chapter 3:

INFECTIOUS DISEASES AND TREATMENTS

OBJECTIVES

After studying this chapter you should be able to:

- describe the types and symptoms of some of the more common infectious diseases;
- outline preventative measures for infectious diseases;
- list the general effects of antibiotics on infectious organisms;
- discuss some general aspects of the management and treatment of specific infectious diseases.

3.1 INTRODUCTION

The presence of virulence factors, described in *Chapter 2*, allows pathogenic microorganisms to infect specific body systems and cause a vast range of diseases. A small number of such organisms are also able to cause **systemic** disease, that is one affecting the whole body. A pathogen must be transmitted from a source to the patient. Direct contact between hosts is the most obvious form of transmission but coughs and sneezes (aerosols), food, water and arthropod vectors are all used by various pathogens. The long-term survival of pathogenic microorganisms also depends on them maintaining their infectivity during transmission from host to host. The sources of pathogens can be abiotic, soil, water for example, or animals or other humans. Diseases that infect animals and humans are called **zoonoses**, while humans who harbor a pathogen but are symptomless are called **carriers**.

In a text of this length, it is simply not possible to describe all diseases caused by microorganisms. This chapter will concentrate on selected, representative examples of infections of the major body systems and those microorganisms that can produce a generalized infection. In addition, the ways in which infectious diseases are prevented, investigated and treated will be outlined.

3.2 INFECTIONS OF THE SKIN

The skin is a major element of the innate immune defense (*Chapter 4*). It is normally colonized by a variety of microorganisms although the numbers and types vary between different areas of the body. In normal circumstances it forms an effective barrier to invading microorganisms.

Papillomaviruses can infect epidermal cells and stimulate their proliferation to form warts. Numerous herpes viruses have been described and at least eight of them, the **Human Herpes Viruses** (**HHV1-8**), infect humans and can cause clinical disease. Largely due to historical reasons, they are also known by other names (*Table 3.1*). For example, *Herpes simplex* viruses 1 and 2 may infect skin of the genitalia causing Herpes labialis and Herpes genitalis respectively and *Varicella zoster (Figure 2.5*) causes chickenpox and shingles.

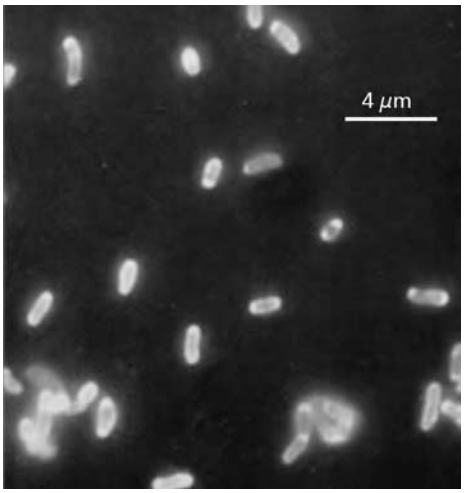


Figure 3.1 ***Propionibacterium acnes*, a bacterium associated with skin infections such as acne.** Courtesy of Dr S. Patrick, Queen's University Belfast, UK.

Figure 3.2 Electron micrograph of *Staphylococcus aureus*. Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

Human herpes viruses	Historical name
HHV1	*Herpes simplex* virus 1 (HSV1)
HHV2	*Herpes simplex* virus 2 (HSV2)
HHV3	*Varicella zoster* virus (VZV)
HHV4	Epstein Barr virus (EBV)
HHV5	Cytomegalovirus (CMV)
HHV6	B-lymphotropic virus (B-LV)
HHV7	T-lymphotropic virus (T-LV)
HHV8	Kaposi's sarcoma virus (KSV)

Table 3.1 Nomenclatures of the Herpes viruses 1–8

Bacterial skin infections normally occur only if the normal balance between the skin environment and these organisms is disturbed. The outbreaks of acne caused by *Propionibacterium* species (*Figure 3.1*) during the hormonal changes associated with puberty are typical. Breaks in the skin from wounds or surgery or lesions from insect bites or chickenpox may also lead to infections of the skin by *Staphylococcus aureus* (*Figure 3.2*) and *Streptococcus pyogenes*. In children especially, they may cause impetigo contagiosa, an extremely contagious skin infection. *Staphylococcus aureus* can colonize hair follicles leading to inflammations that can develop into abscesses (boils) or even, in extreme cases, **carbuncles**: an amalgam of several abscesses. Enterobacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes,* are all associated with a variety of skin problems following weakening of the patient, for example, by diabetes (*Chapter 7*), by a deficiency of the immune system (*Chapter 5*), by **nosocomial**, or hospital acquired, infection of surgical wounds or burning of the skin. *Mycobacterium leprae* is the causative organism of the dreaded disease leprosy, which, although now virtually eliminated in developed countries, still affects many thousands in the developing world.

Fungi are associated with a variety of infections, particularly of the skin (*Chapter 2* and *Figure 2.11*).

3.3 INFECTIONS OF THE EYES, EARS AND CENTRAL NERVOUS SYSTEM

Eyelid infections generally involve the lid margins, eyelid glands or follicles causing styes or **hordeolums**. They are usually associated with *Staphylococcus*

aureus infections. The conjunctiva is particularly susceptible to infection. The epithelial surface enclosed by the eyelids is a warm moist enclosed environment in which microorganisms can rapidly become established. However, microorganisms must avoid being rinsed away by tears and some pathogens, such as *Chlamydia trachomatis* (*Figure 3.3*), attach specifically to conjunctival cells. An estimated 500 million people are infected with different serotypes of *Chlamydia trachomatis*, making trachoma the most significant eye infection worldwide. The disease blinds approximately 1% of infected individuals while many others suffer visual impairment. *Chlamydia trachomatis* is transmitted by contact with contaminated flies, fingers and towels although trachoma itself results from chronic repeated infections. This is much more likely to occur in regions where restricted access to water prevents regular washing of the hands and face. Chlamydia is also a sexually transmitted disease (*Section 3.6*) and there is evidence that untreated chlamydial infections can lead to premature delivery and babies born to infected mothers can be infected in their eyes and respiratory tracts. Chlamydia is a leading cause of early infant pneumonia and conjunctivitis (pink eye) in the newborn.

A laboratory diagnosis of trachoma can be carried out using samples of conjunctival fluid or scrapings. The usual treatment is with oral or topical antibiotics, such as tetracycline or doxycycline (*Section 3.11*). *Chlamydia* infections account for only a fifth of cases of conjunctivitis; others are caused by bacteria such as *Streptococcus pneumoniae* and *Leptospira spp*.

Serious infections of the inner eye with *Pseudomonas aeruginosa* may follow trauma or after invasion by the protozoan *Toxoplasma gondii* causing chorioretinitis and possible blindness. This widespread protozoan is not a serious threat unless acquired *in utero* or when an individual is immunosuppressed, perhaps as a result of taking drugs to prevent transplant rejection (*Chapter 6*). Infection occurs by swallowing oocysts released by infected cats or by eating meat containing tissue cysts.

The eyes may also be infected by parasitic worms, for example, larval forms of the tapeworm *Echinococcus granulosus* that is transmitted by eggs passed from infected dogs. Infection by the larvae of the nematode *Toxocara canis*, which occurs naturally in the intestine of dogs, is, however, more common. An infection can lead to the detachment of the retina. River blindness in Africa and Central America is caused by the helminth *Onchocerca volvulus*. Simulium flies carrying larvae obtained from the skin of infected hosts transmit the infection. These flies develop in rivers, hence the name of the disease. River blindness is a serious infection with over 300 000 people infected worldwide. The rates of blindness, which is irreversible, may reach 40%. The usual treatment is with anthelminthic drugs.

Infections of the outer ear may cause pain or irritation and are resistant to some antibiotics. The middle ear can be colonized by viruses and bacteria from the upper respiratory tract causing acute otitis media with swelling and blockage of the Eustachian tube and may lead to deafness, though this is generally temporary. Microorganisms that cause middle ear infections include the virus that causes mumps. This may be followed by secondary infections with *Streptococcus pneumoniae* and *Haemophilus influenzae*. This is very common in children. Indeed, otitis media is the most frequent illness diagnosed in young children. The general symptoms, apart from a devastating earache, are fever, vomiting and diarrhea. The vesicles of the tympanum become dilated, with bulging of the drum itself occurring in the later stages of infection. If treatment is inadequate, then acute attacks may eventually perforate the eardrum, produce chronic discharges and defective hearing. The usual treatment is with oral antibiotics (*Section 3.11*), such as ampicillin, amoxycillin, erythromycin and cefixime.

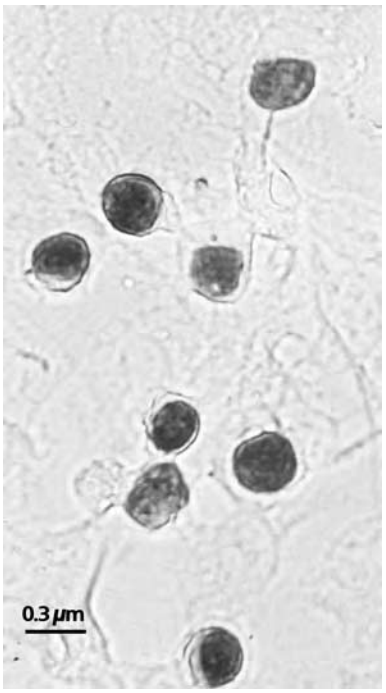


Figure 3.3 Light micrograph of *Chlamydia trachomatis*, growing in cultured eukaryotic cells, stained with iodine. Courtesy of School of Biochemistry and Microbiology, University of Leeds, UK.

Margin Note 3.1 *Haemophilus influenzae* type b

Haemophilus influenzae type b is the cause of 'Hib' meningitis. It is also responsible for childhood epiglottitis, causing the throat to swell alarmingly and breathing difficulties as mucus collects in the throat and fever. The condition is life threatening. *Haemophilus influenzae* type b can also cause pneumonia and other lower respiratory infections. The health risks are mainly associated with children under five years old but adults whose resistance has been weakened by sickle cell anemia (*Chapter 13*), chronic disease of the spleen, alcoholism (*Chapter 12*) or some malignancies (*Chapter 17*) are also at risk. In the developed world, the introduction of an effective vaccine in the 1980s eradicated Hib disease. However, it is still a problem with thousands of children in sub-Saharan countries affected. In 2005, it was reported that a five-year Medical Research Council led program, involving Sanofi Pasteur and the World Health Organization (WHO), of vaccinations against Hib had successfully eliminated the disease in children in the Gambia. Hopefully, this will encourage other countries to adopt a similar practice of routine Hib immunization policies.

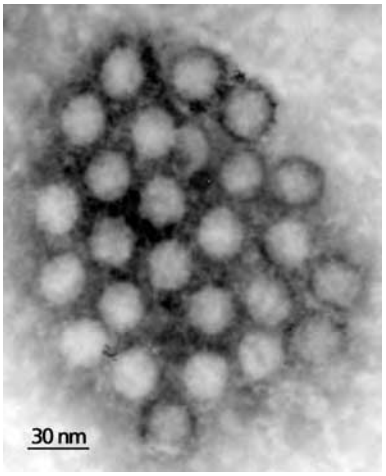


Figure 3.5 Electron micrograph of poliomyelitis virus. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

Infections of the central nervous system (CNS) may affect the meninges, the spinal cord or the brain causing meningitis, myelitis and encephalitis respectively. More than one area may be infected simultaneously. Such infections can also become systemic infections (*Section 3.7*). Pathogens can enter these areas following head injuries, along the axons of neurons or by breaching the blood–brain or blood–cerebrospinal fluid barriers (*Figure 3.4*). The most common causes of viral meningitis are enteroviruses, such as ECHO-, Coxsackie- and, formerly, poliomyelitis viruses (*Figure 3.5*). Viral meningitis is not usually a life-threatening condition. Bacterial meningitis, in contrast, has a mortality greater than 10%. The principal causative organisms are the capsulated bacteria, *Neisseria meningitidis* (*Figure 1.3*), *Streptococcus pneumoniae* and, before the introduction of a vaccine, *Haemophilus influenzae* (*Margin Note 3.1*). Effective vaccines are also available against some serogroups of *Neisseria meningitidis* and a vaccine against *Streptococcus pneumoniae* is being tested.

Encephalomyelitis results from infections by a number of viruses or protozoa. These include some poliovirus types, *Herpes simplex*, measles virus, HIV, toga viruses, which are transmitted by arthropods, and the rabies virus that is transmitted from infected mammals. The protozoan *Toxoplasma gondii* may infect individuals with compromized immune systems while *Trypanosoma brucei* is the causative organism of African sleeping sickness.

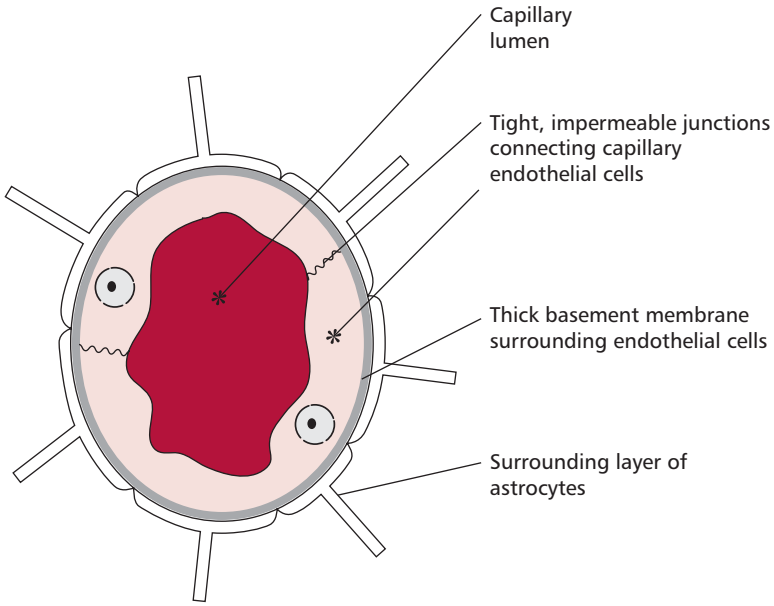


Figure 3.4 Schematic of the blood–brain barrier. Note the tightly associated endothelial cells and thick basement membrane, which prevent materials in the blood entering the brain.

3.4 INFECTIONS OF THE RESPIRATORY SYSTEM

The respiratory system is constantly exposed to inhaled microorganisms but is protected by extensive defenses. The nose filters out particles larger than 10 μm although those smaller than 5 μm may reach the bronchi and alveoli. In addition, there is a host of immune defenses including alveolar macrophages, secretory IgA antibodies, complement proteins, surfactant proteins, secreted defensins and lactoferrin (*Chapter 4*). Despite this, infections of the respiratory tract are frequent causes of illness. The World Health Organization (WHO) has reported that many hundreds of millions of patients suffer acute infections of the lower respiratory tract worldwide. *Figure 3.6* indicates the sites of a number of respiratory diseases.

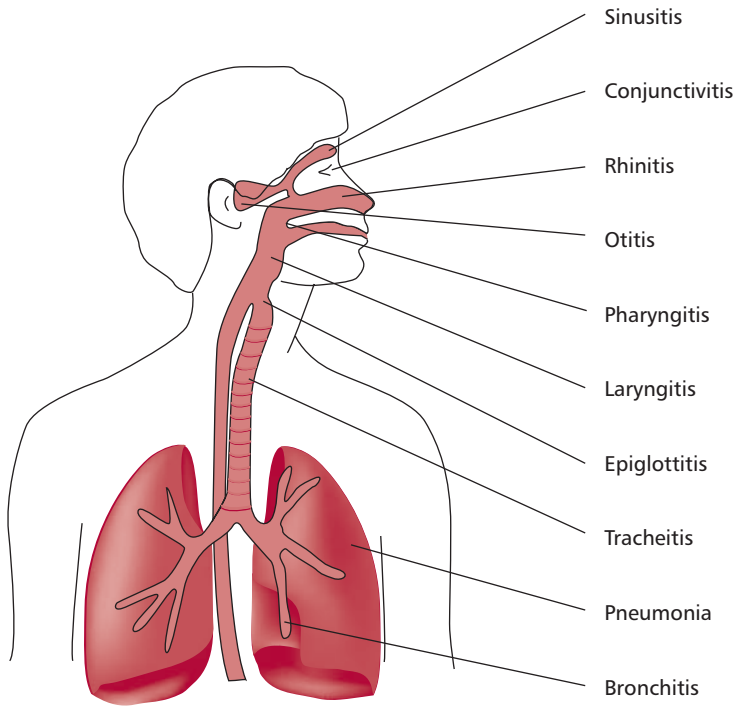


Figure 3.6 Selected infections associated with the head and respiratory system. See text for discussions.

Respiratory viruses are transmitted directly by aerosols or indirectly from contaminated surfaces. The first site of attack is, not surprisingly, the epithelium of the nose and throat. Indeed, the hundreds of corona and rhinoviruses that cause the common cold replicate at 32 to 33°C, the temperature of the mucosal surface lining the nose. The influenza viruses (*Figures 2.4* and *2.8*) infect and replicate in respiratory epithelial cells causing cellular damage. The generalized symptoms that present, such as muscular aches, malaise and anorexia, suggest the virus may spread systemically from the respiratory tract but there is no conclusive evidence for this.

The loss of ciliated and mucus producing epithelial cells impairs clearance of invading microbes and creates conditions for secondary bacterial infections of staphylococci, streptococci or *Haemophilus influenzae*. Bacterial proteases, for example the V8 protease of *Staphylococcus aureus*, can enhance the infectiveness of the influenza virus by improving virus adhesion.

Corynebacterium diphtheriae (*Figure 3.7*) and *Bordetella pertussis* (*Figure 3.8*) are obligate bacterial pathogens. *Corynebacterium diphtheriae* infects the nasopharynx and the tonsils and may lead to a lethal systemic infection affecting the heart, liver and kidneys. *Bordetella pertussis* is the causative agent of whooping cough. It adheres to the epithelial cells lining the trachea and bronchi where it interferes with ciliary action and releases toxins (*Chapter 2*) and substances that damage and kill cells and irritate the surface, causing the characteristic cough. Effective vaccines are available against both organisms although 40 million infections of whooping cough occur annually worldwide. In contrast, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Moraxella catarrhalis*, make up to 60% of the normal bacterial population of the nasopharyngeal mucous membrane in healthy individuals. They can become opportunistic pathogens in immunosuppressed individuals

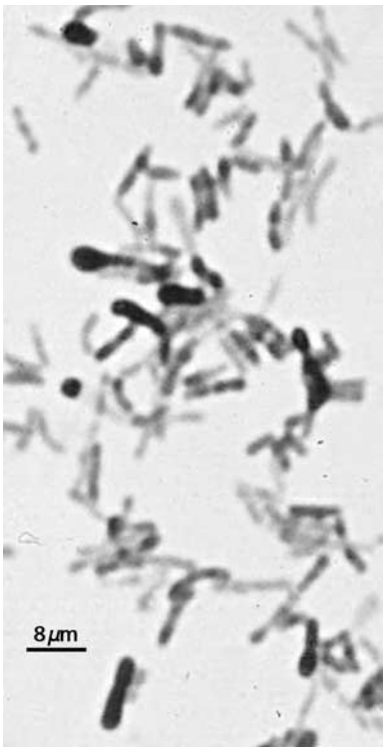


Figure 3.7 Light micrograph of *Corynebacterium diphtheriae*. Courtesy of School of Biochemistry and Microbiology, University of Leeds, UK.

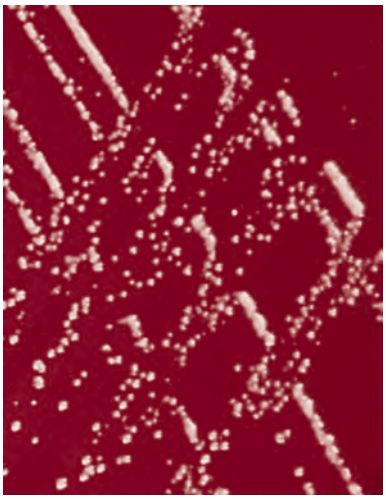


Figure 3.8 Characteristic small 'metallic' colonies of *Bordetella pertussis* growing on agar enriched with potato starch, glycerol and blood. Charcoal has been added to this medium to absorb bacterial metabolites that would inhibit the growth of the pathogen. Courtesy of School of Biochemistry and Microbiology, University of Leeds, UK.

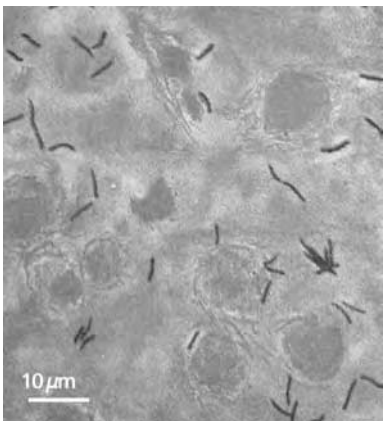


Figure 3.9 Light micrograph of *Mycobacterium tuberculosis* in a specimen of sputum. Courtesy of Public Health Image Library, Centers for Disease Control and Prevention, USA.

or following changes to the bacterium that render it increasingly virulent. The commonest form of bacterial pneumonia is lobar pneumonia caused by *Streptococcus pneumoniae* and results in a massive inflammation of one lobe of the lung. *Staphylococcus aureus* may cause bronchopneumonia, while *Haemophilus influenzae* can infect the epiglottis.

Mycobacterium tuberculosis (*Figure 3.9*) causes tuberculosis (TB) of the lung and may be considered a rather special case of bacterial infection of the lower respiratory tract. The bacteria enter the alveoli in inhaled air and are phagocytozed by macrophages where they escape being killed and multiply (*Chapter 4*). Mycobacteria can then enter the lymphatic system and invade a neighboring lymph node. The healing of local lesions leads to calcification of the lung tissues. In immunodeficient individuals, the lymph nodes and tissues may be progressively affected until eventually the mycobacteria are spread by the blood. Also with impaired immunity, dormant *Mycobacterium tuberculosis* can be reactivated causing a severe form of pneumonia.

Atypical pneumonias can result from infections with *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*. These infections are associated with 'flu-like' symptoms, such as high temperatures and coughing, although bronchial secretions and sputum do not contain pus as would be expected of a typical bacterial lobar pneumonia.

Generally, pathogenic fungi do not produce toxins but damage tissues directly or disturb normal metabolic functions and can induce hypersensitivity responses (*Chapter 5*). Fungi can cause respiratory infections; *Aspergillus fumigatus* can invade the respiratory system and lead to one of several types of diseases. It may simply grow in the mucus of the bronchi and induce a hypersensitive state but may invade old wound cavities of the lungs, such as those resulting from TB, and grow as a solid mass called an **aspergilloma**. Aspergillosis may also result from an invasive growth in the lungs and other tissues. Generally, the infective dose of spores is extremely large although the invasive form may be secondary to other systemic diseases. Similarly, *Pneumocystis carinii* can cause a serious pneumonia (PCP) in AIDS compromized patients (*Box 3.1*). The yeast, *Candida albicans*, is also an opportunistic agent in sufferers of AIDS.

3.5 INFECTIONS OF THE GASTROINTESTINAL TRACT

All regions of the gastrointestinal tract (GIT) are subject to infection. Saliva traps and removes many pathogens and these can also be killed by stomach acid (*Chapter 11*). Unfortunately new ones are constantly introduced through breathing and eating.

Infections of the oral cavity (*Figure 3.6*) differ in type and symptoms to those of the stomach and intestines. Inflammation of oral tissues caused by fungal infection, **actinomycosis**, often occurs following injuries, such as an accidental bite to the lining of the mouth or fracture of the jaw. Immunosuppression resulting from viral infections, AIDS, cancer treatment or treatment with broad spectrum antibiotics can all allow the yeast *Candida albicans* (*Figure 3.10*) to invade and colonize the mucous membrane, eventually producing a thick layer of yeast cells called candidiasis or thrush.

Some bacteria can resist removal by saliva and become immobilized by binding to surface receptors of cells in the mouth, eventually forming biofilms and microcolonies. Oral streptococci, such as *Streptococcus sanguis* and *Streptococcus mutans* (*Figure 3.11*), secrete glycosyltransferases that mediate their adhesion to extracellular carbohydrates on tooth surfaces leading to the formation of dental plaque, which is a complex mixture of bacteria and extracellular materials. These bacteria, together with *Actinomyces* species, can

cause caries by forming plaque on the tooth enamel, where they catabolize sugars to produce acid that demineralizes enamel and allows the dentine to be eroded. Abscesses of the roots of teeth can also be caused by mixed bacterial infections.

Periodontal (gum) diseases are inflammatory conditions that attack the gums, bone, and other supporting structures of the teeth. The extent of the inflammatory response depends upon the types of pathogens involved and the effectiveness of the immune response. However, they are major causes of tooth loss in adults. Gingivitis is the earliest form of periodontal disease and occurs when plaque accumulates on the teeth near the gums, which become inflamed and bleed easily. If detected and treated early, gingival tissues will return to normal without long-lasting damage. Untreated gingivitis progresses to periodontitis, which is also known as pyorrhea. Plaque hardens and extends from the gum line to the tooth root causing the gums to detach from the teeth and form pockets. Periodontal pockets create room for greater bacterial activity, particularly of facultative and obligate anaerobic bacteria leading to a progressive cycle of tissue damage until eventually the bone supporting the teeth is destroyed resulting in their loss.

Stomach and intestinal infections are caused by viruses, bacteria, protozoa and worms, all of which may be transmitted in food, contaminated drinking water or by fecal–oral contact. The need for strict personal hygiene is emphasized because these are the most frequent infections of children under five years of age. Approximately 40% of cases of diarrhea (*Chapter 11*) in children are caused by rotaviruses (*Figure 3.12*). In the very young this is potentially lethal and the WHO has estimated that out of the nearly two billion annual diarrhea diseases worldwide, three million end fatally.

Figure 3.13 indicates a number of pathogens that can infect the GIT. The acidic environment and proteolytic enzymes of the stomach kill most

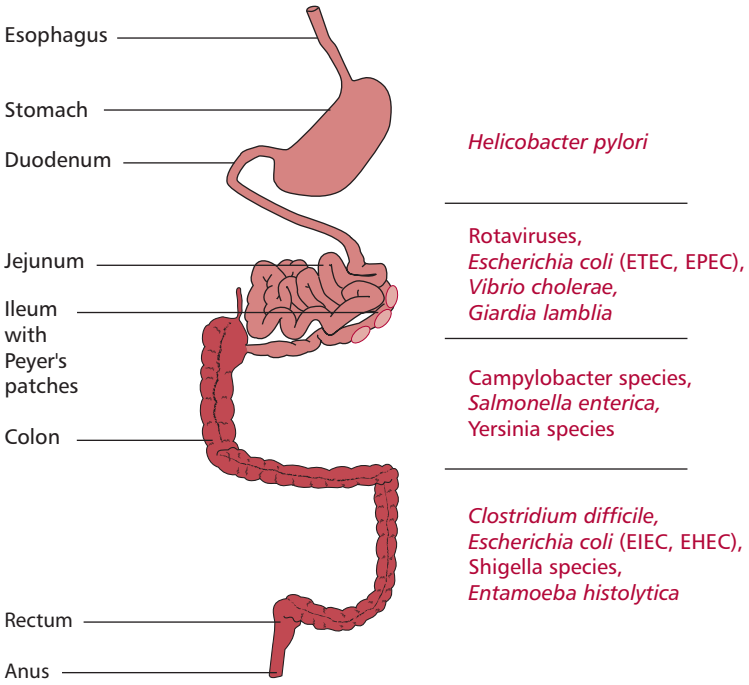


Figure 3.13 Some infectious organisms associated with the gastrointestinal tract. See text for discussions.

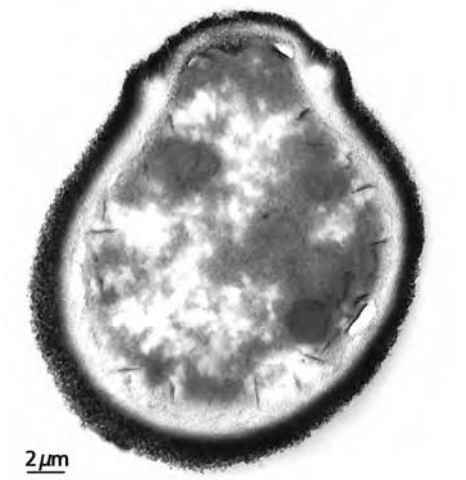


Figure 3.10 Electron micrograph of *Candida albicans*. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

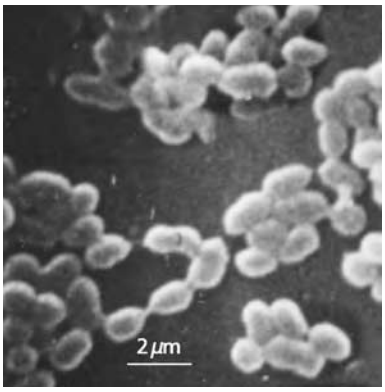


Figure 3.11 Electron micrograph of *Streptococcus mutans*. Courtesy of Professor J. Verran, School of Biology, Chemistry and Health Science, Manchester Metropolitan University, UK.

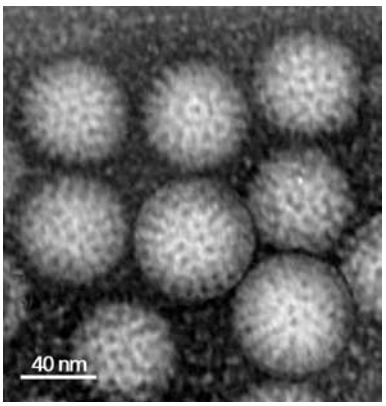


Figure 3.12 Electron micrograph of rotavirus. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

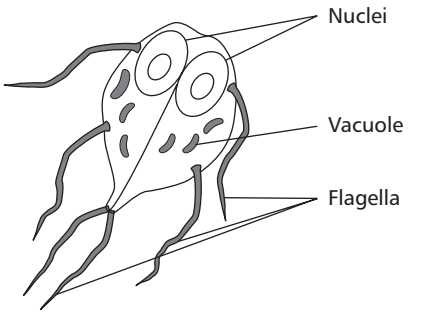


Figure 3.14 Schematic structure of *Giardia lamblia* based on several light and electron micrographs.

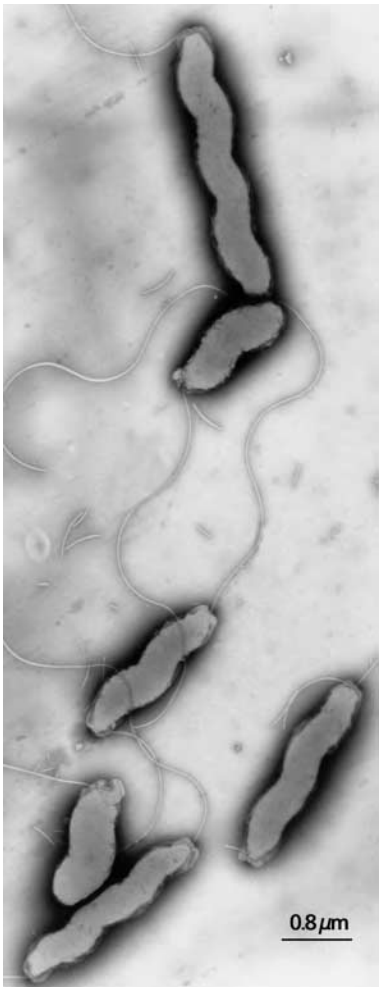


Figure 3.15 Electron micrograph of *Campylobacter jejuni*. Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

ingested microorganisms. However, the motile bacterium, *Helicobacter pylori* (*Chapter 11*) has specific receptors enabling it to bind to the gastric epithelium. It secretes urease, which catalyzes the hydrolysis of urea releasing ammonia that neutralizes stomach acid, and cytotoxins that damage the cells. This causes chronic inflammation of the gastric mucosal membrane and can lead to stomach and duodenal ulcers (*Chapter 11*). The partially digested food (chyme) in the stomach is made alkaline in the small intestine by secretions of the gut and pancreas and by bile salts (*Chapter 11*). In the ileum and jejunum, nonenveloped viruses, such as rotaviruses and adenoviruses, may infect enterocytes (*Chapter 11*) and damage the intestinal mucous membrane with disruption of water and electrolyte resorption. This can result in intestinal cramps, vomiting, watery diarrhea and a raised temperature. Enteropathogens, such as *Vibrio cholerae* and forms of *Escherichia coli* that are enterotoxic (ETEC) or enteropathogenic (EPEC) all have similar effects. The protozoan parasites *Giardia lamblia* and *Cryptosporidium parvum* are water-borne parasites that can infect the GIT. *Giardia lamblia* (*Figure 3.14*) frequently causes chronic disease, with watery diarrhea and, in some cases, a subfebrile temperature leading to malnutrition in children as a result of malabsorption. *Cryptosporidium parvum* can adhere to the epithelium of the small intestine and cause a shortening of the villi, which may be the cause of the diarrhea.

The lower portion of the ileum has areas of lymphoid tissue called Peyer's patches (*Figure 3.13*) composed of so-called M (microfold) cells, rather than the usual enterocytes and goblet cells (*Chapter 11*). These cells are able to translocate materials directly to the lymph follicles found beneath the mucosal surface. Invasive bacteria such as *Campylobacter jejuni* (*Figure 3.15*), Salmonellae and Yersiniae can use M cells to enter the submucosal area. Here they can multiply and destroy the adjacent epithelium, form abscesses and spread through the lymph and blood systems into the mesenteric lymph nodes, spleen and liver. The infection can also spread into the colon, causing inflammation of the colon or **colitis**. The ileum and colon can also be attacked by the bacteria *Yersinia enterocolitica*, *Salmonella enterica* and *Campylobacter jejuni* resulting in abdominal cramps, vomiting, watery, occasionally bloody, diarrhea and fever. *Shigella dysenteriae* and *Escherichia coli* pathotypes, EHEC (enterohemorrhagic) and EIEC (enteroinvasive) can cause a hemorrhagic colitis with bloody stools and subfebrile to febrile temperatures. The pathogenic protozoan *Entamoeba histolytica* is thought to infect 50 million people and kill about 100 000 per year worldwide due to amebic liver abscesses. Lastly, *Clostridium difficile*, a normal inhabitant of the gut, is an opportunistic pathogen. It is especially common in older people in hospitals and nursing homes and has been implicated in iatrogenic infections following medical interventions, such as antibiotic therapy. Infection with *Clostridium difficile* is now recognized as the major causative agent of colitis and diarrhea, which may occur following antibiotic intake and can be fatal in older patients.

3.6 INFECTIONS OF THE UROGENITAL SYSTEM

The urinary system and the genital systems are subject to infections by specific respective pathogens (*Figure 3.16*). Urinary tract infections (UTIs) are frequent in the developed world, with many millions of cases occurring each year. A number of factors, including diabetes mellitus (*Chapter 7*), scarring, kidney stones, use of catheters or anatomical peculiarities of the urinary tract all predispose individuals to UTIs. These originate in the perianal area and move up the urethra into the bladder causing a short-lived, acute infection

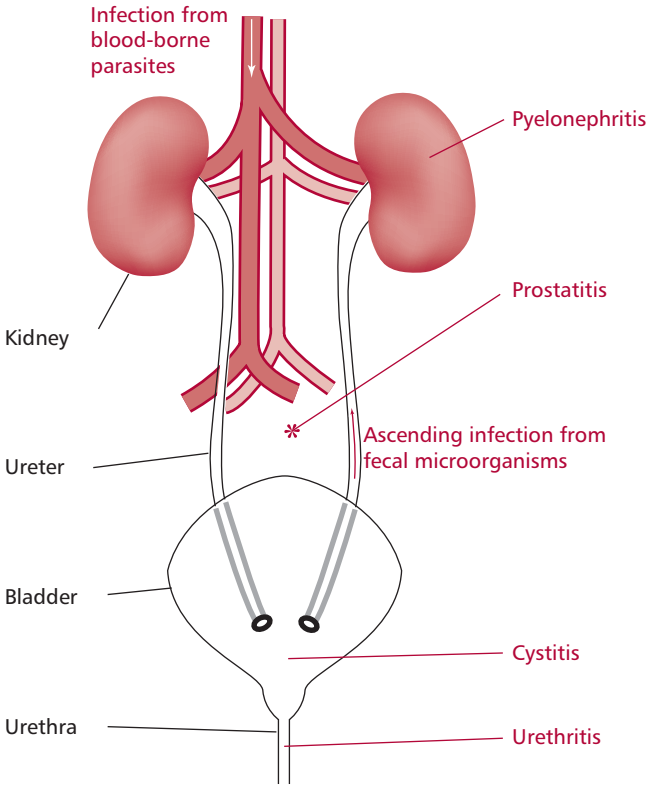


Figure 3.16 Some infections associated with the urogenital system. See text for discussions.

called cystitis. *Escherichia coli* is the most common agent causing up to 80% of the relatively uncomplicated UTIs, like cystitis. Women are more prone than males to cystitis because of their relatively short urethra and the hormonal changes associated with the menstrual cycle (*Chapter 7*). Cystitis is readily treated by increasing the uptake of fluids, particularly of acid drinks like cranberry juice, which causes increased flushing of the bladder, and by the use of antibiotics. Unfortunately, reinfections are frequent.

Pseudomonas aeruginosa (*Figure 2.1*) has been known to cause UTIs following hospitalization. Similarly, infections by *Enterococcus faecalis* and *Klebsiella pneumoniae* have followed organ transplants (*Chapter 6*). Unfortunately, these organisms often show multidrug resistance against antibiotics (*Box 3.4*). Thrush in the bladder, caused by the yeast *Candida albicans* can also occur following antibiotic treatment.

There has been a large increase in the incidence of sexually transmitted diseases (STDs) in the UK in recent years. Sexually transmitted diseases can affect the urinary system and the genital tract and are caused by a wide range of different pathogens. Human papilloma virus (HPV) can cause anogenital warts (condyloma acuminata). Strains 16 and 18 of HPV cause lesions in the cervix that are involved in the development of cervical carcinoma. Infection with *Herpes simplex* virus (HHV-2) can lead to genital herpes with painful ulcers and vesicular lesions of the genital mucous membrane. Infection with human immunodeficiency virus (*Box 3.1*) leads to acquired immunodeficiency syndrome (AIDS).

BOX 3.1 HIV and AIDS

In March 1981, physicians in New York reported eight cases of the previously rare Kaposi's sarcoma (KS), in a form that was markedly more aggressive than usual. All the affected were young gay men. Also in 1981, the Communicable Disease Centers (CDC) in Atlanta, Georgia, began to investigate an increase in requests from New York and Los Angeles for pentamidine, a drug used to treat *Pneumocystis carinii* pneumonia (PCP), an extremely serious fungal infection. In addition to PCP and/or KS, patients suffered a variety of opportunistic pathogens that eventually caused their death. The combination of infections and KS appeared to be the result of a total breakdown of the immune system and became known as Acquired Immunodeficiency Syndrome (AIDS). Subsequently, AIDS was seen in intravenous drug abusers and in a number of recipients of blood transfusions, and a viral cause was suspected.

In 1983, a virus, variously named Lymphadenopathy Virus (LAV) and Human T cell Lymphotropic Virus Type III (HTLV III), was isolated. Following international agreement the virus was renamed Human Immunodeficiency Virus (HIV) (*Figure 3.17*) in 1986. A number of strains of the virus have since emerged; HIVs I and 2 are the most prevalent. HIV is a human retrovirus, that is, its nucleic acid is RNA that on infection is transcribed into DNA by the viral enzyme reverse transcriptase (*Figure 3.18*). An electron micrograph of the virus and an illustration of its replication cycle are shown in *Figures 3.17* and *3.19* respectively. The virus infects the CD4+ Helper T lymphocyte, a key regulatory cell of the immune system (*Chapter 4*), rendering the patient susceptible to a whole range of infections with all microbial groups, from dysentery and diarrhea to pneumonia. For example, the protozoan *Cryptosporidium parvum* can cause a moderate to severe diarrhea that would soon be resolved in healthy people. In HIV infected patients, cryptosporidial diarrhea is among the commonest clinical presentations during the transition to full-blown AIDS, particularly in developed countries. The diarrhea is severe, protracted and may become life threatening. Death is commonly caused by pneumonia associated with the fungus PCP among a host of opportunistic infections. A list of infections common in AIDS patients is shown in *Table 3.2*.

Since the emergence of HIV, the virus has spread worldwide with an estimated 38 million people, including 2.3 million children, living with HIV/AIDS. It is thought that 25 million people have died of AIDS up to the end of 2005. The virus has had devastating effects on communities, particularly in sub-Saharan Africa, where about 8% of the adult population are estimated to be living with HIV/AIDS.

There is no effective treatment that completely clears the body of the virus. Drugs have been developed to inhibit replication of HIV in positive individuals to prevent them developing AIDS. These drugs target enzymes needed at different stages in the replication of the virus and include inhibitors of reverse transcriptase, for example azidothymidine (AZT), and antiproteases, such as amprenavir which prevent the virus from budding from an infected cell as shown in *Figures 1.4* and *2.7*. The combined use of a number of drugs with different actions has been extremely effective at preventing the development of AIDS but aggressive therapy can lead to problems such as HAART (*Margin Note 16.1*). Infected people can now expect to live relatively healthy lives following initial infection. However, combination therapy has to be taken for the remainder of the patient's life. The drugs have a number of side effects and are also extremely expensive. There is evidence that some multiple drug resistance is emerging amongst HIV strains. Since the virus was identified in 1983, the search for a successful vaccine for HIV has been ongoing. All the latest scientific technology has been used in this effort but, to date, no vaccine has been successful.

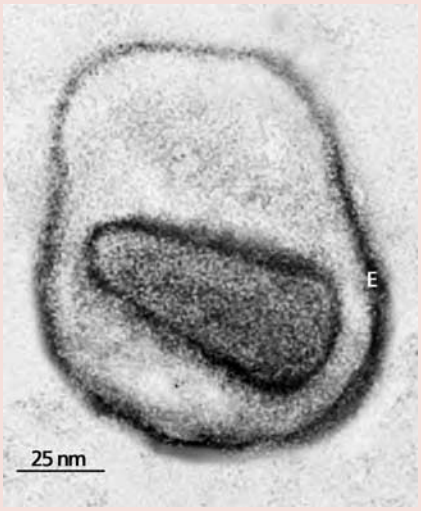


Figure 3.17 Electron micrograph of a single HIV virion. E denotes the envelope.

Disease	Caused by	Infectious agent
Pneumonia	*Pneumocystis carinii*	fungus
Tuberculosis	*Mycobacterium tuberculosis, Mycobacterium avium*	bacteria
Kaposi's sarcoma	Kaposi's sarcoma virus (HHV8)	virus
Lymphoma	*Epstein-Barr virus* (HV4)	virus
Mucocutaneous thrush	*Candida albicans*	yeast
Diarrhea and dysentery	*Cryptosporidium*	protozoan
Shingles	*Varicella zoster* (HHV3)	virus
Cryptococcosis	*Cryptococcus*	fungus

Table 3.2 Some diseases associated with AIDS

Figure 3.18 Molecular model of the HIV 2 reverse transcriptase. PDB file 1MU2.

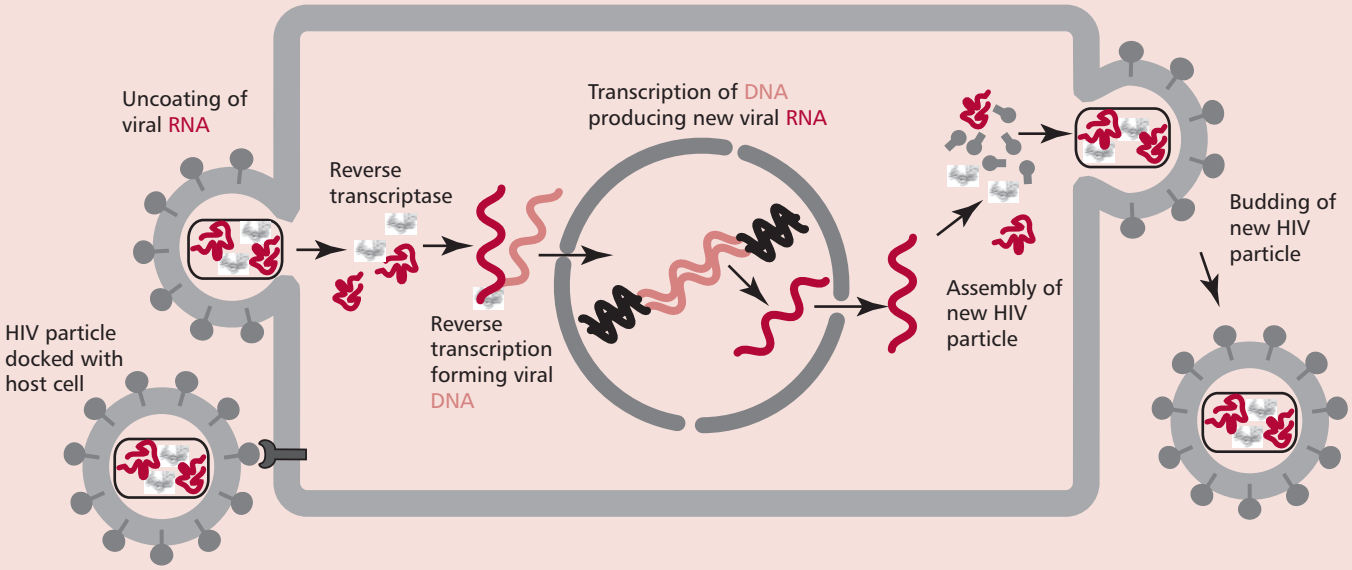


Figure 3.19 The replication cycle of HIV. See text for explanation.

Some types of *Chlamydia trachomatis* are among the commonest causes of STDs producing local inflammations of the urethra and cervix. In contrast, other types are highly invasive and infiltrate the lymphatic system leading to necrosis (lymphogranuloma venereum).

The most widely known STDs are probably gonorrhea and syphilis caused by the bacteria *Neisseria gonorrhoeae*, a Gram-negative diplococcus, and *Treponema pallidum* a spirochete (*Figure 3.20 (A)* and *(B)*) respectively. Gonorrhea, a common STD, is a pelvic inflammatory disease whose major symptoms include a purulent inflammation of the uterine cervix and urethritis. In some women, however, the infection may be relatively asymptomatic and may go unnoticed. Syphilis was thought to have originated in the Americas and been brought to Europe by sailors on the Columbian expeditions. More recent evidence suggests that it was present in the Old World long before this. *Treponema* spirochetes can enter through mucous membranes or minute abrasions in the skin during sexual acts. The infection shows three stages of pathogenesis. Initially, an ulcer called a **chancre** develops at the site of infection. The infection then spreads to nearby lymph nodes causing them to swell and harden. Secondary syphilis develops after one to three months. It is characterized by the presence of highly infectious lesions on various parts of the body. The disease may lie dormant for many years but, if not treated with antibiotics, will develop into tertiary syphilis causing inflammations of the aorta and CNS. Dementia, heart attacks and death can all result. Patients with tertiary syphilis cannot infect others with the disease. Some patients may develop benign late syphilis, which is usually rapid in onset but does respond well to treatment. It usually begins three to 10 years after infection and is characterized by the development of **gummas**. These are tumor-like growths of a rubbery consistency that are most likely to affect the skin or long bones but can also develop in the eyes, mucous membranes, throat, liver and stomach lining. However, since the use of antibiotic treatments for syphilis they are increasingly uncommon.

The protozoan *Trichomonas vaginalis* (*Figure 3.21*) is a frequent colonizer of the mucosal membrane of the urogenital system. It is generally asymptomatic

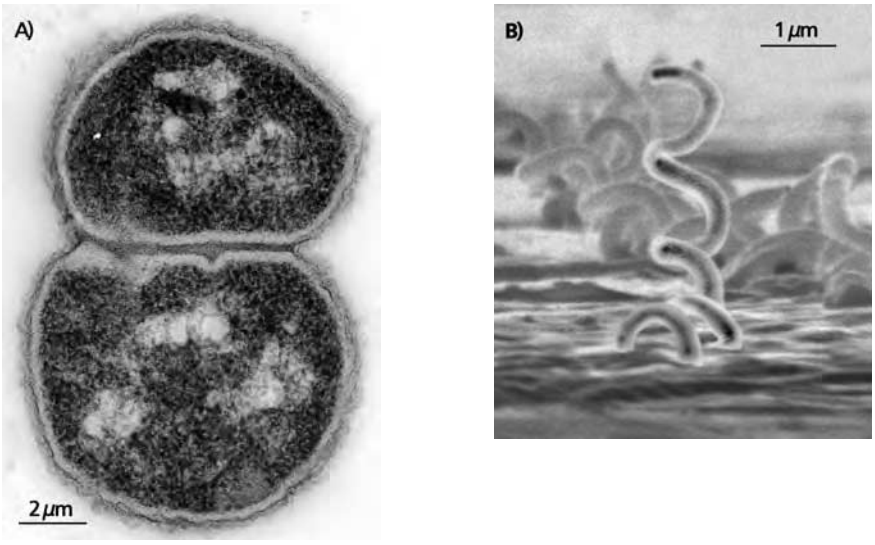


Figure 3.20 Electron micrographs of (A) *Neisseria gonorrhoea* and (B) *Treponema pallidum*. (A) Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK and (B) Public Health Image Library, Centers for Disease Control and Prevention, USA.

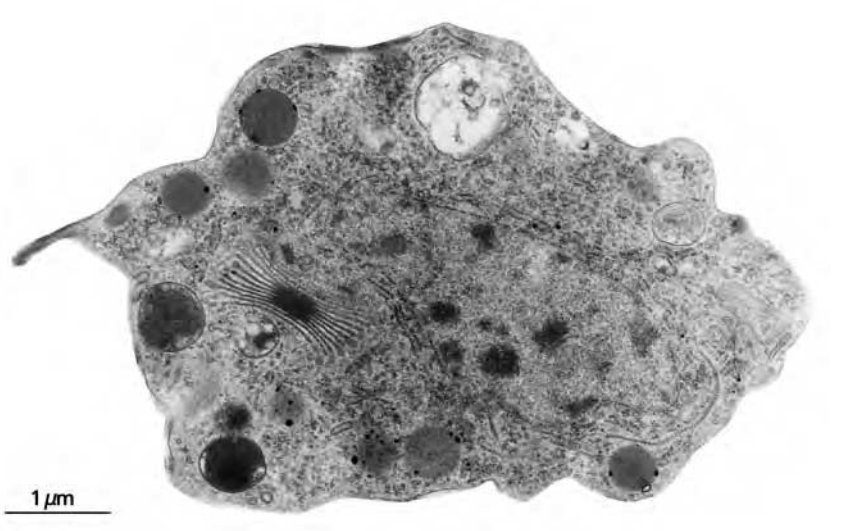


Figure 3.21 **Electron micrograph of *Trichomonas vaginalis*.** Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

but an inflammatory reaction, trichomoniasis vaginitis, may result in a frothy cream-colored discharge.

A number of arthropods may be sexually transmitted. These include crab lice and the scabies mite (Figure 2.14(A)).

3.7 SEPSIS AND SYSTEMIC INFECTIONS

A relatively small number of pathogens are able to enter the body in the lymphatic or circulatory systems and produce a generalized or systemic infection involving numerous body organs: brain, bone marrow, kidneys, liver, lungs and spleen. Local skin infections, such as with *Streptococcus pyogenes* or *Staphylococcus aureus* or infections of the GIT or urogenital system with Enterobacteriaceae can unfortunately progress to acute generalized infections within only a few days, given appropriate conditions. For example, *Streptococcus pyogenes* or *Staphylococcus aureus* secrete toxins called **superantigens** (*Chapters 2* and *4*) that stimulate the release of cytokines from immune cells and produce an excessive inflammatory response called **systemic inflammatory response syndrome** (**SIRS**) consisting of four characteristic stages. In stage I, respiration, heart rate and body temperature all increase. The leukocyte count may be increased or decreased. Stage II refers to the presence of the organism in the blood of the patient; this is called **sepsis**. Normally less than 50% of cases can be identified. Stage II may be cured intrinsically by the immune system or extrinsically by the administration of antibiotics. If unsuccessful, the condition progresses to stage III: serious sepsis or multiorgan dysfunction syndrome (MODS) characterized by lactic acidosis, falling blood pressure, hypoxia and oliguria. If clinical measures fail to stabilize the patient, then stage IV, septic shock, develops with irreversible organ failure and the death of the patient in most cases.

In contrast to the possible acute developments of localized infections, generalized infections may have incubation times of up to three weeks. A classical example of such a systemic infection is typhoid fever caused by *Salmonella typhi* (*Figure 3.22*). The pathogen may be ingested and can enter the body through the tonsils in the throat and Peyer's patches in the gut. The bacterium is distributed to various organs in the lymph and blood, infects

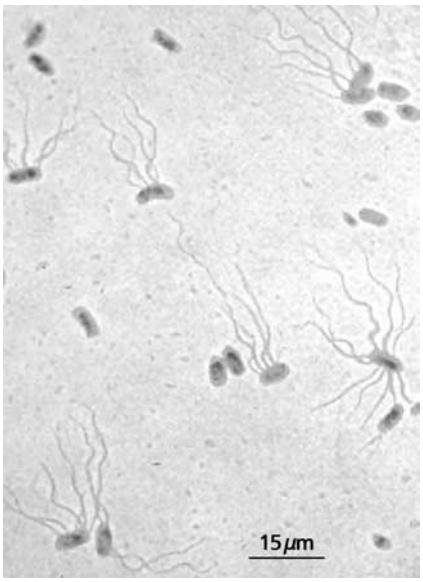


Figure 3.22 **Light micrograph of *Salmonella typhi*.** Courtesy of Public Health Image Library, Centers for Disease Control and Prevention, USA.

Margin Note 3.2 *Salmonella typhimurium*

Salmonella typhimurium (*Figure 3.23*), as its name suggests, causes a lethal, systemic infection that resembles typhoid fever in mice. However, in humans it does not cause as severe a disease as ***Salmonella typhi*** although it is a major cause of food poisoning. This rarely leads to fatalities, other than in the elderly or very young or in patients with depressed immune systems who are not treated with antibiotics. The disease is characterized by symptoms of nausea and vomiting, abdominal cramps and diarrhea that generally last up to seven days.

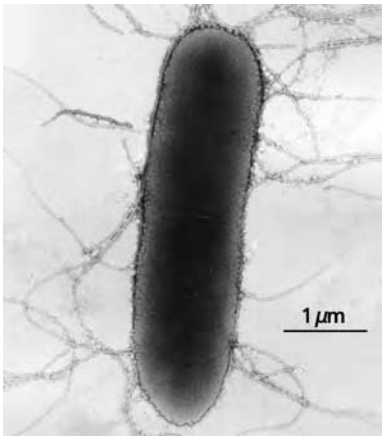


Figure 3.23 Electron micrograph of *Salmonella typhimurium*. Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

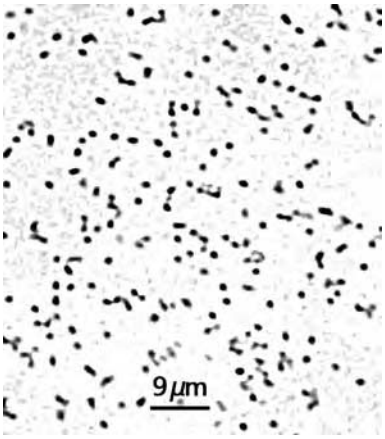


Figure 3.24 Light micrograph of *Brucella* species. Courtesy of L. Stauffer, Public Health Image Library, Centers for Disease Control and Prevention, USA.

cells and grows intracellularly. The major symptoms are fevers of up to 40°C and the development of abscesses in the intestine that may cause perforations and a fatal peritonitis. Even when the patient recovers, a relapse may occur. *Salmonella typhi* infects about 60 million patients annually, mainly in the developing world. If untreated, it can be fatal. Some recovered patients retain the pathogen in the gall bladder and excrete bacteria in their feces, becoming carriers, although remaining healthy themselves.

The zoonosis, brucellosis, caused by various species of *Brucella* (*Figure 3.24*), is also a primary systemic infection. This is a common infection of cattle (*Brucella abortus*), sheep and goats (*Brucella melitensis*) and can infect humans if they drink contaminated milk, if they inhale the bacteria into the lungs or if there is direct contact through skin lesions. People who work in direct contact with animals, such as farmers, veterinarians, shepherds, goatherds and abattoir workers, are most at risk. The pathogen colonizes and grows in the main abdominal organs and bone marrow producing periodic attacks of relapsing fever over an extended period.

3.8 INVESTIGATING INFECTIOUS DISEASES

To diagnose an infectious disease, two criteria must be satisfied. First, signs and symptoms compatible with the suspected infectious agent must be apparent. Secondly, the pathogen must be recovered from the infected site of the patient or there must be evidence of the pathogen being present at that site.

Infectious diseases can affect any organ or system and can cause a wide variety of symptoms and signs (*Chapter 1*). The clinical history and examination should aim to identify the sites of infection and the causative organism. The clinical history focuses on aspects relevant to infectious disease, such as recent travel history, food and water intake, occupational exposure, sexual activity and any use of intravenous drugs. The clinical examination involves identifying fever, skin rashes, swollen lymph nodes (lymphadenopathy) and investigations of the eyes, ears, mouth and throat. Fever is a typical symptom of infection but not all patients with fever have an infection and not all infectious diseases present with fever. Investigations of the vagina, rectum and penis are necessary in sexually transmitted diseases.

The history and clinical examination is often supported by tests to assess health and identify the organs affected. These tests include imaging techniques such as X-rays, ultrasound, computerized tomography (CT), magnetic resonance imaging and blood tests (*Chapters 18* and *13*). The blood tests involve a full blood count where eosinophilia is an important finding in parasitic infections and lymphocytosis is usually found in viral infections. The erythrocyte sedimentation rate and C-reactive protein (*Chapter 4*) levels are nonspecific tests of value in monitoring the course of infectious diseases. Other tests involve assessing liver and renal functions (*Chapters 11* and *8*), which may be disrupted by an infection.

Microbiological tests to identify the infectious agents are especially helpful. Specimens for microbiological investigations include blood, cerebrospinal fluid (CSF), feces, pus, sputum and urine (*Chapter 1*). Microbiological investigations use a variety of techniques, including culture, serological, biochemical and molecular biological tests.

CULTURE

The suspected microorganism from the patient is grown outside the body usually on or in growth media, such as nutrient agar or broth, or in selective media which support the growth of particular microorganisms, until growth

is detectable. In the case of bacteria, characterization is based on their microscopic appearance and the shape, texture and color of colonies. To some extent, bacteria can be identified by their ability to grow in specific media, such as blood agar or Sabouraud's agar. Indicator media that include some substance that visibly changes as a result of the metabolic activities of particular microorganisms are also of use in identification. Fungi and mycoplasmas are cultured in similar ways to bacteria. However, they require a greater use of microscopic and colonial morphology for identification. Certain microorganisms, such as chlamydiae, and all human viruses, are intracellular pathogens and their growth requires the inoculation of cultured eukaryotic cells. When viruses infect and replicate within cultured cells, pathological changes are produced that are characteristic of particular viruses and can be used for identification purposes. Moreover, electron microscopy of supernatants from these cultures, or even on patient samples, can show the presence of particular viruses. For example, Norwalk virus (*Figure 3.25*), which causes outbreaks of vomiting and diarrhea, has been identified in stool samples of affected individuals. The diagnosis of parasitic infections, protozoa and helminths, may involve growing them in culture. More frequently, parasites are identified directly from specimens isolated from infected patients and/or indirectly by examining the cysts or eggs of the parasite.

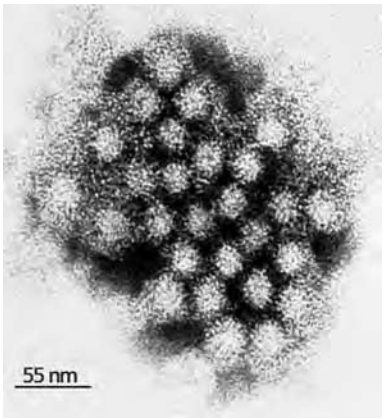


Figure 3.25 Electron micrograph of Norwalk virus. Courtesy of Public Health Image Library, Centers for Disease Control and Prevention, USA.

SEROLOGICAL TESTS

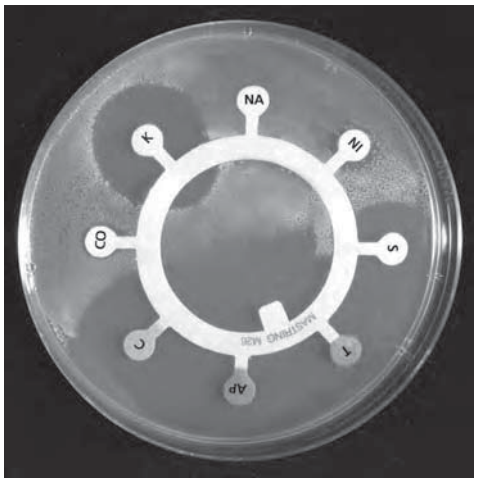
Serological tests involve identifying infectious agents indirectly by measuring serum antibodies in the affected individual. Antibody levels against a pathogen increase in the early stages of the disease and then fall during recovery. Such tests are particularly useful in situations where it is impossible to isolate the infectious agent and are used to make a diagnosis of, say, HIV infection. It is preferable to take a blood sample early in the infection (acute serum) and 10–14 days later (convalescent serum). A fourfold or greater rise in antibody

BOX 3.2 API strips

The API system, named from the parent company *Appareils et Procédés d'Identification*, for identifying bacteria and yeasts was first developed in the 1970s. Although the tests themselves were not new, the system used standardized and miniaturized versions of the existing biochemical tests on a single strip. Each test strip contains a number of separate compartments that contain the dehydrated reagents necessary for each specific test. The test is typically performed by forming a homogeneous suspension of the microorganism to be identified in 0.85% NaCl solution. Samples of the suspension are then added to each of the wells and this also rehydrates the reagents. The organisms will produce some observable change in the wells, for example color changes due to pH differences or enzyme activities or form end products that can be identified. Any one well will, of course, give a positive (+) or negative (–) result. A number of tests are necessary to identify a species or strain and the tests on any one API strip give a profile or numerical identifier that is the sequence of positive and negative test results (*Figure 3.26*). The organisms can then be named from a codebook that correlates this sequence with the bacterial species or strain or, more usually, identifications can be made with a computerized database. Organisms can generally be identified accurately and reliably in four to 48 h, depending upon the strips used and the species of microorganism concerned.

API tests include 15 identification systems for almost all groups of bacteria and over 600 different species. These include Gram-negative and Gram-positive bacteria, such as species and subspecies of Enterobacteriaceae, bacilli, *Campylobacter*, Corynebacteria, enterococci, Listeria, micrococci, Staphylococci and Streptococci and some anaerobic bacteria and yeasts.

Figure 3.26 A developed API strip showing positive (darker colored) and negative (clear) results for each of the test wells.



AP Ampicillin
C Chloramphenicol
CO Colistin sulfate
K Kanamycin
NA Nalidixic acid
NI Nitrofurantoin
S Streptomycin
T Tetracycline

Figure 3.27 **A multidisc containing a different antibiotic in each lobe as indicated, which can be used to assess the antibiotic susceptibility or resistance of bacteria. The bacteria in this case are a strain of *Escherichia coli* that cannot grow in the area surrounding an antibiotic to which they are susceptible.**

titer in the second serum is diagnostic of an acute infection. Antibodies to bacteria can be detected by their ability to agglutinate these microorganisms. Alternatively, a variety of immunotechniques are available which are outlined in *Chapter 4*.

BIOCHEMICAL AND MOLECULAR BIOLOGICAL TECHNIQUES

A variety of biochemical and molecular biological tests are available to help identify microorganisms. The simple Gram stain can immediately eliminate many possible bacteria. The abilities of some bacteria specifically to ferment some carbohydrates, use different substrates, express restricted enzyme activities or form specific products in enzyme catalyzed reactions can all aid in identification. The susceptibility of bacteria to different antibiotics (*Figure 3.27*) can also be useful and has the added utility of indicating possible therapy.

The GC ratio of the DNA of bacteria is generally expressed as a percentage 100(G + C) / (A + T + G + C) and varies from 20 to nearly 80%. Any one particular group of bacteria has a characteristic value. Specific genes from pathogenic organisms have been cloned and sequenced. That for the 16S ribosomal RNA has received considerable attention and, indeed, differences in the sequences of this gene have allowed evolutionary relationships between different groups of bacteria to be deduced. The entire genomes of a number of viruses, pathogenic bacteria and parasitic protozoa have been sequenced and the number is growing rapidly. Nucleic acid probes can be designed to detect characteristic sequences of pathogens and identify the organism in body fluids or tissues. This technique has been enhanced by the development of the **polymerase chain reaction**.

The polymerase chain reaction

The polymerase chain reaction (PCR) was devised by Mullis in 1983. He was awarded the Nobel Prize in Chemistry in 1993; the shortness of time between the two dates is indicative of the perceived importance of PCR. Indeed, it is hard to exaggerate the impact of PCR because it has revolutionized molecular biological techniques. The PCR is an elegantly simple *in vitro* method for increasing in an exponential manner the number of relatively short strands of specific DNA fragments, normally of 500 to 5kbp, although longer lengths of up to 40 kbp can also be amplified. These may be whole genes but are more usually only a fragment of one. DNA consists of two polynucleotide strands arranged together in the now familiar double helical structure. The strands run in opposite directions: one in the 3′ → 5′ direction, the other with a 5′ → 3′ orientation. The bases of the nucleotides of each strand associate together such that they form complementary pairs, with an adenine (A) of one strand paired with a thymine (T) of the other, and guanine (G) pairing with cytosine (C). The base pairs, and therefore the polynucleotide strands, are held by hydrogen bonds. It is the sequence of bases in the DNA strand(s) that is unique and specific to a particular gene.

The PCR is used to replicate the original DNA sample (template DNA) using a DNA dependent DNA polymerase, usually abbreviated to DNA polymerase. This enzyme copies the template DNA to produce new strands with complementary sequence. Some DNA polymerases can proofread, that is correct any mistakes in the newly formed strand to ensure the fidelity of its sequence. Crucially, DNA polymerases cannot begin the new strand *de novo*, but can only extend an existing piece of DNA. Thus two primer molecules are required to initiate the copying process. The primers are artificial oligonucleotides, short DNA strands of fewer than 50 nucleotides that are complementary to regions that flank the section of the template DNA of interest. Hence the primer determines the beginning of the region to be amplified. Primers are usually made to order by commercial suppliers who must, of course, be supplied with the required sequence.

The PCR consists of a series of cycles, each of which consists of three identical steps (*Figure 3.28*). First, the double-stranded DNA has to be heated to 90–96°C to break the hydrogen bonds that connect the two strands and allow them to separate. This denaturation is called melting. Prior to the first cycle, the DNA is often heated for an extended time, called a 'hot start', of up to 5 to 10 min to ensure that the template DNA and the primers are fully melted. In subsequent cycles, 30 s to 3 min at 94 to 96°C is normally sufficient for melting. Placing a heated lid on top of the reaction tubes or a layer of oil on the surfaces of the reaction mixtures prevents evaporation. The second step is primer annealing at temperatures of 50 to 65°C for 30 to 60 s, during which the primers bind to their complementary bases on the now single-stranded DNA templates. The primers must be present in excess of the target DNA otherwise its strands will simply rejoin. The design of the length of the primers requires careful consideration. Primer melting temperature, which must not be confused with that of the template DNA itself, increases with the length of the primer. The optimum length for a primer is generally 20 to 40 nucleotides that have melting temperatures of 60 to 75°C, although this depends upon their G/C content. If the primers are too short they will anneal at random positions on the relatively long template and result in nonspecific amplification. However, too long a primer and the melting temperature would be so high, that is above 80°C, that the DNA polymerase would have reduced

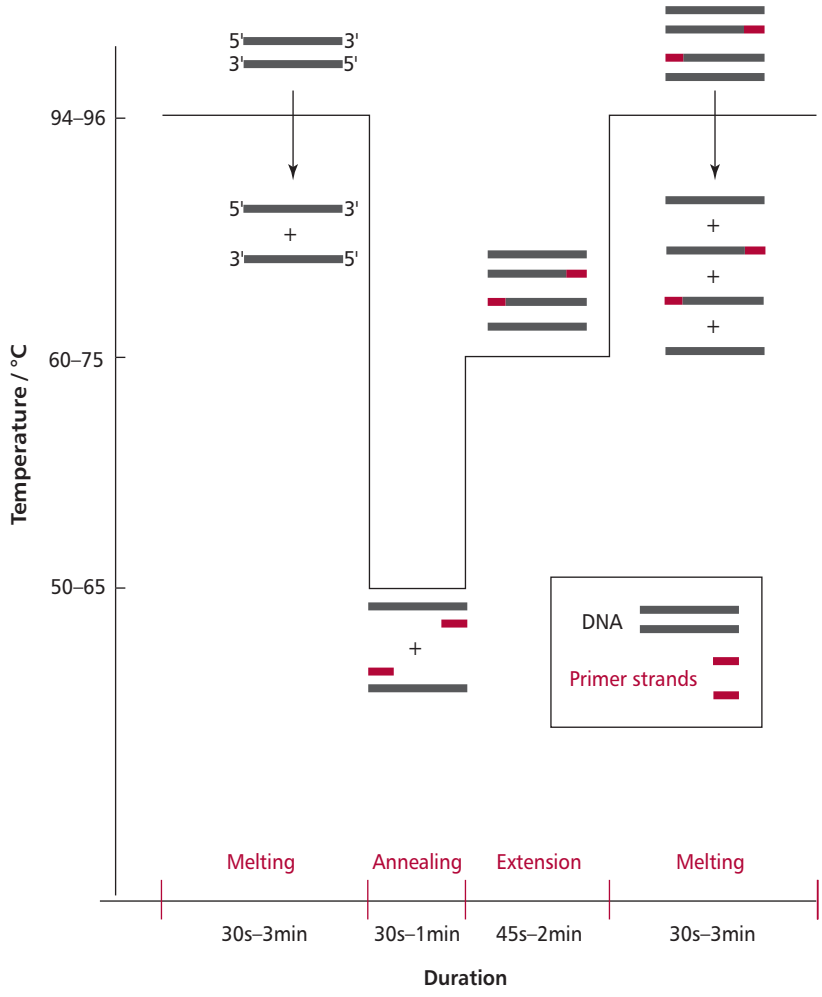


Figure 3.28 Outline of the steps associated with the cycles of the polymerase chain reaction. See text for details.

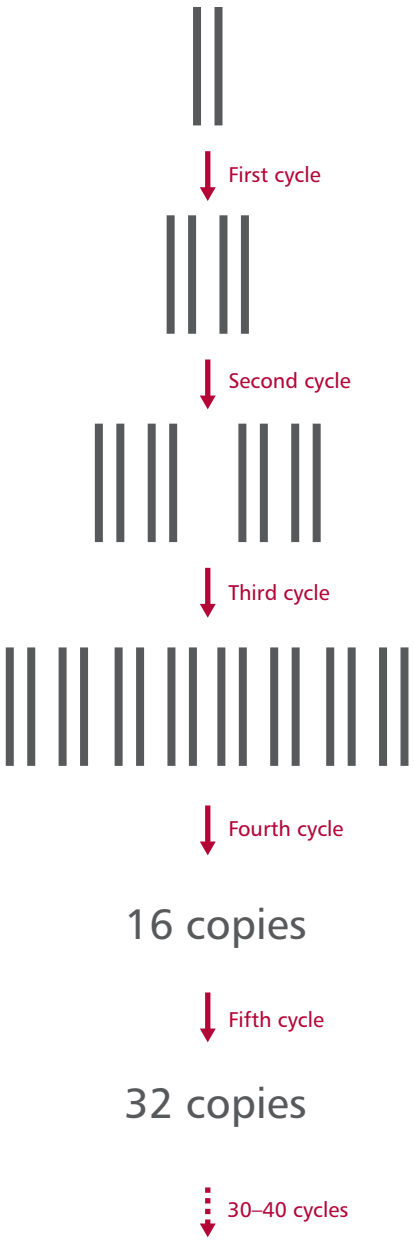


Figure 3.29 The amplification of DNA molecules during cycles of the polymerase chain reaction.

activity. The third step is the synthesis of DNA in reactions catalyzed by the polymerase and which extend the primers using the complementary strands as templates. This usually requires 45 to 120 s at 72°C. The extended portion of DNA is a complement of the template strand. Given the high temperatures of the reactions, DNA polymerases from thermophilic organisms are preferred. The *Taq* polymerase from *Thermus aquaticus* is widely used although it has the disadvantage of lacking proofreading capabilities and therefore of introducing errors (mutations) of 1 in 400 to 500 nucleotides in the new DNA. Polymerases such as *Pwo* or *Pfu*, obtained from Archea, have proofreading mechanisms that significantly reduce mutations and are used in 'long range' PCR of up to about 30 kbp.

The result of the first cycle is two helices that are usually overextensions of the target sequences. Each is composed of one of the original strands plus its newly assembled complementary strand and its associated primer, for each of the original template DNA molecules. The cycle is usually repeated 20 to 30 times in identical conditions with a doubling of the amount of DNA present for every cycle (*Figure 3.29*). Hence after 30 cycles the amount of DNA in the original sample has increased over 2^{30} (10^9 fold)! A PCR experiment normally terminates with a 10 min incubation at 72°C to ensure that all of the amplified DNA molecules are fully extended by the polymerase. Note that extremely stringent conditions are necessary to prevent any unwanted DNA contaminating PCR experiments, since this would also be amplified.

The great advantages of PCR are the increase in sensitivity and its automation. Samples of DNA from even a single cell or from samples many years old can be amplified and then analyzed. The PCR reaction is automated in a thermocycler, which automatically heats and cools the reaction tubes to the appropriate temperatures, for the desired times and the required numbers of cycles.

The products of PCR are identified by determining their base sequences and/or their sizes using agarose or polyacrylamide gel electrophoresis against a known sample. The size of the product can be estimated by comparison with the electrophoretic mobility of fragments of DNA of known size (*Figure 3.30*).

The biomedical sciences apply PCR in four main areas, which are the identification of infectious disease organisms for diagnostic purpose, in the detection of variations and mutations in hereditary diseases (*Chapters 15* and *16*), detecting acquired mutations that lead to cancers (*Chapter 17*) and in tissue typing (*Chapter 6*). It is especially useful in diagnosing diseases caused by organisms that are difficult or impossible to culture. Its ability to amplify incredibly small amounts of DNA means that PCR-based tests can identify sources of infection more accurately, reliably, rapidly and cheaply than previous methods. For example, it can detect three different sexually transmitted

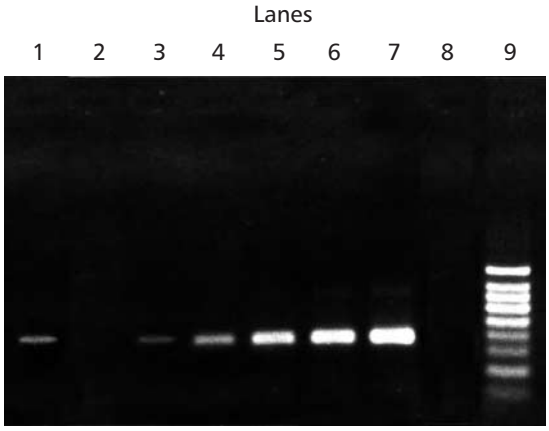


Figure 3.30 **A polyacrylamide gel electrophoresis of the products of a polymerase chain reaction experiment. Lane 1 shows the DNA sample of size 390 bp (positive control) which has been amplified by 10, 20, 25, 30, 35 and 40 cycles of polymerase chain reactions in lanes 2 to 7. Lane 8 shows the results of a negative control in which the DNA sample was replaced by water and amplification did not occur. Lane 10 shows the separation of DNA molecules of known sizes between 100 and 1000 bp as a calibration marker.** Courtesy of Dr Q. Wang, School of Biology, Chemistry and Health Science, Manchester Metropolitan University, UK.

disease agents, *Herpes*, papillomaviruses and *Chlamydia* on a single swab. Polymerase chain reaction tests can even distinguish the specific strains of papillomaviruses that predispose individuals to cervical cancer. Diagnostic tests are also available for the viruses involved in AIDS, viral hepatitis and viral meningitis. The PCR is the most sensitive and specific test for *Helicobacter pylori*, the main cause of stomach ulcers (*Chapter 11*). In England and Wales, 47% of cases of meningococcal infections in 2002 were diagnosed using PCR tests for meningococcal DNA in clinical samples. Bacterial infections in middle ear fluid from children suffering otitis media are detectable by PCR, indicating an active infection, even when standard culture methods fail. The Lyme disease bacterium, *Borrelia burgdorferi*, is often difficult to diagnose accurately on the basis of general symptoms but PCR can amplify its DNA in body fluids.

3.9 PREVENTING INFECTIOUS DISEASES

Infections are the commonest cause of human morbidity and mortality. In developing countries at the beginning of the last century diseases, such as TB, pneumonia and bacterial infections secondary to influenza, were major causes of death. However, in the developed world their impact has been lessened by public health measures such as improved housing, better sanitation and advanced social and economic conditions. Unfortunately, in developing countries infectious diseases, such as malaria, TB, respiratory and GIT infections, are still major causes of death, particularly in children.

The prevention of infectious disease is achieved by the use of vaccines (*Chapter 4*). Vaccination works by stimulating the immune system to produce antibodies against the pathogenic organism by introducing bacteria or viruses that have been rendered 'harmless' in some way. The simplest, though not necessarily the most effective, method of preparing vaccines is by killing whole microorganisms. These are then injected into the host to induce an immune response. An example of a 'killed' vaccine is that used to protect against whooping cough caused by *Bordetella pertussis*. In some instances the immune response produced against dead organisms is insufficient to induce good immunity. This is usually because killing the microorganism often involves denaturing their proteins so while the immune response recognizes the denatured proteins it does not react to the native proteins on the pathogen. To overcome this problem, live but attenuated (weakened) microorganisms may be used. These microorganisms are less virulent and, in most cases, stimulate an effective immune response in the host. This type of approach is used for the combined vaccine against measles, mumps and rubella (MMR) and for the oral vaccine against poliomyelitis (*Margin Note 3.4*).

Some patients do not develop an effective immune response to these weakened microorganisms and, unfortunately, the weakened microorganisms can become virulent again, a phenomenon known as **reversion**. To overcome this problem, selected proteins, from, for example, bacterial capsules or viral envelopes, are extracted from the microorganism and used as vaccines. These are known as subunit vaccines. Unfortunately, bacterial capsular polysaccharide is often poor at stimulating immunity and a recent development is to render the vaccine more immunogenic by attaching the polysaccharide to an immunogenic protein. Such vaccines are known as **conjugate** vaccines: examples include the most recent vaccines against *Neisseria meningitidis* serogroup C, and *Haemophilus influenzae*.

Subunit vaccines against viral proteins can now be produced more cheaply by employing recombinant DNA techniques. In such cases, nucleic acid coding for the protein in question is isolated and cloned. This DNA is then transfected into a suitable microorganism which can be cultured and induced to synthesize

Margin Note 3.3

The extreme sensitivity of PCR means it can even diagnose the diseases on old and therefore largely degraded samples. The former USA vice president and presidential candidate Hubert H. Humphrey (1911–1978) underwent tests for bladder cancer in 1967. However, these gave negative results and, untreated, he died of the disease. A urine sample taken in 1967 and a tissue sample from his cancer-ridden bladder obtained in 1976 were analyzed retrospectively with PCR amplification in 1994. The DNA of both samples showed identical mutations in the p53 gene (*Chapter 17*). This is a well-established tumor suppressing gene. Had it been possible to diagnose the cancer in 1967, then Humphrey could have received the benefits of the then current treatments and his life may have been extended. This is, of course, only one of many examples where advances in biomedical sciences have greatly improved clinical practice.

Margin Note 3.4 Baby and childhood vaccination programmes

Most countries of the developed world have baby and childhood vaccination programmes that protect against a variety of potentially life-threatening conditions. In early 2006, the UK government announced it was amending its vaccination programme for children less than two years old by adding a new vaccine to its programme that protects against the Pneumococcus bacterium, which causes ear infections, pneumonia and meningitis. This would give the UK a fairly typical baby vaccination programme, as follows. At the age of two months, the baby is given a vaccine against Pneumococcus and a five-in-one vaccine that protects against Hib, diphtheria, polio, tetanus and whooping cough. At three months, a meningitis C vaccine and a five-in-one booster are administered. These are followed a month later with another five-in-one booster and boosters for meningitis C and Pneumococcus. When the baby is one year old, he or she receives a combined Hib/meningitis C vaccine, which is followed a month later with the MMR (combined mumps, measles and rubella) vaccine and another Pneumococcal booster.

the protein *in vitro*. An example is the recombinant vaccine against Hepatitis B produced in the yeast *Saccharomyces cerevisiae* that have been transfected with a gene encoding the S (surface) protein of the virus. More recently, DNA vaccines, which consist of viral genes transfected into bacterial plasmids and injected directly into muscle, have been undergoing clinical trials, although, as yet, none of these is routinely available.

For those infectious diseases where toxins (*Chapter 2*), rather than the microorganism, are responsible for the disease, vaccines may be prepared against chemically modified or heat inactivated toxins. These inactive toxins, known as **toxoids**, are then used for vaccination purposes and, indeed, this is the approach used for diphtheria and tetanus vaccines.

3.10 CONTROLLING THE SPREAD OF PATHOGENS

Controlling the spread of an infectious agent is a complex process, requiring a number of interventions, some of which will depend on the microorganism involved. Epidemiological investigations (*Chapter 1*) can help to pinpoint the source of the infection. Strict hygiene precautions, for example thorough cleaning and disinfection of contaminated materials, and hand washing, can prevent the spread of pathogens between patients. The boiling of drinking water may be necessary, depending on the source of the outbreak. Routine microbiological investigations may be carried out to ensure that the microbe is under control. For the patient, antibiotics or antiviral drugs (*Section 3.11*) may be given, while patient contacts may be offered vaccination and/or drug treatment. Examples of this multifactorial approach can be seen following outbreaks of *Neisseria meningitidis*, in schools and colleges, where all contacts with infected patients are offered antibiotics and vaccines, depending on the serotype.

3.11 TREATMENT OF INFECTIOUS DISEASES

The treatment of infectious disease is almost entirely pharmacological although nursing care is obviously necessary in many cases and surgery may sometimes be used. The development and extensive uses of antibiotics and other drugs over the last 60 years in particular has had an enormous impact in reducing the number and effects of infectious diseases. Viruses and bacteria are the most important causes of infectious diseases in developed countries, while fungi, protozoa and helminths are of increased importance in the tropical climates of developing countries.

ANTIVIRAL DRUGS

Viruses are acellular and can only replicate by utilizing the metabolic processes of their host cell. It is difficult to target viruses inside host cells without damaging both infected and normal host cells hence there are relatively few effective antiviral agents. To be fully effective, antiviral drugs should ideally inhibit viral replication but not affect the reproduction of the host cell. Unfortunately, current antiviral drugs are not fully effective as all interfere to some extent with reproduction of the host cell and so produce adverse effects. Antiviral agents do not normally directly 'kill' the virus but, rather, inhibit their replication. Therefore, they must be administered for sufficient time to allow natural immune mechanisms to eradicate all the virions present. Thus antiviral therapy may well fail in severely immunocompromized patients.

Most antiviral agents act by disrupting one of the steps in the replication cycle of the target virus. They may prevent viral internalization into the host cell. If the virion enters its host cell, other antiviral agents can interfere with

the release of the viral nucleic acid from the capsid. Some antiviral agents prevent the synthesis of the viral nucleic acid or its proteins. Even if the virus is successfully synthesized, agents that interfere with its release from the host cell can prevent its dissemination. Lastly, some antivirals can help promote a more effective immune response against viral infection. *Table 3.3* lists examples of each type of antiviral agents.

Mode of action	Example of agent
Prevent internalization	γ-globulin, zanamovir
Inhibit uncoating	p133
Prevent viral nucleic acid synthesis	amantadine, gancyclovir, rimantadine, vidarabine
Inhibit viral protein synthesis	indinavir, ritonavir, saquinavir
Interfere with virion release	4-guanidino-Neu5Ac2en
Promote immune response	α-interferon

Table 3.3 The mode of action of antiviral agents with selected examples

Viruses can become resistant to specific antiviral agents (*Box 3.4*). Hence a therapy combining several agents, each of which acts at a different stage in the replication cycle is likely to be a more effective treatment. For example, HIV treatment is typically a combination of the antiHIV proteases (*Figure 3.31*) amprenavir, ritonavir and the antiHIV reverse transcriptase inhibitor AZT, which is a ribonucleoside analog (*Figure 3.32* and *Box 3.1*).

Figure 3.31 Molecular model of HIV protease with a bound inhibitor. PDB file 1HII.

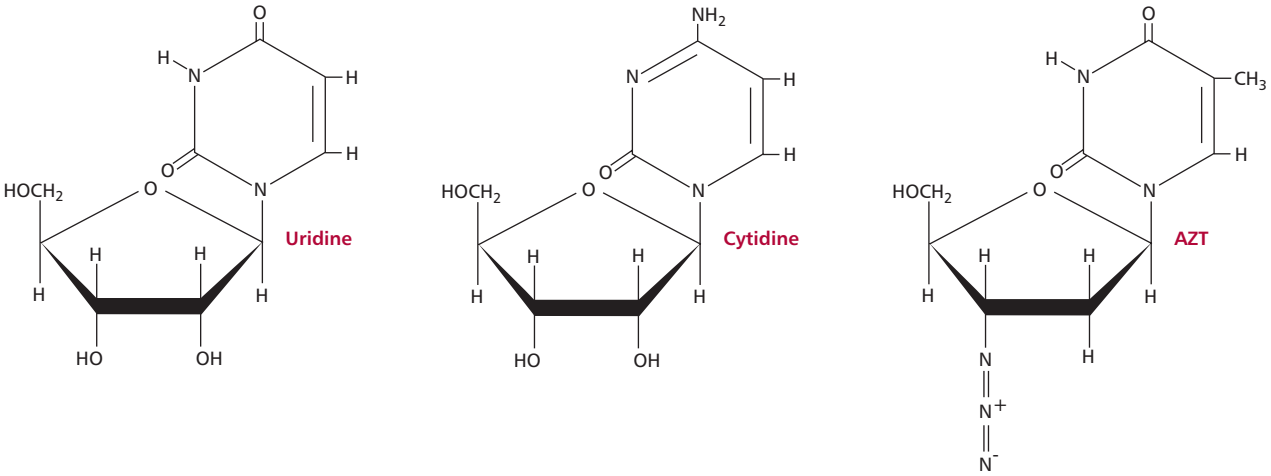


Figure 3.32 Structures of the ribonucleosides uridine and cytidine and the antiHIV drug, AZT.

ANTIBACTERIAL DRUGS

Drugs to treat bacterial diseases are generally called **antibiotics**. They are the most significant in clinical practice because bacteria are responsible for the greater proportion of infectious diseases. Most antibiotics are derived from the products of the metabolism of microorganisms, such as certain bacteria and fungi. Their actions usually rely on differences between microbial and host cells. Ideally, antibiotic drugs should kill the target bacterium, that is, they should be **bactericidal**. **Bacteriostatic** drugs prevent the bacteria replicating and must be administered for sufficient time to allow the immune defenses of the body to eliminate the pathogen.

Figure 3.33 Molecular model of rifampicin (a rifamycin antibiotic) shown in red, bound to an RNA polymerase. The spheres represent magnesium and zinc atoms. PDB file 1I6V.

Not all antibiotics are effective against all pathogenic bacteria. Some are only effective against Gram-negative bacteria, whereas others are effective against Gram-positive only (*see later*). Other antibiotics can be used to treat a range of both Gram-positive and negative organisms and are referred to as **broad spectrum** antibiotics. Antibacterial drugs generally act in one of four major ways: they may inhibit the synthesis of the bacterial nucleic acid, proteins or the cell wall or they may act in a variety of rather miscellaneous and, in some cases, unknown mechanisms.

Many drugs inhibit both replication and transcription at several points. These drugs fall into a number of general families. Sulphonamides inhibit the formation of folate which is essential for the synthesis of precursors of nucleic acids. Nitroimidazoles bind directly to DNA and denature its helical structure such that it is no longer a substrate for DNA-binding proteins. Clofazimine, a drug which is used to treat leprosy, also binds to DNA preventing replication and transcription, though it is not a nitroimidazole. Quinolones, nalidixic acid and norfloxin, and the synthetic antibiotic ciprofloxacin, fluoroquinolones, offloxacin, norfloxacin and others, are inhibitors of DNA topoisomerase II, an enzyme essential for the replication and transcription of DNA. The rifamycins are inhibitors of RNA polymerases (*Figure 3.33*) and suppress transcription.

Protein synthesis begins with the translation of messenger RNA molecules by ribosomes to form polypeptides. Translation is broadly similar in both bacterial and mammalian cells though there are some significant differences between the two types of cells. For instance, bacterial ribosomes consist of 30S and 50S subunits (*Figure 3.34*), whereas eukaryotic ones have larger 60S and 40S subunits. A number of the protein translation factors necessary for translation also differ. These differences are exploited by a number of antibacterial drugs.

The main antibacterial antibiotics that interfere with protein synthesis are the aminoglycosides, lincosamides, macrolides and tetracyclines. The streptogramin quinupristin-dalfopristin, a relatively newly introduced drug, also interferes with protein synthesis. Aminoglycosides, such as streptomycin and gentamicin, are bactericidal and have complex effects following irreversible binding to specific proteins of the 30S subunit. They inhibit protein synthesis by interfering with initiation, inhibiting an essential checking step called proofreading performed by the ribosome so incorrect amino acids are inserted into the polypeptide leading to the production of nonfunctional or toxic peptides, inhibit elongation and prevent ribosomes associating together as functional polyribosomes. Clindamycin and lincomycin are lincosamides. These antibiotics can be bacteriostatic or bactericidal. They interfere with the first and subsequent steps in translation within the ribosome. Macrolides may be bacteriostatic or bactericidal. Examples include the widely used erythromycin and azithromycin and clarithromycin. They prevent translocation, that is, the movement of the peptide-tRNA complex within the ribosome. The tetracyclines are a well known group of drugs and include the parent tetracycline itself, as well as other antibiotics, such as doxycycline and oxytetracycline. Their action is bacteriostatic in that they inhibit the binding of the aminoacyl-tRNA complex to the 30S subunit and so slow down translation. Streptogramins type A and B (dalfopristin and quinupristin respectively), produced by *Streptomyces pristinaepiralis*, are chemically modified to give the drug quinupristin-dalfopristin. Quinupristin and dalfopristin alone each show weak bacteriostatic activities, however, together their actions are synergistic since they both target different site/actions of the 50S ribosome of bacteria, inhibit protein synthesis and lead to the release of incomplete polypeptides. Dalfopristin binds directly within the peptidyl transferase center of the ribosome interfering with the binding of tRNA molecules and inhibiting

elongation of the polypeptide, while quinupristin blocks the exit tunnel of the polypeptide from the ribosome. Quinupristin-dalfopristin was licensed for use in the UK and USA in the late 1990s for treating severe infections with Gram-positive organisms, including nosocomial pneumonia and infections related to the use of intravascular catheters. It is particularly useful for treating complicated skin infections with methicillin-resistant *Staphylococcus aureus* and *Streptococcus pyogenes* and life-threatening infections of vancomycin-resistant *Enterococcus faecium* (*Box 3.4*). Quinupristin-dalfopristin has poor activity against *Enterococcus faecalis* compared with *Enterococcus faecium*. The latter is generally a less serious pathogen and other treatments are available, even though the former is the more prevalent clinically. However, strains of *Enterococcus faecium* resistant to quinupristin-dalfopristin are being found.

Several other antibiotics that interfere with protein synthesis are also in clinical use. Fusidic acid, a bactericidal agent that is used only in the treatment of gonorrhea, inhibits translocation. Chloramphenicol is a widely known antibiotic although its action is bacteriostatic. It inhibits the formation of peptide bonds by the ribosome. Lastly, spectinomycin, used in the treatment of penicillin-resistant staphylococcal infections, prevents translocation.

Human cells, like all animal cells, lack a cell wall. This means that metabolic processes in the bacterial cell concerned with wall synthesis are excellent targets for specific antibacterial agents. The bacterial cell wall forms a protective bag around each microbial cell that prevents osmotic lysis. The wall contains layers of peptidoglycan, each of which consists of rows of amino sugars linked together by short peptides. Gram-negative bacterial cell walls have only a single layer of peptidoglycan. In contrast, those of Gram-positive organisms may have as many as 40.

Bacterial growth involves cell division and entails the breakdown of the cell wall by bacterial enzymes, followed by synthesis of new peptidoglycan. Antibiotics, such as the β-lactams, that inhibit cell wall synthesis are almost all bactericidal since their presence stops new peptidoglycan formation and therefore affects cell wall synthesis while bacterial enzymes continue to break down the existing cell wall. β-Lactams are the largest, most widely used class of antibacterial antibiotics and contain the best known antibiotics, the penicillins and cephalosporins. They are, of course, only active against growing bacterial cells. All contain a chemical structure known as a β-lactam ring (*Figure 3.35*) responsible for their antibacterial effects. β-Lactams are irreversible inhibitors of transpeptidase, the enzyme that catalyzes the cross-link between the sugar residues and peptides in the peptidoglycan layer(s). A number of non-β-lactam antibiotics also reduce the efficiency of bacterial cell wall synthesis. They inhibit a number of different enzyme catalyzed steps in the synthesis at a wide variety of sites; hence they have no common mechanism of action. Examples of these drugs include cycloserine, vancomycin, fosfomycin and isoniazid (*Figure 10.29*) among others.

A rather miscellaneous grouping of antibiotics includes the polymyxins, nitrofurantoin, pyrazinamide and metronidazole. Polymyxins are effective against Gram-negative bacteria where they disrupt the structure of the cell membrane. Nitrofurantoin may be considered a prodrug given the need for bacterial enzymes to metabolize it to an active form that is thought to damage bacterial DNA. Pyrazinamide is used to treat TB and acts by an unknown mechanism although TB treatment, in general, requires prolonged therapy with a combination of antibiotics (*see later*). Metronidazole is an effective drug against anaerobic bacteria and some protozoan parasites. The unionized form of metronidazole is readily taken up by these organisms, which possess electron transport systems able to reduce it to an active form that disrupts the helical structure of DNA, inhibiting bacterial nucleic acid

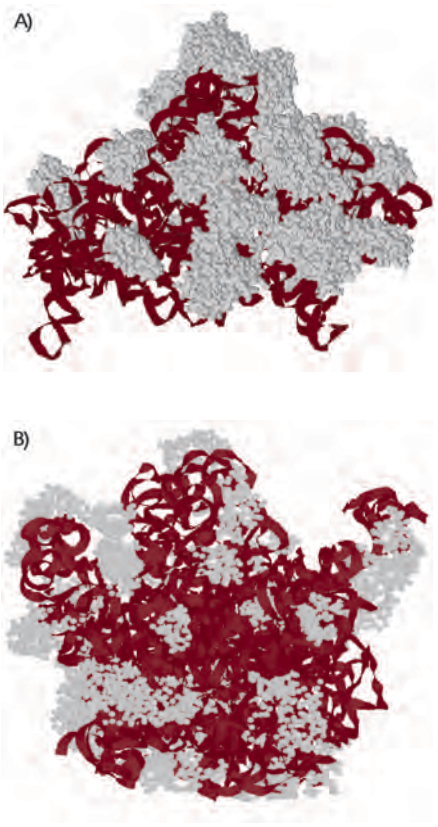


Figure 3.34 Molecular models of the (A) small and (B) large ribosomal subunits of *Escherichia coli*. PDB files 1P87 and 1P86 respectively.

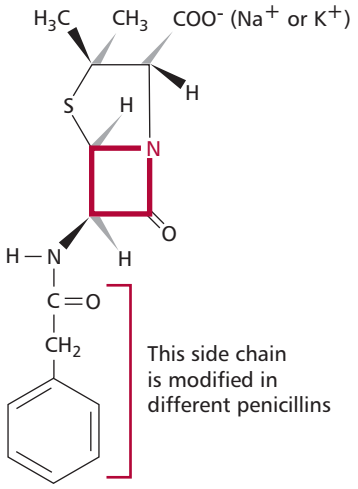


Figure 3.35 Natural penicillin. The β-lactam ring is shown in red.

Margin Note 3.5 Fleming and penicillin

The antibacterial activity of penicillin was discovered by Fleming (1881–1955) in 1928 at St Mary's Hospital in London. During this period, Fleming went on holiday and left some cultures of staphylococci on agar plates unwashed. When he returned, he noticed a fungal contaminant growing on one of the plates that was inhibiting the growth of the bacteria around it. Fleming realized that the mold was secreting a substance into the agar which was preventing the bacteria from growing. He called this substance **penicillin** after the contaminating mold, *Penicillium notatum* (*Figure 3.36*). However, Fleming was unable to isolate the substance and its purification did not occur until many years later following extensive work by Chain and Florey in Oxford during World War II. The significance of Fleming's discovery of the first antibiotic was quickly appreciated and, among many honors, Fleming was elected Fellow of the Royal Society in 1943 and knighted in 1944. In 1945 Fleming, Chain and Florey were jointly awarded the Nobel Prize in Physiology or Medicine for their discovery of penicillin and its curative effects.

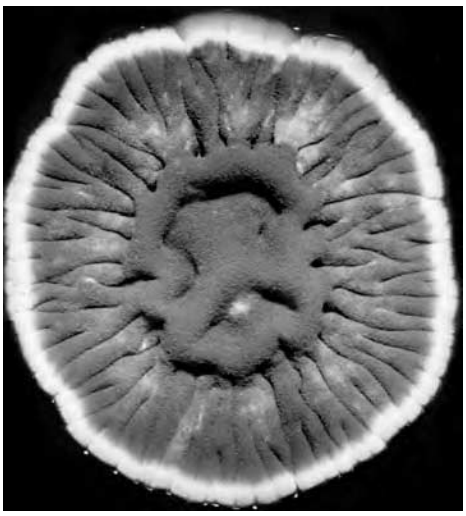


Figure 3.36 Penicillium mold growing on agar.

synthesis. Metronidazole is used alone or combined with other antibiotics to treat abscesses in the liver, pelvis, abdomen and brain caused by susceptible anaerobic bacteria and colon infections caused by *Clostridium difficile*, *Giardia* infections of the small intestine, amebic liver abscesses and amebic dysentery and *Trichomonas vaginalis* vaginal infections, and both male and female carriers of trichomonas.

Despite their specificity, antibiotics can cause toxicity in humans including allergic responses to the penicillins and sulphonamides, ear and kidney damage by aminoglycosides (*Box 3.3*), while chloramphenicol can show liver and bone marrow toxicities causing serious hematological diseases, particularly aplastic anemia. This can cause the death of rare susceptible individuals.

ANTIFUNGAL, ANTIPROTOZOAL AND ANTHELMINTHIC DRUGS

Fungi, protozoa and helminth parasites are responsible for many infections, particularly in the developing world. Given that they are all eukaryotic, then drugs to treat them are prone to act against host cells and they often have side effects. These drugs may kill the parasite or simply inhibit its growth. In the latter case, therapy must be continued for sufficient time to allow the host immune system to eradicate the organism.

Most antifungal drugs are not fungistatic but are fungicidal. One fungistatic drug, griseofulvin, inhibits intracellular transport and mitosis in fungi by interfering with the functions of their microtubules. A comparatively large number of fungicidal drugs suppress the synthesis of the essential cell membrane constituent, ergosterol. These include the allylamine antifungals, terbinafine and naftifine; the imidazoles, clotrimazole, econazole, ketoconazole and miconazole and the triazoles fluconazole and itraconazole. Ciclopiroxolamine inhibits the synthesis of fungal cell membrane proteins. The polyene antifungals, amphotericin and nystatin insert into plasma membranes of susceptible fungi. This increases the permeability of the membranes, allowing water and ions to leak and kill the parasite. Fluorocytosine inhibits the synthesis of fungal DNA.

In many cases, the precise biochemical mechanisms of antiprotozoal drugs are not known in any great detail. However, atovaquone inhibits electron transport in mitochondria. Pentamidine and isethionate interfere with the synthesis of protozoal macromolecules, while metronidazole, nifurtimox and tinidazole are thought to denature existing macromolecules. A number of other antiprotozoal therapeutic drugs are in clinical use. These variously affect protozoal enzymes, inhibit glycolysis and fatty acid oxidation or inhibit the synthesis of precursors of nucleic acids.

In general, the therapeutic bases of anthelminthic drugs are poorly understood. A number of commonly used drugs interfere with muscle contractions in the worms producing flaccid or spastic paralysis. This kills the parasite or makes it susceptible to attack by the host immune system. Paralysis is achieved by several overlapping mechanisms. Metriphonate inhibits cholinesterase leading to spastic paralysis while ivermectin potentiates inhibitory γ aminobutyric acid mediated peripheral neurotransmission and levamisole and pyrantel block nerve transmission at the neuromuscular junction. Diethylcarbamazine and piperazine cause hyperpolarization of muscle membranes. Praziquantel acts directly on muscle cells and increases the permeability of muscle membranes to Ca^{2+}.

Other anthelminthic agents act through different mechanisms. Oxamniquine interacts with helminth DNA and disrupts its structure. Niclosamide inhibits mitochondrial oxidative phosphorylation in helminth parasites. The anthelminthic agents albendazole, mebendazole and thiabendazole disrupt the microtubules of the cytoskeleton of the helminth.

BOX 3.3 Ototoxicity and Ménière's disease

Approximately 5–10% of patients treated with aminoglycosides, of which the best known examples are streptomycin and gentamicin, experience side effects, involving hearing, balance and the renal functions. **Ototoxicity**, that is, drug or chemical damage to the inner ear (*Figure 3.37*), can result in hearing loss or tinnitus and can lead to a loss of balance and feelings of dizziness. The extent of ototoxicity varies with the drug, its dose and other clinical conditions. In the majority of cases the damage is minor and reversible once medication ceases. In other cases, the extent of damage is limited, for example high-frequency hearing loss, where the damage to the ear makes it difficult to hear high pitched musical notes, but does not affect the ability to hear the spoken word or converse. In extreme cases, there may be complete and permanent deafness.

Ototoxicity is obviously undesirable; however, the ear damage associated with aminoglycosides can help some patients who suffer from Ménière's disease. Ménière's disease is named after the French physician Ménière (1799–1862). In the 1860s he theorized that attacks of vertigo, tinnitus and hearing loss arose from problems in the inner ear. The disease is an idiopathic syndrome, although some patients can identify triggers that can induce or aggravate symptoms, of endolymphatic hydrops, a condition in which abnormally large amounts of endolymph collect in the inner ear. Its symptoms are recurring episodes of hearing loss, tinnitus, rotational vertigo (a form of dizziness), nausea and a sense of pressure in the middle ear. Ménière's disease affects adults from the age of 20 years but is commonest in patients in their 40s and 50s. Given its generalized symptoms, the criteria used in diagnosing Ménière's disease are variable and estimating its incidence is difficult, although it is thought to be 0.5 to 7.5 per 1000. The incidence varies by ethnic background and is commoner in Britain and Sweden but it is known to affect black and Oriental ethnic groups.

Betahistine may be used to control Ménière's disease. Avoidance of triggers can reduce the frequency and duration of symptoms and episodes but not all episodes can be attributed to triggers. Conservative treatments for Ménière's disease involve a reduced sodium diet and diuretics to control water retention and reduce inner ear fluid pressure, and medications to reduce the vertigo, nausea/vomiting, or both during an episode. Vestibular rehabilitation therapy to help retrain the body and brain to process balance information can help with the poor balance that afflicts patients between attacks. Devices that deliver a series of low-pressure air pulses designed to displace inner ear fluid may also be used. In 20–40% of patients, conservative treatments are ineffective and a chemical labyrinthectomy may be performed with ototoxic aminoglycosides to control the vertigo associated with the affected ear but cause less damage to the hearing mechanism than some traditional treatments. Gentamicin is injected through the tympanic membrane into the middle ear from where it can diffuse into the inner ear and destroy some or all of the balance cells. In patients with Ménière's disease of both ears, streptomycin can be given intramuscularly and will have an effect on both ears. About 10% of patients require surgery, which can be used to relieve the pressure on the inner ear or to block the transfer of information from the affected ear to the brain.

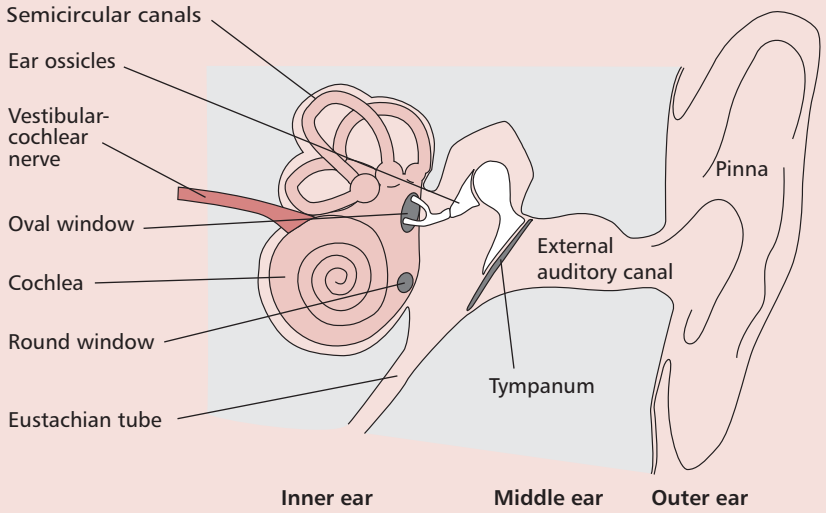


Figure 3.37 Schematic showing structure of the ear with the outer, middle and inner parts clearly separated.

Margin Note 3.6 Necrotizing fasciitis

The condition necrotizing fasciitis is an infection with the so-called 'flesh-eating bacteria', for example Group A Streptococci (GAS), most commonly *Streptococcus pyogenes*. The prevalence and incidence of this condition are both extremely rare but can begin after surgery, particularly abdominal or gynecological interventions. However, it may also develop from complications of childbirth, burns or following relatively minor traumas, for example bites and abscesses. The bacteria produce extracellular enzymes that attack soft tissues, often in an extremity, destroying muscle, fat and skin, causing an extensive necrosis of subcutaneous tissue. The lysosomal hydrolytic enzymes (*Chapter 16*) released from damaged cells of the patient may exacerbate the bacterial damage. The condition can be diagnosed by culturing the bacteria from blood samples or aspirations of pus from affected sites although surgical exploration may be necessary. Necrotizing fasciitis can have such a sudden and rapid onset that extent of destruction of soft tissues may quickly kill the patient. Hence an early diagnosis and prompt medical and surgical interventions are necessary to reduce the risk of death. Treatment often includes intravenous penicillin and clindamycin, along with aggressive surgical **debridement**, the removal of infected tissue, which can be very extensive. For those with severe illness, confinement in an intensive care unit is needed. Limb amputation may be necessary. Unfortunately, approximately 20% of patients who suffer necrotizing fasciitis die of the condition.

COMBINATION THERAPY

Combination therapy is the treatment of infections with two or more drugs usually to increase the clinical efficacy of the treatment, for example as described above for quinupristin-dalfopristin, or to minimize the development of resistant strains of the infective organism. Where the infection is of unknown origin, then multiple therapies are advisable to fight the most likely pathogens. With mixed infections involving two or more known pathogens, it is desirable to target each microorganism with one or more different antibiotics. Combination therapy may be used even if only a single infective pathogen is present as using combinations of drugs can prevent or delay the development of resistance to the drugs being used (*Box 3.4*). For example, some bacteria are resistant to β-lactams because they produce a β-lactamase that catalyzes the breakdown of the β-lactam ring. Combining an inhibitor of β-lactamase, such as clavulanic acid, with the β-lactam antibiotic helps preserve the drug *in vivo*. Other drugs commonly combined in therapeutic use are sulphamethoxazole and trimethoprim that synergistically inhibit the synthesis of folate by blocking different steps in its synthesis. The cocktail of isoniazid, rifampicin and pyrazinamide is used in the treatment of TB, while clofazimine, dapsone and rifampicin are used in combination in the therapy of leprosy.

SURGERY

Most infectious diseases can be treated using drug therapies. However, surgical intervention may be required in instances when the pathogen is resistant to available treatments or where it is the only means to contain an infection that is spreading to other areas of the body, as, for example, in gangrene caused by *Clostridium perfringens* (*Figure 3.38*) or necrotizing fasciitis (*Margin Note 3.6*). In other cases, surgery might be desirable because access to the affected site by the antimicrobial agents is limited, as in the case of some abscesses or appendectomy where surgical drainage or removal of necrotic tissue respectively can enhance the recovery process.

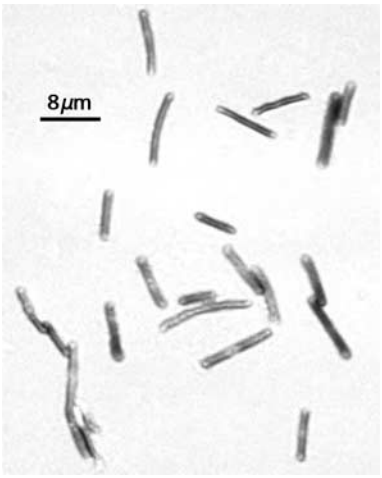


Figure 3.38 Light micrograph of *Clostridium perfringens* growing in Schaedler's broth. Courtesy of D. Stalons, Public Health Image Library, Centers for Disease Control and Prevention, USA.

BOX 3.4 Development of antidrug resistance

Drug resistance refers to the loss of effectiveness of a pharmacological agent against a pathogen. The pathogen or parasite may, of course, be initially nonsusceptible to a particular drug and this is referred to as primary resistance. Secondary or acquired resistance is that which develops over a period of time in a previously sensitive organism.

The development of secondary resistance by viruses following the chronic use of antiviral drugs is common and is normally the result of spontaneous mutations in viral genes. Many antiviral drugs target the same viral enzyme. Hence a mutation in the gene for that enzyme may render the virus resistant to several antiviral agents. In other cases, resistance to two antivirals is acquired by discrete mutations. The strain of virus that acquires resistance to one specific drug may be more susceptible to another antiviral agent. This is one reason why combination therapy involving two or more antiviral drugs maximizes the therapeutic effects, as for example, in the treatment of AIDS (*Box 3.1*). Unfortunately, given time, viral strains resistant even to combinations of antiviral drugs are likely to develop. Strains of the HIV virus resistant to the commonly used zidovudine, lamivudine and ritonavir are now present.

Like viruses, secondary or acquired resistance can also develop in previously sensitive bacteria. Indeed, the development of microorganisms that are resistant to drug treatment is well illustrated in bacteria, a number of which are now resistant to commonly used antibiotics. For example, sulphonamide resistance can be caused by a single mutation in the gene coding for dihydropterate synthase. Genes that confer resistance against a particular drug may be acquired as a result of spontaneous mutations. It may, however, occur by acquiring new genetic material from other bacteria. This genetic material may be chromosomal, most commonly transposons, or extrachromosomal, usually plasmids. Plasmids are especially significant in the transfer of antibiotic resistance between resistant and nonresistant bacteria. Further, a single plasmid may contain the genetic determinants for resistance to several drugs.

Antibiotic resistance in bacteria is achieved by four major biochemical mechanisms. There may be a decrease in the uptake or increase in the efflux of the drug by the bacterial cell. Streptococci and some anaerobe bacteria, for example, lack the electrochemical gradient across the cell membrane necessary to allow aminoglycoside entry. Some bacteria develop enzymes that are able to modify and inactivate certain drugs. The production of β-lactamase by some bacteria that renders them resistant to β-lactams has already been mentioned in the main text. However, in the 1980s it was found that *Klebsiella* spp and in some cases *Escherichia coli* were producing new β-lactamases that could hydrolyze the extended spectrum cephalosporins. These enzymes were collectively named **extended spectrum β-lactamases** (**ESBLs**).

When an antibiotic blocks a single reaction, for example, by inhibiting an enzyme, then resistance may be acquired by the bacterium acquiring mechanisms to bypass that reaction. This is the principal mechanism of resistance to sulphonamides. Bacteria may also acquire the ability to modify chemically the targets of specific drugs. Examples of the target modification include topoisomerase II, the pharmacological target of quinolones and fluoroquinolones, and ribosomal proteins targeted by aminoglycosides.

Bacteria may differ in the way they resist the actions of a drug while, in some cases, they may use more than one way of nullifying the effects of a single drug. Several clinically significant bacteria are now resistant to a number of antibiotics and, unfortunately, are sometimes spread to vulnerable patients within hospitals. An example of such a bacterium is the methicillin-resistant *Staphylococcus aureus* (MRSA). In addition to producing β-lactamase, MRSA is resistant to more than 40 β-lactams in clinical use because they contain a gene coding for an additional penicillin binding protein (PBP) which has only low affinity for the β-lactams. This allows MRSA to continue the synthesis of cell wall material during treatment with β-lactam drugs.

Enterococci are normally found in the large intestine and the female urogenital system. They can contaminate hands, equipment and the patient care environment. The recovery of enterococci from the hands of health care workers indicates that fecal-hand contact may be a major means of transmission to hospitalized patients with intravascular devices. This can lead to life-threatening bloodstream infections. Vancomycin was the drug of choice for treating such patients; however, vancomycin-resistant enterococci (VRE) were isolated in 1994. These bacteria are also resistant to many of the antibiotics previously used in treatment and patients may be affected for many months. Not surprisingly, VRE infections have become a serious health care issue. The vancomycin-resistant gene of VRE can be transmitted to other bacteria, for example *Staphylococcus aureus* and strains of this organism partially resistant to vancomycin, vancomycin-resistant *Staphylococcus aureus* (VRSA), were discovered in Japan in 1996, and in the USA and France in 1997.

Antibiotic resistance can also, of course, develop in the eukaryotic pathogens, fungi, protozoa and helminths. Resistance in the malarial parasite has been mentioned in *Chapter 2*. In all cases, the continual emergence of resistant strains of pathogens means there is a need to keep developing new pharmacological agents that are capable of treating infectious diseases.

CASE STUDY 3.1

Andrew, a 48-year-old male, presented at the Accident and Emergency Department of his local hospital. He told the examining doctor that the previous day he had experienced pain on the side of his right calf. Within 6 h, the area was septic and pus could be expressed. Over the day, the swelling and redness (inflammation) had gradually extended to cover the area from the ankle to the knee. Physical examination showed respiration, pulse, blood pressure and temperature were all within reference values. There was no obvious area of pus at the site and pus did not drain on puncturing with a 20-gauge needle. Andrew was treated with intramuscular and oral ceftriaxone. Two days later, Andrew returned with increased pain and slightly increased temperature. The center of inflammation showed an obvious pus-filled area. Culture of the pus showed the presence of clusters of Gram-positive cocci on nutrient agar and yellowish-colored colonies on 5% sheep blood agar. The area was removed by aspiration and the area excised and drained.

Questions

(a) Describe the history of this case.

(b) Why do you think antibiotic treatment alone was insufficient and that Andrew required drainage and excision at the site of infection?

CASE STUDY 3.2

Chris, a six-year-old boy, complained of a sore throat and had a temperature of 38.6°C. His mother kept him home from school and treated him with a proprietary pediatric painkiller. He slept well but awoke with similar symptoms. His mother took him to their family doctor. Physical examination showed reddening of the nasopharyngeal area and tonsils and a slight enlargement of the cervical lymph nodes. The skin was clear.

Questions

(a) What are the most likely organisms causing this disease?

(b) How could these organisms be detected?

(c) What treatment is desirable and why is this so?

CASE STUDY 3.3

After several days of recurring attacks of repeated severe coughing that left her gasping for breath and which were eventually associated with bouts of vomiting, Neha, a six-week-old baby girl, was transferred to hospital. Physical examination showed a normal trachea. A radiograph showed the chest to be clear of infection. Clinical examination showed the following data (reference values in parentheses):

- pulse of 155 min^{-1} (normal 72 for adults, but higher for babies and children)
- respiratory rate 71 min^{-1} (18 for adults, rather higher for babies and children)
- white blood cell count of 15 000 000 cm^{-3} (4 000 000 to 12 000 000 cm^{-3}).

A nasopharyngeal swab was taken. Organisms did not culture on standard media. Bacterial plaques with a mercury-like appearance were visible when charcoal blood agar was used as culture medium.

Questions

(a) What is Neha suffering from?

(b) What is the causative organism?

3.12 SUMMARY

Pathogenic microorganisms cause clinical conditions from infections of single organs or systems to systemic disease. Infections of the skin may be caused by viruses, such as papilloma and *Herpes simplex* types. Skin bacteria cause boils and abscesses and severe and chronic conditions such as leprosy. Fungi also cause a variety of skin infections, although these are not usually severe, unless the patient is immunocompromized. Severe infections of the eye include trachoma, a chlamydial disease that is the most common eye infection worldwide. Helminths, such as *Toxocara canis* can cause blindness. Viruses and bacteria may cause painful ear infections which, if recurrent, may damage hearing. Both groups of organisms are capable of infecting the CNS, sometimes resulting in encephalomyelitis by *Herpes simplex* or meningitis by *Neisseria menigitidis*. A number of viruses and bacteria cause significant respiratory infections, some of which, for example TB, are chronic. All varieties of microorganisms can infect the GIT, causing illnesses ranging from short-term gastroenteritis to stomach ulcers. An extensive number of microorganisms can infect the genital tract or are transmitted sexually. Several may cause systemic diseases.

The diagnosis of infectious disease involves a combination of laboratory tests to identify the microorganism present in specimens typically by culturing them. However, the presence of antibodies to the organisms in the patient may be used for identification and to compare levels in acute and convalescent patients. An increasing number of biochemical and molecular biological techniques have been introduced, many of which rely on identifying bacterial or viral nucleic acid in clinical specimens.

Infectious disease may be prevented by vaccination and public health measures or treatment with drugs. Depending on the infectious agent, these may be antiviral, antibacterial (antibiotics), antifungal or anthelminthic. Drugs may be given singly, or in combinations, as, for example, in the treatment of HIV patients.

QUESTIONS

1. Briefly review the causes of infections associated with the genital tract.

2. List some of the methods used in the diagnosis of infectious diseases.

3. A 19-year-old student attended a barbecue to celebrate his brother's 21st birthday. He ate a very rare steak. Two days late he began to experience pains on defecation, and to suffer from persistent diarrhea that rapidly increased in frequency and produced bloody stools. What is the student suffering from? Suggest how this condition should be treated.

4. Examine *Figure 3.27*. To which antibiotics is this strain of *Escherichia coli* resistant?

5. Compare and contrast the actions of griseofulvin and albendazole.

6. In what ways may antibiotics prevent the growth of bacterial pathogens?

7. In two words state a major way of delaying the development of antidrug resistance in pathogens.

FURTHER READING

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A useful web site on HIV and AIDS:

http://www.avert.org/historyi.htm