I
Introduction

1 Microbiology: What, Why and How?

Microorganisms (or microbes) inhabit every corner of the globe, and are essential for the maintenance of the world's ecosystems. They include organisms responsible for some of the most deadly human diseases, and others that form the basis of important industrial processes. Yet until a few hundred years ago, nobody knew they existed! This book offers an introduction to the world of microorganisms, and in this opening chapter, we offer some answers to three questions:

- *What* is microbiology?
- *Why* is it such an important subject?
- *How* have we gained our present knowledge of microbiology?

1.1 What is microbiology?

Things aren't always the way they seem. On the face of it, 'microbiology' should be an easy word to define: the science (*logos*) of small (*micro*) life (*bios*), or to put it another way, the study of living things so small that they can't be seen with the naked eye. Bacteria neatly fit this definition, but what about fungi and algae? These two groups each contain members that are far from microscopic. On the other hand, certain animals, such as nematode worms, can be microscopic, yet are not considered to be the domain of the microbiologist. Viruses represent another special case; they are most certainly microscopic; indeed, most are submicroscopic, but by most accepted definitions they are not living (why? – see Chapter 10 for an explanation). Nevertheless, these too fall within the remit of the microbiologist.

Essential Microbiology, Second Edition. Stuart Hogg.

In the central section of this book you can read about the thorny issue of microbial classification and gain some understanding of just what is and what is not regarded as a microorganism.

1.2 Why is microbiology important?

To the lay person, microbiology means the study of sinister, invisible 'bugs' that cause disease. As a subject, it generally tends to impinge on the popular consciousness in news coverage concerning the latest 'health scare'. It may come as something of a surprise therefore to learn that the vast majority of microorganisms coexist alongside us without causing any harm; indeed, at least a thousand different species of bacteria are to be found on human skin! In addition, many microorganisms are positively beneficial, performing vital tasks such as the recycling of essential elements, without which life on our planet could not continue, as we'll examine in Chapter 14. Other microorganisms have been exploited by humans for our own benefit, for instance in the manufacture of antibiotics (Chapter 17) and foodstuffs (Chapter 18). To get some idea of the importance of microbiology in the world today, just consider the following list of some of the general areas in which the expertise of a microbiologist might be used:

- medicine
- environmental science
- food and drink production
- fundamental research
- agriculture
- pharmaceutical industry
- genetic engineering

The popular perception among the general public, however, remains one of infections and plagues. Think back to the first time you ever heard about microorganisms; almost certainly, it was when you were a child and your parents impressed on you the dangers of ingesting 'germs' from dirty hands or putting things in your mouth after they'd been on the floor. In reality, only a couple of hundred out of the half million or so known bacterial species give rise to infections in humans; these are termed *pathogens*, and have tended to dominate our view of the microbial world.

In the next few pages we shall review some of the landmark developments in the history of microbiology, and see how the main driving force throughout this time, but particularly in the early days, has been the desire to understand the nature and cause of infectious diseases in humans.

A *pathogen* is an organism with the potential to cause disease.

1.3 How do we know? Microbiology in perspective: to the Golden Age and beyond

We have learnt an astonishing amount about the invisible world of microorganisms, particularly over the last century and a half. How has this happened? The penetrating insights of brilliant individuals are rightly celebrated, but a great many 'breakthroughs' or 'discoveries' have only been made possible thanks to some (frequently unsung) development in microbiological methodology. For example, on the basis that 'seeing is believing', it was only when we had the means to *see* microorganisms under a microscope that we could prove their existence.

Microorganisms had been on the Earth for some 4000 million years when Antoni van Leeuwenhoek started his pioneering microscope work in 1673. Leeuwenhoek was an amateur scientist who spent much of his spare time grinding glass lenses to produce simple microscopes (Figure 1.1). His detailed drawings make it clear that the 'animalcules' he observed from a variety of sources included representatives of what later became known as protozoa, bacteria and fungi. Where did these creatures come from? Arguments about the origin of living things revolved around the long-held belief in *spontaneous generation*, the idea that living organisms could arise from non-living matter. In an elegant experiment, the Italian Francesco Redi (1626–1697) showed

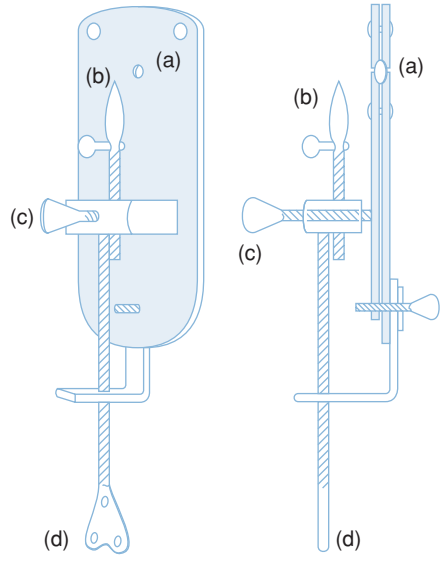


Figure 1.1 Leeuwenhoek's microscope. The lens (a) was held between two brass plates and used to view the specimen, which was placed on the mounting pin (b). Focusing was achieved by means of two screws (c) and (d). Some of Leeuwenhoek's microscopes could magnify up to 300 times. Original source: antoni van Leeuwenhoek and his little animals by CE Dobell (1932).

that the larvae found on putrefying meat arose from eggs deposited by flies, and not spontaneously as a result of the decay process. This can be seen as the beginning of the end for the spontaneous generation theory, but many still clung to the idea, claiming that while it may not have been true for larger organisms, it must surely be so for minute creatures such as those demonstrated by Leeuwenhoek. Despite mounting evidence against the theory, as late as 1859 fresh 'proof' was still being brought forward in its support. Enter onto the scene Louis Pasteur (1822–95), still arguably the most famous figure in the history of microbiology. Pasteur trained as a chemist, and made a lasting contribution to the science of stereochemistry before turning his attention to spoilage problems in the wine industry. He noticed that when lactic acid was produced instead of alcohol in wine, rod-shaped bacteria were always present as well as the expected yeast cells. This led him to believe that while the yeast produced the alcohol, the bacteria were responsible for the spoilage, and must have originated in the environment. Exasperated by continued efforts to substantiate the theory of spontaneous generation, he set out to disprove it once and for all. In response to a call from the French Academy of Science, he carried out a series of experiments that led to the acceptance of *biogenesis*, the idea that life arises only from already existing life. Using his famous swan-necked flasks (Figure 1.2), he demonstrated that as long as dust particles (and the microorganisms carried on them) were excluded, the contents would remain sterile. This also disproved the idea held by many that there was some element in the air itself that was capable of initiating microbial growth. In Pasteur's words '….the doctrine of spontaneous generation will never recover from this mortal blow. *There is no known circumstance in which it can be affirmed that microscopic beings came into the world without germs, without parents similar to themselves*' [author's italics]. Pasteur's findings on the role of microorganisms in wine contamination led inevitably to the idea that they may also be responsible for diseases in humans, animals and plants.

The notion that some invisible (and therefore presumably extremely small) living creatures were responsible for certain diseases was not a new one. Long before microorganisms had been shown to exist, the Roman philosopher Lucretius (~98–55 BC) and much later the physician Girolamo Fracastoro (1478–1553) had supported the idea. Fracastoro wrote 'Contagion is an infection that passes from one thing to another' and recognised three forms of transmission: by direct contact, through inanimate objects and via the air; we still class transmissibility of infectious disease in much the same way today (see Chapter 15). The prevailing belief at the time, however, was that an infectious disease was due to something called a *miasma*, a poisonous vapour arising from dead or diseased bodies, or to an imbalance between the four humours of the body (blood, phlegm, yellow bile and black bile).

During the nineteenth century, many diseases were shown, one by one, to be caused by microorganisms. In 1835, Agostino Bassi showed that a disease

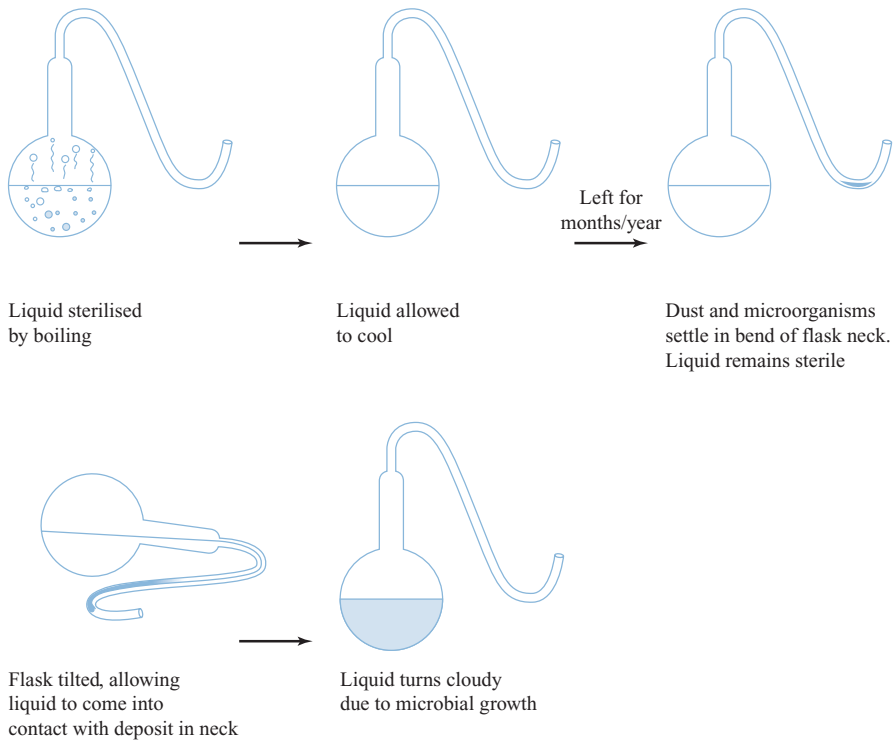


Figure 1.2 Pasteur's swan-necked flasks. Broth solutions rich in nutrients were placed in flasks and boiled. The necks of the flasks were heated and drawn out into a curve, but kept open to the atmosphere. Pasteur showed that the broth remained sterile because any contaminating dust and microorganisms remained trapped in the neck of the flask as long as it remained upright.

of silkworms was due to a fungal infection, and 10 years later, Miles Berkeley demonstrated that a fungus was also responsible for the great Irish potato blight. Joseph Lister's pioneering work on antiseptic surgery provided strong, albeit indirect, evidence of the involvement of microorganisms in infections of humans. The use of heat-treated instruments and of phenol both on dressings and actually sprayed in a mist over the surgical area, was found greatly to reduce the number of fatalities following surgery. Around the same time, in the 1860s, the indefatigable Pasteur had shown that a parasitic protozoan was the cause of another disease of silkworms called 'pébrine', which had devastated the French silk industry.

The definitive proof of the germ theory of disease came from the German, Robert Koch, who in 1876 showed the relationship between the cattle disease anthrax and a bacillus we now know as *Bacillus anthracis*. This was also the first demonstration of the

A *bacillus* is a rod-shaped bacterium.

Box 1.1 Koch's postulates

1. The microorganism must be present in every instance of the disease and absent from healthy individuals.
2. The microorganism must be capable of being isolated and grown in pure culture.
3. When the microorganism is inoculated into a healthy host, the same disease condition must result.
4. The same microorganism must be re-isolated from the experimentally infected host.

involvement of bacteria in disease. Koch infected healthy mice with blood from diseased cattle and sheep, and noted that the symptoms of the disease appeared in the mice, and also, crucially, that rod-shaped bacteria could be isolated from their blood. These could be isolated and grown in culture, where they multiplied and produced spores. Injection of healthy mice with these spores (or more bacilli) led them too to develop anthrax, and once again the bacteria were isolated from their blood. These results led Koch to formalise the criteria necessary to prove a causal relationship between a specific disease condition and a particular microorganism. These criteria became known as *Koch's postulates* (Box 1.1), and are still in use today.

Despite their value, it is now realised that Koch's postulates do have certain limitations. It is known for example that certain agents responsible for causing disease (e.g. viruses, prions: see Chapter 10) can't be grown *in vitro,* but only in host cells. Also, the healthy animal in Postulate 3 is seldom human, so a degree of extrapolation is necessary – if agent X doesn't cause disease in a laboratory animal, can we be sure it won't in humans? Furthermore, some diseases are caused by more than one organism, and some organisms are responsible for more than one disease. On the other hand, the value of Koch's postulates goes beyond just defining the causative agent of a particular disease, and allows us to ascribe a specific effect (of whatever kind) to a given microorganism.

The term *in vitro* (= 'in glass') is used to describe procedures performed outside of the living organism in test tubes, etc. (cf. *in vivo*).

Critical to the development of Koch's postulates was the advance in microbial culturing techniques, enabling the isolation of pure cultures of specific microorganisms. These are discussed in more detail in Chapter 4. The development of pure cultures revolutionised

A *pure* or *axenic culture* contains one type of organism only, and is completely free from contaminants.

Table 1.1 The discovery of some major human pathogens

Year	Disease	Causative agent	Discoverer
1876	Anthrax	*Bacillus anthracis*	Koch
1879	Gonorrhoea	*Neisseria gonorrhoeae*	Neisser
1880	Typhoid fever	*Salmonella typhi*	Gaffky
1880	Malaria	*Plasmodium* spp.	Laveran
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch
1883	Cholera	*Vibrio cholerae*	Koch
1883/4	Diphtheria	*Corynebacterium diphtheriae*	Klebs and Loeffler
1885	Tetanus	*Clostridium tetani*	Nicolaier and Kitasato
1886	Pneumonia (bacterial)	*Streptococcus pneumoniae*	Fraenkel
1892	Gas gangrene	*Clostridium perfringens*	Welch and Nuttall
1894	Plague	*Yersinia pestis*	Kitasato and Yersin
1896	Botulism	*Clostridium botulinum*	Van Ermengem
1898	Dysentery	*Shigella dysenteriae*	Shiga
1901	Yellow fever	Flavivirus	Reed
1905	Syphilis	*Treponema pallidum*	Schaudinn and Hoffman
1906	Whooping cough	*Bordetella pertussis*	Bordet and Gengou
1909	Rocky Mountain spotted fever	*Rickettsia rickettsii*	Ricketts

microbiology, and within 30 years or so of Koch's work on anthrax, the pathogens responsible for the majority of common human bacterial diseases had been isolated and identified. Not without just cause is this period known as the 'golden age' of microbiology! Table 1.1 summarises the discovery of some major human pathogens.

Charles Chamberland, a pupil of Pasteur's, invented the *autoclave*, contributing greatly to the development of pure cultures.

Koch's greatest achievement was in using the advances in methodology and the principles of his own postulates to demonstrate the identity of the causative agent of tuberculosis, which at the time was responsible for around one in every seven human deaths in Europe. Although it was believed by many to have a microbial cause, the causative agent had never been observed, either in culture or in the affected tissues. We now know this is because *Mycobacterium tuberculosis* (the tubercle bacillus) is very difficult to stain by conventional methods due to the high lipid content of the cell wall surface. Koch developed a staining technique that enabled it to be seen, but realised that in order to satisfy his own postulates, he must isolate the organism and grow it in culture. Again, there were technical difficulties, since even under favourable conditions, *M. tuberculosis* grows slowly, but eventually Koch was able to demonstrate the infectivity of the cultured organisms towards guinea pigs. He was then able to isolate them again from the diseased animal and use them to cause disease in uninfected animals, thus satisfying the remainder of his postulates.

Although most bacterial diseases of humans and their aetiological agents have now been identified, important variants continue to evolve and sometimes emerge; examples in recent decades include Lyme disease and legionellosis (legionnaire's disease); the latter is an acute respiratory infection caused by the previously unrecognised genus, *Legionella*. Also, *Helicobacter pylori,* only discovered in the 1980s, has been shown to play an important (and previously unsuspected) role in the development of stomach ulcers. There still remain a few diseases that some investigators suspect are caused by bacteria, but for which no pathogen has been identified.

Aetiology is the cause or origin of a disease.

Another cause of infectious diseases are viruses, and following their discovery during the last decade of the nineteenth century, it was soon established that many diseases of plants, animals and humans were caused by these minute, non-cellular agents.

The major achievement of the first half of the twentieth century was the development of antibiotics and other antimicrobial agents, a topic discussed in some detail in Chapter 17. Infectious diseases that previously accounted for millions of deaths became treatable by a simple course of therapy, at least in the affluent West, where such medications were readily available.

If the decades either side of 1900 have become known as the golden age of microbiology, the second half of the twentieth century will surely be remembered as the golden age of molecular genetics. Following on from the achievements of others such as Griffith and Avery, the publication of Watson and Crick's structure for DNA in 1953 heralded an extraordinary period of achievement in this area, culminating at the turn of the twenty-first century in the completion of the Human Genome Project.

You may ask, what has this genetic revolution to do with microbiology? Well, all the early work in molecular genetics was carried out on bacteria and viruses, as you'll learn in Chapter 11, and microbial systems have also been absolutely central to the development of the techniques of genetic engineering. In addition, as part of the Human Genome Project, the genomes of many microorganisms have been decoded, something that has now become almost routine, thanks to methodological advances made during the project. Having this information will help us to understand in greater detail the disease strategies of microorganisms, and to devise ways of countering them.

The *Human Genome Project* is an international effort to map and sequence all the DNA in the human genome. The project has also sequenced the genomes of many other organisms.

As we have seen, a recurring theme in the history of microbiology has been the way that advances in knowledge have followed on from methodological or technological developments, and we shall refer to a number of such developments during the course of this book. To conclude this introduction to microbiology, we shall return to the instrument that, in some respects, started it all. In any microbiology course, you are sure to spend some time

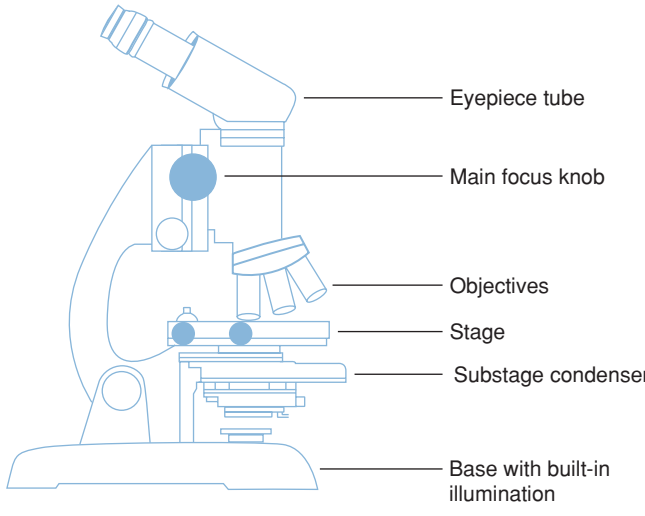


Figure 1.3 The compound light microscope. Modern microscopes have a built-in light source. The light is focused onto the specimen by the condenser lens, and then passes into the body of the microscope via the objective lens. Rotating the objective nosepiece allows different magnifications to be selected. The amount of light entering the microscope is controlled by an iris diaphragm. Light microscopy allows meaningful magnification of up to around 1000 ×.

looking down a microscope, and to get the most out of the instrument it is essential that you understand the principles of how it works. The following pages attempt to explain these principles.

1.4 Light microscopy

Try this simple experiment. Fill a glass with water, then partly immerse a pencil and observe from above; what do you see? The apparent 'bending' of the pencil is due to rays of light being slowed down as they enter the water, because air and water have different *refractive indices*. Light rays are similarly retarded as they enter glass, and all optical instruments are based on this phenomenon of *refraction*.

The compound light microscope consists of three sets of lenses (Figure 1.3):

The *refractive index* of a substance is the ratio between the velocity of light as it passes through that substance and its velocity in a vacuum. It is a measure of how much the substance slows down and therefore refracts the light.

- the *condenser* focuses light onto the specimen to give optimum illumination;
- the *objective* provides a magnified and inverted image of the specimen;
- the *eyepiece* adds further magnification.

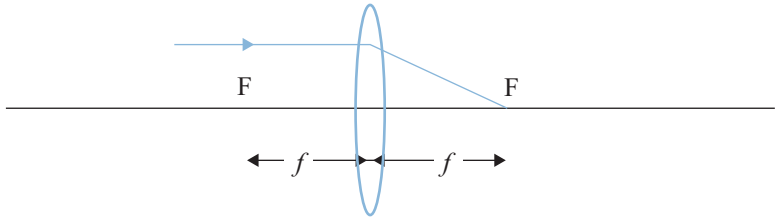


Figure 1.4 Light rays parallel to the axis of a convex lens pass through the focal point. The distance from the centre of the lens to the focal point is called the *focal length* of the lens (f).

Most microscopes have three or four different objectives, giving a range of magnifications, typically from $10\times$ to $100\times$. The total magnification is obtained by multiplying this by the eyepiece value (usually $10\times$), thus giving a maximum magnification of $1000\times$.

In order to appreciate how this magnification is achieved, we need to understand the behaviour of light passing through a convex lens:

- rays parallel to the axis of the lens are brought to a focus at the *focal point* of the lens (Figure 1.4);
- similarly, rays entering the lens from the focal point emerge parallel to the axis;
- rays passing through the centre of the lens from any angle are undeviated.

Because the condenser is not involved in magnification, it need not concern us here. Consider now what happens when light passes through an objective lens from an object AB situated slightly beyond its focal point (Figure 1.5a). Starting at the tip of the object, a ray parallel to the axis will leave the lens and pass through the focal point; a ray leaving the same point and passing through the centre of the lens will be undeviated. The point at which the two rays converge is an image of the original point formed by the lens. The same thing happens at an infinite number of points along the object's length, resulting in a *primary image* of the specimen, A′B′. What can we say about this image, compared to the original specimen AB? It is *magnified* and it is *inverted* (i.e. it appears upside down).

This primary image now serves as an object for a second lens, the eyepiece, and is magnified further (Figure 1.5b); this time the object is situated within the focal length. Using the same principles as before, we can construct a ray diagram, but this time we find that the two lines emerging from a point don't converge on the other side of the lens, but actually get further apart. The point at which the lines do

A *real image* is one that can be projected onto a flat surface such as a screen. A *virtual image* does not exist in space and cannot be projected in this way. A familiar example is the image seen in a mirror.

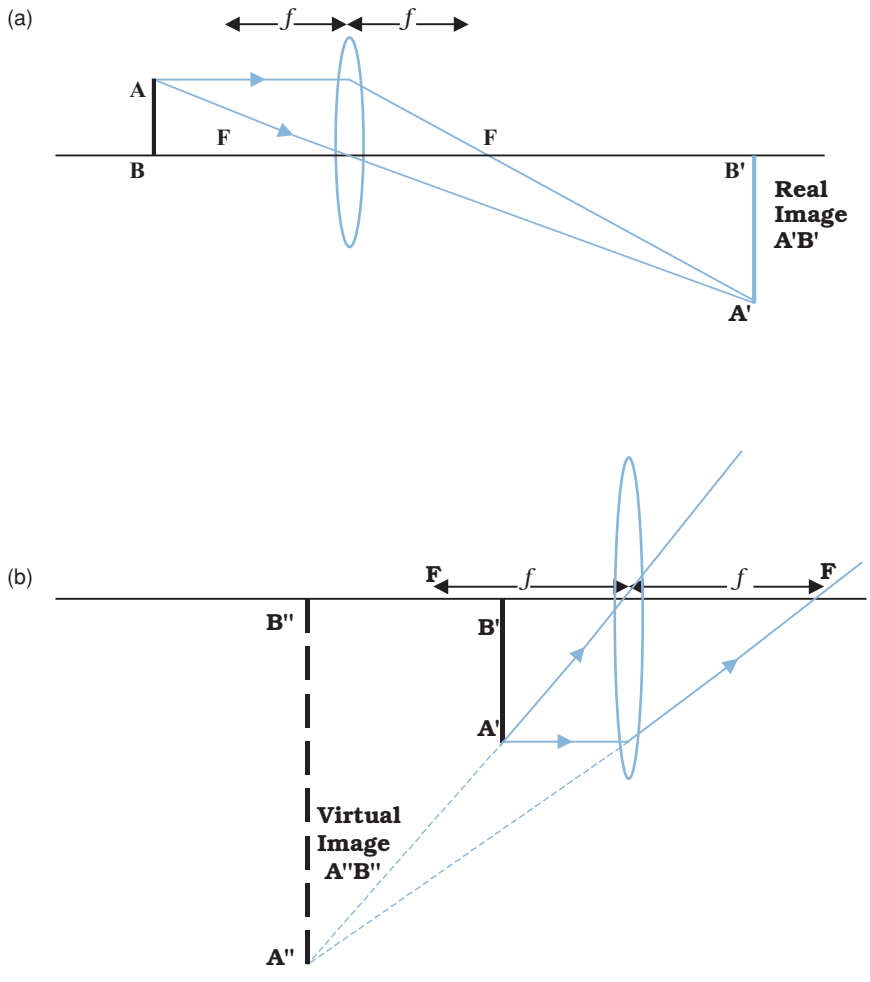


Figure 1.5 The objective lens and eyepiece lens combine to produce a magnified image of the specimen. (a) Light rays from the specimen AB pass through the objective lens to give a *magnified*, *inverted* and *real* primary image, A′B′. (b) The eyepiece lens magnifies this further to produce a *virtual* image of the specimen, A″B″.

eventually converge is actually 'further back' than the original object! What does this mean? The *secondary image* only *appear*s to be coming from A″B″, and isn't actually there. An image such as this is called a *virtual image*. Today's readers, familiar with the concept of virtual reality, will probably find it easier to come to terms with this than some of their predecessors! The primary image A′B′, on the other hand, is a *real image*; if a screen was placed at that position, the image would be projected onto it. If we compare A″B″ with A′B′, we can see that it has been further magnified, but not further inverted, so it is still upside down compared with the original. The rays of light

emerging from the eyepiece lens are focused by the lens of the eye to form a real image on the observer's retina.

So a combination of two lens systems allows us to see a considerably magnified image of our specimen. To continue magnifying an image beyond a certain point, however, serves little purpose, if it is not accompanied by an increase in detail. This is termed empty magnification, since it does not provide us with any more information. The *resolution* (resolving power, d) of a microscope is its capacity for discerning detail. More specifically, it is the ability to distinguish between two points a short distance apart, and is determined by the equation:

Immersion oil is used to improve the resolution of a light microscope at high power. It has the same refractive index as glass and is placed between the high-power objective and the glass slide. With no layer of air, more light from the specimen enters the objective lens instead of being refracted outside of it, resulting in a sharper image.

$$d = \frac{0.61\lambda}{n \sin \theta}$$

where:

λ= the wavelength of the light source;
n = the refractive index of the air or liquid between the objective lens and the specimen;
θ = the aperture angle (a measure of the light-gathering ability of the lens).

The expression $n \sin \theta$ is called the *numerical aperture* and for good quality lenses has a value of around 1.4. The lowest wavelength of light visible to the human eye is approximately 400 nm, so the maximum resolving power for a light microscope is approximately:

$$d = \frac{0.61 \times 400}{1.4} = 0.17\ \mu\text{m}$$

that is, it cannot distinguish between two points closer together than about 0.2 microns. For comparison, the naked eye is unable to resolve two points more than about 0.2 mm apart.

For us to be able to discern detail in a specimen, it must have *contrast*; most biological specimens, however, are more or less colourless, so unless a structure is appreciably denser than its surroundings, it will not stand out using conventional light microscopy. This is why preparations are commonly subjected to *staining*

A *nanometre* (nm) is one-millionth of a millimetre. There are 1000 nanometres in 1 *micron* (μm), which is therefore one-thousandth of a millimetre.
1 mm = 10^{-3} metre
1 μm = 10^{-6} metre
1 nm = 10^{-9} metre

procedures prior to viewing. The introduction of coloured dyes, which bind to certain structures, enables the viewer to discern more detail.

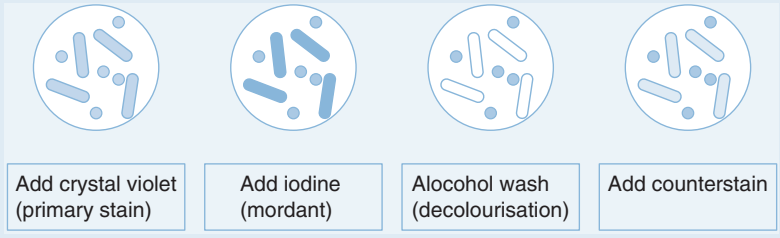
Since staining procedures involve the addition and washing off of liquid stains, the sample must clearly be immobilised or *fixed* to the slide if it is not to end up down the drain. The commonest way of doing this is to make a *heat fixed smear*; this kills the cells and attaches them to the glass microscope slide. A thin aqueous suspension of the cells is spread across the slide, allowed to dry, then passed (sample side up!) through a flame a few times. Excessive heating must be avoided, as it would distort the natural structure of the cells.

Using simple stains, such as methylene blue, we can see the size and shape of bacterial cells, for example, and their arrangement, while the binding properties of *differential stains* react with specific structures, helping us to distinguish between bacterial types. Probably the most widely used bacterial stain is the *Gram stain* (see Box 1.2), which for more than 100 years has been an invaluable first step in the identification of unknown bacteria.

The Gram stain is a differential stain, which only takes a few minutes to carry out, and which enables us to place a bacterial specimen into one of two groups – Gram-positive or Gram-negative. The reason for this differential reaction to the stain was not understood for many years, but is now seen to be a reflection of differences in cell wall structure, discussed in more detail in Chapter 3.

Box 1.2 The Gram stain

The Gram stain involves the sequential use of two stains. The critical stage is step 3; some cells will resist the alcohol treatment and retain the crystal violet, while others become decolourised. The counterstain (safranin or neutral red) is weaker than the crystal violet, and will only be apparent in those cells that have been decolourised.



Specialised forms of microscopy have been developed to allow the viewer to discern detail in living, unstained specimens; these include *phase-contrast* and *dark-field* microscopy. We can also gain an estimate of the number of microorganisms in a sample by directly counting them under the microscope. This is discussed along with other enumeration methods in Chapter 5.

Phase-contrast microscopy exploits differences in thickness and refractive index of transparent objects such as living cells to give improved contrast.

Dark-field microscopy employs a modified condenser. It works by blocking out direct light, and viewing the object only by the light it diffracts.

1.5 Electron microscopy

From the equation shown in the previous section, you can see that if it were possible to use a shorter wavelength of light, we could improve the resolving power of a microscope. However, because we are limited by the wavelength of light visible to the human eye we are not able to do this with the light microscope. The *electron microscope*, however, is able to achieve greater magnification and resolution because it uses a high-voltage beam of electrons, whose wavelength is very much shorter than that of visible light. Consequently we are able to resolve points that are much closer together than is possible even with the very best light microscope. The resolving power of an electron microscope may be as low as 1–2 nm, enabling us to see viruses, for example, or the internal structure of cells in considerable detail. The greatly improved resolution means that specimens can be meaningfully magnified over 100 000 × .

Electron microscopes, which were first developed in the 1930s and 40s, use ring-shaped electromagnets as 'lenses' to focus the beam of electrons onto the specimen. Because the electrons would collide with, and be deflected by, molecules in the air, electron microscopes require a pump to maintain a vacuum in the column of the instrument. There are two principal types of electron microscope: the *transmission electron microscope* (TEM) and the *scanning electron microscope* (SEM).

Figure 1.6 shows the main features of a TEM. As the name suggests, in TEM, the electron beam passes *through* the specimen and is scattered according to the density of the different parts. Due to the limited penetrating power of the electrons, extremely thin sections (<100 nm, or less than one-tenth of the diameter of a bacterial cell) must be cut, using a diamond knife. To allow this, the specimen must be fixed and dehydrated, a process that can introduce shrinkage and distortion to its structure if not correctly performed.

After being magnified by an objective 'lens', an image of the specimen is projected onto a fluorescent screen or photographic plate. Denser areas, which scatter the beam, appear dark, and those which allow it to pass through are light. It is often necessary to enhance contrast artificially, by means of

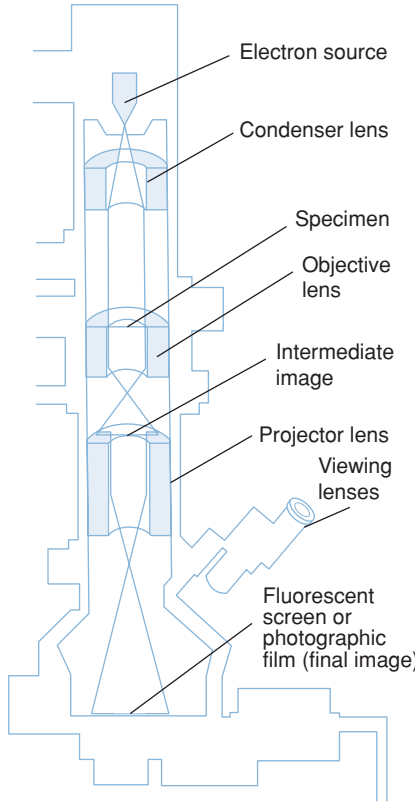


Figure 1.6 The transmission electron microscope (TEM). Electrons from a tungsten filament pass through a vacuum chamber and are focused by powerful electromagnets. Passage through the specimen causes a scattering of the electrons to form an image that is captured on a fluorescent screen. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

'staining' techniques that involve coating the specimen with a thin layer of a compound containing a heavy metal, such as osmium or palladium. It will be evident from the foregoing description of sample preparation and use of a vacuum that electron microscopy cannot be used to study living specimens.

The TEM has been invaluable in advancing our knowledge of the fine structure of cells, microbial or otherwise. The resulting image is, however, a flat, two-dimensional one, and of limited use if we wish to learn about the surface of a cell or a virus. For this, we turn to SEM. The scanning electron microscope was developed in the 1960s and provides vivid, sometimes startling, three-dimensional images of surface structure. Samples are dehydrated and coated with gold to give a layer a few nanometres thick. A fine beam of electrons probes back and forth across the surface of the specimen

and causes secondary electrons to be given off. The number of these, and the angle at which they are emitted, depends on the topography of the specimen's surface. SEM does not have quite the resolving power of the TEM, and therefore does not operate at such high magnifications.

Between them, SEM and TEM have opened up a whole new world to microbiologists, allowing us to put advances in our knowledge of microbial biochemistry and genetics into a structural context.

2
Biochemical Principles

All matter, whether living or non-living, is made up of *atoms*; the atom is the smallest unit of matter capable of entering into a chemical reaction. Atoms can combine together by bonding, to form *molecules*, which range from the small and simple to the large and complex. The latter are known as *macromolecules*; major cellular constituents such as carbohydrates and proteins belong to this group, and it is with these that this chapter is mainly concerned (Table 2.1). In order to appreciate how these macromolecules operate in the structure and function of microbial cells, however, you need to appreciate the basic principles of how atoms are constructed and how they interact with one other.

2.1 Atomic structure

All atoms have a central, positively charged *nucleus*, which is very dense and makes up most of the mass of the atom. The nucleus is made up of two types of particle, *protons* and *neutrons*. Protons carry a positive charge, and neutrons are uncharged, hence the nucleus overall is positively charged. It is surrounded by much lighter, and rapidly orbiting, *electrons* (Figure 2.1). These are negatively charged, with the charge on each electron being equal (but of course opposite) to that of the protons; however, the electrons have only 1/1840 of the mass of either protons or neutrons. It is the attractive force between the positively charged protons and the negatively charged electrons that holds the atom together.

The number of protons in the nucleus is called the *atomic number*, and ranges from one to over one hundred. The combined total of protons and neutrons is known as the *mass number*. All atoms have an equal number of protons and electrons, so regardless of the atomic number, the overall charge on the atom will always be zero.

Essential Microbiology, Second Edition. Stuart Hogg.

Table 2.1 Biological macromolecules. Important examples of each of the four major classes of macromolecule found in biological systems

Proteins	Carbohydrates	Lipids	Nucleic acids
Enzymes	Sugars	Triacylglycerols	DNA
Receptors	Cellulose	(fats)	RNA
Antibodies	Starch	Phospholipids	
Structural		Waxes	
proteins		Sterols	

Atoms having the same atomic number have the same chemical properties; such atoms all belong to the same *element*. An element is made up of one type of atom only and cannot be chemically broken down into simpler substances; thus pure copper, for example, is made up entirely of copper atoms. There are 92 of these elements, 26 of which commonly occur in living things. Each element has been given a universally agreed symbol; examples that we shall encounter in biological macromolecules include carbon (C), hydrogen (H) and oxygen (O). The atomic numbers of selected elements are shown in Table 2.2.

The relationship between neutrons, protons, atomic number and mass number is illustrated in Table 2.3. We have used carbon as an example, since all living matter is based upon this element. The carbon represented can be expressed in the form:

$$\overset{12}{\underset{6}{\mathrm{C}}} = \begin{matrix}(\text{mass number})\\(\text{element symbol})\\(\text{atomic number})\end{matrix}$$

The number of neutrons in an atom can be deduced by subtracting the atomic number from the mass number. In the case of carbon, this is the same

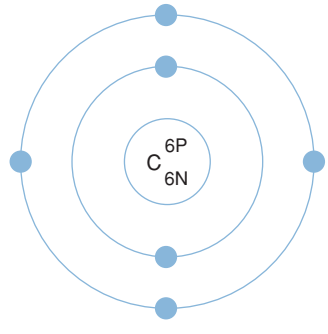


Figure 2.1 Atomic structure. The nucleus of a carbon atom contains six protons and six neutrons, surrounded by six electrons. Note how these are distributed between inner (2) and outer (4) electron shells.

Table 2.2 Symbols and atomic numbers of some elements occurring in living systems

Element	Symbol	Atomic no.
Hydrogen	H	1
Carbon	C	6
Nitrogen	N	7
Oxygen	O	8
Sodium	Na	11
Magnesium	Mg	12
Phosphorus	P	15
Sulphur	S	16
Chlorine	Cl	17
Potassium	K	19
Iron	Fe	26

as the number of protons (6), but this is not always so; phosphorus for example has 15 protons and 16 neutrons, giving it an atomic number of 15 and a mass number of 31.

2.1.1 Isotopes

Although the number of protons in the nucleus of a given element is always the same, the number of neutrons can vary, giving different forms, or *isotopes*, of that element. Carbon-14 (^{14}C) is a naturally occurring but rare isotope of carbon that has eight neutrons instead of six, hence the atomic mass of 14. Carbon-13 (^{13}C) is a rather more common isotope, making up around 1% of naturally occurring carbon; it has seven neutrons per atomic nucleus. The *atomic mass* (or atomic weight) of an element is the average of the mass numbers of an element's different isotopes, taking into account the proportions in which they occur. Carbon-12 is by far the predominant form of the element in nature, but the existence of small amounts of the other (slightly heavier) forms means that the atomic mass is 12.011 rather than exactly 12. Some isotopes are stable, while others decay spontaneously, with the release of subatomic particles. The latter are called *radioisotopes*; ^{14}C is a radioisotope, while the other two forms of carbon are stable isotopes. Radioisotopes have been an extremely useful research tool in a number of areas of molecular biology.

Table 2.3 The vital statistics of carbon

No. of protons	No. of neutrons	Atomic number	Mass number	Atomic mass
6	6	6	12	12.011

Box 2.1 How heavy is a mole?

When you work in a laboratory, something you'll need to come to grips with sooner or later is the matter of quantifying the amounts and concentrations of substances used. Central to this is the *mole*, so before we go any further, let's define this:

A mole is the molecular weight of a compound expressed in grams.

(The *molecular weight* is simply the sum of the atomic weights of all the atoms in a compound.)

So, to take sodium chloride as an example:

Molecular formula	=	NaCl (one atom each of sodium and chlorine)
Atomic weight of sodium	=	22.99
Atomic weight of chlorine	=	35.45
∴ Molecular weight	=	58.44

Thus one mole of sodium chloride equals 58.44 grams (58.44 g)

Concentrations are expressed in terms of mass per volume, so here we introduce the idea of the *molar solution*. This is a solution containing one mole dissolved in a final volume of one litre of an appropriate solvent (usually water).

Molar solution = one mole per litre

A one molar (1 M) solution of sodium chloride therefore contains 58.44 g dissolved in water and made up to one litre. A 2 M solution would contain 116.88 g in 1 litre, and so on.

In biological systems, a molar solution of anything is actually rather concentrated, so we tend to deal in solutions that are so many millimolar (mM, one-thousandth of a mole per litre) or micromolar (μM, one-millionth of a mole per litre).

Why bother with moles?

So far, so good, but why can't we just deal in grams, or grams per litre?

Consider the following example:

You've been let loose in the laboratory, and been asked to compare the effects of supplementing the growth medium of a bacterial culture with several different amino acids. 'Easy', you think. 'Add X milligrams of each to the normal growth medium, and see which stimulates growth the most'.

The problem is that although you may be adding the same *weight* of each amino acid, you're not adding the same number of *molecules*, because each has a different molecular weight. If you add the same number of moles (or millimoles or micromoles) of each instead, you would be comparing the effect of the same number of molecules of each, and thus obtain a much more meaningful comparison. This is because *a mole of one compound contains the same number of molecules as a mole of any other compound*. This number is called *Avogadro's number*, and is 6.023×10^{23} molecules per mole.

The electrons that orbit around the nucleus do not do so randomly, but are arranged in a series of *electron shells*, radiating out from the nucleus (see Figure 2.1). These layers correspond to different energy levels, with the highest energy levels being located furthest away from the nucleus. Each shell can accommodate a maximum number of electrons, and they always fill up starting at the innermost one, that is, the one with the lowest energy level. In our example, carbon has filled the first shell with two electrons, and occupied four of the eight available spaces on the second.

The chemical properties of atoms are determined by the number of electrons in the outermost occupied shell. Neon, one of the so-called 'noble' gases, has an atomic number of 10, completely filling the first two shells, and is chemically unreactive or *inert*. Atoms that achieve a similar configuration are all stable. If, on the other hand, such an arrangement is not achieved, the atom is unstable, or reactive. Reactions take place between atoms that attempt to achieve stability by attaining a full outer shell. These reactions may involve atoms of the same element or ones of different elements; the result in either case is a molecule or ion. Figure 2.2 shows how atoms combine to form a molecule. A substance made up of molecules containing two or more different elements is called a *compound*. In each example, the

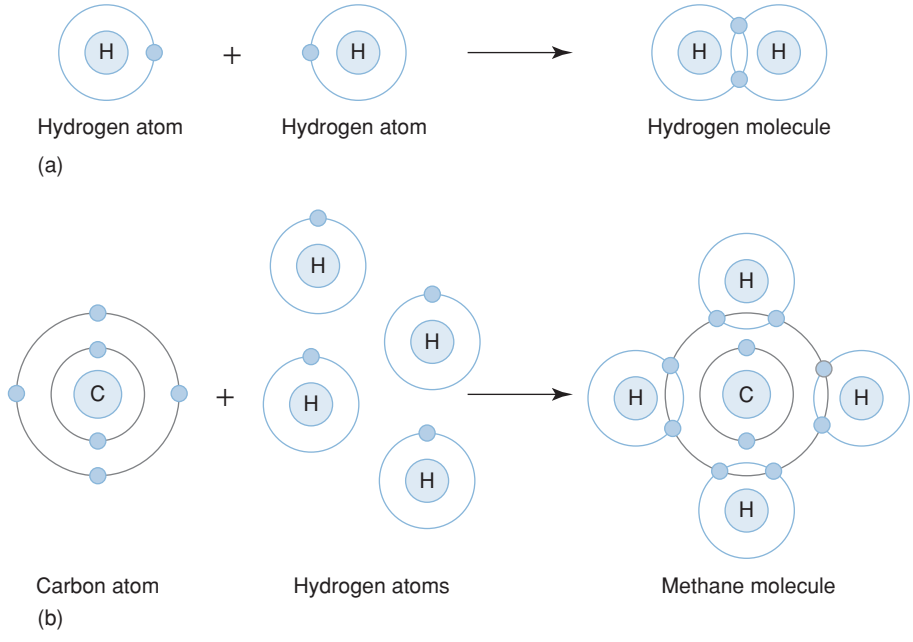


Figure 2.2 Atoms join to form molecules. The formation of (a) hydrogen and (b) methane by covalent bonding. Each atom achieves a full set of electrons in its outer shell by sharing with another atom. A shared pair of electrons constitutes a covalent bond.

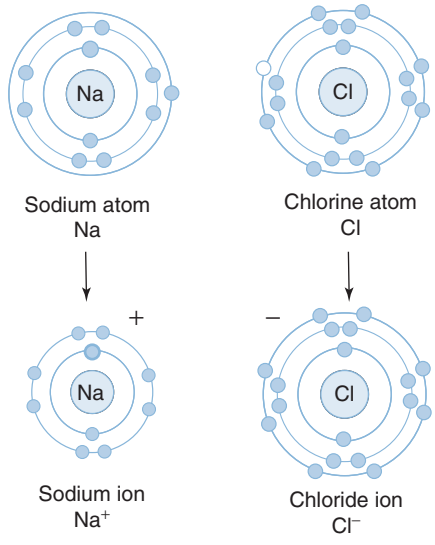


Figure 2.3 Ion formation. Sodium achieves stability by losing the lone electron from its outermost shell. The resulting sodium ion Na^+ has 11 protons and 10 electrons, hence it carries a single positive charge. Chlorine becomes ionised to chloride (Cl^-) when it gains an electron to complete its outer shell.

product of the reaction has a full outer electron shell; note that some atoms are donating electrons, while others are accepting them.

If most of the spaces in the outermost electron shell are full, or if most are empty, atoms tend to strive for stability by gaining or losing electrons, as shown in Figure 2.3. When this happens, an *ion* is formed, which carries either a positive or negative charge. Positively charged ions are called *cations* and negatively charged ones *anions*. The sodium atom, for example, has 11 electrons, meaning that the inner two electron shells are filled and a lone electron occupies the third shell. When it loses this last electron, it has more protons than electrons, and therefore has a net positive charge of one; when this happens, it becomes a sodium ion, Na^+ (see Figure 2.3).

2.1.2 Chemical bonds

The force that causes two or more atoms to join together is known as a *chemical bond*, and several types are found in biological systems. The interaction between sodium and chloride ions shown in Figure 2.4 is an example of *ionic* bonding, where the transfer of an electron from one party to another means that both achieve a complete outer electron shell. There is an attractive force between positively and negatively charged ions, called an ionic bond. Certain elements form ions with more than a single charge, by gaining or losing two

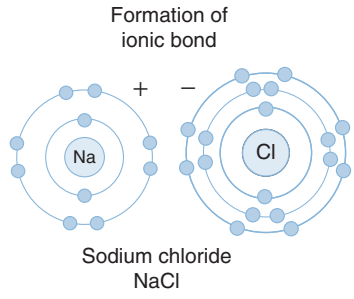


Figure 2.4 Ionic bonding. A positively charged Na^+ and negatively charged Cl^- attract each other, and an ionic bond is formed. The result is a molecule of sodium chloride.

or more electrons in order to achieve a full outer electron shell; thus calcium ions (Ca^{2+}) are formed by the loss of two electrons from a calcium atom.

The goal of stability through a full complement of outer shell electrons may also be achieved by means of *sharing* one or more pairs of electrons. Consider the formation of methane (see Figure 2.2); a carbon atom, which has four spaces in its outer shell, can achieve a full complement by sharing electrons from four separate hydrogen atoms. This type of bond is a *covalent* bond.

Sometimes, a pair of atoms share not one but two pairs of electrons (Figure 2.5). This involves the formation of a *double bond*. Triple bonding, through the sharing of three pairs of electrons, is also possible, but rare.

In the examples of covalent bonding we've looked at so far, the sharing of the electrons has been equal, but this is not always the case because sometimes the electrons may be drawn closer to one atom than another (Figure 2.6a). This has the effect of making one atom slightly negative and another slightly positive. Molecules like this are called *polar* molecules and the bonds are polar bonds. Sometimes a large molecule may have both polar and nonpolar areas. Polar molecules are attracted to each other, with the

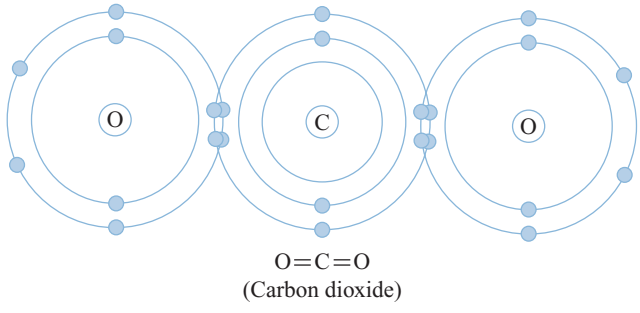


Figure 2.5 Double bond formation. In the formation of carbon dioxide, the carbon atom shares *two* pairs of electrons with each oxygen atom.

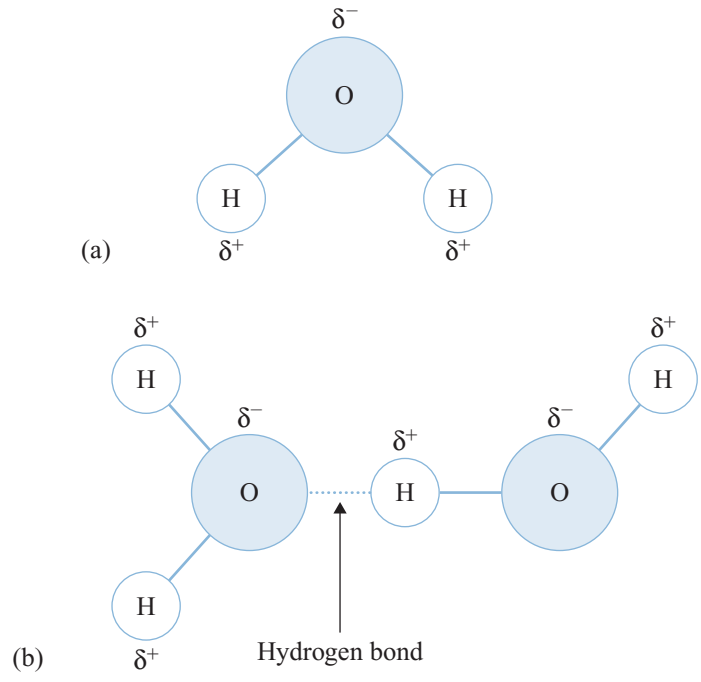


Figure 2.6 Water is a polar molecule. (a) The electrons of the hydrogen atoms are strongly attracted to the oxygen atom, causing this part of the water molecule to carry a slightly negative charge, and the hydrogen part a slightly positive one. (b) Because of their polar nature, water molecules are attracted to each other by hydrogen bonding. Hydrogen bonding is much weaker than ionic or covalent bonding, but plays an important role in the structure of macromolecules such as proteins and nucleic acids.

negative areas of one molecule attracted to the positive areas of another (Figure 2.6b). In water, hydrogen atoms bearing a positive charge are drawn to the negatively charged oxygens.

This attraction between polar atoms is called *hydrogen bonding*, and can take place between covalently bonded hydrogen and any electronegative atom, most commonly oxygen or nitrogen. Hydrogen bonds are much weaker than either ionic or covalent bonds; however, if sufficient of them form in a compound, the overall bonding force can be appreciable. Each water molecule can form hydrogen bonds with others of its kind in four places. In order to break all these bonds, a substantial input of energy is required, explaining why water has such a relatively high boiling point, and why most of the water on our planet is in liquid form.

Another weak form of interaction is brought about by *van der Waals forces,* which occur briefly when two nonpolar molecules (or parts of molecules) come into very close contact with one another. Although transient, and generally even weaker than hydrogen bonds, they occur in great numbers

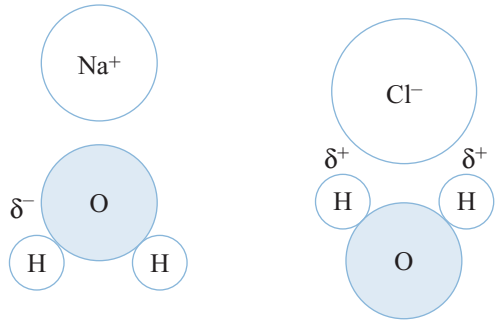


Figure 2.7 Ions dissolve in water. An ionic compound such as sodium chloride dissociates in water to its constituent ions. Water molecules form hydration shells around both Na^+ and Cl^- ions.

in certain macromolecules and play an important role in holding proteins together (see Section 2.3.3).

Water is essential for living things, both in the composition of their cells and in the environment surrounding them. Organisms are made up of 60–95% water by weight, and even inert dormant forms like spores and seeds have a significant water component. This dependence on water is a function of its unique properties, which in turn derive from its polar nature.

Water is the medium in which most biochemical reactions take place; it is a highly efficient solvent; indeed, more substances will dissolve in water than in any other solvent. Substances held together by ionic bonds tend to dissociate into anions and cations in water, because as individual solute molecules become surrounded by molecules of water, *hydration shells* are formed, in which the negatively charged parts of the solute attract the positive region of the water molecule, and the positive parts the negative region (Figure 2.7). The attractive forces that allow the solute to dissolve are called *hydrophilic* forces, and substances that are water-soluble are hydrophilic (water-loving). Other polar substances such as sugars and proteins are also soluble in water by forming hydrophilic interactions.

Molecules such as oils and fats are nonpolar, and because of their non-reactivity with water are termed *hydrophobic* ('water-fearing'). If such a molecule is mixed with water, it will be excluded, as water molecules 'stick together'. This very exclusion by water can act as a cohesive force among hydrophobic molecules (or hydrophobic areas of large molecules). This is often called hydrophobic bonding, but it isn't really bonding as such, rather a shared avoidance of water. All living cells have a hydrophilic interior surrounded by a hydrophobic membrane, as we'll see in Chapter 3.

An *amphipathic* substance is one that is part polar and part nonpolar. When such a substance is mixed with water, *micelles* are formed (Figure 2.8); the nonpolar parts are excluded by the water and group together as described above, leaving the polar groups pointing outwards into the water, where they are attracted by hydrophilic forces. Detergents exert their action by trapping

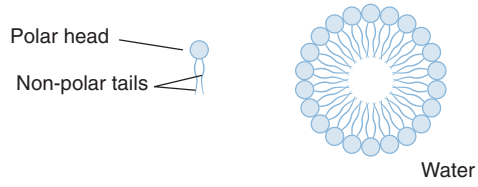


Figure 2.8 Amphipathic molecules form micelles in water. In an aqueous environment, amphipathic substances align their molecules so that the nonpolar parts are hidden away from the water. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

insoluble grease inside the centre of a micelle, while interaction with water allows them to be rinsed away (see Chapter 16).

Water plays a role in many essential metabolic reactions, and its polar nature allows for the breakdown to hydrogen and hydroxyl ions (H^+ and OH^-), and resynthesis as water. Water acts as a reactant in hydrolysis reactions such as:

$$XY + H_2O \rightarrow XH + YOH$$

or as a product in certain synthetic reactions, like

$$XH + YOH \rightarrow XY + H_2O$$

2.2 Acids, bases and pH

Only a minute proportion of water molecules, something like one in every 500 million, are present in the dissociated form, but as we've already seen, the H^+ and OH^- ions play an important part in cellular reactions.

A solution becomes acid or alkaline if there is an imbalance in the amount of these ions present. If there is an excess of H^+, the solution becomes *acid*, whilst if OH^- predominates, it becomes *alkaline*. The *pH* of a solution is an expression of the molar concentration of hydrogen ions; it is expressed thus:

$$pH = -\log_{10}[H^+]$$

In pure water, hydrogen ions are present at a concentration of 10^{-7} M, thus the pH is 7.0. This is called *neutrality*, where the solution is neither acid nor alkaline. At higher concentrations of H^+, such as 10^{-3} M (1 millimolar), the pH value is *lower*, in this case 3.0, so acid solutions have a value *below* 7. Conversely, alkaline solutions have a pH *above* 7. You will see from this example that an increase of 10^4 (10 000)-fold in the $[H^+]$ leads to a change of only four points on the pH scale. This is because it is a *logarithmic scale*, thus a solution of pH 10 is 10 times more alkaline than one of pH 9, and 100 times more than one of pH 8. Figure 2.9 shows the pH value of a number of familiar substances.

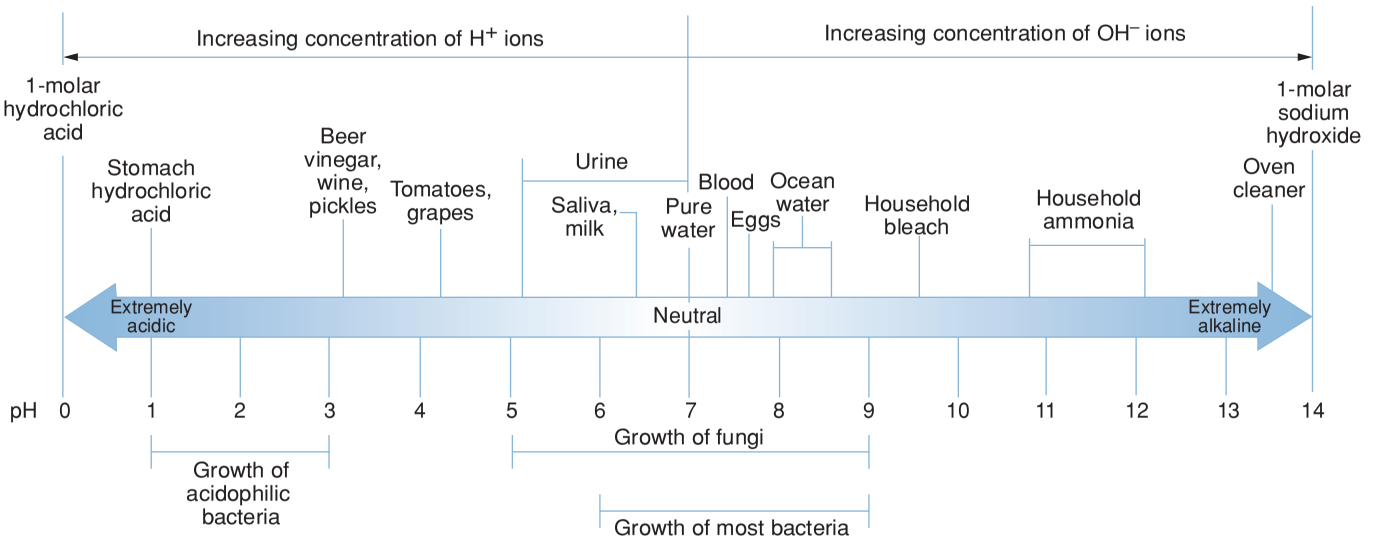
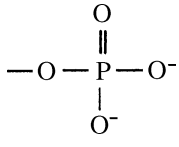


Figure 2.9 The pH value of some common substances. Most microorganisms exist at pH values around neutrality, but representatives are found at extremes of both acidity and alkalinity. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

Table 2.4 Occurrence and characteristics of some functional groups

Functional group	Formula	Type of molecule	Found in:	Remarks
Hydroxyl	–OH	Alcohols	Sugars	Polar group, making organic molecules more water soluble
Carbonyl		Aldehydes	Sugars	Carbonyl at end of chain
		Ketones	Sugars	Carbonyl elsewhere in chain
Carboxyl	–COOH	Carboxylic acids	Sugars, fats, amino acids	
Amino	$-NH_2$	Amines	Amino acids, proteins	Can gain H^+ to become NH_3^+
Sulphydryl	–SH	Thiols	Amino acids, proteins	Oxidises to give S=S bonds
Phosphate			Phospholipids, nucleic acids	Involved in energy transfer

Most microorganisms live in an aqueous environment, and the pH of this is very important. Most will only tolerate a small range of pH, and the majority occupy a range around neutrality, although as we shall see later in this book, there are some startling exceptions to this.

Most of the important molecules involved in the chemistry of living cells are *organic*, that is, they are based on a skeleton of covalently linked carbon atoms. Biological molecules have one or more *functional groups* attached to this skeleton; these are groupings of atoms with distinctive reactive properties, and are responsible for many of the chemical properties of the organic molecule. The possession of one or more functional groups frequently makes an organic molecule more polar and therefore more soluble in water. Some of the most common functional groups are shown in Table 2.4. It can be seen that the functional groups occur in simpler organic molecules as well as in the more complex macromolecules we consider below.

2.3 Biomacromolecules

Many of the most important molecules in biological systems are *polymers*, that is, large molecules made up of smaller subunits joined together by covalent bonds, and in some cases in a specific order.

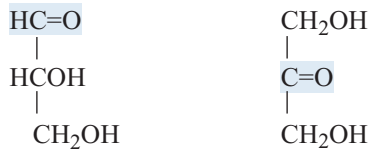
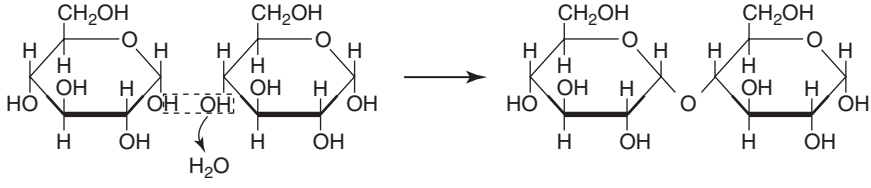


Figure 2.10 Monosaccharides may be aldoses or ketoses. The three-carbon sugars (a) glyceraldehyde and (b) dihydroxyacetone share the same molecular formula, but have different functional groups. The two molecules are isomers (see Box 2.2).

2.3.1 Carbohydrates

Carbohydrates are made up of just three different elements: carbon, hydrogen and oxygen. The simplest carbohydrates are *monosaccharides*, or simple sugars; these have the general formula $(CH_2O)_n$. They are classed as either aldoses or ketoses, according to whether they contain an aldehyde group or a ketone group (Figure 2.10). Monosaccharides can furthermore be classified on the basis of the number of carbon atoms they contain. The simplest are trioses (three carbons) and the most important biologically are hexoses (six carbons) (see Boxes 2.2 and 2.3).



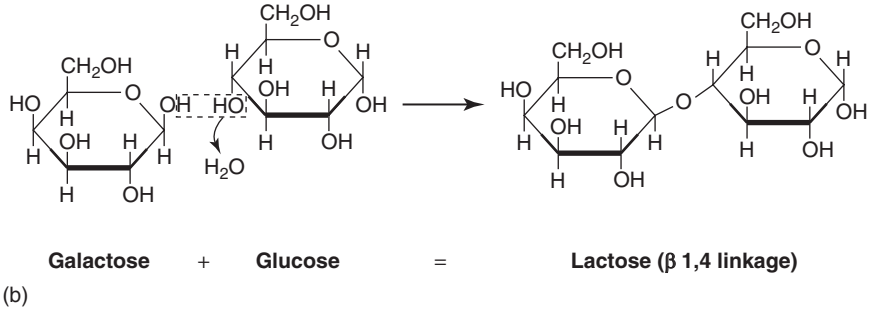


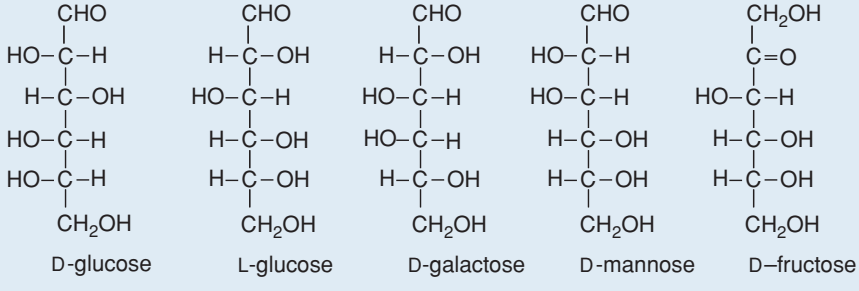
Figure 2.11 Monosaccharides can be joined by a glycosidic linkage. The result is a condensation reaction, in which a molecule of water is lost. α- and β- linkages result in different orientations in space.

Monosaccharides are generally crystalline solids that are soluble in water and have a sweet taste. They are all reducing sugars, so called because they are able to reduce alkaline solutions of copper ions from the cupric (Cu^{2+}) to the cuprous (Cu^{+}) form.

A *disaccharide* is formed when two monosaccharides (which may be of the same type or different), join together with a concomitant loss of a water molecule (Figure 2.11). Further monosaccharides can be added, giving chains of three, four, five or more units. These are termed *oligosaccharides* (*oligo* = a few), and chains with many units are *polysaccharides*. The chemical bond

Box 2.2 Isomers: same formula, different structure

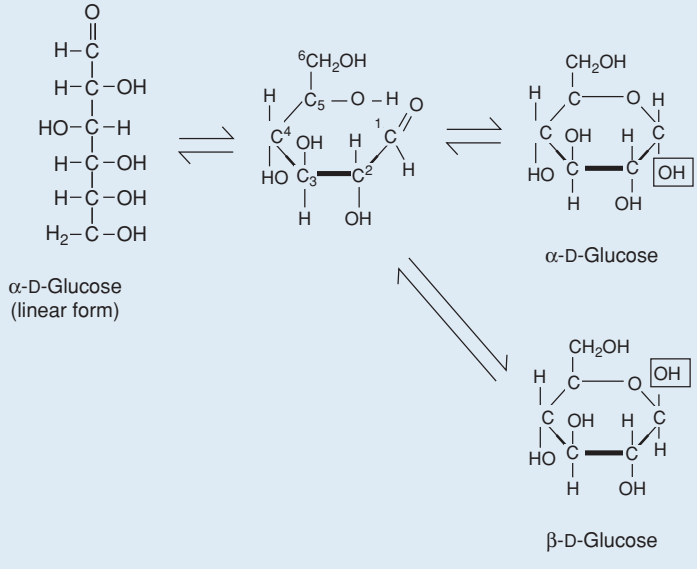
The simplest monosaccharides are the trioses glyceraldehyde and dihydroxyacetone (see Figure 2.10). Look carefully at the structures, and you will see that although they both share the same number of carbons (3), hydrogens (6) and oxygens (3), the way in which these atoms are arranged is different in the two sugars. Molecules such as these, which have the same chemical formula but different structural formulas, are said to be structural *isomers*. The different groupings of atoms lead to structural isomers having different chemical properties. When we come to look at the *hexoses* (six-carbon sugars), we see that there are many structural possibilities for the general formula $C_6H_{12}O_6$; some of these are shown below.



Note that some of these structures are identical apart from the orientation of groups around the central axis; D-glucose and L-glucose, for example, differ only in the way H atoms and –OH groups are arranged to the right or left. They are said to be *stereoisomers* or optical isomers, and are mirror images of each other, just like your right and left hands. (D- and L- are short for *dextro-* and *laevo*-rotatory, meaning that the plane of polarised light is turned to the right and left respectively when passed through a solution of these substances). Generally, living cells will only synthesise one or other stereoisomer, not both.

Box 2.3 Sugars are more accurately shown as ring structures

When dissolved in water, the aldehyde or ketone group reacts with a hydroxyl group on the fifth carbon to give a cyclic form. D-glucose is shown in both forms below. The cyclic form of the molecule is shown below as a *Haworth projection*. The idea is that the ring is orientated at 90° to the page, with the edge that is shown thicker towards you, and the top edge away from you. Notice that there are even two forms of D-glucose! Depending on whether the –OH on carbon-1 is below or above the plane of the ring, we have α- or β-D-glucose.



joining the monosaccharide units together is called a *glycosidic linkage*. The bond between the two glucose molecules that make up maltose is called an α-glycosidic linkage; in lactose, formed from one glucose and one galactose, we have a β-glycosidic linkage. The two bonds are formed in the same way, with the elimination of water, but they have a different orientation in space. Thus disaccharides bound together by α- and β-glycosidic linkages have a different overall shape and as a result the molecules behave differently in cellular reactions.

Biologically important molecules such as starch, cellulose and glycogen are all polysaccharides. Another is dextran, a sticky substance produced by some bacteria to aid their adhesion. They differ from monosaccharides in being generally insoluble in water, not tasting sweet and not being able to reduce

cupric ions. Most polysaccharides are made up from either pentose or hexose sugars, and, like di- and oligosaccharides, can be broken down into their constituent subunits by hydrolysis reactions.

2.3.2 Proteins

Of the macromolecules commonly found in living systems, proteins have the greatest structural diversity, a fact reflected in their wide range of biological functions.

The five elements found in most naturally occurring proteins are carbon, hydrogen, oxygen, nitrogen and sulphur. In addition, other elements may be essential components of certain specialised proteins such as haemoglobin (iron) and casein (phosphorus).

Proteins can be very large molecules, with molecular weights of tens or even hundreds of thousands. Whatever their size, and in spite of the diversity referred to above, all proteins are made up of a collection of 'building bricks' called *amino acids* joined together. Amino acids are thought to have been among the first organic molecules formed in the early history of the Earth, and many different types exist in nature. All these, including the 20 commonly found in proteins, are based on a common structure, shown in Figure 2.12. It comprises a central carbon atom (known as the α-carbon) covalently bonded to an amino (NH_2) group, a carboxyl (COOH) group and a hydrogen atom. It is the group attached to the fourth and final valency bond of the α-carbon that varies from one amino acid to another; this is known as the *'R'-group*.

The 20 amino acids found in proteins can be conveniently divided into five groups, on the basis of the chemical nature of their 'R'-group. These range from a single hydrogen atom to a variety of quite complex side chains (Figure 2.13). It is unlikely nowadays that you would need to memorise the precise structure of all 20 as the author was asked to do in days gone by, but it would be advisable to familiarise yourself with the groupings and examples from each of them. The groups differentiate on the basis of a polar/nonpolar nature and on the presence or absence of an ionisable 'R'-group.

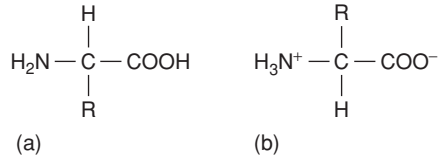


Figure 2.12 Amino acid structure. (a) The basic structure of an amino acid. (b) In solution, the amino and carboxyl groups become ionised, giving rise to a *zwitterion* (a molecule with spatially separated positive and negative charges). All the 20 amino acids commonly found in proteins are based on a common structure, differing only in the nature of their 'R' group (see Figure 2.13).

Box 2.4 Amino acid shorthand

It is sometimes necessary to express in print the sequence of amino acids that make up the primary structure of a particular protein; clearly it would be desperately tedious to express a sequence of hundreds of bases in the form 'glycine, phenylalanine, tryptophan, methionine, . . . etc.', so a system of abbreviations for each amino acid has been agreed. Each amino acid can be reduced to a three-letter code, thus you might see something like:

1	2	3	4	5	6	7	8	9	10	11	
Gly	Phe	Tyr	Met	His	Lys	Gly	Ala	His	Val	Glu	. . . and so on.

Note that each residue has a number; this *numbering always begins at the N-terminus*.

Each amino acid can also be represented by a single letter. The abbreviations using the two systems are shown below.

A	Ala	Alanine	M	Met	Methionine
B	Asx	Asparagine/aspartic acid	N	Asn	Asparagine
C	Cys	Cysteine	P	Pro	Proline
D	Asp	Aspartic acid	Q	Gln	Glutamine
E	Glu	Glutamic acid	R	Arg	Arginine
F	Phe	Phenylalanine	S	Ser	Serine
G	Gly	Glycine	T	Thr	Threonine
H	His	Histidine	V	Val	Valine
I	Ile	Isoleucine	W	Trp	Tryptophan
K	Lys	Lysine	Y	Tyr	Tyrosine
L	Leu	Leucine	Z	Glx	Glutamine/glutamic acid

Note that one amino acid, *proline*, falls outside the main groups. Closer inspection of its molecular structure reveals that this differs from the other amino acids by having one of its N–H linkages replaced by an N–C, which forms part of a cyclic structure (see Figure 2.13). This puts certain conformational constraints upon proteins containing proline residues.

As can be seen from Figure 2.13, the simplest amino acid is glycine, whose R-group is simply a hydrogen atom. This means that the glycine molecule is symmetrical, with a hydrogen atom on opposite valency bonds. All the other amino acids, however, are asymmetrical: the α-carbon acts as what is called a chiral centre, giving the molecule right or left 'handedness'. Thus two *stereoisomers*, known as the D- and L- forms, are possible for each of the amino acids except glycine. Note that all the amino acids found in naturally occurring proteins have the L-form; the D-form also occurs in nature but only in certain specific, nonprotein contexts.

Proteins, as we've seen, are made up of amino acids; these are joined together by means of *peptide bonds*, involving the $-NH_2$ group of one amino

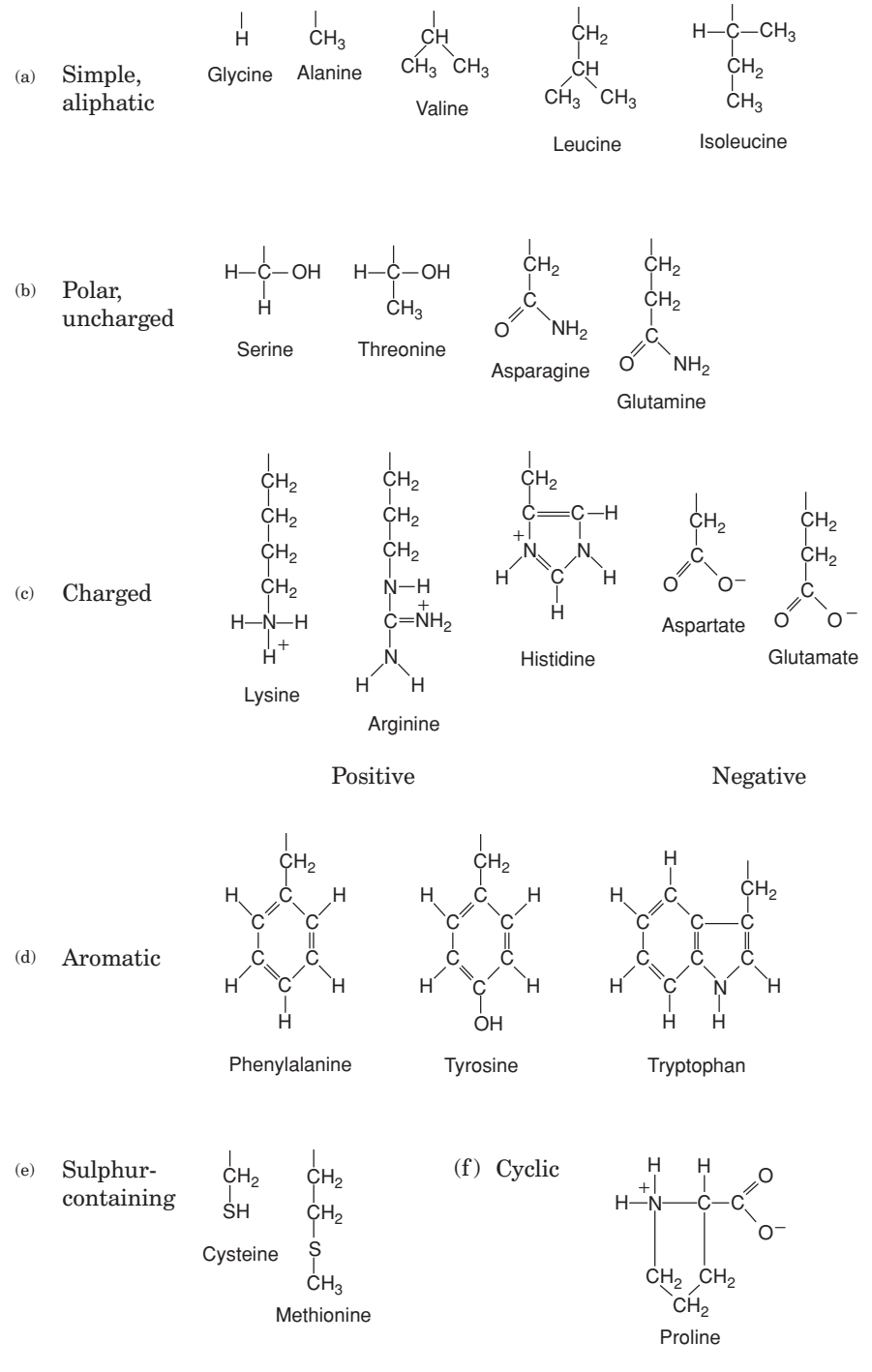


Figure 2.13 The 20 amino acids found in proteins. The 'R' group of each amino acid is shown. These range from the simplest, glycine, to more complex representatives such as tryptophan.

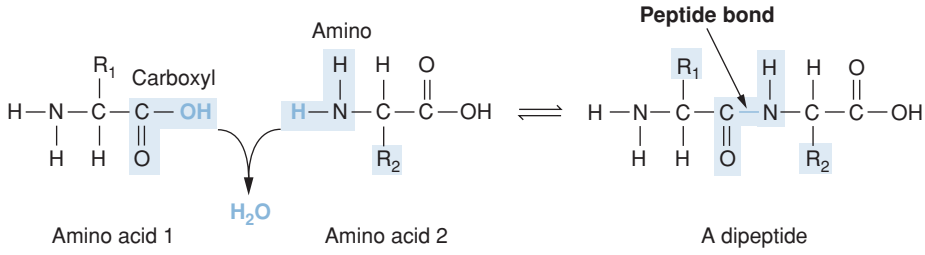


Figure 2.14 Amino acids are joined by peptide linkage. The carboxyl group of one amino acid is joined to the amino group of another. This is another example of a condensation reaction (cf. Figure 2.11). No matter how many amino acids are added, the resulting structure always has a free carboxyl group at one end and a free amino group at the other.

acid and the –COOH group of another. The formation of a peptide bind is a form of *condensation reaction* in which water is lost (Figure 2.14). The resulting structure of two linked amino acids is called a *dipeptide,* and retains an $–NH_2$ at one end and a –COOH at the other. If we were to add another amino acid to form a tripeptide, this would still be so, and if we kept on adding them until we had a *polypeptide*, we would still have the same two groupings at the extremities of the molecule. These are referred to as the *N-terminus* and the *C-terminus* of the polypeptide. Since a water molecule has been removed at the formation of each peptide bond, we refer to the chain so formed as being composed of amino acid *residues*, rather than amino acids. Generally, when a polypeptide reaches 50 residues in length it is termed a protein, although the actual distinction is not clear-cut.

So far, we can think of proteins as long chains of many amino acid residues, rather like a string of beads. This is called the *primary structure* of the protein; it is determined by the relative proportions of each of the 20 amino acids, and also the order in which they are joined together. It is the basis of all the remaining levels of structural complexity, and it *ultimately determines the properties of a particular protein.* It is also what makes one protein different from another. Since the 20 types of amino acid can be linked together in any order, the number of possible sequences is astronomical, and it is this great variety of structural possibilities that gives proteins such diverse structures and functions.

In theory, there are 20^{100} or some 10^{130} different ways in which 20 different amino acids could combine to give a protein 100 amino acid residues in length!

2.3.3 Higher levels of protein structure

The structure of proteins is a good deal more complicated than just a linear chain of amino acids. A long thin chain would be a very fragile structure;

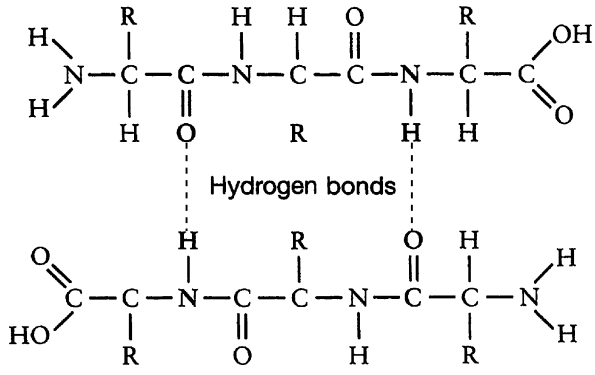


Figure 2.15 Secondary structure in proteins. Hydrogen bonding occurs between the –CO and –NH groups of amino acids on the backbone of a polypeptide chain. The two amino acids may be on the same or different chains.

proteins that have undergone folding are more stable and compact. The results of this folding are the secondary and tertiary structures of a protein.

The *secondary structure* is due to hydrogen bonding between a carbonyl (–CO) group and an amido (–NH) group of amino acid residues on the peptide backbone (Figure 2.15). *Note that the 'R'-group plays no part in secondary protein structure.* Two regular patterns of folding result from this; the α-helix and the β-pleated sheet.

The *α-helix* occurs when hydrogen bonding takes place between amino acids close together in the primary structure. A stable helix is formed by the –NH group of an amino acid bonding to the –CO group of the amino acid four residues further along the chain (Figure 2.16a). This causes the chain to twist into the characteristic helical shape. One turn of the helix occurs every 3.6 amino acid residues, and results in a rise of 5.4 Å; this is called the *pitch height* of the helix. The ability to form a helix like this is dependent on the component amino acids; if there are too many with large R-groups, or R-groups carrying the same charge, a stable helix will not be formed. Because of its rigid structure, proline (see Figure 2.13) cannot be accommodated in an α-helix. Naturally occurring α-helices are always *right-handed*, that is, the chain of amino acids coils round the central axis in a clockwise direction. This is a much more stable configuration than a left-handed helix, due to the fact that there is less steric hindrance (overlapping of electron clouds) between the R-groups and the C=O group on the peptide backbone. (Note that if proteins were made up of the D-form of amino acids, we would have the reverse situation, with a left-handed form favoured). In the *β-pleated sheet,* the hydrogen bonding occurs between amino acids either on

Very small distances within molecules are measured in Angstrom units (Å). One Angstrom unit is equal to one tenbillionth (10^{-10}) of a metre.

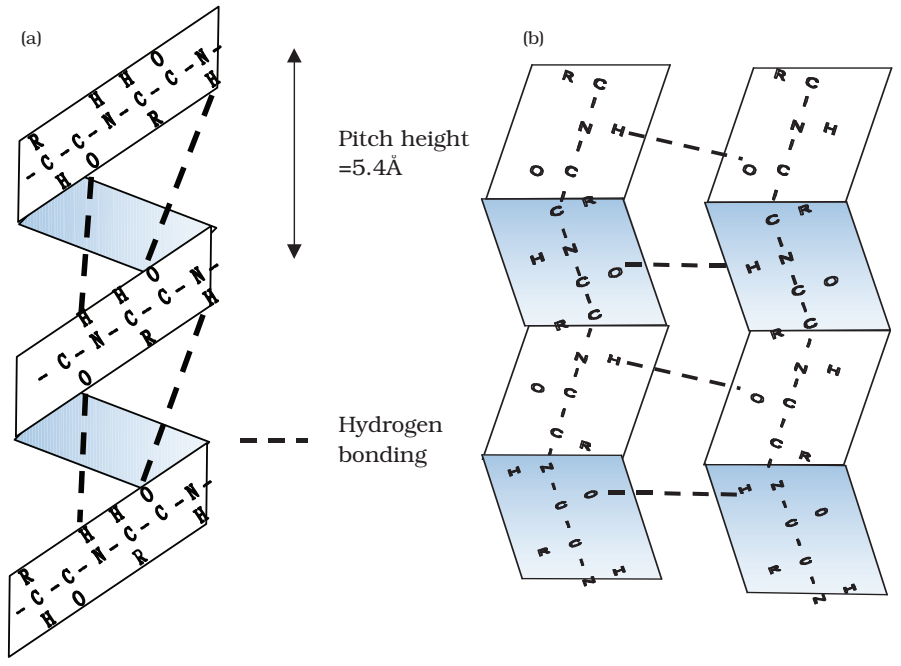


Figure 2.16 Secondary structure in proteins: the α-helix and β-pleated sheet. (a) Hydrogen bonding between amino acids four residues apart in the primary sequence results in the formation of an α-helix. (b) In the β-pleated sheet hydrogen bonding joins adjacent chains. Note how each chain is more fully extended than in the α-helix. In the example shown, the chains run in the same direction (parallel).

separate polypeptide chains or on residues on the same chain but far apart in the primary structure (Figure 2.16b). The chains in a β-pleated sheet are fully extended, with 3.5 Å between adjacent amino acid residues (cf. α-helix, 1.5 Å). When two or more of these chains lie next to each other, extensive hydrogen bonding occurs between the chains. Adjacent strands in a β-pleated sheet can either run in the same direction (e.g. N → C), giving rise to a parallel β-pleated sheet, or in opposite directions (antiparallel β-pleated sheet).

A common structural element in the secondary structure of proteins is the β-*turn*. This occurs when a chain doubles back on itself, such as in an antiparallel β-pleated sheet. The –CO group of one amino acid is hydrogen bonded to the –NH group of the residue three further along the chain. Frequently, it is called a *hairpin turn*, for obvious reasons (Figure 2.17). Numerous changes in direction of the polypeptide chains result in a compact, globular shape to the molecule.

Typically about 50% of a protein's secondary structure will have an irregular form. Although this is often referred to as random coiling, it is only random in the sense that there is no regular pattern; it still contributes towards

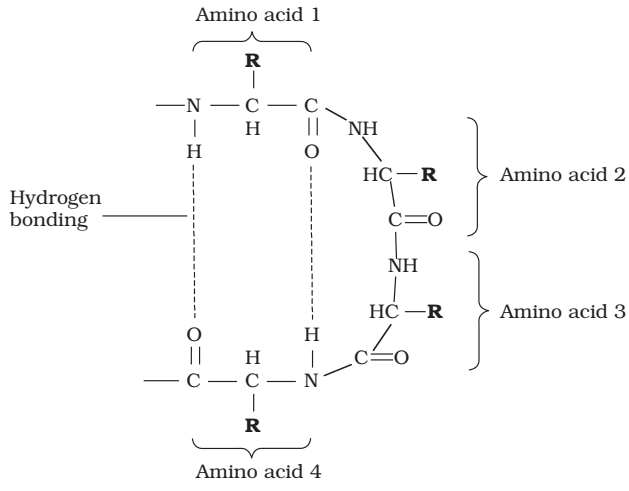


Figure 2.17 The β-turn. The compact folding of many globular proteins is achieved by the polypeptide chain reversing its direction in one or more places. A common way of doing this is with the β-turn. Hydrogen bonding between amino acid residues on the same polypeptide stabilises the structure.

the integrity of the molecule. The proportions and combinations in which α-helix, β-pleated sheet and random coiling occur vary from one protein to another. Keratin, a structural protein found in skin, horn and feathers, is an example of a protein entirely made up of α-helix, whilst the lectin (sugar-binding protein) concanavalin A is mostly made up of β-pleated sheets.

The *tertiary structure* of a protein is due to interactions between side chains, that is, R-groups of amino acid residues, resulting in the folding of the molecule to produce a thermodynamically more favourable structure. The structure is formed by a variety of weak, non-covalent forces; these include hydrogen bonding, ionic bonds, hydrophobic interactions, and van der Waals forces. The strength of these forces diminishes with distance, therefore the formation of a compact structure is encouraged. In addition, the –SH groups on separate cysteine residues can form a covalent –S–S– linkage. This is known as a *disulphide bridge* and may have the effect of bringing together two cysteine residues that are far apart in the primary sequence (Figure 2.18).

In globular proteins, the R-groups are distributed according to their polarities; nonpolar residues such as valine and leucine nearly always occur on the inside, away from the aqueous phase, while charged, polar residues including glutamic acid and histidine generally occur at the surface, in contact with the water.

The protein can be *denatured* by heating or treatment with certain chemicals; this causes the tertiary structure to break down and the molecule to

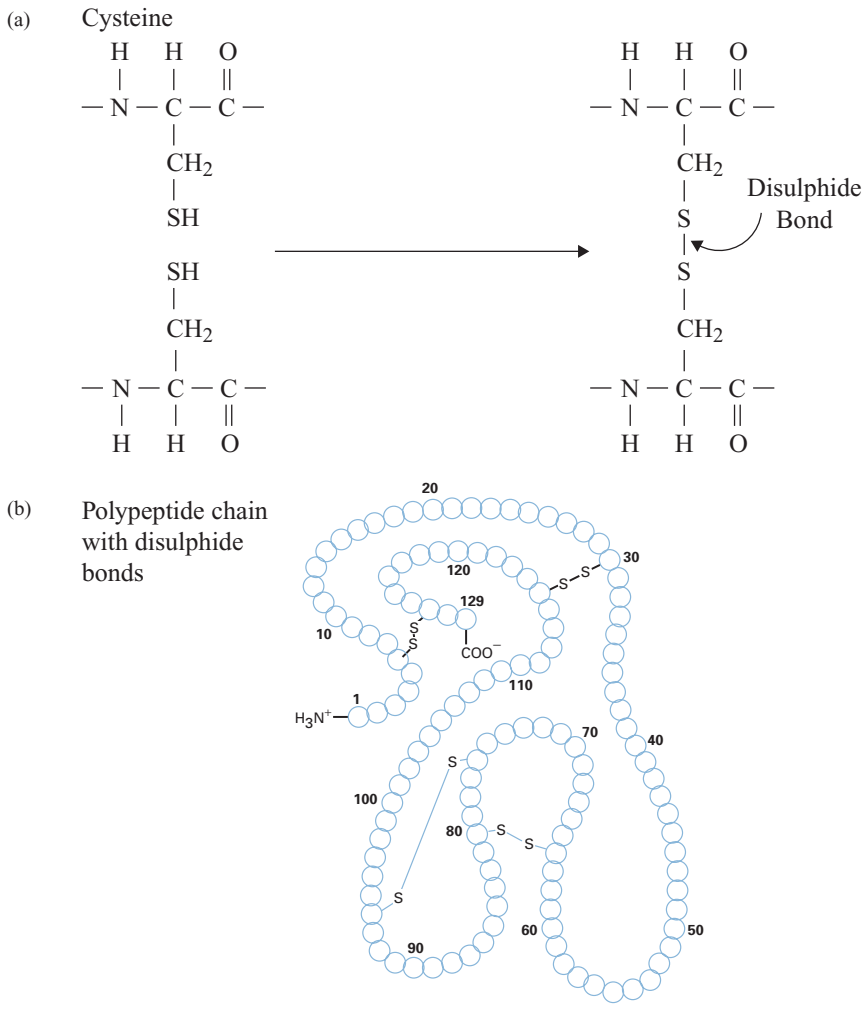


Figure 2.18 Disulphide bond formation. (a) Disulphide bonds formed by the oxidation of cysteine residues result in cross-linking of a polypeptide chain. (b) This can have the effect of bringing together residues that lie far apart in the primary amino acid sequence. Disulphide bonds are often found in proteins that are exported from the cell, but rarely in intracellular proteins.

unfold, resulting in a loss of the protein's biological properties. Cooling, or removal of the chemical agents, will lead to a restoration of both the tertiary structure and biological activity, showing that both are entirely dependent on the primary sequence of amino acids.

Even the tertiary structure may not be the ultimate level of organisation of a protein, because some are made up of two or more polypeptide chains, each with its own secondary and tertiary structure, combined together to give the

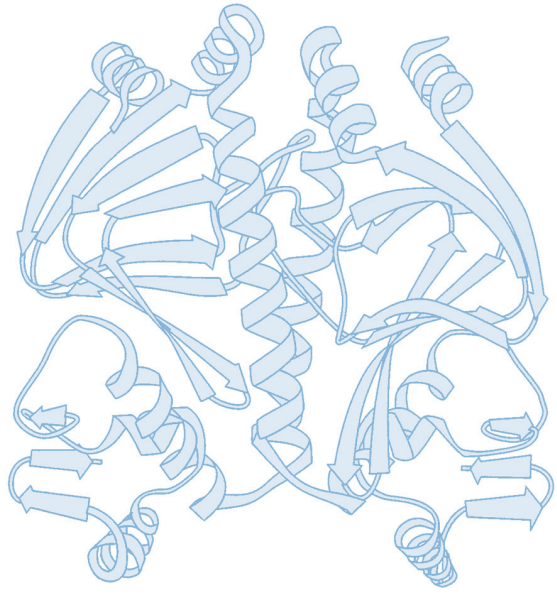


Figure 2.19 Polypeptide chains may join to form quaternary structure. The example shown comprises two identical polypeptide subunits. Coils indicate α-helical sequences, arrows are β-pleated sheets. Reproduced from Bolsover, SR, et al. (1997) From Genes to Cells, with permission from John Wiley & Sons.

quaternary structure (Figure 2.19). These chains may be identical or different, depending on the protein. As with the tertiary structure, non-covalent forces between R-groups are responsible, the difference being that this time they link amino acid residues on separate chains rather than on the same one.

Such proteins lose their functional properties if dissociated into their constituent units; the quaternary joining is essential for their activity. The enzyme phosphorylase A is an example of a protein with a quaternary structure. It has four subunits, which have no catalytic activity unless joined together as a tetramer.

Although all proteins are polymers of amino acids existing in various levels of structural complexity as we've seen above, some have additional, non-amino acid components. They may be organic, such as sugars (glycoproteins) or lipids (lipoproteins) or inorganic, including metals (metalloproteins) or phosphate groups (phosphoproteins). These components, which form an integral part of the protein's structure, are called *prosthetic groups*.

2.3.4 Nucleic acids

The third class of polymeric macromolecules are the *nucleic acids*. These are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and both are polymers of smaller molecules called nucleotides.

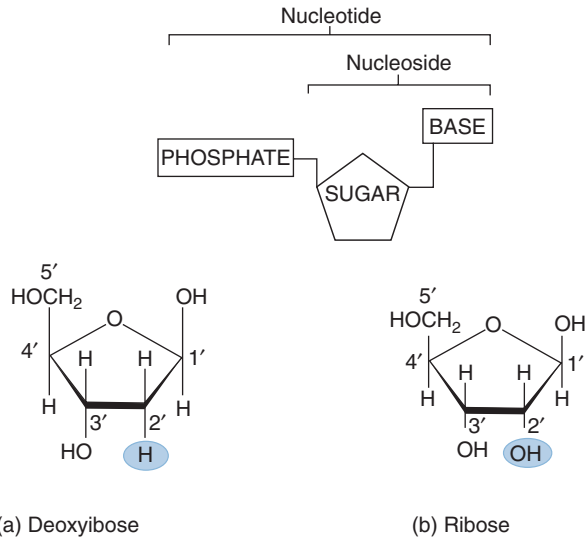


Figure 2.20 A nucleotide has three parts: a pentose sugar, a phosphate group and a nitrogenous base (see Figure 2.21). Note the difference between the sugars (a) deoxyribose (DNA) and (b) ribose (RNA).

As we shall see, there are important differences both in the overall structures of RNA and DNA and in the nucleotides they contain, so we shall consider each of them in turn.

The structure of DNA The composition of a DNA nucleotide is shown in Figure 2.20(a). It has three parts: a five-carbon sugar called *deoxyribose*, a phosphate group and a base. This base can be any one of four molecules; as can be seen in Figure 2.21, these are all based on a cyclic structure containing nitrogen. Two of the bases, *cytosine* and *thymine*, have a single ring and are called *pyrimidines*. The other two, *guanine* and *adenine*, have a double ring structure; these are the *purines*. For convenience, the four bases are often referred to by their initial letter only: A, C, G and T.

One nucleotide differs from another by the identity of the base it contains; the rest of the molecule (sugar and phosphate) is identical. You will recall from the previous section that the properties of a protein depend on the order in which its constituent amino acids are linked together; we have exactly the same situation with nucleic acids, except that instead of an 'alphabet' of 20 'letters', here we have one of only four. Nevertheless, because nucleic acid molecules are extremely long, and the bases

Erwin Chargaff measured the proportions of the different nucleotides in a range of DNA samples. He found that T always = A and C always = G. Watson and Crick interpreted this as meaning that the bases always paired up in this way.

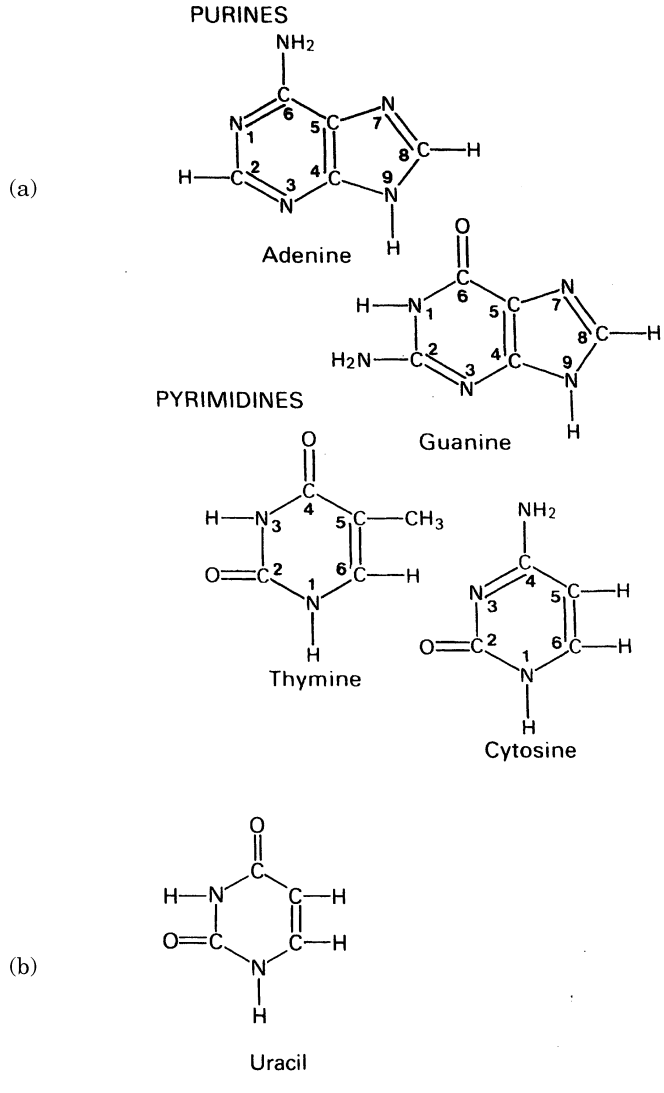


Figure 2.21 Bases belong to two classes. Nucleotides differ from each other in the identity of the nitrogenous base. (a) In DNA these are adenine (A), cytosine (C), guanine (G) or thymine (T). The purines (A and G) have a two-ring structure, while the pyrimidines (C and T) have only one ring. (b) In RNA, thymine is replaced by a similar molecule, uracil (U).

can occur in almost any order, an astronomically large number of different sequences is possible.

The nucleotides join together by means of a *phosphodiester bond* that links the phosphate group of one nucleotide to an –OH group on the 3-carbon of the deoxyribose sugar of another (Figure 2.22). The chain of nucleotides therefore has a free –OH group on a 3-carbon (the *3′ end*) and a phosphate

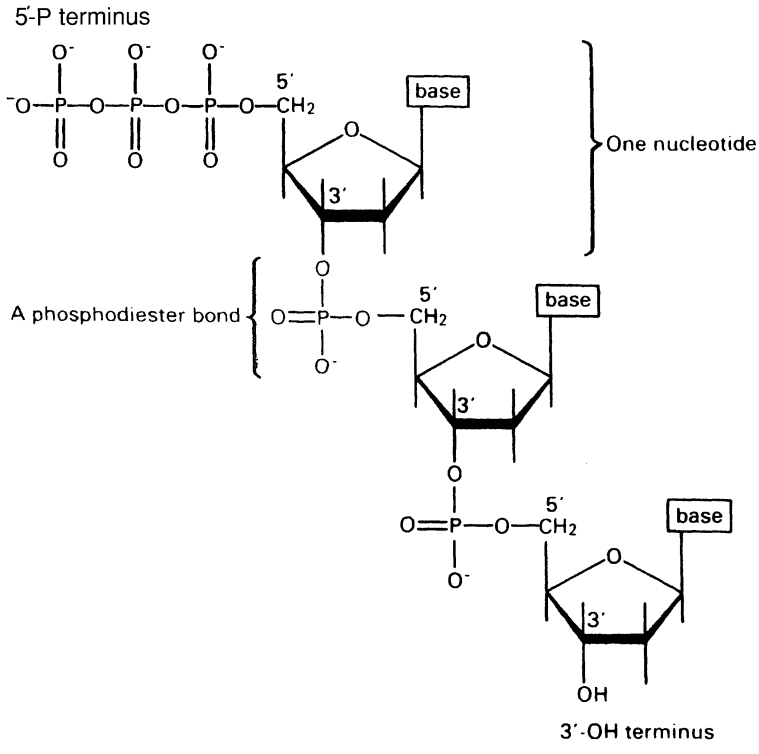


Figure 2.22 The phosphodiester bond. A chain of DNA is made longer by the addition of nucleotides containing not one but three phosphate groups; on joining the chain, two of these phosphates are removed. Nucleotides are joined to each other by a phosphodiester bond, linking the phosphate group on the 5-carbon of one deoxyribose to the –OH group on the 3-carbon of another. (These carbons are known as 5′ and 3′ to distinguish them from the 3- and 5-carbon on the nitrogenous base.) Note that the resulting chain, however many nucleotides it may comprise, always has a 5′(PO_4) group at one end and a 3′(OH) group at the other.

group on a 5-carbon (the *5′ end*). This remains the case however long the chain becomes.

However, the structure of DNA is not just a single chain of linked nucleotides, but two chains wound around each other to give the famous *double helix* form proposed by James Watson and Francis Crick in 1953 (Figure 2.23; see also Chapter 11). If we compare this to an open spiral staircase, alternate sugar and phosphate groups make up the 'skeleton' of the staircase, while the inward-facing bases pair up by hydrogen bonding to form the steps. Notice that each nucleotide pair always comprises three rings, resulting from a combination of one purine and one pyrimidine base. This means that the two strands of the helix are always evenly spaced. The way in which the bases pair up is further governed by the phenomenon of complementary base

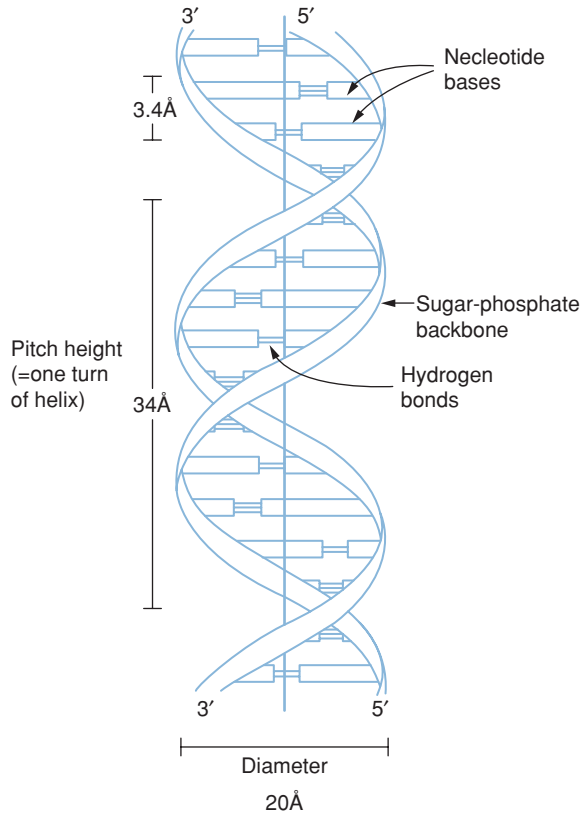


Figure 2.23 DNA is a double helix. The model proposed by Watson and Crick has two chains of nucleotides joined together by hydrogen-bonded base pairs pointing inwards towards the centre of the helix. The rules of complementary base pairing mean that the sequence of one chain can be predicted from the sequence of the other. Note how the chains run in opposite directions (antiparallel).

pairing. *A nucleotide containing thymine will only pair with one containing adenine, and likewise guanine always pairs with cytosine* (Figure 2.24). Thus the sequence of nucleotides on one strand of the double helix determines that of the other, as it has a complementary structure. Figure 2.23 shows how the two strands of the double helix are *antiparallel*, that is, they run in opposite directions, one 5′ → 3′ and the other 3′ → 5′. In Chapter 11 we shall look at how this structure was used to propose a mechanism for the way in which DNA replicates and genetic material is copied.

The structure of RNA In view of the similarities in the structure of DNA and RNA, we shall confine ourselves here to a consideration of the major

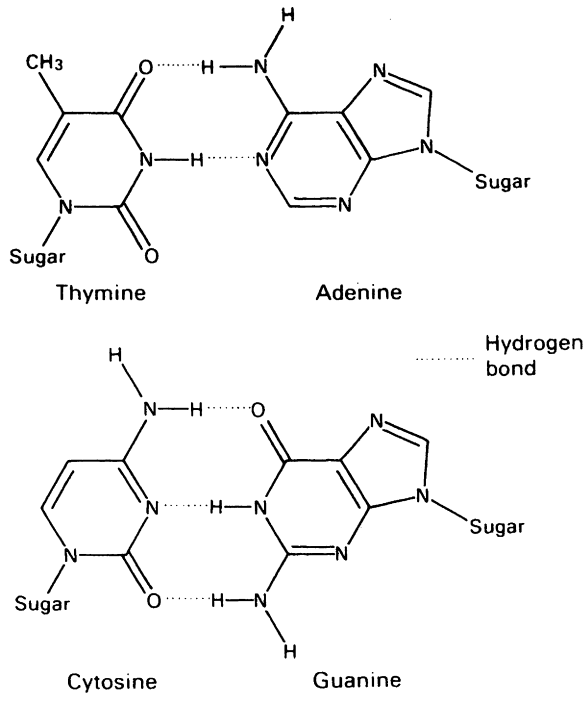


Figure 2.24 DNA nucleotides obey strict rules of base-pairing. Adenine pairs only with thymine, and guanine with cytosine, thus if the sequence of bases in one strand of a DNA molecule is known, that of the other can be predicted. This critical feature of Watson and Crick's model offers an explanation for how DNA is able to replicate itself. Note that GC pairs are held together by three hydrogen bonds, while AT pairs only have two.

differences. There are two important differences in the composition of nucleotides of RNA and DNA. The central sugar molecule of RNA is not deoxyribose, but *ribose*; as shown in Figure 2.20, these differ only in the possession of an H atom or an –OH group attached to carbon-2. Second, although RNA shares three of DNA's nitrogenous bases (A, C and G), instead of thymine it has *uracil*. Like thymine, this can form pairs specifically with adenine.

The final main difference between RNA and DNA is the fact that RNA generally comprises only a single polynucleotide chain, although this may be subject to secondary and tertiary folding as a result of complementary base pairing within the same strand. The roles of the three different forms of RNA will be discussed in Chapter 11.

2.3.5 Lipids

Although lipids can be large molecules, they are not regarded as macromolecules because unlike proteins, polysaccharides and nucleic acids, they

are not polymers of a basic subunit. Moreover, lipids do not share any single structural characteristic; they are a diverse group structurally, but have in common the fact that they are *insoluble in water,* but soluble in a range of organic solvents. This nonpolar nature is due to the predominance of covalent bonding, mainly between atoms of carbon and hydrogen.

Fats are simple lipids, whose structure is based on *fatty acids*. Fatty acids are long hydrocarbon chains ending in a carboxyl (–COOH) group. They have the general formula:

$$CH_3 - (CH_2)_n - COOH$$

where '*n*' is usually an even number. They combine with glycerol according to the basic reaction:

$$\text{Alcohol} + \text{Acid} \rightarrow \text{Ester}$$

The bond so formed is called an ester linkage, and the result is an acylglycerol (Figure 2.25). One, two or all three of the –OH groups may be esterified with a fatty acid, to give respectively mono-, di- and triacylglycerols (or mono-, di- and triglycerides). Natural fats generally contain a mixture of two or three different fatty acids substituted at the three positions; consequently, a considerable diversity is possible among fats. Fats serve as energy stores; a higher proportion of C–C and C–H bonds in comparison with proteins or carbohydrates results in a greater energy-storing capacity.

The second main group of lipids to be found in living cells are *phospholipids*. These have a similar structure to triacylglycerols, except that instead of a third fatty acid chain, they have a phosphate group joined to the glycerol (Figure 2.26), introducing a hydrophilic element to an otherwise hydrophobic molecule. Thus phospholipids are an example of an amphipathic molecule, with a polar region at one end of the molecule and a nonpolar region at the other. This fact is essential for the formation of a bilayer when the phospholipid is introduced into an aqueous environment; the hydrophilic phosphate groups point outwards towards the water, while the hydrophobic hydrocarbon chains 'hide' inside (Figure 2.27, and cf. Figure 2.8, micelle formation).

This bilayer structure forms the basis of all biological membranes (see Chapter 3), forming a barrier around cells and certain organelles. Phospholipids generally have another polar group attached to the phosphate; Figure 2.26 shows the effect of substituting choline.

The structural diversity of lipids can be illustrated by comparing fats and phospholipids with the final group of lipids we need to consider, the steroids. As can be seen from Figure 2.28, these have a completely different form,

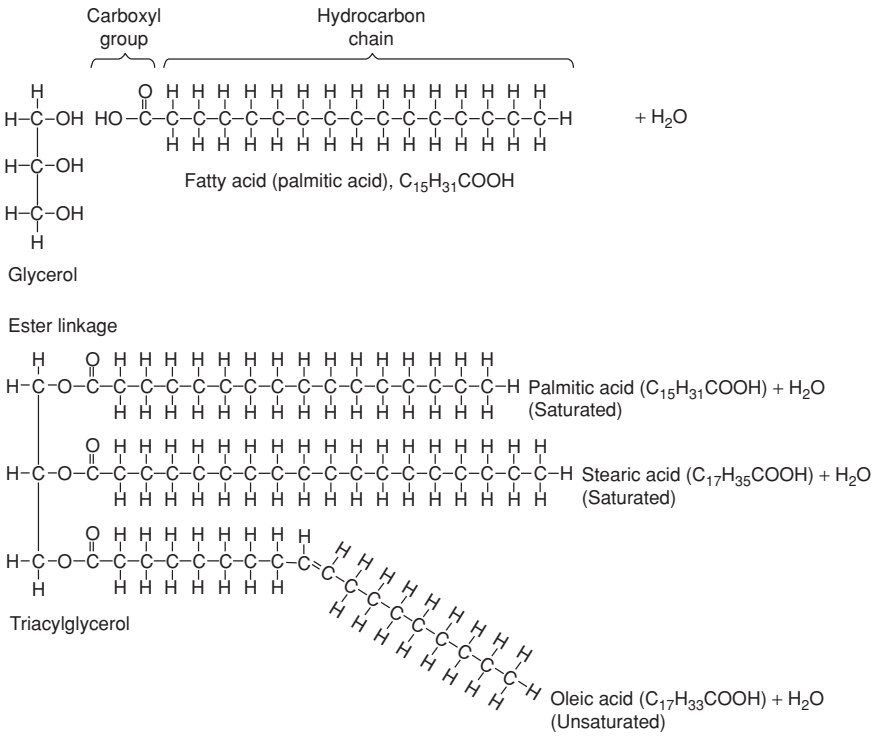


Figure 2.25 Acylglycerol formation. Fatty acids are linked to glycerol to form an acylglycerol. When all three –OH groups on the glycerol are esterified, the result is a triacylglycerol or triglyceride. The three fatty acids may or may not be the same. In the example shown, one of the fatty acids is unsaturated (see Box 2.5).

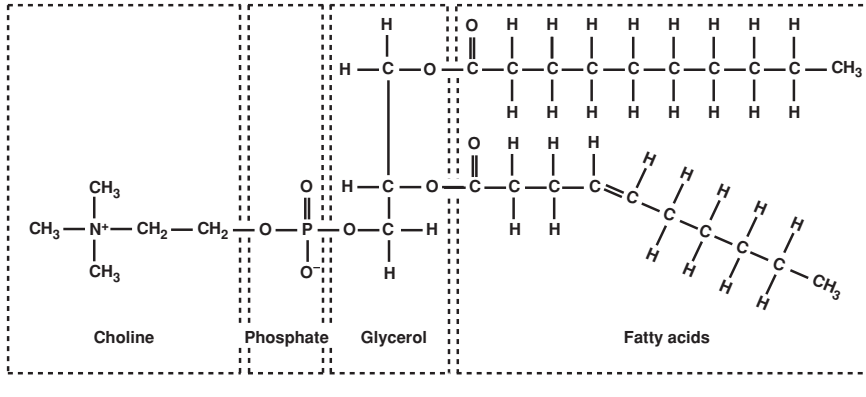


Figure 2.26 Phospholipids. Phospholipids introduce a polar element to acylglycerols by substituting a phosphate at one of the glycerol –OH groups. A second charged group may attach to the phosphate group; the phospholipid shown is phosphatidylcholine.

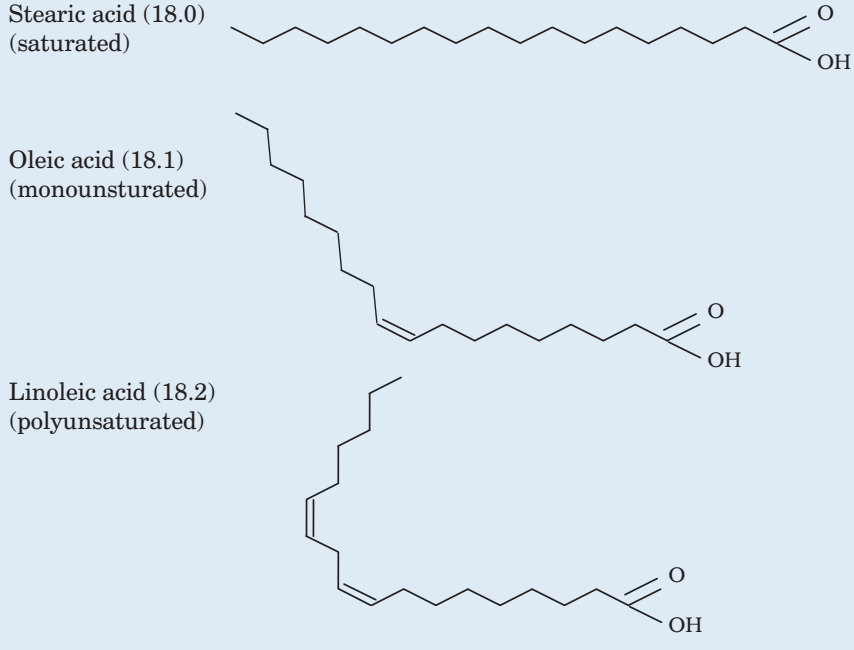
Box 2.5 Saturated or unsaturated?

You may well have heard of saturated and unsaturated fats in the context of the sorts of foods we should and shouldn't be eating. This terminology derives from the type of fatty acids that make up the different fats.

Each carbon atom in the hydrocarbon chain of a *saturated* fatty acid such as stearic acid is bonded to the maximum possible number of hydrogen atoms (i.e. it is saturated with them).

Fatty acids containing one or more double bonds have fewer hydrogen atoms and are said to be *unsaturated*.

Compare the structures of stearic acid and oleic acid below. Both have identical structures except that oleic acid has two fewer hydrogen atoms and in their place a C=C double bond. A *kink* or bend is introduced into the chain at the point of the double bond; this means that adjacent fatty acids do not pack together so neatly, leading to a drop in the melting point. The presence of unsaturated fatty acids in membrane phospholipids makes the membrane more fluid.



but still share in common the property of hydrophobicity. The four-ring planar structure is common to all steroids, with the substitution of different side groups producing great differences in function. Cholesterol is an important component of many membranes.

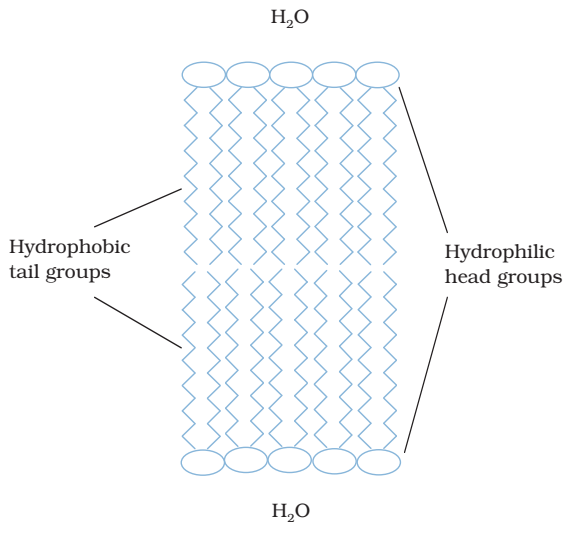


Figure 2.27 Phospholipids can form a bilayer in aqueous surroundings. A 'sandwich' arrangement is achieved by the polar phosphate groups facing outwards and burying the fatty acid chains within. Water is thus excluded from the hydrophobic region, a key property of biological membranes (see Figure 3.5).

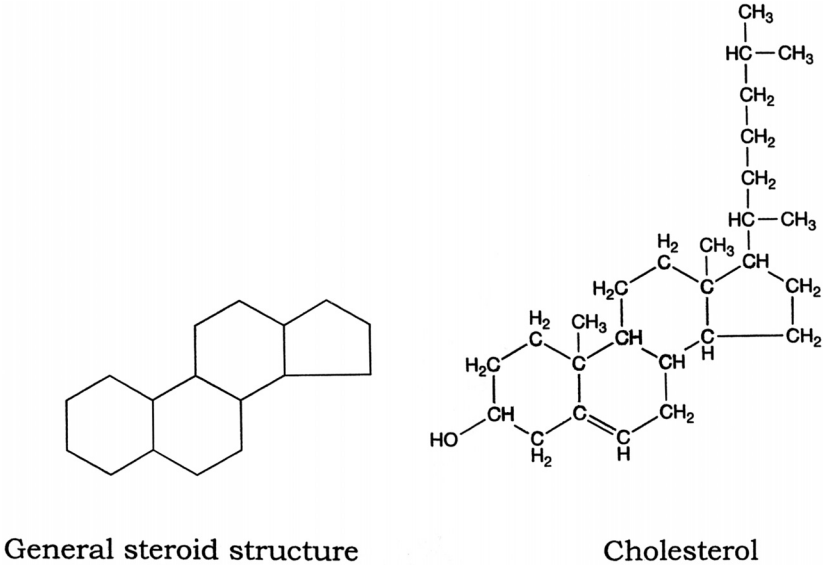


Figure 2.28 Steroids. All steroids are based on a four-ringed structure. The presence of an –OH group on the lower left ring makes the molecule a *sterol*. Cholesterol plays an important role in the fluidity of animal membranes by interposing itself among the fatty acid tails of phospholipids. The only bacterial group to contain sterols are the mycoplasmas; however, some other groups contain *hopanoids*, which have a similar structure and are thought to play a comparable role in membrane stability.

It would be wrong to gain the impression that living cells contain only molecules of the four groups outlined above. Smaller organic molecules play important roles as precursors or intermediates in metabolic pathways (see Chapter 6), and several inorganic ions such as potassium, sodium and chloride play essential roles in maintaining the living cell. Finally, some macromolecules comprise elements of more than one group, for example, lipopolysaccharides (carbohydrate and lipid) and glycoproteins (protein and carbohydrate).

3
Cell Structure and Organisation

The basic unit of all living things is the cell. The *cell theory* is one of the fundamental concepts of biology; it states that:

- all organisms are made up of cells;
- all cells derive from other, pre-existing cells.

As we shall see in this chapter, there may exist within a cell many smaller, subcellular structures, each with its own characteristics and function, but these are not capable of independent life.

An organism may comprise just a single cell (unicellular), a collection of cells that are not morphologically or functionally differentiated (colonial), or many distinct cell types organised into tissues or organs (multicellular). Among microorganisms, all bacteria and protozoans are unicellular; fungi may be unicellular or multicellular, while algae may exist in all three forms. There is, however, one way that organisms can be differentiated from each other that is even more fundamental than whether they are uni- or multicellular. It is a difference that is greater than that between a lion and a mushroom or an earthworm and an oak tree, and it exists at the level of the individual cell. All organisms are made up of one or other (but definitely not both!) of two very distinct cell types, which we call *prokaryotic* and *eukaryotic* cells, both of which exist in the microbial world. These differ from each other in many ways, including size, structural complexity and organisation of genetic material (Table 3.1).

The names given to the two cell types derive from Greek words:
Prokaryotic = 'before nucleus'
Eukaryotic = 'true nucleus'

Essential Microbiology, Second Edition. Stuart Hogg.

Table 3.1 Similarities and differences between prokaryotic and eukaryotic cell structure

Similarities	
Cell contents bounded by a *plasma membrane*	
Genetic information encoded on *DNA*	
Ribosomes act as site of protein synthesis	
Differences	
PROKARYOTIC	EUKARYOTIC
Size	
Typically 1–5 microns (μm)	Typically 10–100 μm
Genetic material	
Free in cytoplasm	Contained within a membrane-bound *nucleus*
Single circular chromosome or *nucleoid*	Multiple chromosomes, generally in pairs
Histones absent	DNA complexed with histone proteins
Internal features	
Membrane-bound organelles absent	Several membrane-bound organelles present, including *mitochondria, Golgi body, endoplasmic reticulum* and (in plants and algae) *chloroplasts*
Ribosomes smaller (70S), free in cytoplasm	Ribosomes larger (80S), free in cytoplasm or attached to membranes
Respiratory enzymes bound to plasma membrane	Respiratory enzymes located in mitochondria
Cell wall	
Usually based on *peptidoglycan* (not Archaea)	When present, based on cellulose or chitin
External features	
Cilia absent	Cilia may be present
Flagella, if present, composed of flagellin. Provide rotating motility	Flagella, if present, have complex (9 + 2) structure. Provide 'whiplash' motility
Pili may be present	Pili absent
Outside layer (slime layer, capsule, glycocalyx) present in some types	Pellicle or test present in some types

The most fundamental difference between prokaryotic and eukaryotic cells is reflected in their names; eukaryotic cells possess a true *nucleus*, and several other distinct subcellular *organelles* that are bounded by a membrane. Prokaryotes have no such organelles. Most of these differences only became apparent after the development of the electron microscopy techniques described in Chapter 1.

Table 3.2 Principal groups of prokaryotic and eukaryotic organisms

Prokaryotes	Eukaryotes
Archaea	Fungi
Bacteria	Algae
	Protozoa
	Plants
	Animals

As can be seen from Table 3.2, the prokaryotes comprise the simpler and more primitive types of microorganisms; they are generally single-celled, and arose much earlier in evolutionary history than the eukaryotes. Indeed, as discussed later in this chapter, it is widely accepted that eukaryotic cells actually developed from their more primitive counterparts. Note that the viruses do not appear in Table 3.2, because they do not have a cellular structure at all, and are incapable of independent replication. They are not therefore considered to be living organisms. (See Chapter 10 for a fuller discussion of the viruses.)

Modern nucleic acid sequencing methods used to determine *phylogenetic* relationships between organisms have revealed that within the prokaryotes there is another fundamental division. One group of bacteria were shown to differ greatly from all the others; we now call these the *Archaea*, to differentiate them from the true Bacteria. These two groups, together with the eukaryotes, are thought to have evolved from a common ancestor, and represent the three *domains* of life (Figure 3.1). The Archaea comprise a wide range of mostly anaerobic bacteria, including many of those that inhabit extreme environments such as hot springs. In this book we shall largely confine our discussions to the Bacteria; however, in Chapter 7 there is a discussion of the principal features of the Archaea and their main taxonomic groupings.

Phylogenetic: relating to the evolutionary relationship between organisms.

Despite their differences, *Archaea* and *Bacteria* are both prokaryotes.

Taxonomy is the science of classifying living (and once-living) organisms.

3.1 The prokaryotic cell

Bacteria are much smaller than eukaryotic cells; most fall into a size range of about 1–5 μm, although some may be larger than this. Some of the smallest bacteria, such as the Mollicutes (mycoplasmas), measure less than 1 μm, and are too small to be resolved clearly by an ordinary light microscope. Because of their extremely small size, it was only with the advent

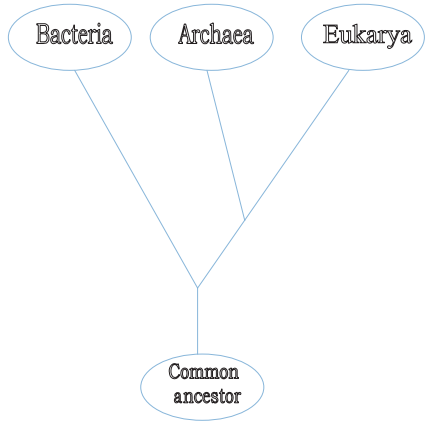


Figure 3.1 The three domains of life. All life forms can be assigned to one of three domains on the basis of their ribosomal RNA sequences. The Archaea are quite distinct from the true bacteria and are thought to have diverged from a common ancestral line at a very early stage, before the evolution of eukaryotic organisms. The scheme illustrated is the one most widely accepted by microbiologists, but alternative models have been proposed.

of the electron microscope that we were able to learn about the detailed structure of bacterial cells. Using the light microscope, however, it is possible to recognise differences in the shape and arrangement of bacteria. Although a good deal of variation is possible, most have one of three basic shapes (Figure 3.2):

In recent years, square, triangular and star-shaped bacteria have all been discovered!

- rod-shaped (bacillus; pl. bacilli);
- spherical (coccus; pl. cocci);
- curved: these range from comma-shaped (vibrio) to corkscrew-shaped (spirochaete).

All these shapes confer certain advantages to their owners; rods, with a large surface area, are better able to take up nutrients from the environment, while the cocci are less prone to drying out. The spiral forms are usually motile; their shape aids their movement through an aqueous medium.

As well as these characteristic cell shapes, bacteria may also be found grouped together in particular formations. When they divide, they may remain attached to one another, and the shape the groups of cells assume reflects the way the cell divides. Cocci, for example, are frequently found as chains of cells, a reflection of repeated division in one plane (Figure 3.2). Other cocci may form regular sheets or packets of cells, as a result of division

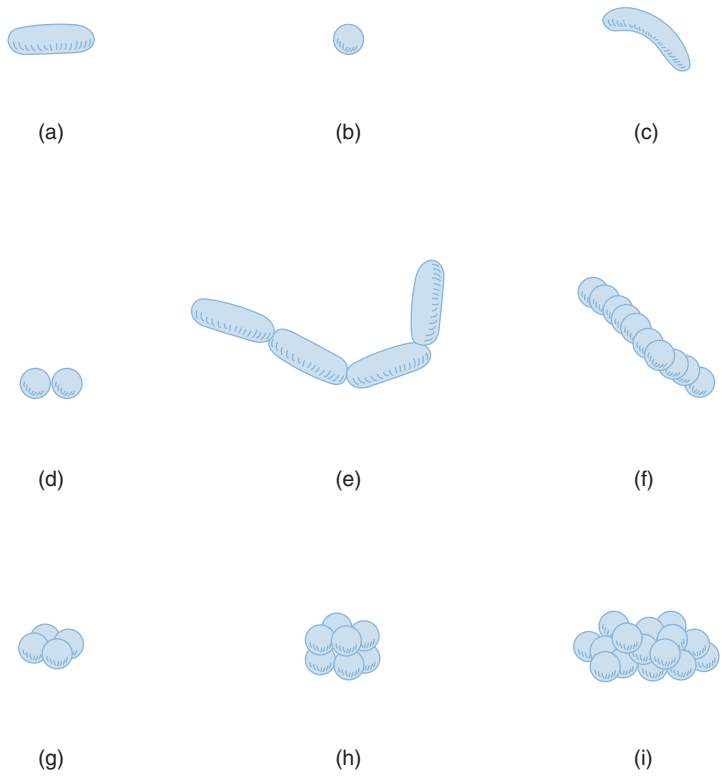


Figure 3.2 Bacterial shapes. Most bacteria are (a) rod-shaped, (b) spherical or (c) curved. These basic shapes may join to form (d) pairs, (e and f) chains, (g) sheets, (h) packets and (i) irregular aggregates.

in two or three planes. Yet others, such as the staphylococci, divide in several planes, producing the irregular and characteristic 'bunch of grapes' appearance. Rod-shaped bacteria only divide in a single plane and may therefore be found in chains, while spiral forms also divide in one plane, but tend not to stick together. Blue-greens form filaments; these are regarded as truly multicellular rather than as a loose association of individuals.

3.1.1 Prokaryotic cell structure

When compared with the profusion of elaborate organelles encountered inside a typical eukaryotic cell, the interior of a typical bacterium looks rather empty. The only internal structural features are:

- a bacterial chromosome or *nucleoid*, comprising a closed loop of double-stranded, supercoiled DNA; there may also be additional DNA in the form of *plasmids*;

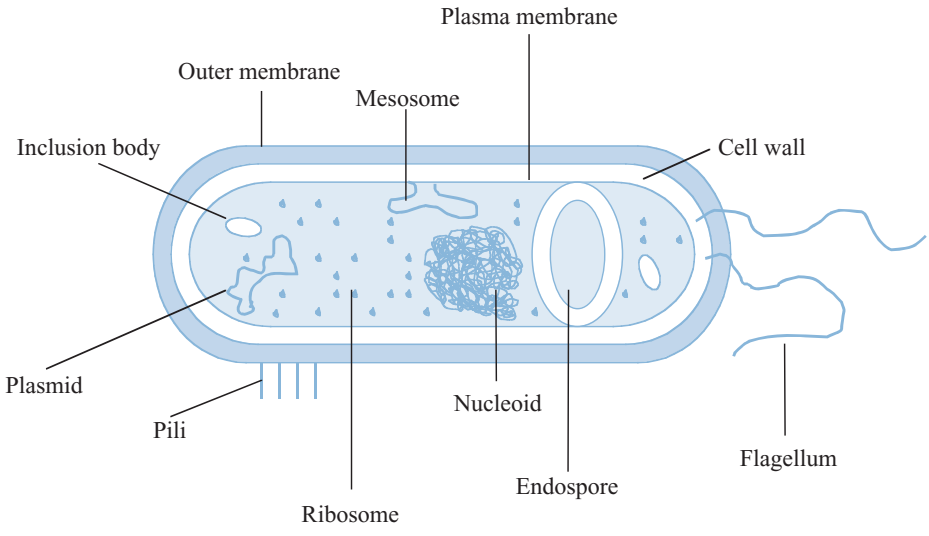


Figure 3.3 Structure of a generalised prokaryotic cell. Note the lack of complex internal organelles (cf. Figure 3.13). Gram-positive and Gram-negative bacteria differ in the details of their cell wall structure (see Figures 8.8 and 8.9).

- thousands of granular *ribosomes*;
- a variety of granular *inclusions* associated with nutrient storage.

All of these are contained in a thick aqueous soup of carbohydrates, proteins, lipids and inorganic salts known as the *cytoplasm*, which is surrounded by a *plasma membrane*. This in turn is wrapped in a *cell wall,* whose rigidity gives each bacterial cell its characteristic shape. Depending on the type of bacterium, there may be a further surrounding layer such as a *capsule* or *slime layer* and/or structures external to the cell associated with motility (*flagella*) or attachment (*pili/fimbriae*). Figure 3.3 shows these features in a generalised bacterial cell. In the following pages we shall examine these features in a little more detail, noting how each has a crucial role to play in the survival or reproduction of the cell.

3.1.2 Genetic material

Although it occupies a well-defined area within the cell, the genetic material of prokaryotes is not present as a true nucleus, as it lacks a surrounding nuclear membrane (cf. the eukaryotic nucleus, Figure 3.13). The *nucleoid* or bacterial chromosome comprises a closed circle of double-stranded DNA, many times the length

Not all bacteria conform to the model of a single circular chromosome; some have been shown to possess two with genes shared between them, while examples of linear chromosomes are also known.

of the cell and supercoiled to form a highly compact structure. (The common laboratory bacterium *E. coli* is around 3–4 μm in length, but contains a DNA molecule some 1400 μm in length!) The DNA may be associated with certain bacterial proteins, but these are not the same as the histones found in eukaryotic chromosomes.

Some bacteria contain additional DNA in the form of small, self-replicating extrachromosomal elements called *plasmids*. These do not carry any genes essential for growth and reproduction, and thus the cell may survive without them. They can be very important, however, as they may carry genes encoding toxins or resistance to antibiotics, and can be passed from cell to cell (see Chapter 12). Under certain environmental conditions, possession of a plasmid may confer an advantage to a bacterial cell.

3.1.3 Ribosomes

Apart from the nucleoid, the principal internal structures of prokaryotic cells are the ribosomes. There may be many thousands of these in an active cell, lending a speckled appearance to the cytoplasm. Ribosomes are composed of a complex of protein and RNA, and are the site of protein synthesis in the cell.

Although they carry out a similar function, the ribosomes of prokaryotic cells are smaller and lighter than their eukaryotic counterparts. Ribosomes are measured in Svedberg units (S), a function of their size and shape, and determined by their rate of sedimentation in a centrifuge; prokaryotic ribosomes are 70S, while those of eukaryotes are 80S. Some types of antibiotic exploit this difference by targeting the prokaryotic form and selectively disrupting bacterial protein synthesis (see Chapter 17).

All ribosomes comprise two unequal subunits (in prokaryotes, these are 50S and 30S, in eukaryotes 60S and 40S: Table 3.3). Each subunit contains its own RNA and a number of proteins (Figure 3.4). Many ribosomes may be attached simultaneously to a single mRNA molecule, forming a threadlike polysome. The role of ribosomes in bacterial protein synthesis is discussed in Chapter 11.

A *polysome* is a chain of ribosomes attached to the same molecule of mRNA.

Table 3.3 Comparison of prokaryotic and eukaryotic ribosomes

	Prokaryotic	Eukaryotic
Overall size	70S	80S
Large subunit size	50S	60S
Large subunit RNA	23S and 5S	28S, 5.8S and 5S
Small subunit size	30S	40S
Small subunit RNA	16S	18S

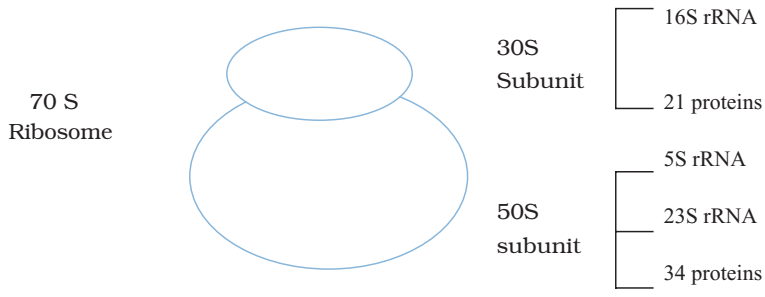


Figure 3.4 The bacterial ribosome. Each subunit comprises ribosomal RNA (rRNA) and proteins. The nucleotide sequence of small subunit (16S) rRNA is widely used in determining the phylogenetic (evolutionary) relationship between bacteria (see Chapter 7).

3.1.4 Inclusion bodies

Certain bacteria may contain within their cytoplasm granular structures known as inclusion bodies. These act as food reserves, and may contain organic compounds such as starch, glycogen or lipid. In addition, sulphur and polyphosphate can be stored as inclusion bodies, the latter being known as *volutin* or metachromatic granules. Two special types of inclusion body are worthy of mention. *Magnetosomes*, which contain a form of iron oxide, help some types of bacteria to orientate themselves downwards into favourable conditions, whilst *gas vacuoles* maintain buoyancy of the cell in blue-greens and some halobacteria.

3.1.5 Endospores

Certain bacteria such as *Bacillus* and *Clostridium* produce *endospores*. They are dormant forms of the cell that are highly resistant to extremes of temperature, pH and other environmental factors, and germinate into new bacterial cells when conditions become more favourable. The spore's resistance is due to the thick coat that surrounds it.

Endospores of pathogens such as *Clostridium botulinum* can resist boiling for several hours. It is this resistance that makes it necessary to autoclave at 121°C in order to ensure complete sterility.

3.1.6 The plasma membrane

The cytoplasm and its contents are surrounded by a plasma membrane, which can be thought of as a bilayer of phospholipid arranged like a sandwich, together with associated proteins (Figure 3.5).

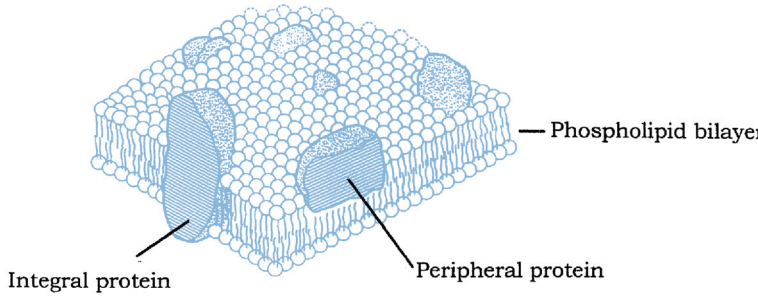


Figure 3.5 The plasma membrane. Phospholipid molecules form a bilayer, with the hydrophobic hydrocarbon chains pointing in towards each other, leaving the hydrophilic phosphate groups to face outwards. Proteins embedded in the membrane are known as integral proteins, and may pass part of the way or all of the way through the phospholipid bilayer. The amino acid composition of such proteins reflects their location; the part actually embedded among the lipid component of the membrane comprises non-polar (hydrophobic) amino acids, while polar ones are found in the aqueous environment at either side. Reproduced from Singleton, P (1999) Bacteria in Biology, Biotechnology and Medicine, 5th edn, with permission from John Wiley & Sons.

The function of the plasma membrane is to keep the contents in, while at the same time allowing the selective passage of certain substances in and out of the cell (it is a semipermeable membrane).

Phospholipids comprise a compact, hydrophilic (water-loving) head and a long hydrophobic tail region (see Figure 2.27); this results in a highly ordered structure when the membrane is surrounded by water. The tails 'hide' from the water to form the inside of the membrane, while the heads project outwards. Also included in the membrane are a variety of proteins; these may pass right through the bilayer or be associated with the inner (cytoplasmic) or outer surface only. These proteins may play structural or functional roles in the life of the cell. Many enzymes associated with the metabolism of nutrients and the production of energy are associated with the plasma membrane in prokaryotes. As we'll see later in this chapter, this is fundamentally different from eukaryotic cells, where these reactions are carried out at specialised internal organelles. Proteins involved in the active transport of nutrients (see Chapter 4) are also to be found associated with the plasma membrane. The model of membrane structure as depicted in Figure 3.5 must not be thought of as static; in the widely accepted *fluid mosaic model*, the lipid is seen as a fluid state, in which proteins float around, rather like icebergs in an ocean.

The majority of bacterial membranes do not contain sterols (cf. eukaryotes: see later); however, many do contain molecules called hopanoids that are derived from the same precursors. Like sterols, they are thought to assist in maintaining membrane stability.

A comparison of the lipid components of plasma membranes reveals a distinct difference between members of the Archaea and the Bacteria.

3.1.7 The bacterial cell wall

Bacteria have a thick, rigid cell wall, which maintains the integrity of the cell and determines its characteristic shape. Since their cytoplasm contains high concentrations of dissolved substances, bacteria generally live in a hypotonic environment (i.e. one that is more dilute than their own cytoplasm). There is therefore a natural tendency for water to flow into the cell, and without the cell wall the cell would fill and burst (this can be demonstrated by using enzymes to strip off the cell wall, leaving the naked *protoplast*).

A *protoplast* is a cell that has had its cell wall removed.

The major component of the cell wall, which is responsible for its rigidity, is a substance unique to bacteria, called *peptidoglycan* (murein). This is a high molecular weight polymer whose basic subunit is made up of three parts: *N*-acetylglucosamine, *N*-acetylmuramic acid and a short peptide chain (Figure 3.6). The latter comprises the amino acids L-alanine, D-alanine, D-glutamic acid and either L-lysine or diaminopimelic acid (DAP). DAP is a rare amino acid, only found in the cell walls of prokaryotes. Note that some of the amino acids of peptidoglycan are found in the D-configuration. This is contrary to the situation in proteins, as you may recall from Chapter 2, and confers protection against proteases whose action is specifically directed against L-amino acids.

Precursor molecules for peptidoglycan are synthesised inside the cell, and transported across the plasma membrane by a carrier called bactoprenol phosphate before being incorporated into the cell wall structure. Enzymes called transpeptidases then covalently bond the tetrapeptide chains to one another, giving rise to a complex network (Figure 3.7); it is this cross-linking that gives the wall its mechanical strength. A number of antimicrobial agents exert their effect by inhibiting cell wall synthesis; these will be discussed further in Chapter 17.

Although all bacteria (with a few exceptions) have a cell wall containing peptidoglycan, there are two distinct structural types of cell wall, known as *Gram-positive* and *Gram-negative*. The names derive from the Danish scientist Christian Gram, who, in the 1880s, developed a rapid staining technique that could differentiate bacteria as belonging to one of two basic types (see Box 1.2). Although the usefulness of the Gram stain was recognised for many years, it was only with the advent of electron microscopy that the underlying molecular basis of the test could be explained, in terms of cell wall structure.

Gram-positive cell walls are relatively simple in structure, comprising several layers of peptidoglycan connected to each other by cross-linkages to form a strong, rigid scaffolding. In addition, they contain acidic polysaccharides

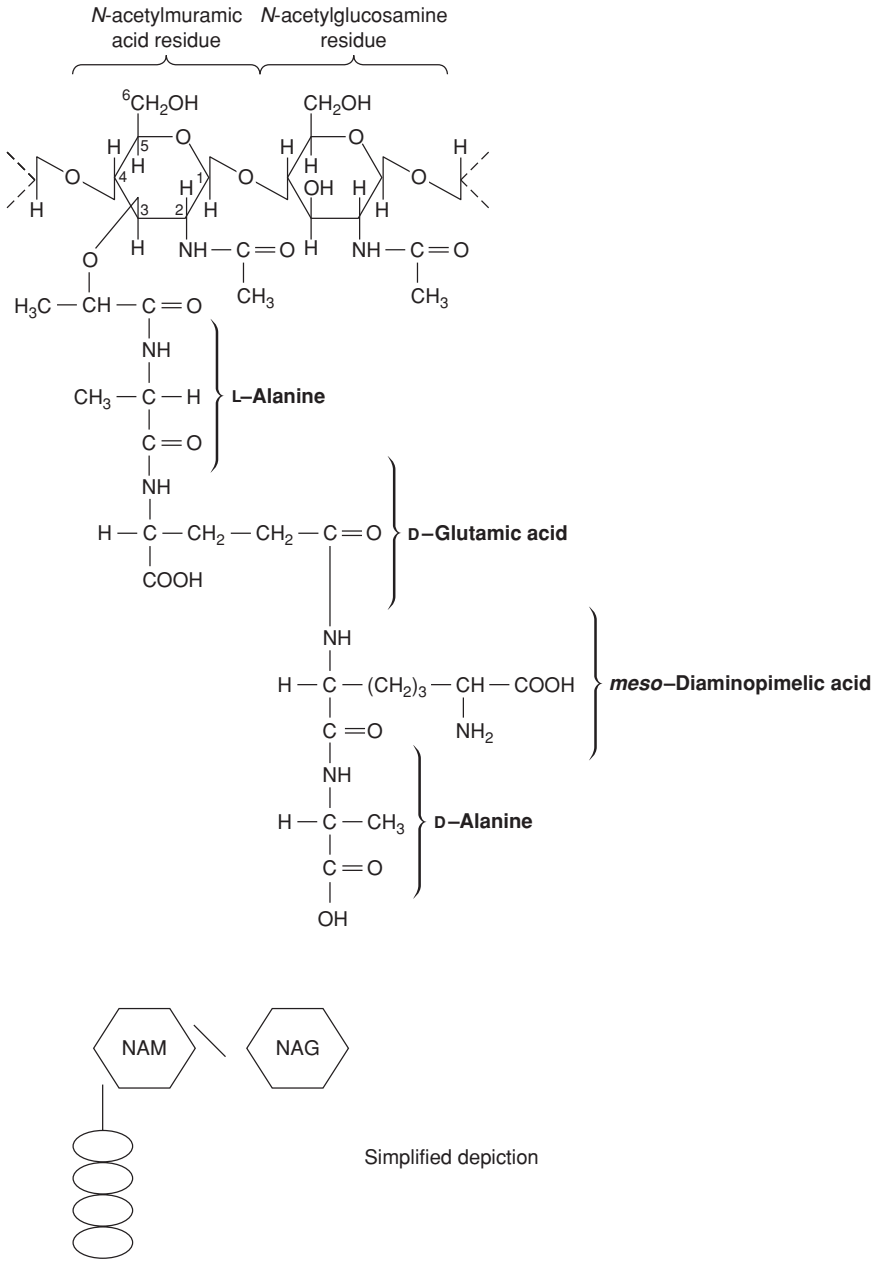


Figure 3.6 Peptidoglycan structure. Peptidoglycan is a polymer made up of alternating molecules of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). A short peptide chain is linked to the NAM residues (see text for details). This is important in the cross-linking of the straight chain polymers to form a rigid network (see Figure 3.7). The composition of *E. coli* peptidoglycan is shown; the peptide chain may contain different amino acids in other bacteria. Partly reproduced from Hardy, SP (2002) Human Microbiology, with permission from Taylor & Francis Group.

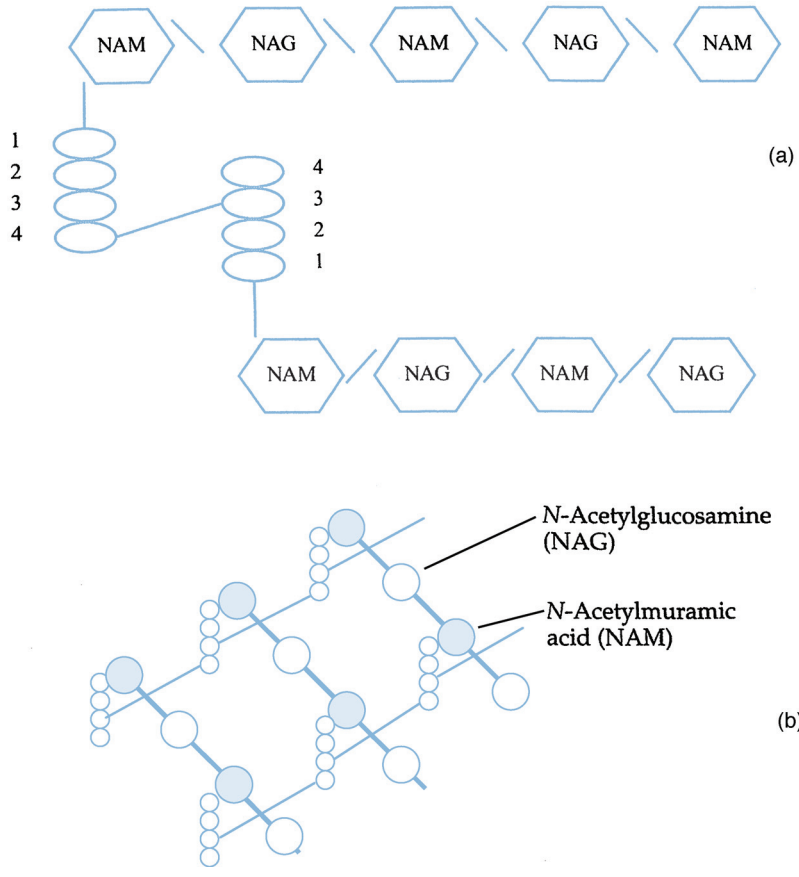


Figure 3.7 Cross-linking of peptidoglycan chains in *E. coli*. (a) The D-alanine on the short peptide chain attached to the *N*-acetylmuramic acid cross-links to a diaminopimelic acid residue on another chain. In other bacteria, the precise nature of the cross-linking may differ. (b) Further cross-linking produces a rigid network of peptidoglycan. The antibiotic penicillin acts by inhibiting the transpeptidase enzymes responsible for the cross-linking reaction (see Chapter 17). Reproduced from Hardy, SP (2002) Human Microbiology, with permission from Taylor & Francis Group.

called teichoic acids; these contain phosphate groups, which impart an overall negative charge to the cell surface. A diagram of the Gram-positive cell wall is shown in Figure 3.8.

Gram-negative cells have a much thinner layer of peptidoglycan, making the wall less sturdy; however, the structure is made more complex by the presence of a layer of lipoprotein, polysaccharide and phospholipid known as the *outer membrane* (Figure 3.9). This misleading name derives from the fact that it superficially resembles the bilayer of the plasma membrane; however, instead of two layers of phospholipid, it has only one, the outer layer being made up of *lipopolysaccharide*. This has three parts: lipid A, core

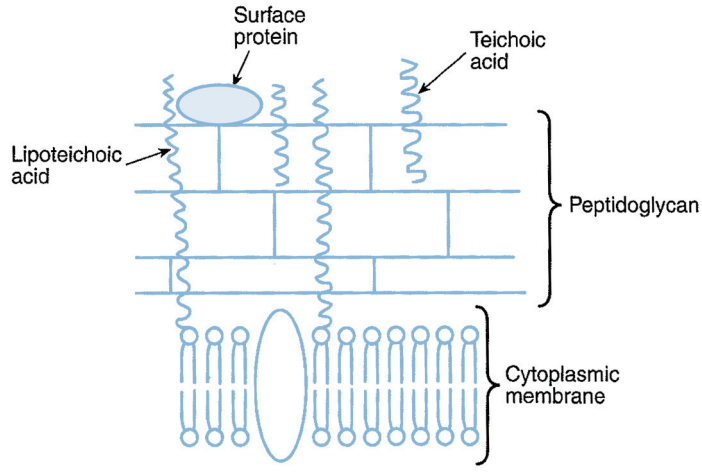


Figure 3.8 The Gram-positive cell wall. Peptidoglycan is many layers thick in the Gram-positive cell wall and may account for 30–70% of its dry weight. Teichoic acids are negatively charged polysaccharides; they are polymers of ribitol phosphate and cross-link to peptidoglycan. *Lipoteichoic acids* are teichoic acids found in association with glycolipids. Reproduced from Henderson, B, et al. (1999) Cellular Microbiology: Bacteria-Host Interactions in Health and Disease, with permission from John Wiley & Sons.

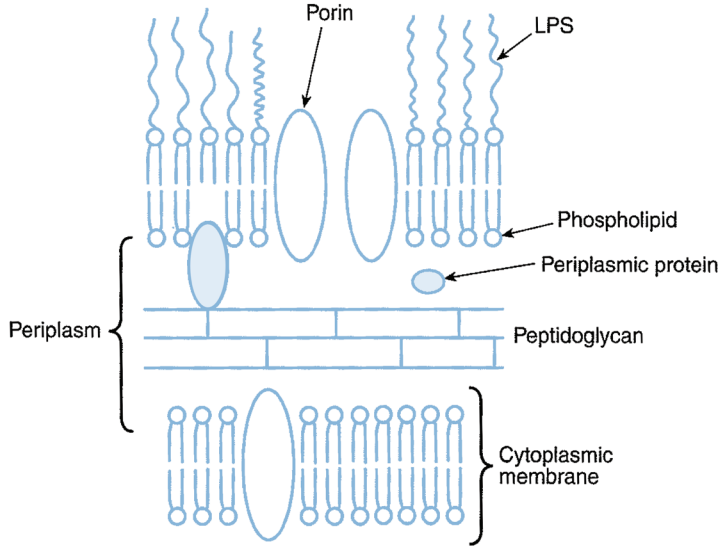


Figure 3.9 The Gram-negative cell wall. Note the thinner layer of peptidoglycan compared to the Gram-positive cell wall (Figure 3.8). It accounts for $<10\%$ of the dry weight. Beyond this lies the outer membrane, with its high lipopolysaccharide (LPS) content. Channels made of *porins* allow the passage of certain solutes into the cell. Reproduced from Henderson, B, et al. (1999) Cellular Microbiology: Bacteria-Host Interactions in Health and Disease, with permission from John Wiley & Sons.

Box 3.1 Mesosomes – the structures that never were?

When looked at under the electron microscope, Gram-positive bacteria often contained localised infoldings of the plasma membrane. These were given the name *mesosomes*, and were thought by some to act as attachment points for DNA during cell division, or to play a role in the formation of cross-walls. Others thought they were nothing more than artefacts produced by the rather elaborate sample preparation procedures necessary for electron microscopy. It is now generally accepted that this is the case.

polysaccharide and an O-specific side chain. The lipid A component may act as an *endotoxin*, which, if released into the bloodstream, can lead to serious conditions such as fever and toxic shock. The O-specific antigens are carbohydrate chains whose composition often varies between strains of the same species. Serological methods can distinguish between these, making them a valuable tool in the investigation, for example, of the origin of an outbreak of an infectious disease. Proteins called *porins* incorporated into the outer membrane and penetrating its entire thickness, form channels, which allow the passage of water and small molecules to enter the cell. Unlike the plasma membrane, the outer membrane plays no part in cellular respiration. Some bacteria, mostly of the Gram-negative type, may have a *periplasmic space* between the plasma membrane and the cell wall. This is the site of metabolic activity, and contains a number of enzymes and transport proteins.

Members of the Archaea have a cell wall chemistry quite different from that described above (see Chapter 7). Instead of being based on peptidoglycan, they have other complex polysaccharides, although a distinction between Gram-positive and Gram-negative types still occurs.

3.1.8 Beyond the cell wall

A number of structural features are to be found on the outer surface of the cell wall; these are mainly involved either with locomotion of the cell or its attachment to a suitable surface.

Perhaps the most obvious extracellular structures are *flagella* (sing. *flagellum*), thin hair-like structures, often much longer than the cell itself, and used for locomotion in many bacteria. There may be a single flagellum, one at each end, or many, depending on the bacteria concerned (Figure 3.10). Each flagellum is a hollow but rigid cylindrical filament made of the protein flagellin, attached via a hook to a basal body, which secures it to the cell wall and plasma membrane (Figure 3.11). The basal body comprises a

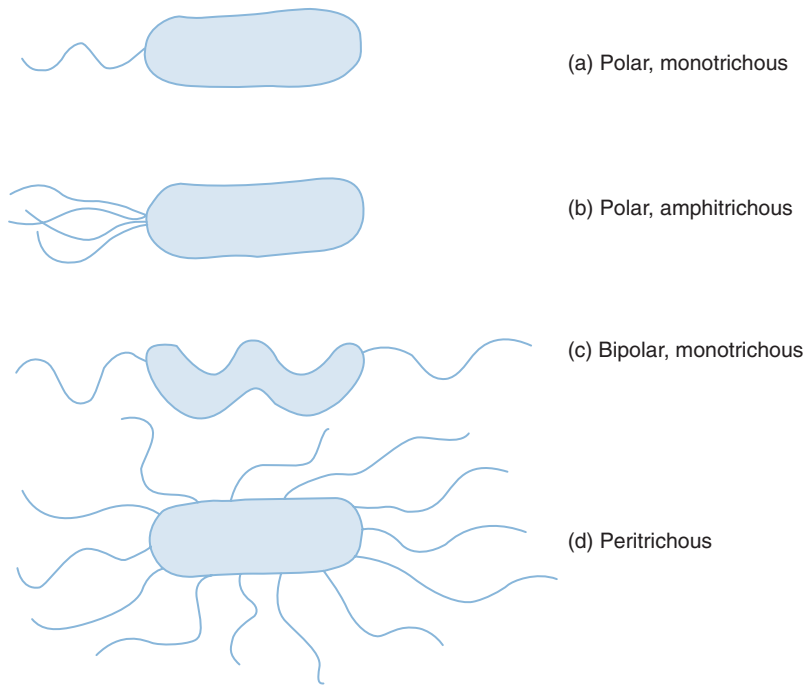


Figure 3.10 The arrangement of flagella. Flagella may be situated at one end (a and b), at both ends (c) or all over the cell surface (d).

series of rings, and is more complex in Gram-negative than in Gram-positive bacteria. Rotation of the flagellum, which may reach several hundred rpm, is an energy-dependent process driven by the basal body, and the direction of rotation determines the nature of the resulting cellular movement. Clockwise rotation of a single flagellum results in a directionless 'tumbling', but if it rotates anticlockwise, the bacterium will 'run' in a straight line (Figure 3.12a). Likewise, anticlockwise rotation causes bunched flagella to 'run' by winding around each other and acting as a single structure, whilst spinning in the opposite direction gives rise to multiple independent rotations and results in tumbling (Figure 3.12b).

Pili (sing. *pilus*) are structures that superficially resemble short flagella. They differ from flagella, however, in that they do not penetrate to the plasma membrane, and they are not associated with motility. Their function, rather, is to anchor the bacterium to an appropriate surface. Pathogenic bacteria have proteins called *adhesins* on their pili, which adhere to specific receptors on host tissues. Attachment pili are sometimes called *fimbriae*, to distinguish them from another distinct type of pilus, the *sex pilus*, which as its name suggests, is involved in the transfer of genetic information by *conjugation*. This is discussed in more detail in Chapter 11.

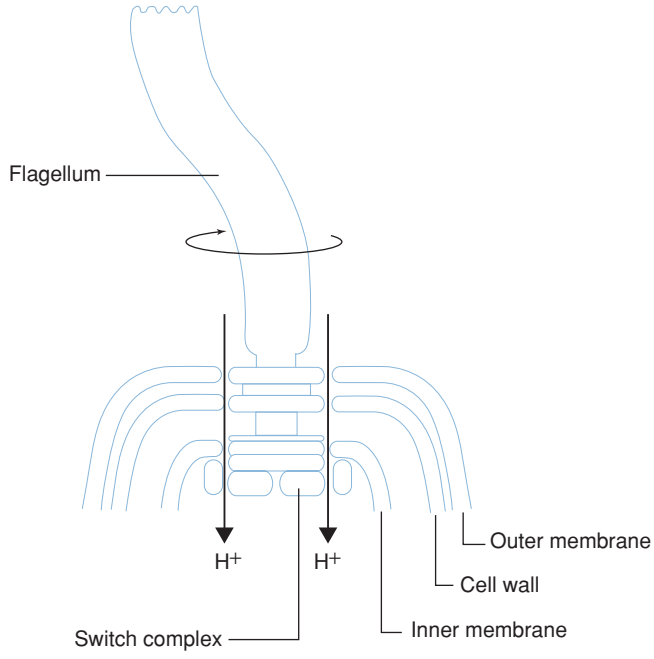


Figure 3.11 Bacterial flagella are anchored in the cell wall and plasma membrane. The filament of the flagellum is anchored by a basal body. In Gram-positive organisms, this comprises two rings inserted in the plasma membrane. In Gram-negative organisms (as shown), there are additional rings associated with the outer membrane and the peptidoglycan layer. Energy for rotation of the flagellum is derived from the proton motive force generated by the movement of protons across a membrane (see Chapter 6). Reproduced from Bolsover, SR, et al. (1997) From Genes to Cells, with permission from John Wiley & Sons.

Outside the cell wall, most bacteria have a polysaccharide layer called a glycocalyx. This may be a diffuse and loosely bound *slime layer*, or a better defined and generally thicker *capsule*. The slime layer helps protect against desiccation, and is instrumental in the attachment of certain bacteria to a substratum (the bacteria that stick to your teeth are a good example of this). Capsules offer protection to certain pathogenic bacteria against the phagocytic cells of the immune system. Both capsules and slime layers are key components of *biofilms*, which form at liquid–solid interfaces, and can be highly significant in such varied settings as wastewater treatment systems, indwelling catheters and the inside of your mouth!

3.2 The eukaryotic cell

We have already seen that eukaryotic cells are, for the most part, larger and much more complex than prokaryotes, containing a range of specialised subcellular organelles (Figure 3.13). Within the microbial world, the major

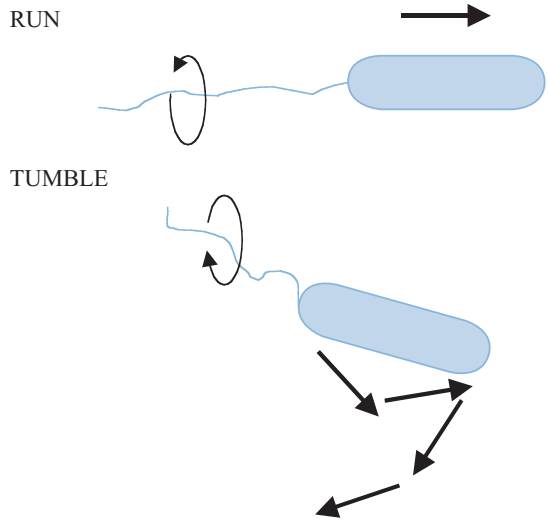


Figure 3.12 Running and tumbling. Anticlockwise rotation of bunched flagella gives rise to 'running' in a set direction. Reversing the direction of rotation causes 'tumbling', and allows the bacterial cell to change direction.

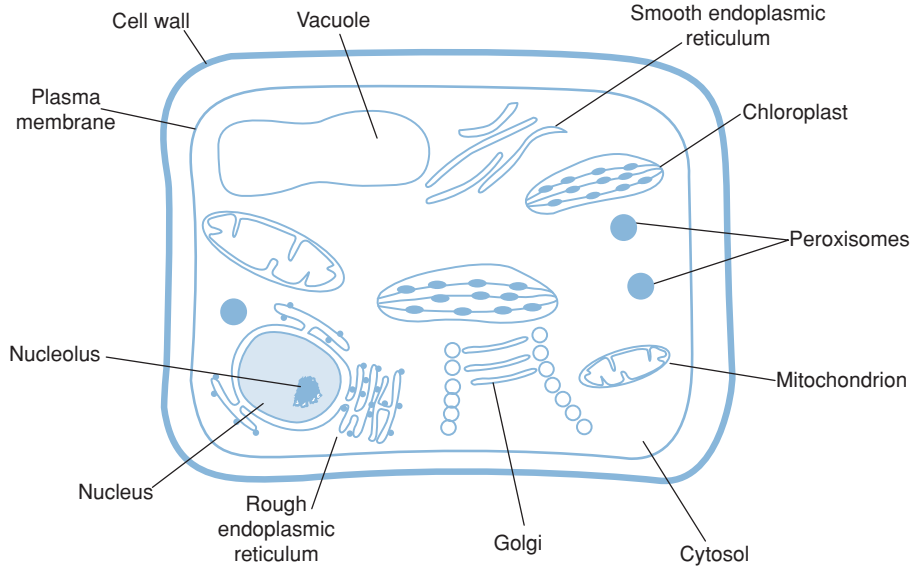


Figure 3.13 The eukaryotic cell. This example of eukaryotic cell structure shows a plant cell. Other eukaryotic cells may differ with respect to the cell wall and the possession of chloroplasts. Note the much more elaborate internal structure compared to a typical prokaryotic cell (cf. Figure 3.3), in particular the presence of membrane-bounded organelles such as mitochondria, chloroplasts, endoplasmic reticulum and a true nucleus. Reproduced from Nicklin, J, et al. (2002) Instant Notes in Microbiology, 2nd edn, with permission from Taylor & Francis Group.

groups of eukaryotes are the fungi and the protists (protozoans and algae); all of these groups have single-celled representatives, and there are multicellular forms in the algae and fungi. These groups are discussed in Chapters 8 and 9.

Our survey of eukaryotic cell structure begins as before with the genetic material, and works outwards. However, since many internal structures in eukaryotes are enclosed in a membrane, it is appropriate to preface our description by briefly considering eukaryotic membranes. These are, in fact, very similar to the fluid mosaic structure we described earlier in this chapter, as depicted in Figure 3.5. The main difference is that eukaryotic membranes contain lipids called *sterols*, which enhance their rigidity. We shall consider the significance of this when we discuss the plasma membrane of eukaryotes below. Cholesterol is a very important sterol found in the membranes of many eukaryotes.

3.2.1 The nucleus

The principal difference between prokaryotic and eukaryotic cells, and the one that gives the two forms their names, lies in the accommodation of their genetic material. Eukaryotic cells have a true nucleus, surrounded by a *nuclear membrane*. This is in fact a double membrane; it contains pores, through which messenger RNA leaves the nucleus on its way to the ribosomes during protein synthesis (see Chapter 11).

The organisation of genetic material in eukaryotes is very different from that in prokaryotes. Instead of existing as a single closed loop, the DNA of eukaryotes is organised into pairs of *chromosomes*. The fact that they occur in pairs highlights another important difference from prokaryotes: eukaryotes are genetically *diploid* in at least some part of their life cycle, while prokaryotes are always *haploid*. The DNA of eukaryotic chromosomes is linear in the sense that it has free ends; however, because there is so much of it, it is highly condensed and wound around proteins called *histones*. These carry a strong positive charge and associate with the negatively charged phosphate groups on the DNA.

A cell containing only one copy of each chromosome is said to be *haploid*. The term is also applied to organisms made up of such cells. The haploid state is often denoted as *N* (cf. *diploid,* or *2N* – containing two copies of each chromosome).

A *histone* is a basic protein found associated with DNA in eukaryotic chromosomes.

As well as the chromosomes, the nucleus also contains the *nucleolus*, a discrete structure rich in RNA, where ribosomes are assembled. The ribosomes themselves have the same function as their prokaryotic counterparts; the differences in size have already been discussed (see Table 3.3). They may be found free

in the cytoplasm or associated with the endoplasmic reticulum (see below), depending on the type of protein they synthesise.

3.2.2 Endoplasmic reticulum

Running throughout the cell and taking up much of its volume, the endoplasmic reticulum (ER) is a complex membrane system of tubes and flattened sacs (*cisternae*). The presence of numerous ribosomes on their surface gives those parts of the ER involved in protein synthesis a granular appearance when seen under the electron microscope, giving rise to the name *rough ER*. Areas of the ER not associated with ribosomes are known as *smooth ER*; this is where the synthesis of membrane lipids takes place. The ER also serves as a communications network, allowing the transport of materials between different parts of the cell.

3.2.3 Golgi apparatus

The Golgi apparatus is another membranous organelle, comprising a set of flattened vesicles, usually arranged in a stack called a *dictyosome*. The function of the Golgi apparatus is to package newly synthesised substances such as proteins and assist in their transport away from the cell. The substances are contained in vesicles that are released from the main part of the complex, and fuse with the cytoplasmic membrane. The Golgi apparatus is poorly defined in certain fungi and protozoans.

3.2.4 Lysosomes

Another function of the Golgi apparatus is to package certain hydrolytic (digestive) enzymes into membrane-bound packets called *lysosomes*. The enzymes, which are needed to digest nutrient molecules that enter the cell by *endocytosis* (Figure 3.14), would break down the fabric of the cell itself if they were not contained within the lysosomes.

Peroxisomes are similar to lysosomes, but smaller, and also contain enzymes such as catalase, which breaks down the potentially toxic hydrogen peroxide generated by other breakdown reactions within the peroxisome.

3.2.5 Mitochondria

Whereas in prokaryotes the reactions involved in energy generation are associated with the plasma membrane, in eukaryotes they take place in specialised organelles called mitochondria. These are generally cigar-shaped and may be present in large numbers. They are enclosed by a double membrane, the inner surface of which is folded into finger-like projections called *cristae*. Respiratory enzymes are located on the increased surface area this provides, while

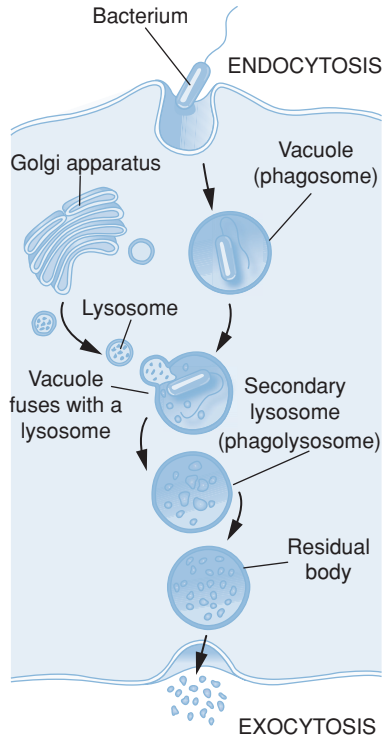


Figure 3.14 Endocytosis. Membrane-bound vacuoles surround a food particle and internalise it in the form of a *phagosome*. This fuses with a lysosome, which releases digestive enzymes, resulting in the breakdown of the contents. The process of endocytosis is unique to eukaryotic cells. The products of digestion are released from the cell by exocytosis. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

other metabolic reactions take place in the semi-fluid matrix (Figure 3.15) (see also Chapter 6). The matrix of mitochondria contains a range of enzymes involved in oxidative metabolism as well as mitochondrial ribosomes. These are very similar to the ribosomes found in prokaryotic cells. The matrix also contains a small amount of DNA; this is typically circular, like the nucleoid of bacteria, and contains a limited number of genes. These mostly carry the code for proteins involved with the respiratory chain, and ribosomal and transfer RNAs.

The mitochondrial cristae of algae, fungi and protozoans each have their own characteristic shapes. Until very recently, a few primitive protozoans, such as *Giardia*, were thought to lack mitochondria completely, and to represent an intermediate stage in the evolution of the eukaryotic condition. Recent research, however, has shown them to possess highly reduced remnants of mitochondria, which have been given the name *mitosomes*. It

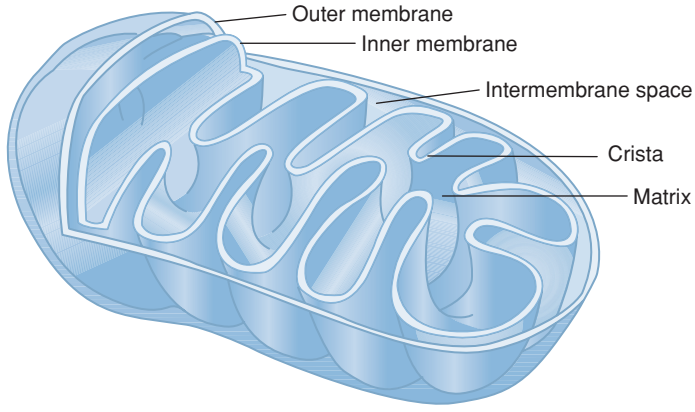


Figure 3.15 Mitochondrial structure. The inner membrane, the location of the electron transport chain in aerobic respiration, is formed by the invagination of the more permeable outer membrane. Mitochondria have similar dimensions to many bacteria (approx. 1–3 μm), but may vary in shape due to the plasticity of their membranes.

seems that such organisms did, after all, once possess mitochondria, but have subsequently lost much of their function – an example of so-called reductive evolution.

3.2.6 Chloroplasts

Chloroplasts are specialised organelles involved in the process of *photosynthesis*, the conversion of light into cellular energy. As such, they are characteristic of green plants and algae. Like mitochondria, chloroplasts are surrounded by a double membrane, and serve as the location for energy-generating reactions. Inside the chloroplast are flattened membranous sacs known as *thylakoids*, which contain the photosynthetic pigment *chlorophyll*. Thylakoids are arranged in stacks called *grana* (Figure 3.16).

Mitochondria and chloroplasts both contain 70S ribosomes (similar to those found in prokaryotes), a limited amount of circular DNA and the means to replicate themselves. This is seen as key evidence for the *endosymbiotic theory* of eukaryotic evolution, which envisages that specialised organelles within eukaryotic cells arose from the ingestion of small prokaryotes, which over a long period of time lost their independent existence.

3.2.7 Vacuoles

Vacuoles are membrane-covered spaces within cells, and derive from the Golgi apparatus. They act as stores for various nutrients, and also for waste products. Some types of vacuole are important in regulating the water content of the cell.

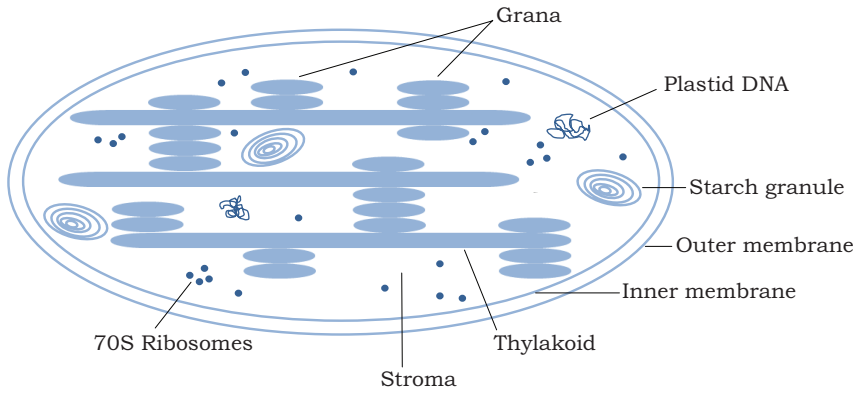


Figure 3.16 Chloroplast structure. Generation of ATP (adenosine triphosphate) from photosynthesis occurs on the thylakoid membranes. In green algae these take the form of discrete structures called grana. The enzyme ribulose bisphosphate carboxylase, responsible for fixing carbon dioxide via the Calvin cycle (see Chapter 6) is located in the stroma. The outer membrane of chloroplasts is relatively permeable, allowing the diffusion of the products of photosynthesis into the surrounding cytoplasm.

3.2.8 Plasma membrane

Many eukaryotes do not have cell walls, so the plasma membrane represents the outermost layer of the cell. The sterols mentioned earlier are important in helping these cells to resist the effects of osmotic pressure. The only prokaryotes to contain sterols are the Mollicutes, which are unusual in not possessing the typical bacterial cell wall. Although the eukaryotic plasma membrane does not have the role in cellular respiration associated with its prokaryotic counterpart, it does have additional functions. The process of *endocytosis* (and its reverse, *exocytosis*), by which particles or large soluble molecules are enveloped and brought into the cell, takes place at the plasma membrane. Also, carbohydrate residues in the membrane act as receptors for cell-to-cell recognition, and may be involved in cell adhesion.

3.2.9 Cell wall

As we have just noted, not all eukaryotes possess a cell wall; among those that do are fungi, algae and plants. Whilst the function, like that of prokaryotes, is to give strength to the cell, the chemical composition is very different, generally being a good deal simpler. The cell walls of plants, algae and lower members of the fungi are based on *cellulose* (Figure 3.17a), a repeating chain of glucose molecules joined by β-1,4 linkages, and may also include pectin and hemicellulose, both also polymers of simple sugars. Many fungi such as yeasts and mushrooms contain *chitin*, a polymer of *N*-acetylglucosamine (Figure 3.17b) We have encountered *N*-acetylglucosamine before, as a

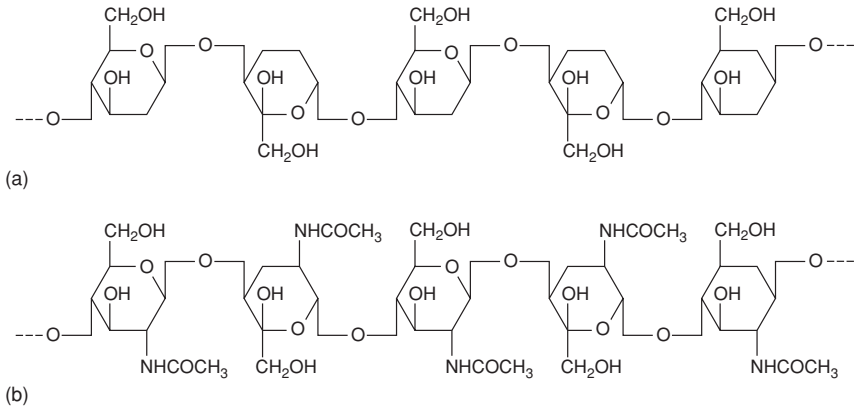


Figure 3.17 Cellulose and chitin. The structures of (a) cellulose and (b) chitin. Cellulose is composed of repeating glucose units joined by β-1,4 linkages, and chitin is a polymer of *N*-acetylglucosamine.

component of peptidoglycan in bacterial walls. Chitin is also to be found as the major component of insect and crustacean exoskeletons, where the function is also to provide strength and rigidity. As in prokaryotes, the cell wall plays little part in the exchange of materials between the cell and its environment, a role fulfilled by the plasma membrane.

Some protozoans and unicellular algae are surrounded by a flexible *pellicle* made of protein.

3.2.10 Flagella and cilia

Motility in eukaryotic cells may be achieved by means of flagella or *cilia*; cilia can be thought of as, essentially, short flagella. Both are enclosed within the plasma membrane and anchored by means of a basal body. Flagellated cells generally have a single flagellum, whereas cilia are often present in very large numbers on each cell. In the microbial world, flagella are found in protozoans and motile algal forms, whilst cilia are mostly found in a class of protozoans called the Ciliophora. Flagella and cilia are not found in members of the Fungi.

Although they share the same thread-like gross morphology, eukaryotic flagella differ considerably from those of prokaryotes in their ultrastructure. Seen in cross-section, they have a very characteristic appearance, made up of two central *microtubules*, surrounded by a further nine pairs arranged in a circle (Figure 3.18). The microtubules are made of a protein called *tubulin*. Flagella in eukaryotes beat in waves, rather than rotating; cilia, which are present in large numbers, beat in a coordinated fashion so that some are in forward motion while others are in the recovery stroke (rather like a 'Mexican wave'!). In animals, ciliary motion has been adapted to move

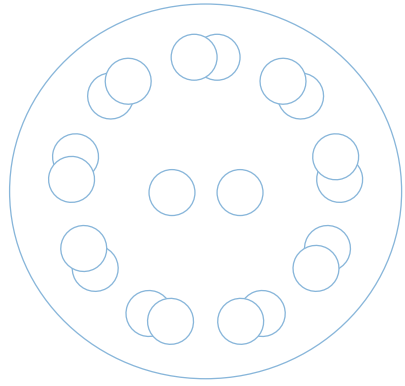


Figure 3.18 Eukaryotic flagella have a characteristic '9 + 2' structure. Although functionally analogous to their prokaryotic counterparts, eukaryotic flagella differ appreciably in their fine structure. A membrane surrounds an arrangement of proteinaceous microtubules, in which nine pairs surround a single central pair. Movement of eukaryotic flagella is by means of an ATP-driven whiplike motion.

particulate matter across a tissue surface; ciliated cells of the respiratory tract, for example, act as a first line of defence in the removal of inhaled particles, such as bacteria from the airways.

3.3 Cell division in prokaryotes and eukaryotes

In unicellular prokaryotes, cell division by *binary fission* leads to the creation of new individuals. Growth occurs in individual cells until a maximum size is achieved, and a cross-wall is formed. Before cell division takes place, the genetic material must replicate itself (see Chapter 11), and one copy pass to each new daughter cell (Figure 3.19).

Cell division in eukaryotes also results in two identical daughter cells. In the case of unicellular eukaryotes, this results in two individual organisms (asexual reproduction), while in multicellular forms there is an increase in overall size. Cell division is preceded by a process of nuclear division called *mitosis*, which ensures that both daughter cells receive a full complement of chromosomes. The principal phases of mitosis are summarised in Figure 3.20a. In *interphase*, the chromosomes are not clearly visible under the microscope; DNA replication takes place during this period. The duplicated chromosomes, held together as sister *chromatids* by the centromere, move towards the centre of the cell during *prophase*. A series of microtubules form a spindle between the centrioles, and the chromosomes line up along this during *metaphase*. By this time, the nuclear membrane has broken down, allowing the paired sister chromatids to separate and migrate away from the centre to opposite ends of the spindle. This stage is called *anaphase*. Finally, in *telophase*, new nuclear membranes surround the two sets of chromosomes,

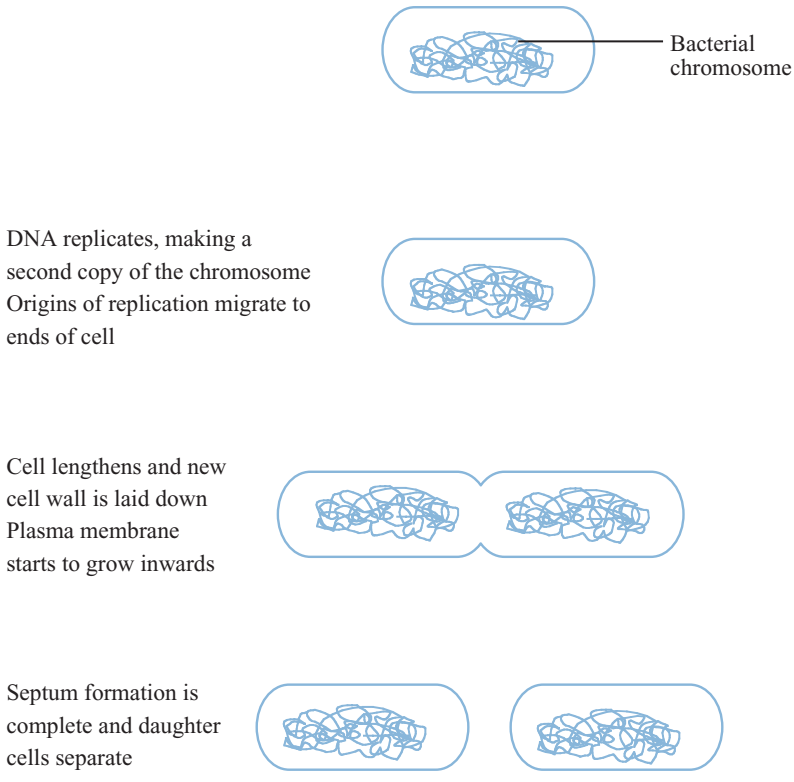


Figure 3.19 Binary fission in *E. coli*. Replication of the single circular chromosome is accompanied by an increase in cell size. The plasma membrane invaginates, and a new cross-wall is synthesised, resulting in two new daughter cells.

to form two nuclei. Mitosis is followed by cell division. Overall, the process of mitosis results in two identical nuclei containing the original (diploid) chromosome number.

At various stages of eukaryotic life cycles, a process of *meiosis* may occur, which halves the total number of chromosomes, so that each nucleus only contains one copy of each. In sexual reproduction, the haploid gametes are formed in this way, and the diploid condition is restored when two different gametes fuse. In some eukaryotes, not only the gametes but a substantial part of the life cycle may occur in the haploid form (see Chapters 8 and 9). Meiosis (Figure 3.20b) comprises two nuclear divisions, the second of which is very similar to the process of mitosis just described. In the first meiotic division, following DNA replication, homologous chromosomes (i.e. the two members of each pair) line up on the spindle together and eventually migrate to opposite poles. While they are together, it is possible for *crossing over* to occur, a process by which the two members of a chromosome pair swap homologous stretches of DNA (Figure 3.21). Since these may not be

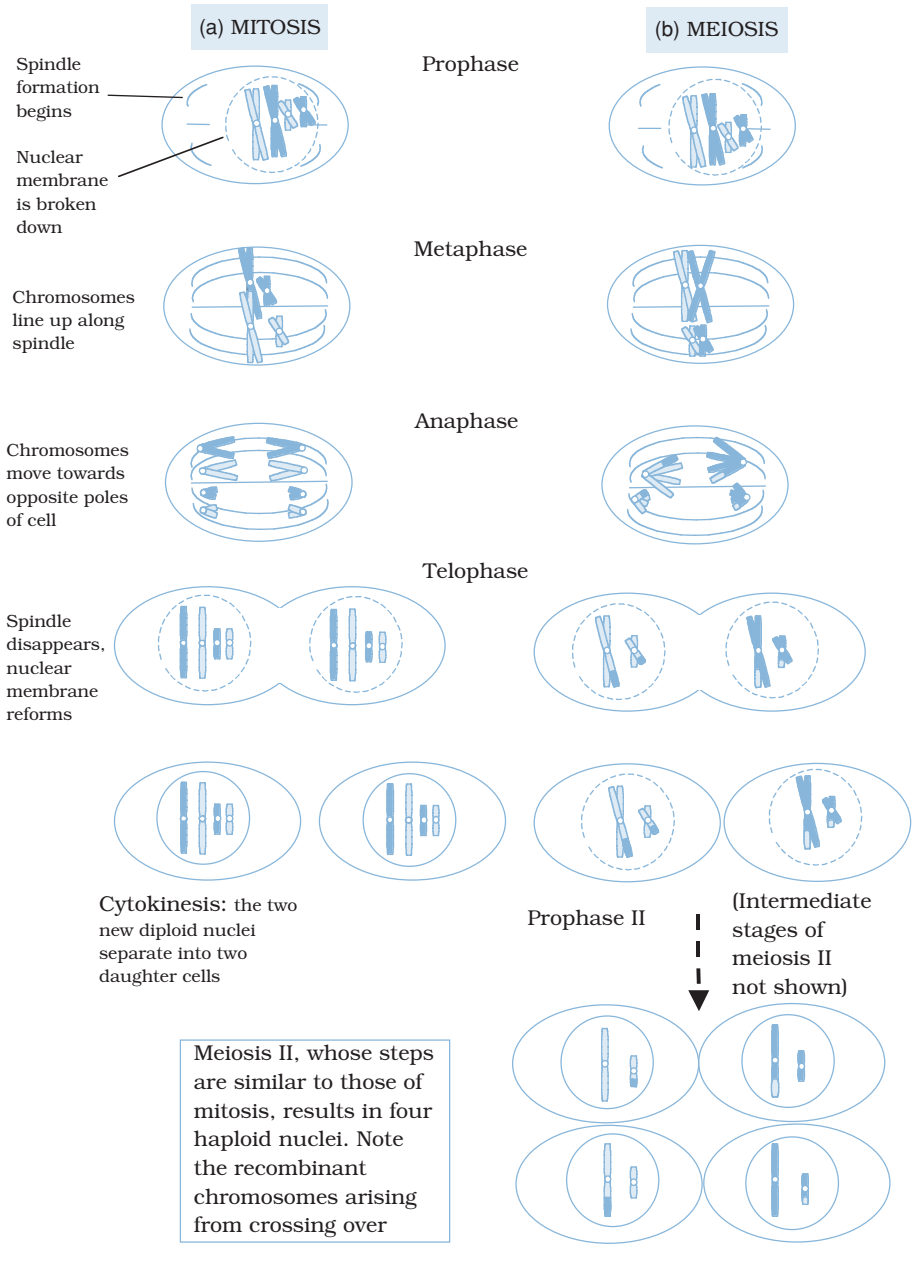


Figure 3.20 Mitosis and meiosis. The main steps of (a) mitosis and (b) meiosis. Mitosis results in two cells identical to the parent. Meiosis results in a reduction in the chromosome number and introduces genetic variation by means of crossing over. For details see the text.

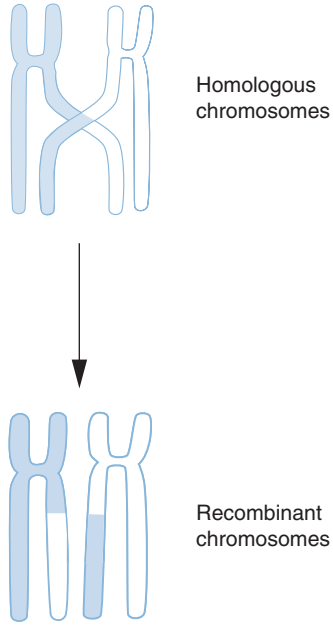


Figure 3.21 Crossing over leads to recombination of genetic material. During crossing over, portions of homologous chromosomes are exchanged. This forms the basis of genetic recombination in eukaryotes, and ensures that offspring contain new combinations of genetic material.

identical in DNA sequence, crossing over serves to introduce genetic variation into the daughter nuclei. In the second meiotic division, sister chromatids separate as before, resulting in four haploid nuclei.

Meiosis ensures that all gametes are subtly different from each other and is a major contributor to the maintenance of genetic variation.