This third edition of the popular Cellular Pathology textbook provides a thorough coverage of all the key areas of histological and cytological techniques. It is written for students studying courses in biomedical sciences, healthcare science or other subjects allied to medicine. The book provides essential information on those techniques that have particular relevance to both the diagnosis of disease and also for research in pathology.

This new edition has been thoroughly updated and extended to:

- include changes in established practice
- accommodate newly emerging techniques such as in molecular diagnostics
- provide an introduction to the latest immunological methods, microscopy techniques, image analysis systems and approaches in liquid-based cytology
- show all images in full colour.

Additionally, the general principles of pathology are given a more rigorous treatment and the approach to good laboratory practice has been expanded.

This edition continues to feature learning objectives, revision notes, recommended further reading and self-evaluation questions provided for each chapter. The book further benefits from an increased number of full-colour photographs that illustrate typical results and techniques.

Cellular Pathology 3rd edition reflects the current requirements of cellular pathology teaching and practice and provides essential reading on any course that relates to cellular pathology, histology and histopathology.

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Cellular Pathology
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An Introduction to Techniques and Applications

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Infective agents and amyloid

Learning objectives

After studying this chapter you should confidently be able to:

- **Outline the limitations of histological identification of bacteria**
  The fixation and processing of tissues means that bacteria will be killed, so many important microbiological methods are not applicable.

- **List the types of organisms found in sections and name examples of each type of organism**
  Organisms may be bacteria, fungi, viruses or parasites.

- **Outline methods for the detection of micro-organisms in tissue sections**
  Only staining and immunotechniques can be used. Staining techniques are limited in scope and selectivity, making definitive identification difficult or impossible. Gram staining using methyl violet and iodine can subdivide the bacteria into Gram-positive and Gram-negative, whilst some methods such as Ziehl–Neelsen staining can identify single groups of organisms. Immunotechniques using antibodies raised against a single species of pathogen can be precise but are limited to confirmation of a diagnosis rather than identifying an unknown organism. Molecular biological techniques have similar advantages and limitations to immunotechniques and can also detect viral DNA in cell nuclei.

- **Describe the nature of prions and associated diseases**
  Prions are abnormally folded proteins which can act as catalysts to cause normal proteins to fold in the same abnormal manner. They can be infective and are classed as transmissible spongiform encephalopathies. Prions cause diseases such as Creutzfeldt–Jakob disease, scrapie and BSE.

- **Describe the nature of amyloid**
  Amyloid is a pathological protein formed by a normal protein being converted into a β-pleated sheet form. The altered protein is not easily degraded in the body and accumulates as amyloid deposits. The proteins altered in this way include immunoglobulins, peptide hormones, membrane glycoproteins and keratin.

- **Describe the major staining methods used to detect amyloid**
  These are Congo red staining combined with polarizing microscopy and staining with the fluorescent dye thioflavine. Other methods are much less useful, although IHC is an option.

Infection of tissue is a common finding in cellular pathology and the ability to recognize the causative agent can be invaluable in diagnosis. Identification from tissue sections is not the best method available and whenever possible identification should be done by microbiological techniques, which allow greater sensitivity and accuracy. Most microbiological identification relies on growing the organism and detecting its growth requirements, sensitivity to inhibitors and reactions to biochemical tests. Identification from histological sections suffers in comparison with these culturing methods since the
organisms are killed by fixation. Formaldehyde fixation in particular is a very effective method of killing infective organisms. All of the useful microbiological culture methods are lost once the tissue is fixed. Identification of the organism may still be possible but is less certain or more difficult, or both.

In infected tissues the causative organism may be relatively uncommon and unevenly spread. This will mean that in an individual section there may be only a few organisms. Unless the staining technique makes these pathogens stand out strongly, it is easy to miss them altogether. The use of fluorescent techniques (see Chapter 17) where the organism is the only material staining against a dark background can help to identify their localization in these cases.

Organisms can also be altered by processing and care may be needed. The simple staining techniques of microbiologists often need adapting to cope with formalin-fixed sections, e.g. Gram-positive bacteria may lose their positive reaction in sections and appear Gram-negative.

Finally there may be the possibility of harmless bacteria proliferating in the tissues unconnected with the disease (see Box 11.1).

**Box 11.1 // Commensals**

Accurate identification is often crucial since bacteria are common in many sites. Non-disease-causing organisms are called commensals and these are common and may even be beneficial. Innocuous commensals need to be eliminated as only the pathogenic organisms are of concern in disease. Rapid fixation of the tissues is needed since many organisms will continue to grow after death and in a few hours or days they can completely destroy the tissue making diagnosis impossible.

Commensal organisms can also mask pathogens by their sheer numbers. One slightly different pathogenic organism in a group of commensals may easily be missed. This can be a problem in post-mortem material where the growth of a harmless commensal may overwhelm the pathogenic organism.

**11.1 Identification of causative organisms**

Although simple histological staining may be limited, there is often other information available or other tests that can be tried if a diagnosis is needed. The attempt to diagnose an agent in fixed tissue can use the strategies detailed below. The following approach is particularly informative when investigating a tissue sample, say a skin biopsy, from an immunocompromised patient which may have several different and unusual infectious agents present.

- **Clinical diagnosis and symptoms** may be helpful. Often a clinical diagnosis may already have been made and all that is needed to identify the organism is to confirm the clinical assessment. The patient’s history may also be helpful with regard to recent travel abroad, ethnic origin, medication and immune status.

- **Appearance of the section.** The body may respond in a characteristic way to certain organisms. Langhans’ giant cells (see Figs 11.1 and 11.2) are very strongly linked to tuberculosis, even if the organism itself is not easily seen in the standard haematoxylin and eosin stain. The appearance of the tissue may not be specific but may limit the possibilities and thus simplify identification.
11.1 Identification of causative organisms

Figure 11.1
Giant cells. These are large cells, often more than 200 μm in diameter, with a large number of peripheral nuclei.

Figure 11.2
A multinucleated giant cell is shown in the centre of the image.

- **Special stains or histochemical tests.** The stains may be fairly general (e.g. Gram stain) and may simply identify whether the organism belongs to one particular group or they may be more specific indicating a limited range of organisms.

- **Immunofluorescent or immunochemical detection.** This method will identify any organism very selectively and can be absolutely specific. The only difficulty is that this can only be used as a confirmatory method since you need to know which antiserum to try. Once you have narrowed the options down to a very limited range of possible organisms, this is probably the best way to identify and confirm an individual organism. The use of immunotechniques is also a good way to spot organisms that are too small to be visualized with the light microscope (e.g. viruses).

- **Nucleic acid hybridization techniques.** In many ways, these resemble the antibody techniques in their applicability. They are excellent confirmatory methods provided you know what you are looking for. They do have the advantage over immunotechniques for virus detection in that they will detect the nucleic acid incorporated into the cell even when no virus particles are present. Some viruses can insert their own nucleic acid into the genome of the host cell resulting in a latent infection. Herpes simplex virus does this and can regularly reactivate, causing repeated bouts of cold sores. These latent infections can be quite important and the use of DNA hybridization allows the detection of the virus even when the infection is latent and not active. Kits are available that will distinguish between types of a particular virus, e.g. HPV 16 and 18 in colposcopic biopsies of cervix.
11.2 Types of micro-organism

Infective agents can be of a variety of types and these vary in their size and life history. Only the major types of organism are dealt with in this chapter although others exist and can be important. Mycoplasmas, for example, are considered to be sufficiently different from the classical bacteria to have their own classification and are important in causing up to 10% of community-acquired atypical pneumonia (caused by *Mycoplasma pneumoniae*).

The main groups of organisms are:

- **Viruses.** These are very small, obligate intracellular parasites. Individual virus particles can usually only be seen with the electron microscope.

- **Bacteria.** These are larger organisms with most being between 1 and 10 µm in size. Bacteria are visible with the light microscope and a few types can be stained selectively, although not usually identified positively, using simple staining methods. They are probably the most important group in histological identification although not necessarily the most important in terms of disease.

- **Fungi.** These are found in two forms: yeasts, which are single-celled forms, and hyphae, which are groups of cells forming threads. They are often larger than bacteria and vary from about 2 to 200 µm. They have a distinctively different cell wall to bacteria.

- **Protozoa.** Tropical diseases such as malaria and sleeping sickness are caused by protozoa. Although once uncommon in Britain, these diseases are becoming increasingly important as a result of immigration and international travel. Some previously minor diseases have also become important in immunosuppressed patients.

- **Helminthic infections.** These are caused by parasitic worms and are more common in countries where hygiene is poor. Helminths are usually quite large and can be recognized by their morphology alone.

**Box 11.2 // Smallpox**

Viral diseases are often more difficult to treat than many other forms of infection, so identification may only be of academic interest rather than clinically useful. Viruses can be prevented from causing disease by immunization. This was extremely effective in the case of smallpox, which has been completely eradicated by a World Health Organization programme that started in 1967 and ended in 1969 after 2 years with no reported cases. The disease was once prevalent in many countries with 15 million cases and up to 2 million deaths. The organism caused a distinctive inclusion body called a Guarnieri body in infected cells.

By contrast, there has been a rise in the incidence of human immunodeficiency virus infections but this virus is best seen in cultured cells and cannot be identified easily in tissue sections.

**Viral infections**

There are many viruses that cause disease (see Box 11.3) and so can occur in tissues. Recognition of viral infections is often possible on haematoxylin and eosin sections since they can have distinctive effects on the cells. Common signs are syncitial (grape-like) accumulations of nuclei following the fusion of cell membranes initiated by viral coat
proteins, and large nuclei with pale areas (halos) at their periphery. Although individual viruses are very small, they may occur as aggregates where the viruses clump together within the tissue forming **viral inclusion bodies**. The inclusion bodies are rich in both proteins and nucleic acids so they stain very strongly. These inclusion bodies can often be better seen with trichrome stains (e.g. Lendrum's phloxine tartrazine) where the differences in acidophilia and basophilia of the inclusion body can be exaggerated. Inclusion bodies often have individual names after the person who first described them. Inclusion bodies can be in the cytoplasm (e.g. Negri bodies in rabies) or in the nucleus (e.g. herpes virus) or in both (e.g. cytomegalovirus). In some cases, such as with the surface antigen of the hepatitis B virus (HBsAg, previously also called Australia antigen), there are more selective methods available. HBsAg can be demonstrated by using an orcein stain after permanganate oxidation, which converts sulphur-containing protein into sulphonate residues that then react with the orcein.

Electron microscopy is one of the most useful methods for detection of viruses as many viruses have distinctive shapes, although this will often only indicate the type of virus involved rather than the individual species. The use of antisera against viral proteins is probably currently the best method in widespread use but the use of *in situ* hybridization techniques may become more important as they can be even more specific and have the advantage of detecting viral DNA hidden inside the nuclei of cells. This is particularly useful because a range of viral infections can progress beyond the simple inflammatory and immune responses when there is a failure to fully clear the embedded viral genome from the DNA of its host. It is estimated that at least 20% of malignancies are associated with an earlier viral infection and many autoimmune reactions are also believed to be triggered by viral infections that generate an abnormal immune response.

The **rickettsiae** are intermediate between the viruses and bacteria in that they are more bacteria-like in structure but are obligatory intracellular parasites. Like viruses, they produce inclusion bodies in the cell rather than extracellular colonies and so their demonstration in tissues is more related to viruses than bacteria.

**Important virus types causing human diseases**

**Herpes viruses.** These are DNA viruses that are often acquired during childhood and then become latent when they integrate their DNA into the host’s DNA. They may re-erupt
into active disease in later life. All are similar in structure with a roughly spherical shape of about 120 nm and are covered with a lipid membrane. Individual diseases include varicella-zoster virus, which initially causes chickenpox but can re-emerge from latency in dorsal root ganglia as shingles; herpes simplex virus 1, which causes cold sores; herpes simplex virus 2, which causes genital herpes; Epstein–Barr virus, which causes glandular fever; and cytomegalovirus, which often occurs as an opportunistic infection in AIDS.

**Retroviruses.** These viruses contain RNA rather than DNA and include human immunodeficiency virus, which causes AIDS, and human T-cell leukaemia virus.

**Paramyxoviruses.** These are RNA viruses and include the viruses that cause measles and mumps.

**Rhinoviruses.** These are RNA viruses and include the common cold virus.

**Togaviruses.** These are RNA viruses and include rubella virus, which causes German measles.

**Rhabdoviruses.** These are RNA viruses and include rabies virus.

**Enteroviruses.** These are RNA viruses and include poliovirus. Although best known for its paralysing effects from infecting the anterior horn cells of the spinal cord, poliovirus is an enteric virus that first infects the gut; only in some cases does it get into the bloodstream and affect the nerve cells.

**Papillomaviruses.** These are DNA viruses and include human papilloma viruses, which cause warts. Some types have also been linked to cervical cancer.

**Bacterial infections**

The principal method used to demonstrate bacteria is the Gram stain or one of its modifications. The Gram stain detects a difference in the cells of the two types of bacteria although there are several possible explanations for the differential staining. The permeability of the cell wall differs (Gram-positive cells have thicker walls); this is the simplest reason and explains the staining well. The dye (usually methyl violet or crystal violet) penetrates the cell wall and is then aggregated by adding iodine. The large dye aggregates are still easily removed from the more permeable Gram-negative bacteria by a decolorizing agent such as alcohol or acetone but are retained by the more impermeable Gram-positive bacteria (see Fig. 11.3). The difference is only in the rate of removal and prolonged washing in acetone will decolorize all bacteria. The Gram-positive nature also alters with fixation and processing. Usually another modified Gram stain is used rather than the simple technique but the method is similar.

**Some bacteria can be stained more selectively**

Mycobacteria can be stained using the Ziehl–Neelsen (ZN) stain for acid-fast bacilli. The original method used hot carbol fuchsin to stain the mycobacteria followed by differentiation in acid/alcohol. The presence of a hydrophobic material (mycolic acid) in the wall of these organisms is the usual explanation for their retention of the dye. The ability of mycobacteria to resist decolorization is called acid fastness and the organisms can be referred to as AFBs (acid-fast bacilli) or AAFBs (alcohol- and acid-fast bacilli). The acid fastness varies with the species. *Mycobacterium tuberculosis* (which causes tuberculosis) is more robust than *Mycobacterium leprae* (which causes leprosy) and will show stronger acid fastness. The ZN technique is often modified to retain the acid fastness
by avoiding strong hydrophobic solvents that would extract the mycolic acid. It has also become popular to use variations of the staining technique that do not involve the use of hot carbol fuchsin and are often referred to as a ‘cold ZN’. The best known of these is the Kinyoun’s modification which uses a 4× strength carbol fuchsin solution.

The demonstration of AFB can also be performed using the fluorescent dyes auramine O and rhodamine. This makes it easier to spot isolated bacteria, which fluoresce bright yellow against a dark background (see Fig. 11.4).

Selective methods have been published for Helicobacter pylori (which can occur in the stomach and is linked to ulceration, gastritis and stomach cancer) and the use of silver staining for spirochetes. Details of these methods are available in many books on histological techniques. Antibodies specific for H. pylori are available commercially and are preferred by some workers when the number of organisms are small and may be difficult to spot using, for instance, a Giemsa stain.

Important organisms seen in tissue sections

Gram-positive organisms. Staphylococcus aureus is a common pathogen and can cause boils, infections in wounds, abscesses and septicaemia. It is a great problem in hospitals where some strains are becoming resistant to several antibiotics including methicillin (methicillin-resistant Staphylococcus aureus or MRSA). The clumps of Gram-positive cocci are fairly distinctive. Streptococcus species are also Gram-positive but form chains rather than clumps and include Streptococcus pneumoniae.

Box 11.4 // Bacterial morphology

The shape of bacteria and their method of associating together can also be useful indicators of the type of organism present. Cocci are rounded, whilst bacilli are rod-shaped. These two forms of bacteria are the commonest. Other shapes such as comma-shaped (vibrio) and spiral forms are less common, so if seen they can be more informative. If the organisms form chains they can be called streptococci (or streptobacilli), whilst clustering indicates staphylococci.

The shape needs to be examined carefully since sections will often slice through the organism so that rods can appear as cocci. If there are many organisms present, then some will lie entirely within the section and the shape and grouping will be identifiable, but if there are only a few bacteria in a section, it becomes more difficult.
Chapter 11  Infective agents and amyloid

*Lactobacillus acidophilus* is a commensal found in the vagina (‘Döderlein’s bacillus’). *Corynebacterium vaginale* may cause cervicitis and is fairly common in cervical tissues (5–7% of women). *Corynebacterium diphtheriae* was once a common disease causing a severe form of laryngitis but effective vaccination has made it a rare disease in modern Britain.

Clostridia species are associated with a variety of diseases including gas gangrene (*Clostridium perfringens*), botulism (*Clostridium botulinum*), tetanus (*Clostridium tetani*) and pseudomembranous colitis (*Clostridium difficile*).

Mycobacteria are also Gram-positive but only weakly so. Although leprosy and tuberculosis are the main mycobacterial diseases in humans, other mycobacterial species may become opportunistic infections in AIDS patients.

**Gram-negative organisms.** The Gram-negative ‘gonococcus’ *Neisseria gonorrhoeae* is the causative organism in gonorrhoea. The organisms are difficult to find in sections of infected tissues although easier to find in smears. *Legionella pneumophila* is a small coccobacillus that causes the pneumonia associated with droplets of water from air-conditioning units. It has a high mortality and is also known as legionnaires’ disease. This is again difficult to see in sections. It is easier to stain with silver techniques than with dyes.

The spirochetes are an unusual group of long, slender, spiral, rod-shaped organisms. They cause the diseases syphilis (*Treponema pallidum*) and Weil’s disease (*Leptospira interrogans*). The slender rods are difficult to see in sections and need to be stained with a silver technique to see them easily. Suitable silver techniques include the Dieterle’s, Warthin–Starry, and Steiner and Steiner methods.

**Fungal infections**

Fungi are extremely common organisms in the environment but only a few are pathogenic in humans (see Box 11.5). The commonest site of infection is on a surface such as skin or in the mouth. The general term for fungal diseases is mycoses. Superficial mycoses...
such as athlete’s foot and ringworm are usually fairly mild diseases but systemic mycoses, where the disease is more widespread inside the body, can be dangerous.

Fungi can occur as a yeast or hyphal form. The hyphae are often distinctive, with features such as the presence or absence of cross-walls (septa) and the degree of branching being useful indicators. Yeast forms are less easy to differentiate on morphological criteria.

Fungi can be stained and demonstrated by the periodic acid–Schiff (PAS) technique, which detects their polysaccharide cell walls. The use of chromic acid as an oxidizing agent (Gridley’s technique) or methanamine silver (Grocott’s technique) are essentially similar to the PAS method but are still used instead of a simple PAS.

**Box 11.5 // Fungal infections**

Fungal infections were once either comparatively mild and usually superficial diseases (e.g. athlete’s foot and thrush) or if they were life-threatening they were unusual and often limited to certain occupations. The increase in immunosuppression and immunodeficiency has changed the situation and several once minor or unusual diseases have become common causes of death. In a normal immunocompetent person, these infections would be countered effectively by the immune system and either eliminated or kept in check as a low-level infection. The loss of immune capability in AIDS demonstrates the importance of the immune system since these diseases have now become killers in AIDS patients. AIDS patients are said to be compromised by the immunosuppression; they are much more susceptible to infection and often succumb to minor diseases. These infections are referred to as **opportunistic infections** since they seize the opportunity of growing and spreading in the compromised host.

**Important fungi seen in tissue sections**

*Aspergillus fumigatus* is a very common organism in soil and can be a commensal in the upper respiratory tract. If it invades the lung, as happens in some AIDS patients (see Box 11.5), it may cause pneumonia.

*Candida albicans* is found in small numbers as a common commensal on wet mucous membranes but can become pathogenic following antibiotic therapy that kills the normal suppressive bacterial flora. It then causes the condition known as **thrush**. In immunosuppressed patients, it can also become systemic.

*Cryptococcus neoformans* and *Histoplasma capsulatum* can both be acquired from bird droppings and cause systemic disease but are only significant in immunosuppressed patients.

*Pneumocystis carinii* again is an organism that has only become important in immunosuppressed patients and is a major cause of death in AIDS patients where it can cause pneumocystis pneumonia or PCP. Originally classified as a protozoan because of an amoeboid trophozoite stage, and also found in rats, this organism has since been reclassified. It is now described as a yeast-like fungus and only *Pneumocystis jirovecii* preferentially colonises human lung, with *P. carinii* colonizing the rat lung. PCP pneumonia and identification of the causative agent is one of four key signs of an HIV +ve status.

**Parasitic infections**

Parasites are large enough and distinctive enough for most diagnoses to be made on the shape and structure of the organism and the nature of the host’s response. A haematoxylin and eosin stain may be supplemented with the Romanowsky method (such as Giemsa) or
stronger haematoxylins (iron or phosphotungstic acid haematoxylin) or even using PAS to show glycogen, but there are no staining techniques specific for the organisms.

*Entamoeba histolytica* causes amoebic dysentery. The organism is up to 50 μm in diameter and may contain the remains of ingested blood cells.

*Toxoplasma gondii* is common in cat faeces and can cause an acute inflammatory condition in lymph nodes (lymphadenopathy) but the infection is often unnoticed (a subclinical infection). In immunosuppressed patients, it can be more dangerous causing brain infections and inflammation of the meninges.

*Plasmodium* species cause the various forms of malaria. Malaria is common in tropical countries and is regularly seen in immigrants and travellers from these countries. The parasites are usually diagnosed from blood films but they can also be seen in tissues (see Fig. 11.5). The associated malarial pigment is similar to formalin pigment and can be removed by alcoholic picric acid. Removal of the pigment allows the malarial parasites to be seen more easily.

*Leishmania donovani* causes the systemic disease kala-azar, whilst *Leishmania tropica* causes the skin disease 'oriental sore'. The infection is spread by the bite of the sandfly and the organisms can be seen in infected areas in the cytoplasm of enlarged macrophages.

*Trichomonas vaginalis* is found in the urinogenital tract of both sexes. It is commonly seen in smears but less often in sections.

*Schistosomiasis mansoni* is found in the lower digestive tract, has a characteristic spike and a ZN positive wall. *Schistosomiasis haematobium* is found in the urinary tract and is ZN negative with no spike.

*Trypanosome* species is found in Africa where it is transmitted by bites from infected Tsetse flies and causes sleeping sickness. It is also endemic in South America where it is spread by reduvid bugs causing Chagas’ disease. It is found in the blood and can be seen in blood films and in tissues such as brain (see Fig. 11.6).

Larger parasites include tapeworms and nematode worms living in the gut lumen (see Fig. 11.7).
Prion diseases

Prion diseases represent a comparatively recently recognised pathology. The name was coined by Stanley Prusiner in the early 1980s from the words *protein* and *infection* to describe the causative agent for a group of transmissible diseases causing brain degeneration, with the brain tissue becoming spongy (transmissible spongiform encephalopathy, TSE). The sheep disease scrapie has been recognised for a long time but its mode of transmission and the nature of the transmissible agent were unknown. No bacteria, fungi or virus could reliably be demonstrated in all cases of the disease. The agent could be transmitted by injection of infected brain tissue, but even when the infected brain tissue was ‘sterilised’ by the usual biocidal agents (disinfectants, formaldehyde, boiling, autoclaving or UV irradiation) the tissue retained its infectivity. This suggested that no known type of infective agent (bacteria, fungi or virus) was involved because none of these could resist all of these agents.
It was suggested in the 1960s by Alper and Griffith that the particle consisted exclusively of a novel self-replicating protein. This was a radical suggestion because nucleic acids were believed to be essential in any biological replicative activity such as infection and so it was strongly opposed at first, with suggestions of ‘virino’ and ‘atypical slow viruses’ being involved, but the ‘protein only’ concept is now widely accepted. In the 1980s Prusiner isolated and purified the infectious protein with no trace of nucleic acid and so confirmed the hypothesis.

It is now believed that the mechanism involves a change in the folding pattern of particular proteins that results in the degeneration and transmissibility. The protein involved has been called PrP (from prion protein) but it is a natural cell component with the gene (the PRNP gene) being found on chromosome 20. The cellular form (PrP<sup>c</sup>) is a membrane protein and is probably a receptor. The protein from scrapie (PrP<sup>Sc</sup>) differs from PrP<sup>c</sup> in having a higher content of beta-pleated sheets and less alpha-helix which represents a change in protein conformation rather than a mutation or change in amino acid sequence. The scrapie protein seems to be able to bind to the normal PrP<sup>c</sup> protein and act as a catalytic template to reconfigure the PrP<sup>c</sup> folding to form more PrP<sup>Sc</sup> which can detach and then both the original and new PrP<sup>Sc</sup> molecules can recruit and convert more normal protein in a chain reaction. The scrapie protein is resistant to the usual proteases and so accumulates and can form a type of amyloid deposit.

There are many forms of TSE in both animals and humans. Human forms of TSE caused by prions are Creutzfeldt–Jakob disease (CJD), fatal insomnia, Kuru, and Gerstmann–Sträussler–Schenker disease. Animal forms include scrapie and bovine spongiform encephalopathy (BSE).

CJD is the most important human form (see Fig. 11.8) and has several subgroups.

* It can be sporadic with no obvious predisposing cause for the disease.
* It can be familial, where the PrP<sup>c</sup> has a mutation that predisposes to the pathological refolding.
* It can be iatrogenic where the prion is transmitted by treatment (from infected instruments, tissues such as corneal transplants and blood transfusions, pituitary hormones).
* Variant CJD (vCJD) is related to the bovine form of scrapie, BSE (also known as ‘mad cow disease’).

![Figure 11.8](https://example.com/figure11.8.png)

*Figure 11.8*
Spongiform encephalopathy. This is brain tissue from a case of the prion disease, CJD.
Amyloid is an abnormal protein that accumulates between cells in many organs and can be associated with different pathological conditions. Fresh amyloid has a waxy appearance and absorbs acid dyes although only very weakly. This name is inaccurate as there is no chemical resemblance to starch and it is mainly protein (see Box 11.7). The cut surface of affected organs when tested at autopsy will, however, give a positive reaction to the iodine starch test.

**Composition and structure of amyloid**

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water and salts</td>
<td>75</td>
</tr>
<tr>
<td>Protein (including plasma protein)</td>
<td>25</td>
</tr>
<tr>
<td>Polysaccharides (mainly mucins)</td>
<td>1–2</td>
</tr>
<tr>
<td>Lipid</td>
<td>0–3</td>
</tr>
</tbody>
</table>

*Amyloid protein is a β-pleated sheet*

Despite always having a very similar appearance and sharing staining properties, amyloid protein is very variable in its amino acid composition. All of the different forms of the protein have a β-pleated sheet structure and this seems to be the common link between these different materials. Although amyloid has little structure when viewed with the light microscope (i.e. it is amorphous), it does form fibrils that are visible with the electron microscope.

**Box 11.6 // Prions and safety**

In the histological laboratory specimens believed to be infected with prions need a great many extra precautions because the causative prion is not inactivated by routine formalin or glutaraldehyde fixation, as is the case with the usual bacterial, viral and fungal infections. Routine formalin fixed TSE material remains infective for long periods (possibly indefinitely). The prions can be inactivated by initial formalin fixation followed by treatment with formic acid (95%) and then given a further formalin fixation. However, preparation of infected material is probably best left to specialist centres rather than being handled in routine histology laboratories.

There are no reports of laboratory acquired infections.

**Box 11.7 // Origin of the term amyloid**

The name amyloid is misleading since it suggests a starch-like material. The eminent pathologist Rudolf Virchow found that amyloid reacted with iodine/sulphuric acid and produced a blue colour and since this is reminiscent of the blue colour given by starch when treated with iodine he introduced the name *amyloid* (*amylo* = starch).

The later finding that it is in fact protein has led to people suggesting other names but in an attempt to be more descriptive these names have often been clumsy. One suggestion was *idiopathic fibrillar glycoprotein*. This name is certainly more accurate than amyloid but such names are unlikely to replace the succinct, if inaccurate, amyloid, which is much easier to say and remember.
microscope. These fibrils are 7.5–10 nm in diameter but are variable in length and are striated at 10 nm intervals. The β-pleated sheet structure is unusual in human proteins (although it is found in silk). This structure is very resistant to enzyme degradation and this probably accounts for its accumulation and deposition in tissues since the enzymes cannot remove the deposits quickly enough.

The different amyloid proteins are related to normal proteins
There are several types of amyloid that have been identified but not all of them are considered to be pathological. ASc-type amyloid is common in old people but does not seem to be linked directly to any disease. Only two of the pathological types are common (AA and AL) and the others are included for completeness. The two common pathological amyloid types occur mainly as a secondary effect to other diseases and amyloidosis may be the first clinical finding that indicates an underlying disease. The different types of amyloid have amino acid sequences that are similar or identical to normal body proteins and each amyloid type has a set of diseases or conditions with which it is often associated. Table 11.1 lists several different types of amyloid.

Amyloid P is associated with all amyloids
In addition to the main fibrillar amyloid, there is a minor protein component that is found in all amyloid deposits except for those in the brain. This component is called amyloid P. It is derived from blood proteins (serum amyloid P, SAP) and is an acute-phase reactive protein. The normal blood form of the protein (SAP) appears to bind to

<table>
<thead>
<tr>
<th>Amyloid type</th>
<th>Normal protein the amyloid resembles</th>
<th>Diseases with which the amyloid is associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Immunoglobulin light chain</td>
<td>Multiple myeloma, Waldenström’s disease</td>
</tr>
<tr>
<td>ASc</td>
<td>Transthyretin (pre-albumin)</td>
<td></td>
</tr>
<tr>
<td>ASc1</td>
<td>Atrial naturetic peptide</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>Serum amyloid A (acute phase reactant, apolipoprotein of high-density lipoprotein)</td>
<td>Rheumatoid arthritis, tuberculosis, Hodgkin’s disease, familial Mediterranean fever</td>
</tr>
<tr>
<td>AE</td>
<td>Peptide hormones (calcitonin, insulin)</td>
<td>Insulinomas, medullary carcinoma of the thyroid</td>
</tr>
<tr>
<td>β-Amyloid</td>
<td>Membrane glycoprotein</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>AS</td>
<td>Transthyretin (pre-albumin)</td>
<td>Old age (80+)</td>
</tr>
<tr>
<td>AF</td>
<td>Transthyretin (pre-albumin)</td>
<td>Familial amyloidosis</td>
</tr>
<tr>
<td>AD</td>
<td>Keratin</td>
<td>Lichen amyloidosis</td>
</tr>
<tr>
<td>Haemodialysis-associated amyloid</td>
<td>β2 microglobulin</td>
<td>Renal failure with haemodialysis</td>
</tr>
<tr>
<td>AScr</td>
<td>Scrapie protein</td>
<td>Transmissible spongiform encephalopathies (CJD, GSS, etc.)</td>
</tr>
</tbody>
</table>
Amyloid deposits, hence its accumulation, but instead of forming fibrils it forms a 9 nm ring structure made up of five subunits of SAP with a central hole of about 4 nm. Amyloid P is immunogenic and has been used to make amyloid-specific antibodies which have some use for demonstrating the ß-amyloid accumulations in the senile plaques seen with Alzheimer’s disease.

Distribution of amyloid

Amyloid distribution varies with the disease

Amyloid can occur in many organs and the exact distribution varies with the associated disease. Three types of distribution have been identified:

1. **Systemic primary.** Amyloid is mainly found in the heart, gastrointestinal tract, tongue, skin and nerves. This is seen in cases of primary amyloidosis and neoplasms of B lymphocytes. The amyloid type is usually AL but this primary distribution pattern can occur with AA amyloid in rheumatoid arthritis.

2. **Systemic secondary.** In this case the amyloid is found in the liver, spleen, kidney, adrenals, gastrointestinal tract and skin. This distribution is associated with cases of chronic inflammatory diseases. The amyloid type that is found is usually the AA variety.

3. **Localized.** Localized tumour-like nodules occur in tongue, bladder, skin or lung and are associated with localized endocrine neoplasms. The ß-amyloid in Alzheimer’s disease is also localized but occurs as plaques rather than nodules.

Amyloid can be found in many organs but the frequency with which a particular organ is involved varies and there is no single site that is always affected. Taking a single biopsy sample is therefore never fully reliable as the organ may not be affected in that patient. Common sites include the liver (48%), kidney (87.5%), gingiva (19%) and rectum (75%). Rectal biopsies have become a popular method of investigating possible cases of amyloidosis because of their less-invasive nature (often an endoscopic biopsy) and frequent involvement.

Amyloid accumulates between cells and causes their death

The first accumulation of amyloid occurs between the cells and often close to the basement membranes of a blood vessel. As the deposit increases, it gradually infiltrates into tissues and can trap and destroy the cells. There is still doubt about the mechanism of cell death. It may be due to simple mechanical ‘strangling’ by cutting off the cells from their blood supply or it may involve a toxic effect of the amyloid. Amyloid has been found to be toxic to nerve cells in cultures but whether it is also toxic *in vivo* is not known.

Effects on organs

**Kidney.** Amyloid is first deposited in the glomerular mesangium and the basement membrane of blood vessels. As the accumulation continues, it eventually obliterates the capillary lumens and destroys the glomerular cells. The amyloid deposit can eventually completely replace the glomerulus. Renal arterioles can also be affected leading to ischaemia and tubular atrophy. The destruction of the renal tissue produces proteinuria and eventually the nephrotic syndrome. Renal involvement is usually fatal (see Box 11.8).

**Spleen.** The spleen becomes enlarged (splenomegaly) and the deposits may be of two types: (i) generalized deposits in both the red and white pulp (‘lardaceous spleen’), and
(ii) deposits only in the white pulp (‘sago spleen’). If only the spleen is infiltrated and other organs are not affected, it is not usually fatal.

Heart. Usually deposits in the heart are smaller than in other organs. The deposits occur in the subendocardium, with more generalized deposits in the myocardium. The deposits may cause pressure atrophy of the heart cells and cardiac amyloid often leads to cardiac arrhythmia particularly if localized within the SA or AV nodes or conducting pathways between.

Liver. The amyloid deposits cause massive enlargement (hepatomegaly) and the liver becomes pale and waxy. Amyloid appears first in the space of Disse and progressively squeezes the adjacent hepatocytes to death. If only the liver is involved, then it is rarely the cause of death.

Detection of amyloid
Targeted sampling is important when attempting to demonstrate the systemic accumulation of amyloid. It is common to take a small rectal biopsy in the first instance, rather than attempting to sample a major organ. It is often easier to demonstrate amyloid deposits in the walls of small blood vessels in the sub-mucosa. Skin and gingival biopsies are easily accessible and so are also favoured for the same reason.

Staining
Since amyloid deposits vary in their composition, they will not all stain in the same way. A technique that works well with one sample of amyloid may stain a different

Box 11.8 // Treatment of patients with amyloid
Amyloid deposition has no direct cure but effective treatment of any underlying disease will often slow down or even stop the deposition. Identification of the type of amyloid can therefore help by indicating which underlying cause is implicated and so suggest possible therapeutic measures. Amyloid diagnosis before death has become much more common than in the past due to better biopsy techniques and a greater awareness of the deposition of amyloid. Most patients are now detected in the early stages rather than late in the disease. Whilst this is often beneficial to the patient, it has made life more difficult for the laboratories. The large deposits in organs that are easy to find and easy to stain are being replaced with small biopsies containing less amyloid, which is more difficult to detect.

Box 11.9 // Origin of deposits
The deposition of amyloid in the tissues probably reflects a generalized problem with the destruction and removal of large amounts of unusual proteins. The wide range of proteins involved and the relationship with excess protein secretion suggests that during the degradation of the extracellular protein, the protein alters its conformation and forms the β-pleated sheet form. The β-pleated sheet form is then more difficult for enzymes to destroy. If other proteins can similarly change their form during degradation, then it is likely that other forms of amyloid will continue to be discovered.
case of amyloidosis only very weakly or not at all. A negative staining reaction should be considered inconclusive rather than definitely negative. If an amyloid deposit fails to stain with one technique, then it may be worthwhile trying a different one, which may give a much better result. It is also believed that the reactivity of amyloid decreases with the length of time between cutting and staining a section which is another source of variability.

There are a variety of methods that have been used to demonstrate amyloid and some of these are outlined below.

**Iodine/sulphuric acid**
This method is mainly of historical interest although some pathologists still use it as a macroscopic method on whole organs whilst performing a post mortem. The mechanism is still unclear, with the original idea of a carbohydrate in the amyloid acting in a similar way to starch being largely replaced by the concept that it is the β-pleated sheet arrangement that traps the iodine.

**Methyl violet metachromasia**
Methyl violet has been used for a long time but how it produces metachromasia is not apparent. Amyloid deposits often contain mucinous materials that might explain the metachromasia but methyl violet does not produce metachromasia with any carbohydrates or mucins. Amyloid is not metachromatic with other dyes such as toluidine blue that give strong metachromasia with mucins. The results with methyl violet are inconsistent and often difficult to observe. This has led to the suggestion that the methyl violet contains an impurity that is simply more selective for amyloid. This would be analogous to the trichrome methods where differences in staining are relative rather than absolutely selective. It is no longer as popular a method as it once was.

**Congo red**
This is the preferred method in most laboratories. Most amyloids react and give some staining. The red colour (see Fig. 11.9) may be weak and some other materials may occasionally stain slightly, but it is still probably the best of the current methods. It is even more specific if the section is examined using crossed polarizers in a polarizing microscope (see Chapter 17). The amyloid fibrils will show up as a dichroic green/yellow colour (see Fig. 11.10). The Congo red attaches using hydrogen bonding and inserts between the fibrils in the β-pleated structure and this results in a regular lattice or crystalline-like arrangement that gives rise to the birefringence. Chitin and cellulose show a similar birefringence but these look quite different to amyloid in sections so there should be no confusion. The section thickness and orientation can be crucial to this colour – too thin and the colour is red, too thick and it becomes yellowish. The fibres need to be oriented correctly relative to the plane of polarization of the light to see the full effect so only a proportion of the amyloid will be seen at any one time (see Fig. 11.10). The remaining amyloid deposits can be seen by rotating the section. A Congo red technique from 1962 uses an alcoholic solution, high salt concentration and high pH to minimize ionic staining and so enhance hydrogen bond staining; this does not require differentiation and is better than simple congo red staining (e.g. Highman's) and is recommended by UK NEQAS. Congo red is also fluorescent so the extent of the amyloid can be seen using this property. (Sirius red is an alternative to Congo red for staining amyloid but is not fluorescent.)
Pre-treatment of the section with potassium permanganate destroys the congophilia of AA amyloid but AL amyloid is resistant so this property can be used to help to distinguish the two types.

**Thioflavine**
This is possibly more sensitive than Congo red because of its intense fluorescence but it can be non-specific. It still remains popular as an alternative or complementary method to Congo red and is excellent for detecting very small deposits that are easily missed with Congo red staining.

**Immunohistochemistry**
Because amyloid is proteinaceous it is possible to raise antibodies against it for use in immunological techniques. A sensitive IHC detecting most types of amyloid utilizes antibodies to Amyloid P protein and IHC for the individual proteins found in amyloid can indicate the type of amyloid.
Proteomics
Proteomics is very sensitive and uses mass spectrometry to analyse protein fragments from amyloid. Congo red-stained amyloid is first isolated from sections by laser micro-dissection and then digested to generate a mixture of protein fragments that can be separated by HPLC and then analysed by mass spectrometry. This can identify the type of proteins present in the amyloid and is also capable of recognising if a new amyloidogenic protein is involved. This is a highly specialised and expensive technique and is likely to be restricted to a few specialist research centres.

Other methods
Other methods include immunotechniques, staining for tryptophan and X-ray crystallography but these tend to be limited to research into amyloid rather than reliable and easy identification techniques.

Infectivity of amyloidosis
The role of prions in the transmissible spongiform encephalopathies that produce amyloid deposits in the brain is widely accepted, but the infectivity or transmissibility (see Box 11.10) of other forms of amyloid is not so well documented or investigated. There is no significant evidence of amyloidosis being transmitted in humans, but in animals some reports of transmissibility have been published. These suggest that injection of amyloid fibrils can accelerate the development of amyloidosis which in turn suggests a catalytic action increasing abnormal protein folding analogous to that of prions in TSEs. Mice are the main model for studying amyloidosis, but whether the results found in mice are relevant to humans has not yet been established.

Box 11.10 // 'Catching' a disease
The possibility of a disease passing from one person to another has generated a lot of descriptive words which are often confusing because their precise meaning is unclear. This lack of clarity applies not only to the general public but even to the medical literature. The words include infective and infectious. In the medical literature these generally mean that an outside agent invades the body and replicates causing the disease. This would include bacterial, fungal, viral, parasitic and prion diseases. The bacteria, virus, etc. would be called infective whilst the disease in an individual would be an infection, and the general term infectious disease would be used for all occurrences of the disease. Infections can spread from one infected individual to another by direct contact, inhalation of infected droplets, ingestion of contaminated foodstuff and a variety of indirect routes.

The term contagious and contagion can be synonymous with infectious, but they have been used to specify that the disease is spread by direct physical contact. Communicable disease is used to cover diseases that can be passed directly from one person to another by any mechanism.

Transmissible is a more widespread description of diseases that can be acquired directly from another person but also diseases that can only be acquired by injection into the body and cannot be simply passed to an uninfected person without the intervention of an infecting (or injecting) vector. This would include diseases such as malaria and Lyme disease in which the route of infection is from an insect bite (i.e. injection) and direct human to human transfer does not occur.
Suggested further reading


Self-assessment questions

1. Explain why it is better to identify bacterial infections from microbiological specimens rather than using paraffin wax sections.
2. Outline the types of method that can be used to help identify microorganisms in tissue sections.
3. What are the major types of infective organism that can occur in sections? Name one example in each group.
4. What is amyloid? Why is amyloid not destroyed in the body?
5. List the different types of amyloid that are usually recognized and give an example of an associated disease or condition for each.