
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 3

# Basic microbiology

An understanding of the basic science of microbiology is essential for the optometrist. It will help him deal with matters such as:

(a) patients with 'red eye';
(b) contact lens solutions and the claims made for them by manufacturers;
(c) preventive measures following contact tonometry and foreign body removal;
(d) the constituents of eye drops and the maintenance of sterility.

The science of microbiology covers organisms invisible to the naked eye. Micro-organisms include protozoa, fungi, bacteria, rickettsia, chlamydia and viruses.

Protozoa and fungi are the only micro-organisms which have eukaryotic cells similar in structure to those of higher organisms. Such cells have inclusions like nuclei and an endoplasmic reticulum. Fungi and protozoa can be either parasitic or free-living. Bacteria are simpler cells (procaryotic cells) but some species are capable of an independent existence. However, many bacteria are parasitic or saprophytic but there are others which can exist in very simple environments. Rickettsia and chlamydiae are more simple and are obligate intracellular parasites. Viruses are the simplest and can only multiply by utilizing the host cell's biochemical systems. Of the above, it is the bacteria that have received most attention.

## Bacteria

Bacteria are important because of their ubiquity, the capability of some types to cause disease and their ability to infect and multiply in varied environments such as eyebrows. In order to avoid problems caused by bacteria, it is important to understand something of their structure, growth, environmental and metabolic requirements, classification, relationship with disease and the particular problems they can cause in the eye.

### Structure (Figures 3.1 and 3.2)

The cytoplasm of bacterial cells is notable because of the absence of discrete structures normally found in eucaryotic cells. There are no mitochondria; the respiratory enzymes are instead located on the cell membrane.

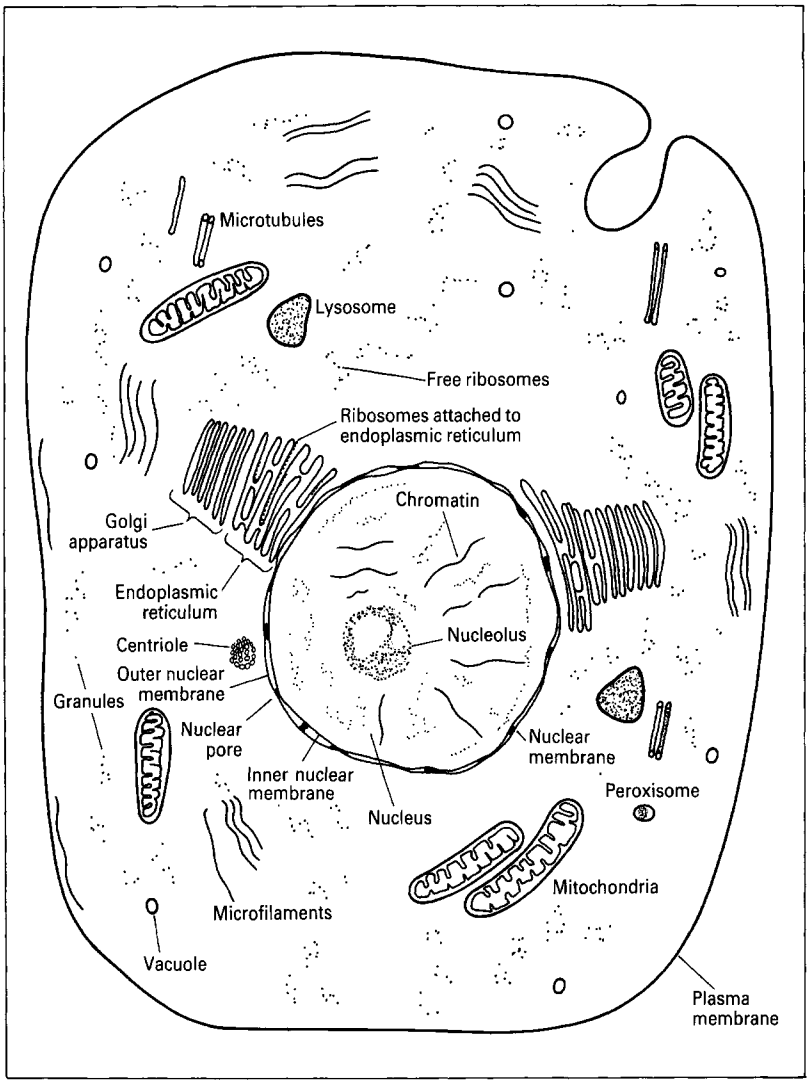


**Figure 3.1** Eucaryotic cell

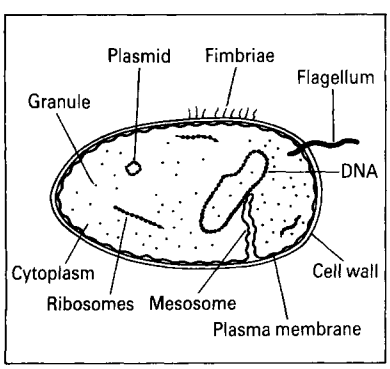


**Figure 3.2** Protocaryotic cell

There is no endoplasmic reticulum and the ribosomes are found free in the cytoplasm. There is also no nucleus and no nuclear membrane, and when the cell divides there is no mitosis. Genetic material is carried on a single strand of DNA.

Surrounding the cytoplasm is a thin, selectively permeable, lipoprotein, elastic membrane — the plasma membrane. It is the site of action of many of the bacterial enzymes and controls entry of substances into the cell.

Being elastic, the cell membrane does not determine the shape of the bacterial cell. This is the function of the cell wall, a rigid, permeable structure principally composed of a substance called murein. Because of the osmotic pressure of the cytoplasm, the cell membrane is usually pushed hard against the inside of the cell wall by a pressure of up to 20 atmospheres. The cell wall is relatively thick, especially in Gram positive bacteria.

In some bacteria the cell wall is surrounded by the capsule or slime layer. This is a poorly organized layer of large molecules such as polysaccharides or polypeptides. The effect of this layer is to impede the ingress of substances (useful and harmful) into the cell wall. The result is that the cells tend to grow and divide at a slower rate but are more resistant to antibacterial chemicals, viruses (bacteriophages), phagocytes and other adverse agents. It may also inhibit antibody formation against the bacteria, thereby rendering the bacteria more harmful to the body. Such a bacterium is *Mycobacterium tuberculosum*, the causative organism of tuberculosis.

On the outside of some types of bacteria are found flagella. The number of flagella per cell is constant for each species. Flagella are long filamentous structures containing a contractile protein, flagellin, which is similar to muscle myosin. The presence of flagella normally confers the ability or motion which it is assumed allows the bacterium to migrate to better environments.

Other bacteria have the ability to move without possessing flagella. These are the spiral forms which move by twisting the whole body.

## Environmental and metabolic requirements

Bacteria are ubiquitous and can exist in many environments that are far too hostile for the cells of higher organisms. More fastidious bacteria have requirements closer to those of the internal environment of animals and hence are more likely to be parasitic and pathogenic.

### *Nutritional requirements*

All organisms have a requirement for carbon hydrogen, oxygen and nitrogen. Since hydrogen and oxygen can be obtained from water, it is the requirement for the other elements that is most critical. Some bacterial species can obtain their nutrient requirements from inorganic sources. They obtain energy from other sources, e.g. bacterial chlorophyll. Others have the ability to utilize inorganic nitrogen providing they are supplied with an organic source of carbon. Such organisms are found in soil and are responsible for maintaining its fertility.

Others require both organic carbon and nitrogen to survive. Pathogenic bacteria need other complicated growth factors and minerals.

### *Oxygen requirement*

Although oxygen can be obtained from water, some types of bacteria have a need for atmospheric oxygen and cannot exist without it. These bacteria are termed

obligate aerobes. Others are the exact opposite and cannot exist in the presence of oxygen, requiring anaerobic situations. The majority, however, are facultative anaerobes, which means they can exist in either the absence or presence of oxygen.

*Physical conditions*

For both pH and temperature different bacteria can exist at both high and low extremes. Pathogens prefer the medium state of pH7 and 37°C. Acidophilic bacteria prefer a low pH, while basophiles like a high one. Thermophilic bacteria grow best at between 55 and 80°C, while the spores of *Bacillus stearothermophilus* can withstand boiling. Psychrophilic bacteria grow at 0°C.

## Growth

Reproduction of bacteria is by binary fission. The cell divides and two equal daughter cells are formed. As there is no nucleus there is no mitosis. The time between a daughter cell being formed and itself dividing to form two new cells is called the generation time and varies greatly between species. It also varies with environmental conditions and the supply of nutrients. Some bacteria multiply very quickly and divide every 20 minutes. Others, like *M. tuberculosus*, take hours or even days.

When a new sterile environment with finite limits is colonized the bacterial cell population goes through four phases (Figure 3.3):

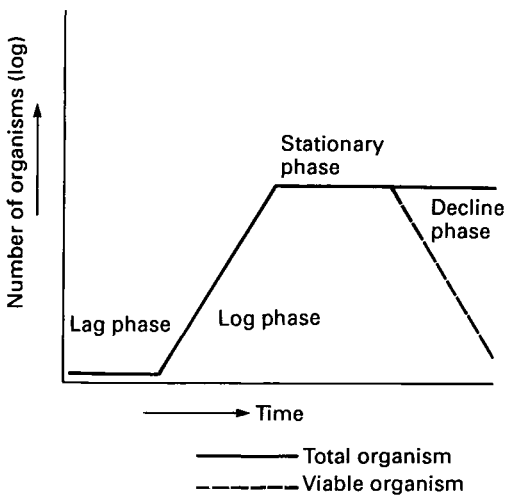


**Figure 3.3** Bacterial growth

(a) Lag phase, when the original innoculum remains dormant and no increase in numbers is seen.

(b) Log phase, during which there is an exponential growth in the number of organisms and the logarithm of the number of cells is directly proportional to time. It is during the early stages of this phase that the bacterial population is most susceptible to antibacterial agents.

(c) Stationary phase represents a time when the number of viable organisms remains constant because the number of new organisms is equal to the number

dying. This phase can be brought about by a depletion of essential nutrients, a change in the oxygen level or an accumulation of metabolites which regulate the growth.

(d) Decline phase, in which the number of viable organisms declines.

*Sporulation*

It is the property of certain bacteria to produce endospores. They are produced inside the vegetative cell as compact masses with a very resistant coat. Once formed, the rest of the cell disintegrates releasing the spore.

Spores have the ability to withstand adverse environments which would be lethal to the vegetative cell. When the conditions are right the spore germinates into one vegetative cell (sporulation is not a form of multiplication).

## Classification

Once a pure colony of an organism has been isolated by successive culturing, it is often necessary to find out which organism is present. Not only genus and species require elucidation but also the particular strain. To elicit this information the following techniques can be used:

(a) Microscopy and staining;
(b) Differential media and biochemical tests;
(c) Serological testing;
(d) Bacteriophage typing.

*Microscopy and differential staining*

Gram's stain divides bacteria into Gram positive and Gram negative bacteria. Bacteria are fixed onto a microscopy slide and stained with a dark purple stain. The slide is then covered with an iodine solution to act as a mordant, i.e. to fix the stain onto the organisms. The next step is the decolourizing process in which the slide is treated with a solvent. A counterstain completes the process and the slide is viewed under the microscope. If the organism has resisted decolourization it is termed Gram positive and will appear purple under the microscope. If the original stain has been lost the colour of the counterstain will show through and the organism will be deemed Gram negative. This is a fundamental method of classifying bacteria.

Other differential stains have been used, e.g. acid fast staining, in which the organisms are subjected to a decolourizing process using acid. Specific stains can be used to show the presence of spore-forming bacteria.

Examination under the microscope not only gives information about the organisms' staining characteristics but, of course, about the shape. Basically, bacteria can be spherical (cocci), rod-shaped (bacilli) or spiral. Cocci can be divided according to their form of aggregation. Some bacteria appear in just one direction and form chains (Streptococci) while others give the appearance of a bunch of grapes (Staphylococci). However, the appearance of aggregations under the microscope can sometimes be deceptive and other tests are necessary to differentiate between Streptococci and Staphylococci.

*Differential media and biochemical tests*

Special media which can be designed to support the growth of some types of bacteria and not others can be useful in bacterial typing. Other tests examine the

organisms's ability to break down hydrogen peroxide, to liquify protein and to ferment certain sugars. Media containing blood are useful in differentiating Streptococci.

*Serological testing*
Bacteria possess many potentially antigenic substances and one of the body's defences against bacterial invasion is to produce antibodies to these antigens. These antibodies are specific to the antigens and this specificity can assist in the determination not only of the genus and species but also the strain of bacteria present.

*Bacteriophage typing*
Bacteriophages are viruses that attack bacteria. They invade the bacterial cell just like any other host cell. Once inside they combine with the bacterial DNA and change the genetic material. This effect can be destructive and the whole cell is taken over, producing new phage particles. Bacteriophages are species specific to the bacterium they invade.

## Viruses

Viruses are much smaller than bacteria (18–300 nm). All known bacteria will be trapped by a 0.22 μm filter (sterilizing filter). Many viruses will pass through, hence the term filtrable viruses. Viruses can infect any form of higher organism and are usually divided into: animal viruses; plant viruses; and bacteriophages.

Viruses consist of either RNA or DNA (never both), surrounded by a layer of protein or capsid (Figure 3.4). The nucleic acid may be single strand or double strand. They contain few if any enzymes and are entirely reliant on the host cell to bring about replication. They vary greatly in size and in the number of genes they carry (from three to several hundred).

### Virus reproduction

Virus reproduction does not take place by binary fission. It generally takes the following pattern:

(a) The virus particle (virion) becomes adsorbed onto the surface of the cell. There are usually specific receptors involved and this leads to the viral preference for certain cells within the host. For example, the HIV (AIDS) virus binds to CD4 receptors which are found on T cells.

(b) The virus particle passes into the cell either with or without its capsid.

(c) The viral RNA (RNA viruses) brings about the production of certain essential enzymes such as RNA-replicase.

(d) These enzymes in turn bring about the production of new nucleic acid and new protein sheaths. The host cell's DNA is unaffected.

(e) Assembly of new virus particles takes place within the host cell and these are then released. The release may bring about disruption of the host cell. The new virus particles are available to infect new cells.

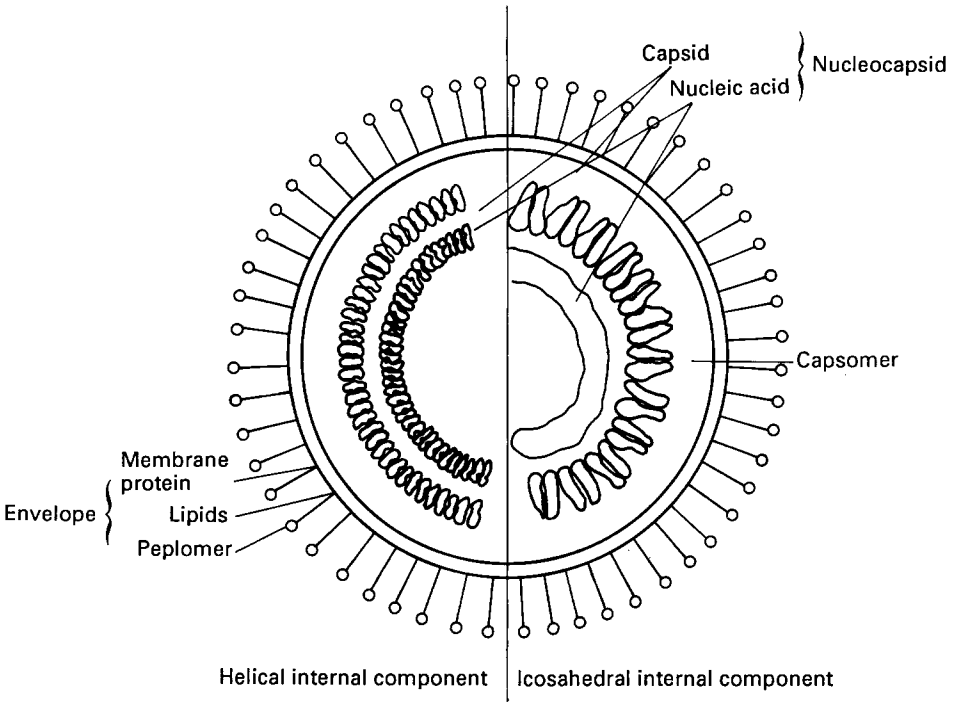


**Figure 3.4** Diagram of a virus

## Classification of viruses

The classification of viruses is more difficult than that of bacteria. Many criteria are used in classifying viruses.

(a) Morphology
(b) Nucleic acid type
(c) Immunological properties
(d) Transmission methods
(e) Host and cell tropisms
(f) Symptomatology and pathology.

The following are the main groups of animal viruses:

*Single strand DNA viruses*
Parvo viruses

*Double strand DNA viruses*
Papova viruses, e.g. papilloma (wart virus)
Adeno viruses, e.g. adeno virus 8 -epidemic keratoconjunctivitis
Herpes viruses, e.g. Herpes simplex
Pox viruses

*Single strand RNA viruses*
Picorna, e.g. poliomyelitis

*Double strand RNA viruses*
Toga viruses, e.g. human respiratory disease
Arena viruses, e.g. Lassa fever
Corona viruses, e.g. human respiratory disease
Retro viruses, e.g. HIV (AIDS)
Bunya viruses, e.g. insect borne diseases
Orthomyxo viruses, e.g. influenza
Paramyxo viruses, e.g. measles, mumps
Rhabdo viruses, e.g. rabies

## Chlamydiae

These organisms are more complex than viruses but less complex than bacteria. They can only multiply in susceptible cells but unlike viruses contain both DNA and RNA. They multiply by binary fission and are susceptible to certain antibiotics.

They exist in two forms: (a) as an elementary body (300 nm) which can exist outside the body and is the infectious unit; and (b) as a reticulate body (1000 nm) which only exists inside the cell and is not infectious.

Chlamydiae attack mucous membranes and inhibit host cell protein synthesis. They rely on the host to provide ATP, which they cannot generate. They synthesize their own nucleic acids and proteins. There are two species, *C. trachomatis* (trachoma, inclusion conjunctivitis) and *C. psittaci* (psittacosis).

## Fungi

As causative organisms of disease, fungi are less important than bacteria and viruses. Out of the tens of thousands of species probably only about 100 are pathogenic. Some of these, however, are capable of producing very severe infections as well as the more trivial, such as athlete's foot. Fungi have the ability to colonize non-living structures and lead to spoilation, e.g. contact lenses.

Fungi are composed of eucaryotic cells with the normal inclusions, e.g. a nucleus and mitochondria. They can be divided into four groups according to their structure.

### Moulds

These grow as a mycelium, which is composed of filamentous multi-cellular structures called hyphae. The mycelium is divided into the vegetative mycelium, which grows into the substrate and assimilates nutrients, and the aerial mycelium, which produces the spores either asexually by budding or sexually by the fusion of two cells.

### Yeasts

These fungi occur as single cells and reproduce by budding.

### Yeast-like fungi

Both hyphae and yeast cells exist together.

### Dimorphic fungi

Dimorphic fungi exist either as yeasts or filaments. If they are grown on artificial media they appear as hyphae. When they inhabit a living host they occur as yeast cells.

## Micro-organisms and disease

The human plays host to a large number of bacteria which normally do no harm and to varying degrees contribute to the body's well-being. For example, bacteria live on the dead surface of the skin and prevent other more potentially dangerous organisms from occupying the site. In the gut, bacteria in exchange for nutrients provide the body with vitamin K essential for the production of prothrombin. These commensal organisms only maintain this mutually advantageous relationship providing they remain in their proper place. One tissue's commensal is another's pathogen.

It is not always the micro-organism itself that causes the harm, but substances that the organism produces. Some bacteria live and multiply in food, producing toxins as they do so. When the food is ingested the toxins produce adverse effects. *Clostridium botulinum* is such an organism and the toxin it produces, botulinum toxin, is fatal in minute quantities. *Staphylococcus aureus* is also capable of bringing about food intoxication.

Most micro-organisms, however, cause disease by acting as parasites on the body, gaining access by a variety of routes.

*Direct contact.* This normally means sexual contact and the disease is classed as a venereal one. This method of transmission favours the very fastidious bacteria which exist only with difficulty outside the human body. *Treponema pallidum* (syphilis) is so fastidious that it cannot be grown on lifeless media.

*Indirect transmission.* Infection is passed from one person to another by an inanimate object (fomite), e.g. bedclothes, used dressings, etc.

*Dust-borne infection.* Dust contains discarded human cells and dried water droplets. These are likely to carry bacteria, especially spores.

*Droplet infection.* Bacteria are present in the fine spray that is exhaled with forced expirations such as coughing and sneezing.

*Water borne infection.* Water is an excellent medium for transmitting infection. Public health and sanitation prevent this until a disaster interrupts the supply of clean drinking water.

*Insect borne infection.* Biting and sucking insects have the ability to take organisms from an infected host and transfer them to a new one.

*Maternal transmission.* In addition to the route mentioned above, infections can also be passed from mother to child either while the child is still in the womb or during birth.

## Microbiology of the eye

The eye is at risk from infection by opportunistic and invasive organisms via a variety of routes. In addition to congenital ocular infections, micro-organisms can gain access as a result of:

(a) Direct contact e.g. Herpes simplex;
(b) Airborne infections;
(c) Insect-borne infections, e.g. trachoma;
(d) Migration of bacteria from the nasopharynx;
(e) Metastatic infection from other loci in the body;
(f) Trauma, especially penetrating injuries;
(g) Infected contact lenses;
(h) Infected eyedrops and lotions;
(i) Infected instruments.

Obviously the lids, cornea and conjunctiva are the most exposed and hence the most at risk from infection. Infections of these tissues are far more common than those of deeper tissues. They are also less serious.

The eye is covered with a medium which contains a number of antimicrobial agents in order to reduce the incidence of infections. The result is a fairly low level of microbial contamination in the fornices. Tears contain immunoglobulins A, G and M (IgA, IgG and IgM) (Reim, 1985) in different proportions to those found in plasma, suggesting that their origin is secretory rather than just the result of filtration.

There are also two agents with marked antibacterial properties, lysozyme and beta-lysin.

Lysozyme is an enzyme capable of dissolving the cell wall of bacteria, especially Gram positive bacteria. The level of lysozyme decreases with age and is reduced in patients with dry eye syndrome (Mackie and Seal, 1976). Beta-lysin, on the other hand, acts principally on the cell membrane (Ford, Delarge and Petty, 1976) and works in concert with lysozyme. Since the cell membrane is the site of action of the bacterial enzymes, the effect is quite marked. Beta-lysin is also present in aqueous humour.

## Some common ocular pathogens

### Gram positive cocci

*Staphylococcus aureus*

Some Staphylococci are normal inhabitants of the skin and mucous membranes, whilst other species are capable of producing conditions such as boils, abscesses and even a fatal septicaemia. They are capable of bringing about a form of food poisoning by the liberation of an enterotoxin. Resistance to certain antibiotics develops easily and the term 'hospital Staph' is applied to some resistant forms.

Staphylococci are differentiated from Streptococci by the presence of an enzyme capable of breaking down hydrogen peroxide (catalase). Pathogenic Staphylococci possess coagulase, which clots blood plasma.

In the eye Staphylococci can cause infections of the lids, lacrimal apparatus, conjunctiva and cornea (Davis, Sarff and Hyndiuk, 1978). Infections of the lash follicle lead to the formation of a stye (hordeolum). Staphylococci can also produce acute or chronic blepharitis. This is sometimes associated with acute conjunctivitis (Brook, 1980; Brook *et al.*, 1979; Brown, 1978). This organism has also been found to be present in a large number of cases of ophthalmia neonatorum in one study (Jarvis, Levine and Askell, 1987). Following septicaemia Staphylococci have caused endophthalmitis (Bloomfield *et al.*, 1978).

Because *Staphylococcus aureus* is so common, it is often employed in the efficiency testing of preservative systems. *Staphylococcus epidermis* is normally considered to be a commensal and is a normal inhabitant of the skin. Unlike *Staph. aureus* it produces white colonies. Maske, Hill and Oliver (1986) found a higher than normal incidence in a group of patients with bacterial corneal ulcers. It has been suggested that *Staph. epidermis* releases a toxin to cause some of the signs of blepharitis and keratophy (McGill *et al.*, 1982).

*Streptococci*

Streptococci lack the enzyme catalase and are characterized by their ability to cause haemolysis. Complete haemolysis is brought about by beta-haemolytic Streptococci while the haemolysis produced by alpha-haemolytic species is incomplete and leads to the formation of a green pigment. There are also non-haemolytic Streptococci.

Streptococci are capable of producing local and general infections. One of the most common local infections of beta-haemolytic Streptococci is the Streptococcal sore throat which in young children can extend into the middle ear to cause otitis media. On the skin they can cause impetigo.

Beta-haemolytic Strept. infections give rise to puerperal fever, wound sepsis and endocarditis. It is fortunate that penicillin continues to be effective against many strains of Streptococci.

In the eye, infections may cause conjunctivitis, dacryoadenitis, dacryocystitis and blepharitis (Brook, 1980).

## Gram negative cocci

*Neisseriae*

The Neisseriae are a group of Gram negative bacteria which include the normal flora of the respiratory system and the pathogens which cause meningitis (*N. meningitidis*), and gonorrhoea (*N. gonorrhoeae*).

In the eye Neisseriae can infect the lids, lacrimal apparatus and conjunctiva but *N. gonorrhoea* is best known as the one-time principal cause of ophthalmia neonatorum, an infection which occurs as the infant passes down the birth canal. The disease becomes manifest between the second and fifth day after birth. The lids become swollen and there is a bilaterial purulent discharge. The lids are tightly closed and difficult to open and the acute phase lasts for 4–6 weeks. The condition is treated with topical and systemic antibacterials. If treatment is not carried out, the cornea may become involved and the eye lost. However other causative organisms and causes are more important today (Jarvis, Levine and Asbell, 1987).

## Gram positive rods

*Corynebacterium diphtheriae*
Corynebacteria are non-motile Gram positive rods which do not form spores. Species are found normally resident in the human respiratory tract. *C. diphtheriae* when infected with the appropriate bacteriophage produces a powerful exotoxin which causes the pathology of diphtheria. The disease results in the growth of a membrane across the throat, leading to suffocation. It can similarly affect the eyelids, with the appearance of such membranes on the inner surface of the lids. The conjunctiva may become involved in the same way. Diphtheroids have been isolated in a proportion of infected conjunctivae (Brooke *et al.*, 1979; Brown *et al.*, 1978).

*Clostridia spp.*
The Clostridia are a group of obligate anaerobes notorious for their pathogenicity. In particular, they include *Cl. botulinum*, which when it infects food produces botulinum toxin. Although botulinum toxin ingestion is potentially fatal, this substance has been used to paralyse the antagonist muscles in cases of paralytic strabismus (Cole and Lee, 1985; Elston and Lee, 1985) and other ocular disorders (Alpar, 1987). *Cl. tetani* is a possible infectant of deep wounds and prophylaxis against the effects of its toxin is routine. Other Clostridia such as *Cl. perfringens*, *Cl. welchii* and *Cl. oedematiens* cause gangrene. Gas gangrene of the lids has been reported (Crock *et al.*, 1985).

## Gram negative rods

This is by far the biggest group of pathogens, most of which are facultative anaerobes.

*Pseudomonas aeruginosa*
Pseudomonas aeruginosa is perhaps the most notorious of bacteria for causing ocular problems and is normally found in small numbers in the gut and on the skin. It is a common contaminant of water and has been cultured from jacuzzis (Brett 1985). Its numbers are kept in check by the presence of other organisms, but since it is resistant to many antibiotics it can gain dominance if the surrounding organisms are suppressed. *Pseudomonas aeruginosa* produces a bluish green colour when grown on media with a characteristic odour, and is pyogenic, the presence of green pus suggesting the presence of a *Pseudomonas* infection.

*Pseudomonas aeruginosa* is an opportunistic organism and is normally kept at bay by the body's defence mechanisms. Once these are breached a serious infection often results. It can infect burns, especially if of large area, and can also gain hold in patients who are immuno-compromised.

*Ps. aeruginosa* is an extremely versatile organism in that it can metabolize fluorescein and hydroxybenzoates as carbon sources for energy, which means that it can survive in conditions which are alien to most other organisms. This organism is only susceptible to antibiotics such as gentamicin and polymixin.

In the eye, *Ps. aeruginosa* can produce meibomitis, conjunctivitis and corneal ulcers and is one of the causes of ophthalmia neonatorum (Cole, Davies and Austin, 1980). Should access be gained to the sterile interior of the eye, then

panophthalmitis may result and indeed has been responsible for causing more than one serious case of hospital acquired disease leading to the loss of an eye (Crompton, 1978). It is an important test organism for contact lens solutions and eyedrop preservative systems.

*Haemophilus spp.*
These are small aerobic organisms which get their name from their requirement for enriched media containing blood for culturing *in vitro*. They include certain important human pathogenic organisms. *H. influenzae* is a secondary invader which helps to produce some of the symptoms of influenza and can produce inflammation in most parts of the respiratory tract. *Bordetella pertussis* is another of this group to affect the respiratory system, causing whooping cough which is transmitted by airborne infections from one person to another. It cannot exist for long periods outside the body. Similarly, *H. ducreyi* is so fastidious in its requirements that it can only be transmitted sexually and consequently is the causative organism of chancroid, a form of venereal disease.

*H. influenzae* and *H. ducreyi* are capable of infecting ocular tissues. There are two members of this group which are particularly noted for their ability to cause conjunctivitis. *H. aegyptius* (*H. conjunctivitidis*, Koch–Weeks bacillus) is often the cause of acute epidemic conjunctivitis, especially in school children. *Moraxella lacunata* (Morax–Axenfeld bacillus) is another well-known causative organism of conjunctivitis.

## Viruses

*Herpes viruses*
The most important members of the Herpes group as far as the eye is concerned are H. zoster, H. simplex and Cytomegalovirus.

H. zoster (varicella) virus leads to chickenpox in children. This is a mild highly contagious disease characterized by a vesicular rash. The disease leaves the patient with a continuing immunity to the disease. In the adult a reactivation of the virus leads to shingles, in which an area of the body becomes covered with a painful rash. Evidently the virus becomes stored in a sensory ganglion, the attack being caused by a migration of the virus along the nerve root. When the nerve affected is the ophthalmic division of the trigeminal nerve, the area served by it exhibits signs, i.e. the eye, the orbit and surrounding areas. This is known as Herpes zoster ophthalmicus, in which the cornea becomes inflamed and oedematous, and sensitivity may be impaired permanently. Secondary infection can occur, leading to ulceration and scarring.

Herpes simplex can be differentiated into Types I and II. Type II is associated with genital herpes and neonatal herpes. Transplacental infection with Type II virus has led to the development of neonatal cataract (Cibis and Burge, 1971). Type I causes cold sores, inflammation of the oral cavity, encephalitis and dendritic ulcers.

Dendritic ulcers are so called because of their branching pattern. As the ulcer extends it may lose this appearance and become amoeboid or geographic. The patient complains of pain, photophobia, blurring of vision and a watery discharge (unlike that from bacterial conjunctivitis). In the early stages infection only affects the epithelium, later progressing to the superficial stroma. The cornea becomes oedematous and there is further loss of stroma and possible vascularization.

The condition is treated with intense local anti-viral therapy. Herpes simplex can also produce a keratoconjunctivitis similar to that caused by adenovirus 8 (Darougar *et al.*, 1978) (see below).

The third virus in this group is Cytomegalovirus, which normally inhabits the female reproductive tract giving rise to congenital infections. Congenital infections can give rise to chorioretinitis, optic atrophy and cataract.

*Adenovirus 8*

Adenovirus 8 gives rise to epidemic kerato-conjunctivitis sometimes called 'eye hospital eye' because of its possible transmission by contaminated instruments. The infection produces severe acute conjunctivitis, which can spread leading to keratitis. Marked discomfort can last for months. Adenovirus was the cause of 8% of cases of acute conjunctivitis in one study (Wishart *et al.*, 1984).

*Pox viruses*

This group of viruses includes smallpox and cowpox. There is a relatively uncommon skin condition affecting young adults and children, Molluscum contagiosum, which is caused by a pox virus. Transparent nodules (2–3 mm in diameter) appear on the skin of the arm, legs, back and face, with possible involvement of the lid margins and conjunctiva. This condition is sometimes seen in patients with AIDS and AIDS-related complex (ARC).

*Toga viruses*

The best known member of this group is rubella (German measles), which can be passed from mother to baby in the uterus, leading to congenital defects in 30% of the children of mothers suffering rubella in the first trimester of pregnancy. Particularly affected are the heart, ears and eyes. Ophthalmic defects lead to microphthalmia, cataracts and congenital glaucoma.

*Retroviruses*

The Human Immunodeficiency Virus (HIV) is present in many of the body fluids of affected individuals, including tears. Although there has been no recorded case of transmission via infected contact lenses, it has become a point of concern of contact lens practitioners. The virus has also been recovered from contact lenses worn by patients with AIDS and ARC (AIDS related complex) (Tervo *et al.*, 1986).

AIDS can have certain ocular manifestations, partially because the patients are from the very nature of the disease more likely to develop opportunistic infections such as Cytomegalovirus retinitis. Conjunctival Kaposi's sarcoma is another ocular complication (Kanski, 1987).

## Fungi

*Candida albicans*

This is a dimorphic opportunistic fungus which is normally found in the mucous membranes of the mouth, vagina, gut and eye (Liotet *et al.*, 1980). It causes oral thrush in newborn infants and terminally ill patients. In the eye it can cause corneal ulcers, conjunctivitis and severe uveitis.

*Aspergillus niger*
This fungus, which is not dimorphic, grows in the form of mycelia. Often found in vegetable matter, it can cause bronchial problems. This fungus is also capable of producing severe local infection in the eye, especially following the injudicious use of local corticosteroids, which tend to mask the clinical signs of the infection, allowing it to get a stronger foothold. *A. niger*, which also can be found in the healthy eye (Liotet *et al.*, 1980), has the ability to infect contact lenses and destroy them. Other species of *Aspergillus* have been implicated in contact lens contamination. For example, Yamaguchi (1984) reported growth on contact lens of *A. flavus* and Filppi, Pfister and Hill (1973) found that *A. fumagatus* penetrated soft contact lenses. Aspergillus species are not the only ones to infect contact lenses. Yamamoto *et al.* (1979) found *Cephalosporium acremonium* growing on a contact lens worn for the treatment of metaherpetic keratitis.

### Chlamydia

*Trachoma (C. trachomatis)*
Trachoma affects a large proportion of the world's population, being endemic in many areas. Where it is endemic, it affects over 90% of the population. Associated with poor living conditions, this organism is passed on by insects and contaminated objects such as bedclothes. It is sometimes a resident of the female genital tract (Barton *et al.*, 1989) and can produce a form of ophthalmia neonatorum (Markham, 1979). The incidence of Chlamydial ophthalmia neonatorum varies. In one study in America, 1.4% of all newborn babies acquired chlamydial conjunctivitis (Schacter *et al.*, 1979) and similar findings were reported in Sweden (Persson *et al.*, 1983). This condition starts as a mild inflammation of the conjunctiva with the development of small follicles which become larger. The cornea is invaded and vascularized, resulting in pannus which can lead to severe scarring and contraction which causes deformity of eyelids. Symblepharon and trichiasis are also seen.

In temperate climates, the organism results in inclusion conjunctivitis.

### Amoebae

As well as the free living forms that are studied by every student of biology, Amoebae have been known for a long time as disease causing organisms. *Entamoeba histolytica* is the causative organism of amoebic dysentery. Amoebic keratitis has been reported as a result of infection with *Acanthamoebae* (Moore *et al.*, 1986). The patients in the report were myopes corrected with soft contact lenses who had used salt tablets dissolved in distilled water during disinfection procedures. This infection has so far proved to be difficult to treat.

## Antimicrobial agents

There are many 'agents' which have the ability to kill or inactivate microorganisms. Within this broad term we encompass the body's defence mechanisms, i.e. the white blood cells and circulating antibodies of the blood, the gastric hydrochloric acid and the lyzosyme and beta-lysin of tears, whilst other microorganisms such as bacteriophages must also be considered as antimicrobial agents. Here we are concerned with three basic groups:

(a) Physical agents capable of rendering objects and chemicals free of contamination.
(b) Antimicrobial preservatives which are incorporated into solutions to maintain sterility.
(c) Chemotherapeutic agents used either to treat or prevent an infection in the body.

It is important to define certain terms which are relevant to this subject and are sometimes used incorrectly.

*Sterilization* means the killing or removal of all viable organisms (including bacterial spores) from an object or pharmaceutical product by the use of chemical or physical agents.

*Disinfection* is a lesser process by which the capacity of an object to cause infection is removed. A disinfected product may not be 'sterile'. *Antisepsis* is a similar degree of decontamination, but refers to solutions and chemicals that are safe to apply to surfaces of the body.

*Chemotherapeutic agents* are described as bacteriocidal or bacteriostatic; the former are actually capable of killing the bacteria (although not necessarily bacterial spores) while bacteriostatic agents prevent bacteria from growing and rely on the body's own defence mechanism to get rid of the organisms.

## Physical agents

All physical agents are forms of energy and the antimicrobial action is dependent on supplying sufficient to cause disruption to the cell. Bacteria and other micro-organisms are far more resistant to adverse situations than animal cells and can withstand environments which would be quickly lethal to us. The effect of antimicrobial agents follows a first order reaction in which the log number of survivors is inversely proportional to the time. The time for 1 log cycle reduction, i.e. the time taken for 90% of the bacteria to be killed, is called the D value or decimal death time, this value reducing as the antimicrobial effect of the antimicrobial increases.

### *Heat*

Heat is one of the best known sterilizing agents. It is used for sterilizing solutions (providing that the substances are thermostable), dressings and some instruments. The effectiveness of heat is increased by the presence of water, especially if the pH is raised, the use of moist heat bringing hydrolysis to bear on the organisms as well as pyrolysis.

Temperatures of around 60°C will kill most viruses as well as the vegetative cells of pathogenic bacteria and fungi, whereas boiling brings about the demise of spores of pathogenic bacteria.

However, there are organisms whose spores will withstand boiling for lengthy periods. Therefore to obtain sterility without compromising the product, autoclaves are used. These work on the 'pressure cooker' principle by heating the product in steam (not air) to a defined temperature and specified time, which is usually 121°C for 15 minutes.

The use of dry heat is far less efficient and temperatures of up to 160°C for 1 hour are needed to kill spores.

Disinfection, as opposed to sterilization, can be brought about by boiling for 10–15 minutes.

Temperatures below boiling can reduce the number of micro-organisms present and are used for materials which cannot withstand heating at high temperatures. Milk is pasteurized at 60–70°C for example. The temperature inside a soft lens storage case, subjected to heating by steam, will not reach boiling but the temperature attained (95°C) is very bacteriocidal. Thermal disinfection of contact lenses is discussed in Chapter 12. With the current concern over AIDS, it is comforting to know that the causative virus is killed at 60°C.

*Freezing*
Freezing the cultures of bacteria will markedly reduce the number of bacteria, as a proportion may be damaged by the formation of ice crystals, but the rest will survive in a dormant state even at temperatures as low as that of liquid nitrogen. Indeed this process is used to store cultures of bacteria.

*Ionizing radiation*
This technique is used for disposable plastic items and for paper products like Fluorets. All types of rays are lethal to micro-organisms (alpha, beta, and gamma rays). Usually it is gamma rays that are used at a dosage of 2.5–3.5 MRad.

*Ultraviolet radiation*
Light is only markedly bacteriocidal at low wavelengths (240–280 nm — the UVC region) and at this level it does not penetrate well, making it suitable only for surface and aerial disinfection.

*Filtration*
Solutions of thermolabile drugs can be sterilized by passing the solution through a 0.22 μm filter which retains all bacteria (the smallest bacterium is about 0.5 μm). The filters, which are sterilized before use, will not remove viral contamination.

*Ultrasonics*
Sound will kill bacteria but high power inputs are required. Ultrasonic cleaners with antibacterial cleaners have been used on contact lenses. Studies on the antimicrobial effectiveness of such devices have yet to be published.

**Antimicrobial preservatives**

There are a whole range of substances incorporated into products to prevent the growth of micro-organisms. These are used in foods and drinks and cosmetics but are most important in multi-dose sterile pharmaceutical solutions to ensure that the product is protected from microbial attack while it is in use.

These compounds are selected for their ability to kill or inhibit the growth of micro-organisms, particularly bacteria and fungi. The rate of kill represented by the D value is dependent upon the concentration of the preservative, but is not always a simple inverse relationship, i.e. with the D value inversely proportional to the concentration of the preservative compound. With some compounds the effect is exponential, with a reduction of concentration to half of the original leading to an increase in D value of a factor of $2^8$ or 256. Such compounds are thus quickly inactivated by dilution.

These agents are capable of producing damage to human cells, and it is necessary to use them in as low a concentration as possible to reduce toxicity, the final

concentration therefore representing a compromise between safety and efficacy. In order to achieve greater efficacy without increasing the toxic effects, mixtures of preservatives are often used.

Many such agents have been used in the past; the following are those in common use.

*Benzalkonium chloride*

Benzalkonium chloride has a detergent action which causes disruption of the cell membrane, and it is by far the most commonly used preservative for eye drops. Benzalkonium chloride has a disruptive effect on the tear film when used in concentrations of 0.01% and greater (Wilson, Duncan and Jay, 1975). This preservative is often found combined with EDTA (ethylene diamine tetra acetic acid, sodium edetate). EDTA is a chelating agent which combines with divalent ions (normally calcium) to form a non-ionizable complex. This agent has a slight antibacterial action of its own but is principally used to enhance the bacteriocidal action of benzalkonium chloride.

*Mercury compounds*

Mercurial compounds include thiomersal and phenylmercuric nitrate. They produce mercury ions which react with sulphydryl groups of essential enzymes. They are slower in action against certain organisms but are less quickly inactivated by dilution than other compounds. Unlike benzalkonium chloride, mercury compounds are not potentiated by the addition of EDTA (Richards and Reary, 1972). In fact, Morton (1985) found that EDTA actually reduces the antimicrobial efficacy of thiomersal. Significant penetration of mercury-containing compounds into the aqueous humour following their use has been recorded by Winder *et al.* (1980). These compounds have been demonstrated to have cytotoxic effects which are time and concentration dependent (Takahashi, 1982), but which are less than those of benzalkonium chloride (Gasset, 1974), and their use in many contact lens solutions has led to the increasing incidence of allergic reactions (Gold, 1983).

*Chlorhexidine gluconate*

Chlorhexidine is a useful alternative to benzalkonium chloride and is used when the latter is incompatible with the active ingredient. It is very toxic to the endothelium of the cornea in concentrations of 20 μg/ml and if the epithelium is perfused the result is a sloughing of the cells without corneal swelling (Green *et al.*, 1980).

*Other compounds*

Other compounds that have been used include chlorbutol, cetrimide, phenylethanol, hydroxybenzoates and chlorocresol.

**Chemotherapeutic agents**

The treatment of infections has evolved somewhat since the treatment of syphilis with mercurial compounds. Developments have led to the introduction of agents which are more effective against the infecting organism and less toxic to the host. Agents to treat infections which are used routinely in ophthalmology are discussed in Chapter 15.

Anti-infectives tend to be specific against groups of organisms, e.g. antibacterials, antifungals, although there is some overlap. Certain antibacterials are effective against chlamydiae.

The mode of action of antibacterial agents varies greatly. Some agents cause disruption to the cell wall, leaving the protoplast at the mercy of phagocytes and to osmotic lysis. The mode of action of penicillin is to prevent the building of the cell wall.

Sulphonamides produce their action by acting as false substrates, interfering with the uptake of essential metabolites.

Many antibiotics work by gene function suppression, interfering with the production of enzymes and other essential proteins.

The administration of antimicrobial agents for the treatment of infection requires the achievement of adequate levels of the antimicrobial agent as quickly as possible. Many antibacterials compete for sites with other compounds. The higher the concentration of the agent the greater will be the effect on the organism.

Failure to achieve these levels may lead to the development of resistant strains. In any population there will be a proportion of organisms which are resistant to the agent. In the presence of the agent these will be selected and will form the majority of the population.

Some bacteria develop resistance by altering their metabolic pathways to avoid those with which the antimicrobial interacts. Other bacteria produce enzymes capable of destroying the chemical, e.g. penicillinase, which is an enzyme produced by certain strains of Staphylococci and is capable of breaking down penicillin.

## Hygiene in practice

Whilst there has been no recorded case of a patient contracting AIDS from a contaminated contact lens, this condition has highlighted the necessity for good practice hygiene. The AIDS virus is not the only organism (opportunist or invasive) that could be transmitted in an optometrist's practice and simple disinfection procedures should be employed in order to protect both practitioner and patient.

The first consideration is one of cleanliness, since clean objects and surfaces are easier to disinfect and will remain uncontaminated for longer. Normal contaminants will harbour bacteria, protect them from antibacterial agents, provide them with nutrients and inactivate disinfectants.

Jacobs (1986) has laid down simple infection control guidelines for optometrists and contact lens practitioners. Basically, anything that can be boiled without adversely affecting its performance should be, e.g. bowls, soft contact lenses. Items of equipment that will touch the eye should be swabbed with 70% alcohol, e.g. tonometer heads, chin rests, trial frames. Working surfaces should be treated with 1% sodium hypochlorite solution, which is effective against bacteria and viruses. At levels as low as 500 ppm (0.05%) it destroys Herpes simplex, Adenovirus 8 and Enterovirus 70 within 10 minutes (Naginton, Satehall and Whipps, 1983). Such procedures will protect the patient more than will a prophylactic eye drop.

In the interests of self protection the practitioner should have no open cuts uncovered, but the added precaution of wearing gloves is only necessary for high risk patients, e.g. patients who are HIV positive.

## References


Alpar, A. I. (1987) Botulinum toxin and its uses in the treatment of ocular disorders. *Am. J. Optometry Physiol. Optics*, **64**, 79–82

Barton, S. E., Thomas, B. J., Taylor-Robinson, D. and Goldmeier, D. (1985) Detection of *Chlamydia trachomatis* in the vaginal vault of women who have had hysterectomies. *Br. Med. J.*, **27**, 250

Bloomfield, S. E., David, D. S., Cheigh, J. S., Kim, Y., White, R. P., Stengel, K. H. and Rubin, A. L. (1978) Endophthalmitis following staphylococcal sepsis in renal failure patients. *Arch. Int. Med.*, **138**, 706–708

Brett, J. (1985) Pseudomonas aeruginosa and whirlpools. *Br. Med. J.*, **April 6**, 1024–1025

Brook, I. (1980) Anaerobic and aerobic bacterial flora of acute conjunctivitis in children. *Arch. Ophthalmol.*, **98**, 833–835,

Brook, I. Pettit, T. H., Martin, W. J. and Finegold, S. M. (1979) Anaerobic and aerobic bacteriology of acute conjunctivitis. *Ann. Ophthalmol.*, **11**, 389–393

Brown, D. H. (1978) The conjunctival flora of nursing home patients and staff. *Ann. Ophthalmol.*, **10**, 333–334

Cibis, A. and Burge, R. M. (1971) Herpes simplex virus induced cataracts. *Arch. Ophthalmol.*, **85**, 220–223

Cole, G. F., Davies, D. P. and Austin D. J. (1980) Pseudomonas ophthalmia neonatorum: a cause of blindness. *Br. Med. J.*, **August 9**, 440–441

Crock, G. W., Heriot, W. J., Janakiraman, P. and Weiner, J. M. (1985) Gas gangrene infection of the eye and orbit. *Br. J. Ophthalmol.*, **69**, 143–148

Crompton, D. O. (1978) Medical ethics and hospital-acquired disease. *Lancet*, **July 15**, 146

Darougar, S., Hunter, P. A., Viswalingam, M., Gibson, J. A., and Jones, B. R. (1978) Acute follicular conjunctivitis and keratoconjunctivitis due to Herpes simplex virus in London. *Br. J. Ophthalmol.*, **62**, 843–849

Davis, S. D. Sarff, L. D. and Hyndiuk, R. A. (1978) Staphylococcal keratitis. *Arch. Ophthalmol.*, **96**, 2114–2116

Elston, J. S. and Lee, J. P. (1985) Paralytic strabismus: the role of botulinum toxin. *Br. J. Ophthalmol.*, **69**, 891–896

Filppi, J. A., Pfister, R. M. and Hill, R. M. (1973) Penetration of hydrophilic contact lenses by *Aspergillus Fumagatus*. *Am. J. Optometry Physiol. Optics*, **50**, 553–557

Ford, L. C., DeLange, R. J. and Petty, R. W. (1976). Identification of a nonlysozymal bacteriocidal factor (beta lysin) in human tears and aqueous humour. *Am. J. Ophthalmol.*, **81**, 30–33

Gasset, A. R., Ishii, Y., Kaufman, H. E. and Miller, I. (1974) Cytotoxicity of ophthalmic preservatives. *Am. J. Ophthalmol.*, **78**, 98–105

Gold, R. M. (1983) The war on thiomersal. *Contemporary Optometry*, **2**, 7–10

Green, K., Livingston, V., Bowman, K. and Hull, D. S. (1980) Chlorhexidine effects on corneal epithelium and endothelium. *Arch. Ophthalmol.*, **98**, 1273–1278

Jarvis, V. N., Levine, R., and Asbell, P. A. (1987) Ophthalmia neonatorum. Study of a decade of experience at the Mount Sinai Hospital. *Br. J. Ophthalmol.*, **71**, 295–300

Jacobs, R. J. (1986) Infection control guidelines for optometrists and contact lens practitioners. *Clin. Exp. Optometry*, **69/2**, 40–45

Kanski, J. J. (1987) Ocular manifestation of AIDS. *The Optician*, **2 March**, 24–25

Liotet, S., Krzywkowski, J. C., Warret, V. N. and Jacqui, C. (1980) Conjunctival flora of healthy people. *J. Francais Ophthalmol.*, **103**, 557–560

Mackie, I. A. and Seal, D. V. (1976) Quantitative tear lysozyme essay in units of activity per microlitre. *Br. J. Ophthalmol.*, **60**, 70–74

Markham, J. G. (1979) Genital tract to eye infection — tissue culture of *Chlamydia trachomatis*. *NZ Med. J.*, **90**, 186–188.

Maske, R., Hill, J. C. and Oliver, S. P. (1986) Management of bacterial corneal ulcers. *Br. J. Ophthalmol.*, **70**, 199–201

Moore, M. B., McCulley, J. P., Kaufman, H. E. and Robin, J. B. (1986) Radial keratoneuritis as a presenting sign in acanthamoeba keratitis. *Ophthalmology*, **93**, 1310–1315

Morton, D. J. (1985) EDTA reduces the antimicrobial efficacy of thiomersal. *Int. J. Pharm.*, **23**, 357–358

McGill, J., Goulding, N. J., Liakos, G., Jacobs, P. and Seal, D. V. (1982) Pathophysiology of bacterial infection in the external eye. *Transact. Ophthalmol. Soc. UK*, **102**, 7–10

Naginton, J., Sutehall, G. M. and Whipp, A. (1983) Tonometer disinfection and viruses. *Br. J. Ophthalmol.*, **67**, 674–676

Persson, K., Ronnerstam, R., Svanberg, L. and Pohla, M-A. (1983) Neonatal chlamydial eye infection: an epidemiological and clinical study. *Br. J. Ophthalmol.*, **67**, 700–704

Reim, M. (1983) Normal and pathological components of tears and conjunctival secretion. *The Ophthalmic Optician*, **May 21**, 346–350

Richards, R. M. E. and Reary, J. M. E. (1972) Changes in antibacterial activity of thiomersal and PMN on autoclaving with certain adjuvants. *J. Pharm. Pharmacol.*, **24**, 84P–85P

Schacter, J., Holt, J., Goodmer, E., Grossman, M., Sweet, R. and Mills, J. (1979). Prospective study of chlamydial infection in neonates. *Lancet*, **August 25**, 377–379

Takahashi, N. (1982) Cytotoxicity of mercurial preservatives in cell culture. *Ophthalmic Research*, **14**, 63–69

Tervo, T., Lahdevirto, J., Vaheria, A., Valle, S. L. and Suni, J. (1986) Recovery of HTLV-III from contact lenses. *Lancet*, **February 15**, 379–380

Wilson, W. S., Duncan, A. J. and Jay, J. (1975) Effect of benzalkonium chloride on the stability of the precorneal tear film in rabbit and man. *Br. J. Ophthalmol.*, **59**, 667–669

Winder, A. F., Astbury, N. J., Sheraidah, G. A. K. and Ruben, M. (1980) Penetration of mercury from ophthalmic preservatives into the human eye. *Lancet*, **2**, 237–239

Wishart, P. K., James, C., Wishart, M. S. and Darougar, S. (1984) Prevalence of acute conjunctivtis caused by chlamydia adenovirus, and Herpes simplex virus in an ophthalmic casualty department. *Br. J. Ophthalmol.*, **68**, 653–655

Yamaguchi, T. (1984) Fungus growth on soft contact lenses with different water contents. *Contact Lens J.*, **10**, 166–171

Yamamoto, G. K., Pavan-Langston, D., Stowe, G. C. and Albert, D. M. (1979) Fungal invasion of a therapeutic soft contact lens and cornea. *Ann. Ophthalmol.*, **11**, 1731–1735

## Further reading

Coster, D. J. (1979) Treacher Collins Prize Essay. Inflammatory diseases of the outer eye. Transactions of the Ophthalmological Societies of the United Kingdom, **99**, 463–480

Coster, D. J. (1978) Herpetic keratitis and corneal destruction. Transactions of the Ophthalmological Societies of the United Kingdom, **98**, 372–376.