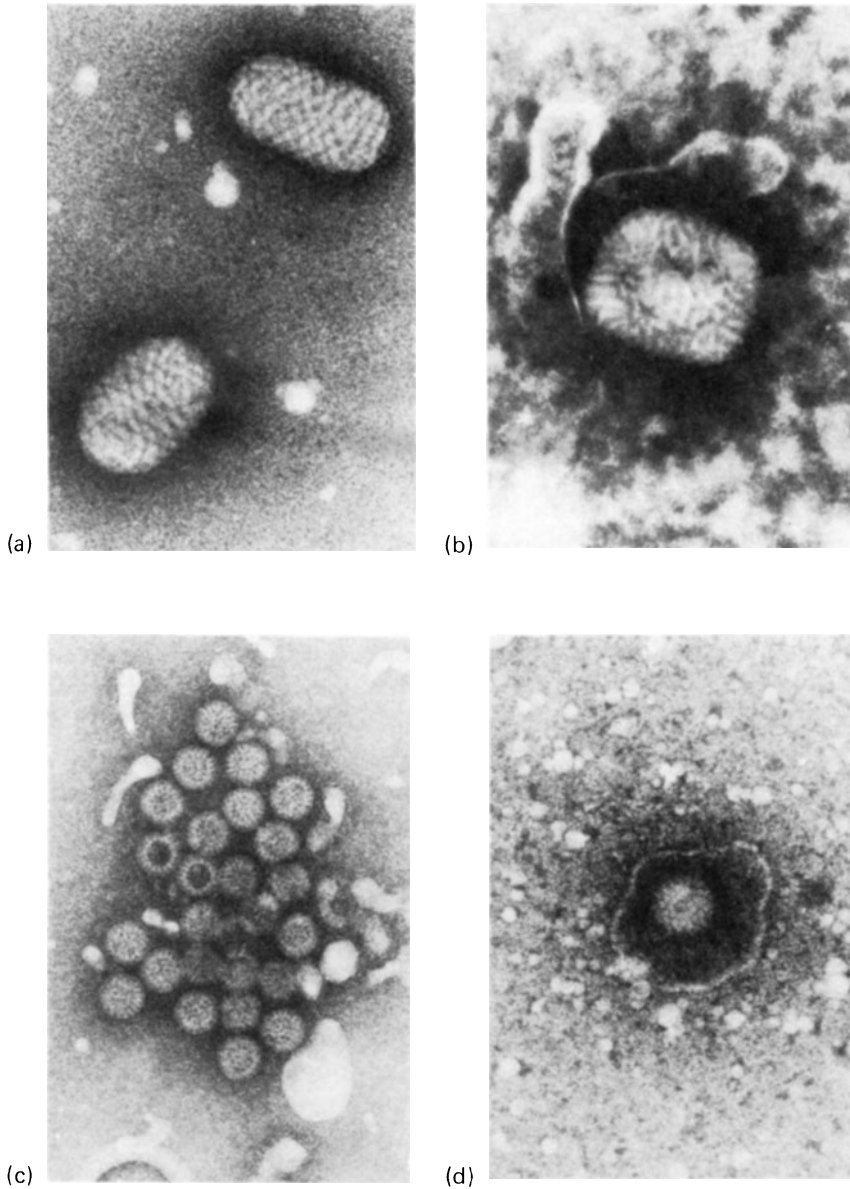Special precautions must be taken by laboratory staff in handling

Figure 1: Electron photomicrographs of viruses from clinical specimens (a) Orf (contagious pustular dermatitis), (b) Vaccinia, (c) Rotavirus, (d) Varicella zoster. All magnifications 75 000 ×

material from vesicular lesions if there is any possibility that the lesion may be caused by smallpox, and such material should be examined only by designated smallpox diagnostic laboratories. This can be ensured only if clinicians inform their usual diagnostic laboratories of such a possibility before collecting the sample.

The second set of diagnostic techniques is based upon culture of viruses. This may be undertaken in laboratory animals, in fertile hens' eggs, or in tissue cultures. The inoculation of animals or eggs for viral diagnostic purposes has been largely supplanted by tissue culture techniques and is now used only for a relatively limited range of diagnostic procedures, such as the differentiation of Coxsackie viruses by injection into the brain of suckling mice. Tissue cultures are monolayer cultures, in bottles, of cell lines derived from mammalian or human tissues which retain their vitality and capacity to divide for lengthy periods provided they are bathed in a suitable fluid, containing nutrients and changed at appropriate intervals. These cell sheets can be infected by viruses from clinical material. As no particular cell culture will be suitable for the culture of all medically important viruses, clinical laboratories undertaking this work maintain several different cell lines to cover most of the likely possibilities. The cost of maintaining cell lines in good condition is high, and so those capable of growing some of the less common of the clinically important viruses are maintained only by specialized laboratories. Some therefore have to be sent on to such laboratories.

In order to obtain satisfactory results, the appropriate specimen must be taken early in the disease. It must be collected directly into a bottle of virus transport medium freshly thawed after removal from the freezer. Viral transport medium contains a mixture of antibiotics designed to kill contaminating bacteria. Cultures in transport medium must be sent rapidly to the laboratory where they will be refrigerated until they can be examined. The fullest possible information must be given as to the nature of the specimen, and of the underlying clinical problem, in order to permit the choice of appropriate cell lines for inoculation.

After tissue cultures have been inoculated and incubated, the multiplication of viruses may be observed by various means.

(1) The production, in the cell sheet, of cytopathic effects visible

under the light microscope. These changes may be specific enough to permit provisional identification.

(2)   The demonstration of viral antigens in the culture fluid.

(3)   The demonstration of viruses by examination under the microscope of culture fluid or disrupted cellular material.

(4)   The adsorption of red blood cells (haemadsorption) onto cell layers infected with myxoviruses, or paramyxoviruses.

(5)   The use of immunofluorescence microscopy.

(6)   The prevention of infection by other viruses (interference).

The many immunological techniques which are used to identify individual viruses will not be discussed here.

Specimens from sites readily colonized by viruses, sent to the laboratory for viral culture, should always be accompanied by the first of two samples of clotted blood (separated by an interval of 10 to 14 days). The serum from this blood can then be tested against any viruses isolated. The mere isolation of a virus from the throat, the nasopharynx or the stools of a patient does not necessarily invest that virus with pathogenic significance: it may be quite unrelated to the patient's illness. If, however, there is a change in the patient's antibody response to the cultured virus, and this change reflects the progress of the infection, that will be useful evidence of the rôle of the virus in causing the disease.

Such serological tests are more commonly used in trying to identify the cause of an infection when cultures have not been attempted, have failed to yield a virus, or are inappropriate. It is important to take two specimens of blood, 10 to 14 days apart, for purposes of comparison, since only changes in antibody level with time have any diagnostic value in most cases. The first of the samples should be collected as soon as the patient is seen, in order to give the best chance of demonstrating a diagnostic rise in antibody level as the infection progresses. The fullest possible clinical information should be given to the laboratory about the features of the disease including details of how long the patient has been unwell. This is necessary because, with many clinical syndromes, any one of a variety of viruses could be implicated and so the laboratory needs as much help as it can get to select an appropriate

range of antigens to react against those antibodies possibly present in the patient's blood. As viral antigens are costly, well conducted laboratories will test only paired sera in this way: they will not normally examine a single blood sample. The numerous different serological techniques used in such tests will not be described here: interested readers will find details in textbooks on diagnostic methods in virology.

In the field of preventive medicine, virology laboratories are now expected to screen all pregnant women to establish whether they are immune to german measles (rubella). This is so that the non-immune can be offered immunization after delivery, so reducing the chances of producing rubella-affected babies in subsequent pregnancies. Any appreciable level of rubella antibodies in the blood ($\geqslant 1:16$) implies previous infection or immunization and hence immunity, whereas an absence of antibodies implies susceptibility to infection. In the course of such testing, some women are found to have very high levels of antibody to rubella. This may be insignificant or may reflect recent infection, potentially a hazard in the first months of pregnancy. Similarly, a woman in early pregnancy may have had contact with someone suffering from rubella, or she may fear that she has. Establishing whether a pregnant woman has, or has just had, rubella is important because it may be felt that the risk of producing a rubella-affected child justifies termination of pregnancy. It is also important that the results of the tests are produced quickly because termination of pregnancy becomes more difficult and more dangerous as the pregnancy proceeds. When the first blood sample tested does not show very high levels of antibody, comparison with a second sample collected 7 to 10 days later will either show a diagnostic rise, confirming current or very recent infection, or will show no change, indicating no risk. Appropriate decisions can then be taken. Alternatively, if very high antibody levels are found in the first sample, it may not subsequently be possible to show significant changes in level. Under such circumstances, the antibody activity can be shown by suitable techniques to be concentrated either in both IgM and IgG fractions of serum proteins or just in the IgG fraction. Such antibody fractionation may be performed on the first specimen to prevent delay. The presence of rubella IgM antibody is strongly suggestive of recent or current infection, and will give rise to appropriate gynaecological advice. The

importance of good clinical information reaching the laboratory is obvious in this case: a history of actual or possible exposure of a pregnant woman to rubella should be clearly communicated, with the relevant dates, and the duration of pregnancy. A pregnant woman found to have rubella antibodies once need not be tested again in the same or in a later pregnancy even if she subsequently comes into contact with rubella.

Another problem causing much clinical anxiety and work for the laboratory is that of the patient with hepatitis. Hepatitis A and hepatitis B may both be spread by contact with infected blood, although the faecal—oral route of spread is usual with hepatitis A. Hepatitis B (serum hepatitis) is usually spread by blood or blood products although sexual spread, particularly in homosexuals, also occurs. There have been a number of outbreaks of hepatitis B in hospitals, attended by several deaths of patients and staff, and these have caused natural anxieties in medical and nursing staff and among organizations representing staff interests. Concurrently, tests have been developed enabling the surface antigen of hepatitis B (HBsAg), the so-called Australia antigen, to be detected in the blood of patients suffering from this disease. The antigen may be detectable in the blood of some patients for many months, or even years, after recovery from the infection.

Experience has shown the wisdom of testing all blood donors for the presence of HBsAg and of refusing to accept as donors those found to carry the antigen. Similarly, all patients admitted to renal dialysis units and the staff of such units should be clear of the antigen. Apart from these very specialized situations, the risks of transmission of hepatitis B between patients or between staff and patients are very small (assuming no sexual activity on the wards). It is, therefore, very ill-advised to undertake screening programmes or semi-systematic testing of patients or staff to identify those who are HBsAg positive, because it causes a group of near outcasts to be identified. There is always a temptation to stop a nurse, a surgeon or a dentist found to carry HBsAg from working. This is, in my opinion, quite unreasonable since the chance of such staff dripping their own blood into a patient's lesion cannot be great. It is better, therefore, not to test staff and so to spare them and their colleagues needless anxiety. I suspect that if HBsAg had not at first been called the Australia antigen it would have seemed less

threatening and would have provoked less anxiety.

The fungi and yeasts

> Can any mortal mixture of earth's mould
> Breathe such divine enchanting ravishment?

Comus. John Milton (1608—1674)

The members of this very large group of ubiquitous micro-organisms are distinctively plant-like, but lack chlorophyll. They cannot synthesize their own nutrients, and so derive their requirements from dead or living organic material. Fungi have rigid cell walls containing chitin, which give the organism its characteristic shape. The cells contain nuclei and often, granules of fat or starch. Single cells are characteristic of the yeasts, but many fungi have filamentous forms (hyphae) with many cells linked in ropes which may become densely packed and matted, giving rise to aggregations known as mycelia.

Although sexual reproduction can occur in some of the unicellular organisms, the usual means of propagation is asexual (e.g. by budding). The multicellular forms may reproduce asexually or sexually. The asexual mechanisms may be vegetative, by the accidental detachment of part of the mycelium and its regrowth in a new situation, or by a more deliberate method in which a variety of spore bearing structures are formed on specialized hyphae. The nomenclature and classification of these structures will not be dealt with here. As the sexual mechanisms of reproduction exemplified by the fungi scarcely concern the medically important members of the group, their description will also be omitted.

Yeasts and fungi can grow in a wide range of temperatures, but those involved in systemic infections of man normally grow at 37 °C, while those which cause superficial infections thrive at a lower temperature. Fungi may grow aerobically or anaerobically. The laboratory diagnosis of infection caused by yeasts and fungi is based on microscopy, culture, biochemical tests and serology.

Direct microscopy of smears from clinical specimens collected for bacteriological examination may reveal the presence of fungi or yeasts as single cells, budding forms, or hyphal forms. The presence of hyphae in a clinical specimen indicates a greater possibility of a patho-

genic rôle for the organism. Sometimes the appearance of the organism is sufficiently characteristic to make the diagnosis, as when yeasts surrounded by large capsules are seen in the cerebrospinal fluid of a patient with meningitis caused by *Cryptococcus neoformans*. Skin scrapings, nail clippings or hair from patients with suspected superficial infections such as ringworm, may be examined microscopically, either directly, or after treatment with potassium hydroxide, in an attempt to recognize morphological characteristics typical of the dermatophytes *Microsporum* spp., *Trichophyton* spp., and *Epidermophyton floccosum*. If microscopy of specimen tissues is not successful, a microscopic examination of fungi cultured from such material will permit the recognition of distinctive features of each dermatophyte. In the case of systemic mycoses, the only specimen available may be tissue removed for biopsy or at postmortem examination. Histological examination of this tissue using suitable staining methods such as silver impregnation may lead to the diagnosis. Unless special staining methods are used, some systemic mycoses may be missed.

The mainstay of the laboratory diagnosis of most fungal infections is the culture of the causative agent. This is sometimes possible without the use of special cultural techniques since some organisms such as *Candida* spp. and *Aspergillus* spp. will grow on routine bacteriological culture media. However, most fungi require special media and even those which will grow on, say, blood agar, will grow more readily and quickly on specially developed media. Generally, fungi and yeasts grown in the laboratory are derived from sites with a rapidly proliferating bacterial flora which will overgrow them unless steps are taken to prevent this. Most fungal culture media used for primary isolation from clinical material contain antibiotics to prevent this bacterial overgrowth. Some of the ringworm fungi grow slowly, taking weeks to produce characteristic colonies. Such cultures must be kept moist and be kept sealed to prevent the seeding of the medium with contaminating fungi from the air. The appearance of colonies of fungi on standard culture media (colour, size and outline of colony, rate of growth, surface appearance) may help in identifying the organism concerned. Examination of the aerial structures of the colony by plate microscopy may be helpful, and microscopic examination of teased out, suitably stained (lactophenol blue) mycelial material may lead to the recognition of characteristic diagnostic
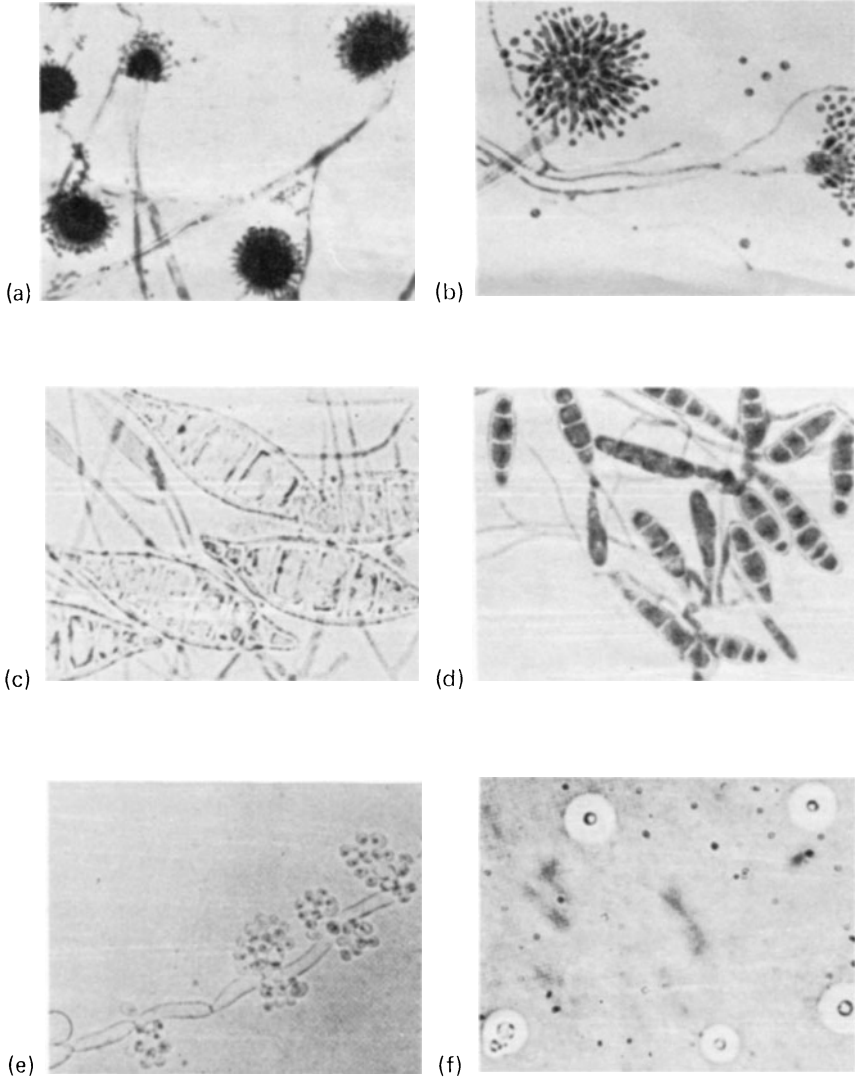
Figure 2:   Photomicrographs of fungi and yeasts. (a) and (b) *Aspergillus* spp., (c) *Microsporum canis*, (d) *Epidermophyton floccosum*, (e) *Candida albicans*, (f) *Cryptococcus neoformans*. (Courtesy of The Upjohn Company, Kalamazoo, Michigan, publishers of a *Scope Monograph on Human Mycoses*)

features. The interested reader is referred to textbooks of mycology for more information. Some characteristic fungal structures are shown in Figure 2.

Biochemical tests on yeasts or fungi cultured in the laboratory play a much smaller part in their identification than do such tests on bacteria. Tests of utilization and fermentation of various carbohydrate substrates, with or without the production of gas, are performed in the identification of yeasts such as *Candida* spp., but few other chemical tests are used in differentiating fungi.

Serological procedures also play a smaller part in the diagnosis of fungal disease than in that of infections caused either by bacteria or by viruses. Serology may, however, be of great value in the diagnosis of systemic infections such as histoplasmosis and cryptococcosis, and may give some indirect evidence of tissue invasion in patients with candidiasis or aspergillosis. All the usual difficulties of interpretation of serological findings discussed in Chapter 2 apply to mycological serology.

The problems of interpretation of mycological observations in a clinical context may be considerable. Yeasts and fungi are plentiful in the environment, and so it may be difficult to decide whether any particular isolate is related to the patient's disease. In some situations, there will be little difficulty, as with the recovery of *Madurella mycetomi* from the depths of the lesion in a patient with Madura foot or of 'sulphur granules' from a patient with actinomycosis. Most clinical mycological situations are not so well defined. The problems are of two kinds: over diagnosis, and under diagnosis.

Over diagnosis happens particularly readily with the opportunistic organisms such as the yeasts and *Aspergillus* spp. The latter are very prevalent and may be found in the air, in soil and in dust. They are often found contaminating bacterial cultures, and are frequently present in diseased airways in man. Various clinical states may be associated with *Aspergillus* infection. These include rare skin infections; otitis externa usually associated with *A. niger*; aspergilloma of the paranasal sinuses generally caused by *A. flavus*, and invasive aspergillosis most often occurring in patients severely ill for other reasons. The main difficulties arise in the diagnosis of pulmonary aspergillosis because, while the isolated fungus may be associated with pulmonary disease, it may equally well be a contamin-

ant of the cultures, or a commensal in the patient. Pulmonary aspergillosis is generally caused by *A. fumigatus* and may present as a hypersensitivity reaction, as an aspergilloma or with invasive disease. Hypersensitivity may take the form of asthmatic attacks, or a chronic process (allergic bronchopulmonary aspergillosis) with fever, pulmonary infiltration, eosinophilia, local and cutaneous immune responses, and even bronchiectasis and localized peribronchial invasion. Aspergilloma is a relatively benign condition in which pre-existing cavities, left for instance by healed tuberculosis, are partially or wholly filled with a ball of *A. fumigatus.* This usually causes little trouble unless a pulmonary bloodvessel is incidentally eroded. Invasive pulmonary disease caused by *A. fumigatus* is rare and occurs in the immune-compromised host, generally proceeding rapidly to a fatal conclusion with little evidence of local inflammatory response.

The assessment of patients with suspected pulmonary aspergillosis is difficult and depends upon clinical and radiological findings as well as upon laboratory methods. Antifungal drugs are often toxic and so it is particularly important to treat the patient and not the pathology report. Mere isolation of *A. fumigatus* from the sputum is not sufficient for diagnosis. The demonstration of an antibody response is common with aspergilloma but less frequent with hypersensitivity reactions. The patient rarely develops a detectable antibody response to systemic invasion by *A. fumigatus.* Precise diagnosis is important because it will determine subsequent management of the patient: inactivity in the commensal state; inactivity or resection of the lesion in aspergilloma; steroid therapy possibly combined with imidazoles for bronchopulmonary aspergillosis, and amphotericin B for invasive aspergillosis.

The tendency to underestimate the significance of fungi is exemplified by an unfortunate patient seen in hospital some years ago. He had an unexplained fever lasting many weeks during which the whole gamut of clinical, radiological and laboratory investigations were undertaken without result. He was given various courses of antibiotic treatment, all to no avail, and he eventually died without anyone having any idea why. His postmortem examination was well attended and revealed massive fungal abscesses of brain, lungs, liver, spleen, lymph glands and bone marrow. The pathologist performing the autopsy was at a loss to know why such extensive fungal invasion had

not resulted in the isolation of the causative organism from blood cultures. Examination of the work records in the bacteriology laboratory showed that more than a dozen sets of blood cultures had been examined and that most of the bottles had been discarded after a few days' incubation because a yeast found growing in them was mistakenly assumed to be a contaminant. Good laboratory supervision is essential.

The choice of antifungal chemotherapy has widened considerably in recent years. There used to be few useful drugs available other than griseofulvin for ringworm, nystatin for yeast infections and the potentially nephrotoxic drug, amphotericin B, for systemic infections. More recently, 5-fluorocytosine and a whole new group of imidazoles (clotrimazole, miconazole, ketoconazole and econazole) have become available. In case of doubt, expert advice should be sought on drug choice and dosage. This may prevent further tragedies such as those reported by Symmers (1973) in which a series of patients with fungal infections were fatally under treated because their doctors were so concerned about the toxicity of amphotericin B that they did not give enough of this potentially life saving drug.

The protozoa and helminths

> While the angels, all pallid and wan
> Uprising, unveiling, affirm
> That the play is the tragedy, 'Man',
> And its hero the Conqueror Worm
>
> *The Conqueror Worm*. Edgar Allan Poe (1809—1849)

The organisms in these two groups are enormously varied. Although transmission is commonly direct from man to man in some, the majority require the intervention of an insect or other vector, and for a number of species there are important animal reservoirs of infection. Complicated life cycles involving two or more hosts and tenuous connecting links often make the chain of transmission extremely inefficient, so that hundreds of thousands of parasites die unfulfilled for every one completing a full life cycle and becoming capable of initiating the next. Nevertheless, aided by poor standards of sanitation and hygiene, the parasites are all too successful. They cause some of the

most prevalent diseases in the world, and the consequences in terms of human misery and economic loss are enormous.

Some of the organisms concerned are, at one stage of their lives, so large that they scarcely fall within the remit of microbiology. The mature pork tape worm *Taenia solium* and the beef tape worm *Taenia saginata* may be several metres long and are easily seen with the naked eye. Common round worms (*Ascaris lumbricoides*), whip worms (*Trichuris trichiura*) and thread worms (*Enterobius vermicularis*), although much smaller than the tape worms, are all, at one stage of their careers, visible without a microscope. For the most part, however, the group is made up of organisms (including those named above) which are very small at the preliminary stages in their development, and which either remain minute in size throughout their cycle, or, despite having grown large at some stage, still require microscopic examination for final identification (such as the differentiation between *T. saginata* and *T. solium*).

Laboratory diagnosis rests upon microscopy, culture and serological methods. The most important of these is microscopy, which commonly involves the phase in the life cycle at which the parasite usually leaves the human host. It is not necessary to know in detail every phase of every cycle.

Examination of faeces for ova or cysts requires the use of appropriate concentration methods. If active trophozoites of *Entamoeba histolytica* are to be sought in material from patients with suspected amoebic dysentery, stool specimens must be examined in the laboratory while they are still quite fresh. In temperate climates this diagnosis is, sadly, not often entertained when there is no history of foreign travel, and the patient may suffer by being treated inappropriately for presumed ulcerative colitis when a simple stool examination or serological test would have given the correct diagnosis. Most experienced medical microbiologists have seen this diagnostic misfortune happen more than once, sometimes with quite disastrous results. The identification of ova or cysts in faeces is a skilled task, best undertaken by those with experience, because the differentiation between pathogenic organisms which may require eradicative chemotherapy (such as *Entamoeba histolytica*) and harmless organisms which should be left alone (such as *Entamoeba coli*) is crucial. Some organisms, such as *Schistosoma haematobium*, may be sought in the urine by micro-

scopic examination.

The examination of suitably stained, thick and thin blood films for malarial parasites is an essential diagnostic investigation for any febrile patient in (or from) an area in which malaria is endemic. Usually it is not too difficult to see malarial parasites within red blood cells if they are there, but occasional patients present great difficulties in this regard. Experience is required to identify the particular stages of development which may be present, and still more expertise is needed to identify the infecting species (*Plasmodium vivax*, *P. malariae*, *P. falciparum* and *P. ovale*), a differentiation which may be crucial because it should be used to guide drug choice. In temperate climates, lives are lost every year because blood smears are not examined for malarial parasites, because the examination is inexpertly done, or because correct laboratory findings do not lead to appropriate drug selection. In case of doubt, expert advice should always be sought.

Microscopic examination of blood collected at night may reveal the presence of microfilariae (*Wucheria bancrofti* or *Brugia malayi*) which cause filariasis, while similar findings in blood collected by day may identify the eye worm *Loa loa*. *Onchocerca* spp. (the cause of river blindness) are found in skin snips.

Histological examination of tissues removed by biopsy, surgically, or at autopsy may reveal lesions recognized as being caused by the tape worm *Taenia solium*; encysted larvae of *Trichinella spiralis*; filarial granulation tissue; cysts of hydatid disease caused by *Echinococcus granulosus* or *E. multilocularis*; the lesions of schistosomiasis; amoebic liver abscesses; damage due to leishmaniasis; pulmonary infection caused by *Pneumocystis carinii*, and many other features of parasitic diseases. In temperate climates, experience of the histological features of many parasitic diseases is difficult to accumulate so that specialist opinions may need to be sought by those histopathologists who do not have a special interest in this field. Species identification can sometimes be made by immunofluorescence of paraffin sections.

Although cultural methods exist for the propagation of some protozoa in the laboratory, relatively little use is made of them in laboratory diagnosis. However, they are important for *Leishmania* spp. which are not always found by direct microscopy. Another exception in some laboratories is the use of culture media to increase

the yield of *Trichomonas vaginalis* from vaginal swabs. However, opinions vary as to the value of such cultures, and many diagnostic laboratories do not use them, preferring to rely upon direct microscopy of vaginal smears. Culture is important for the preparation of antigens for laboratory use and for the testing of new drugs.

A variety of serological techniques have been developed to test the patient's antibody response to invading parasites. Some of these are not generally available because of difficulties in obtaining satisfactory material for the preparation of antigens. With others, notably the round worms (nematodes), the antigens are deplorably non-specific. In some diseases, more than one type of test is necessary to obtain a reliable conclusion. In spite of these problems and those connected with the interpretation of serological tests in general (see Chapter 2), serology may be the only practicable approach to diagnosis in an important range of diseases in which the parasite is frequently inaccessible to the microscope. These diseases include amoebic liver abscess, amoeboma, Chagas's disease, hydatid disease, cysticercosis and pneumocystosis. Test results for some of these can be up to 95% reliable. For other diseases, serology is used as an adjunct to diagnosis or as a test of cure. Skin tests are useful in some types of leishmaniasis, but serology is generally preferable when available. Both are important epidemiological tools.

No account has been given here of the life cycles of medically important parasites, of the control of their spread or of the treatment or prevention of parasitic diseases. The travels of some parasites from habitat to habitat and from host to host match some of the more improbable exploits of Odysseus, and are well worth the attention of romantically inclined readers. These (and others) are recommended to read textbooks and reviews on the subject.