Lower Plants

Anatomy and Activities of Non-flowering Plants and their Allies

C. J. Clegg

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Preface

This book is a concise, largely pictorial account of the complete range of lower plants and their allies studied at Advanced Level and similar courses (TEC Biology II and Cell Biology III). Structural, physiological, biochemical and ecological aspects are covered, along with essential features of taxonomy, reproduction and life-cycle.

I define the lower plants in the widest sense, as all those organisms that botanists have studied other than the angiosperms (angiosperms have received similar treatment in the companion book, *Anatomy and Activities of Plants: a guide to the study of Flowering Plants*, John Murray, 1980). For two or three decades, Advanced Level students have known that fungi were best seen as a separate kingdom, fundamentally different from both plants and animals. More recently we have also learnt to see bacteria and blue-green algae as separate kingdoms and levels of organisation. However, knowledge of these groups is a vital element in our understanding of biology as a whole, so these groups are dealt with here along with the true lower plants.

The great diversity of living things is acknowledged here by considering each organism in the context of the group of organisms that each was chosen to represent. In addition, the major principles of biology that have been observed in and experimented on with particular lower organisms are discussed. For example, our knowledge of photosynthesis in higher plants is based largely on studies of unicellular green algae. Similarly, in ecology, genetics and aspects of biochemistry, lower organisms have been widely used as experimental organisms. Many of the developments in biotechnology are centred on work with prokaryotes, with fungi and with certain smaller eukaryotes. The economic significance of lower plants in industry, in energy studies and in the search for alternative food sources is also given appropriate attention. This economic importance of lower plants and the delightful diversity of structure and function they show will, I hope, be communicated to a new generation of students by the combination of drawings, photographs and text employed in this book.

Acknowledgements

I am most grateful to all who have helped in the preparation of this book. I am particularly indebted to Mr Don Mackean (Welwyn Garden City) and Dr Richard Johnson (Aberdeen University) who painstakingly read the manuscript, and commented, corrected and advised on content, style and presentation. I have also had most helpful advice from Dr Alfred Keys of Rothamsted Experimental Station and Dr David Whitehouse of North East London Polytechnic on specific aspects of biochemistry. However, any remaining errors are my sole responsibility.

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CJC
1984

Safety note

Any experiments involving the use of micro-organisms may be potentially hazardous. They should be carried out under close supervision, following the advice given in *Safeguards in the School Laboratory*, available from The Association for Science Education, College Lane, Hatfield, Hertfordshire AL10 9AA.

Other Books by the same author

Anatomy and Activities of Plants (with Gene Cox)
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Contents

The range of plant life and its classification

1. Classification: its need and role

There are more than one and a half million different types of organisms known and their numbers are continually being added to as a result of new studies. We classify them in order to provide a general plan of the immense diversity of form that exists. Classification aims to use as many characteristics as possible in placing similar organisms together and dissimilar ones apart. Classification is not rigid. It is an artificial creation on the best available evidence using limited knowledge, to reflect fundamental relationships. Classification is on the basis of:

(a) levels of organisation,

(b) evolutionary relationships, although these are known very imperfectly.

As the classification is refined so the more it reflects relationships.

Classification is of great importance in biology because:

(a) it enables communication to occur. Studies cannot be compared unless they are known to refer to exactly the same types of organism,

(b) it enables biologists to make generalisations (and it provides a basis for prediction) about the possible origins, the major role, the usefulness and the consequences of various structures, life-styles and feeding relationships.

In short, classification facilitates biology. The science of classification is known as taxonomy. Classification is standardised throughout the world as a result of the International Code of Botanical Nomenclature (and through the corresponding work of the International Committee on Zoological Nomenclature).

2. Classification: the system

The accepted scheme of classification has been developed from the work of Carl Linnaeus (1707–78), a naturalist who created the binomial system of nomenclature, popularly adopted from 1758. Every species of plant (and animal) is given two Latinised names, e.g. *Ranunculus acris* (Common Buttercup), *Homo sapiens* (Man). The specific or trivial name comes second and follows the generic name which is shared with other related species considered to be sufficiently similar to be grouped in the same genus, e.g. *Ranunculus repens* (Creeping Buttercup). The whole scheme of classification is hierarchical. Above the species level, organisms are placed in wider groupings; the higher the category the more diverse species are included.

Kingdom	the largest and most inclusive group, e.g. plants and animals
Division/ (phylum)	organisms constructed on a similar, general plan and thought to be related, e.g. vascular plants (tracheophytes)
Class	a group within a phylum, e.g. ferns (Filicinae)
Order	a group of apparently related families, e.g. Filicales
Family	a group of apparently related genera, e.g. Polypodiaceae
Genus	a group of similar and closely related species, e.g. *Pteridium*
Species	a group of interbreeding individuals not interbreeding with another such group. A species may include 'races' or 'varieties'. Species have two names, one generic, the other specific, e.g. *Pteridium aquilinum*

There is no uniformity in the size of the categories; a group may contain one or many hundred.

Living things

Prokaryotic organisms	**Eukaryotic organisms**
Blue-green algae, bacteria. The electron microscope has helped show: (i) cell has no distinct nucleus although it contains DNA, (ii) lacks mitochondria, chloroplasts and complex structured flagella, (iii) cell wall of distinct composition and containing mucopeptides (of protein origin).	Plants, animals, fungi, i.e. the majority of living things. The electron microscope has helped show: (i) cell has nucleus separated from the cytoplasm by its nuclear membrane, (ii) cytoplasm compartmentalised by membranes into regions called organelles, e.g. endoplasmic reticulum, mitochondria and chloroplasts, (iii) eukaryotic flagella and cilia are made of a series of tubes arranged in a cylindrical manner, (iv) cell wall (when present) is usually made of cellulose.

Viruses
Agents of disease that require to be transmitted from host to host. They are not 'alive' outside the host cell. Most viruses are constructed from protein and nucleic acid alone. Viruses are only capable of replication within specific host cells.

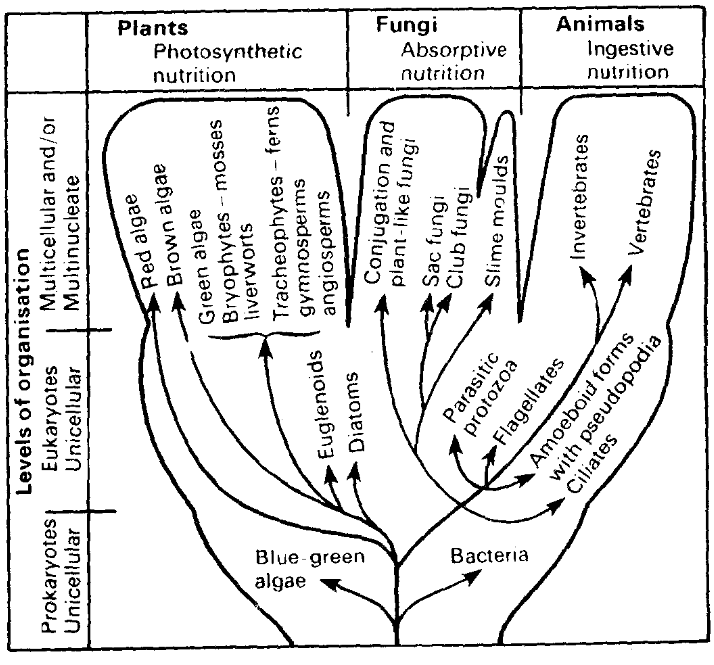
3. Fundamental differences: animals and plants – are there other kingdoms?

Living things have traditionally been classified as animals or plants. Today the eukaryotes (see below) are now divided into three kingdoms:

kingdom of animals, kingdom of plants, kingdom of fungi.

Fungi resemble plants in that, with few exceptions, they have definite cell walls, are usually non-motile (although they may have motile reproductive cells) and they reproduce by spores. Fungi differ from green plants in that they lack chlorophyll, and their cell walls may contain chitin rather than cellulose.

Figure 1.1 The diversity of living things summarised in a matrix contrasting the principle modes of nutrition with levels of organisation



Adapted from R. Whittaker's *Five Kingdom System* and G. Leedales *Four Kingdom System* as presented by M. Tribe, A. Morgan and P. Whittaker in *The Evolution of Eukaryotic Cells*, Arnold Studies in Biology (131) 1981

4. Prokaryotic and eukaryotic organisation

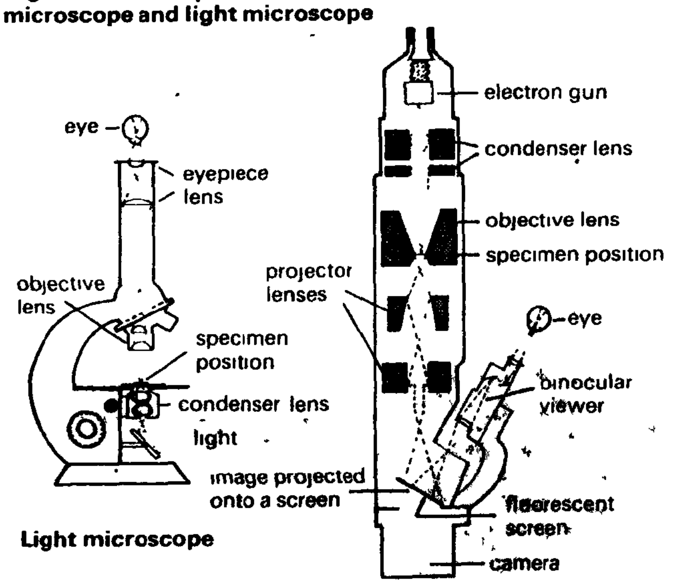
The development of electron microscopy has led to the discovery of two types of cellular organisation, the smaller, simpler prokaryotic cell, and the more complex eukaryotic cell. In addition there are viruses. These are structurally simpler (see page 15), for they contain protein and nucleic acid only, and are unable to multiply outside host cells. It is questionable whether viruses can be recognised as living things.

The electron microscope

The transmission electron microscope employs electrons to produce a magnified image in a similar way to the optical microscope's use of light. Electrons from an electron gun are focused by varying the current passing through circular electromagnetic lenses. The image must be observed on a fluorescent screen because the human eye is not sensitive to electrons as it is to light. A permanent record of the image is made on a photographic plate. Electrons are easily scattered in collision with other atoms, so air must be removed from the path of the electrons, and the inside of the electron microscope is maintained at a very low pressure ('vacuum'). Consequently living or wet specimens cannot be examined, except under very special circumstances. The specimens must be relatively thin with membranes or other structures stained with chemicals that scatter electrons.

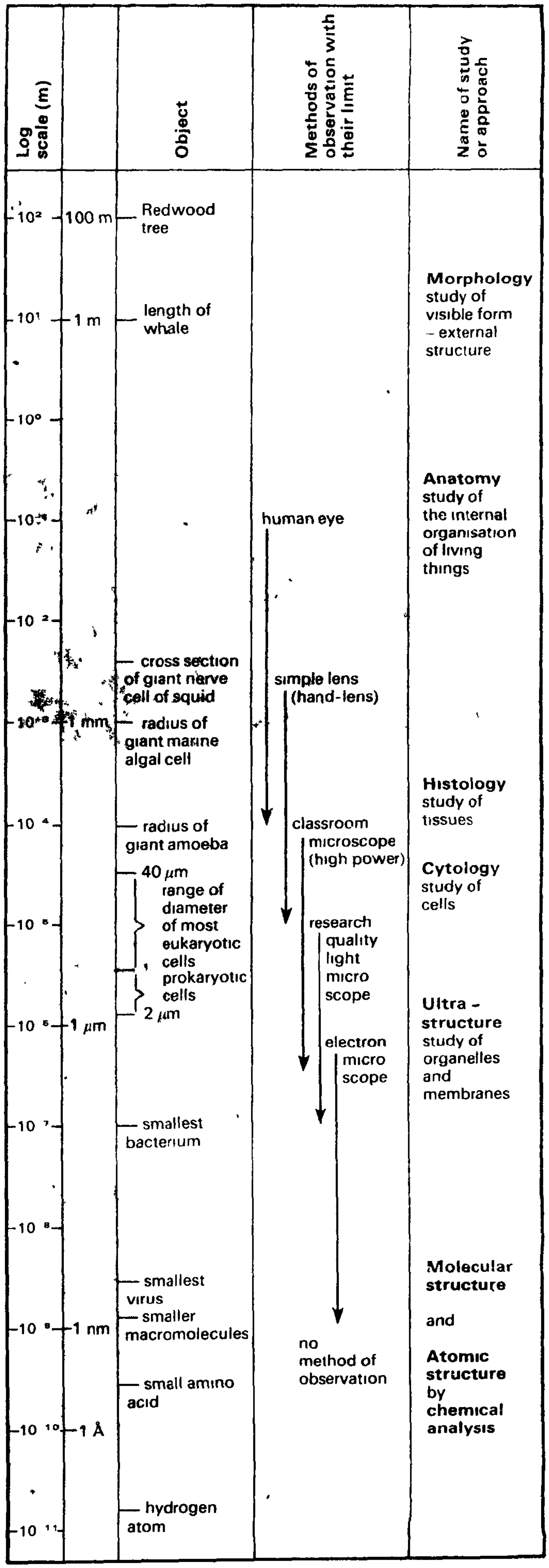
An alternative form of electron microscope, the scanning electron microscope, produces an image from electrons reflected from the surface of the specimen. This usually has a lower resolution than the transmission electron microscope, but a relatively immense depth of field which permits the structure of the surface of solid objects to be observed in focus. The fineness of detail which may be made out is a measure of the resolving power or resolution of a microscope, whereas the magnification tells us about the size of the image (but not the amount of detail). The electron microscope achieves greater resolution than the light microscope and therefore enables greater useful magnification.

Figure 1.2 A comparison of the transmission electron microscope and light microscope



The lenses of the electron microscope are electromagnetic coils that focus the electron beam (negatively charged particles) by the magnetic field they produce. The current passing through the lenses may be varied to change their strength and hence focus.

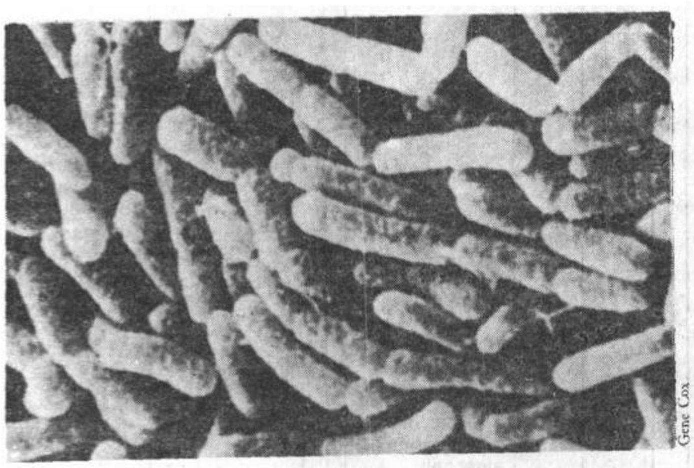
Figure 1.3 Size relationships on a logarithmic scale of biological and chemical levels of organisation



Bacteria

Bacteria are very small and difficult to see, yet they occur in vast numbers. The total mass of living bacteria is estimated to be at least twenty times that of all animal life. Bacteria are simple in structure, and the fossil record indicates that some of the earliest cells were of this relatively simple construction. Bacteria are present in air, soil and water, they contaminate every surface and niche in our homes and on our bodies, and some even occur within parts of our bodies. Bacteria are often associated with disease, yet the bulk of bacteria are far from harmful. They are biochemically very active and fast growing, and their presence, together with that of fungi and green plants, is essential for the maintenance of life on Earth.

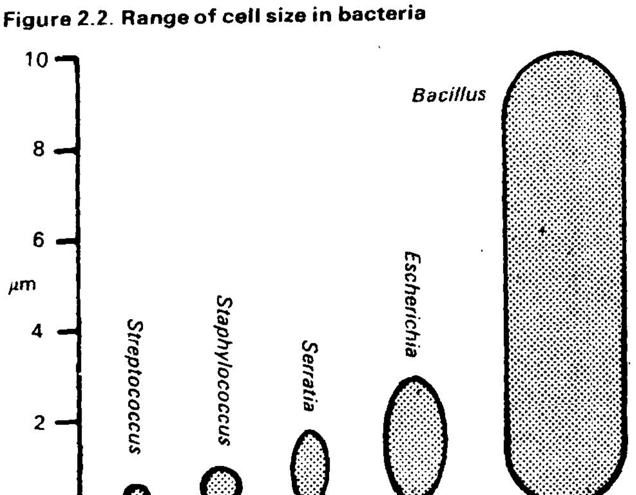
Figure 2.1 Rod-shaped bacteria (*Bacillus* sp.) observed by scanning electron microscopy (x 10 500)



Size, shape and structure

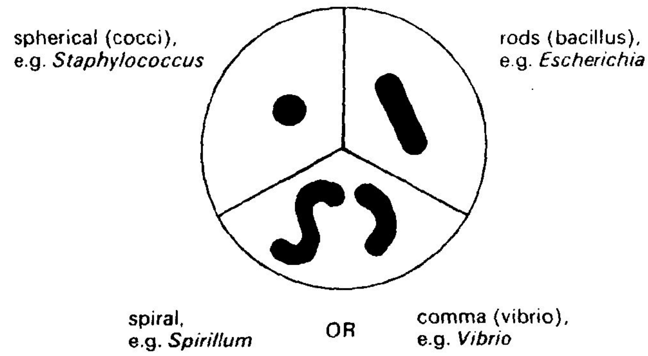
Bacteria are unicellular but often occur clumped together, and in some species the individual cells actually remain joined to form simple filaments. The cells have a variety of sizes and shapes but they are all very small. In any population some cells will have divided recently whilst others will have reached their maximum and be about to divide, and thus there is always a range of sizes present. Because of their small size, the characteristic of shape is the most visible and important in the identification of bacteria. Three distinct forms are recognised, namely spheres, rods and spirals (or curved rods).

Figure 2.2. Range of cell size in bacteria



Most human cells are in the range 5–20 μm
Most flowering plant cells are in the range 10–50 μm

Figure 2.3 The three categories of bacterial shape



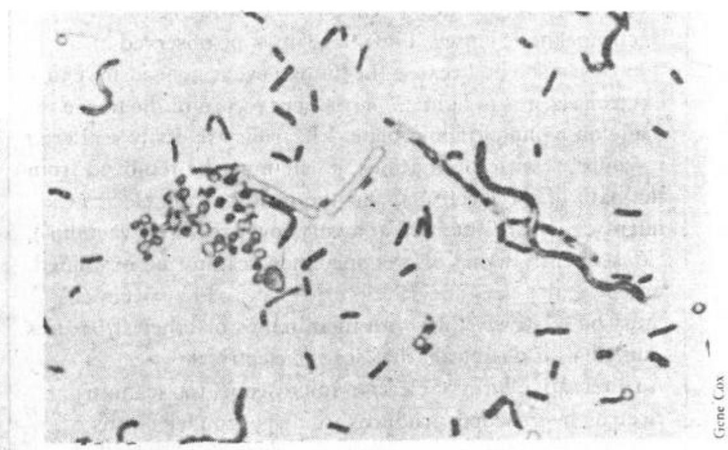


Figure 2.4 Cocci bacteria can be divided into the following generic groups, depending upon the way cells divide and how they subsequently adhere to each other

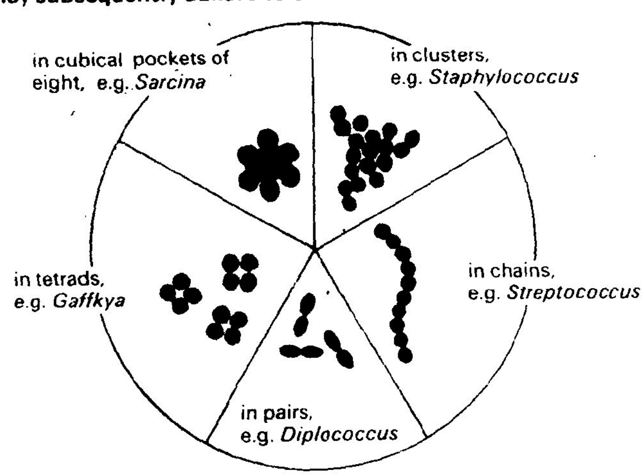


Figure 2.5 *Staphylococcus aureus* observed by scanning electron microscopy. Cells are seen in clusters like bunches of grapes (x 15 600)

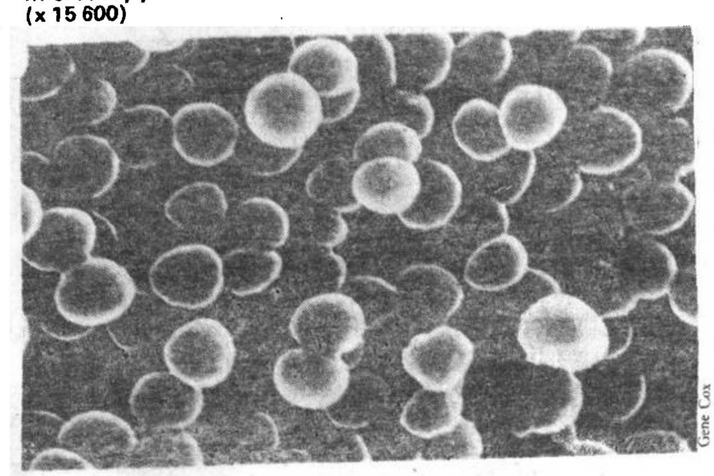
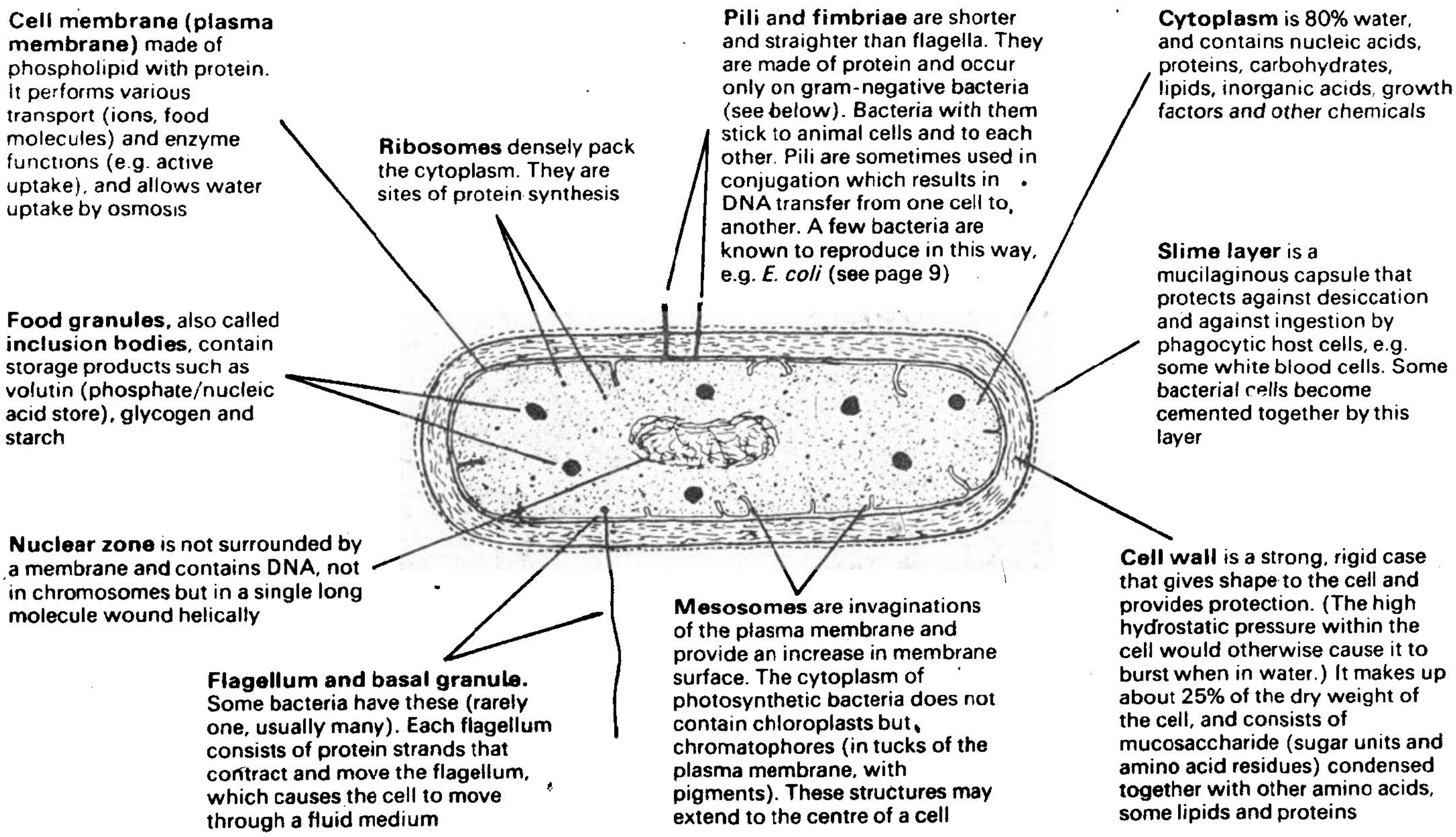


Figure 2.6 Structure of a bacterium based on electron micrographs



The gram stain technique

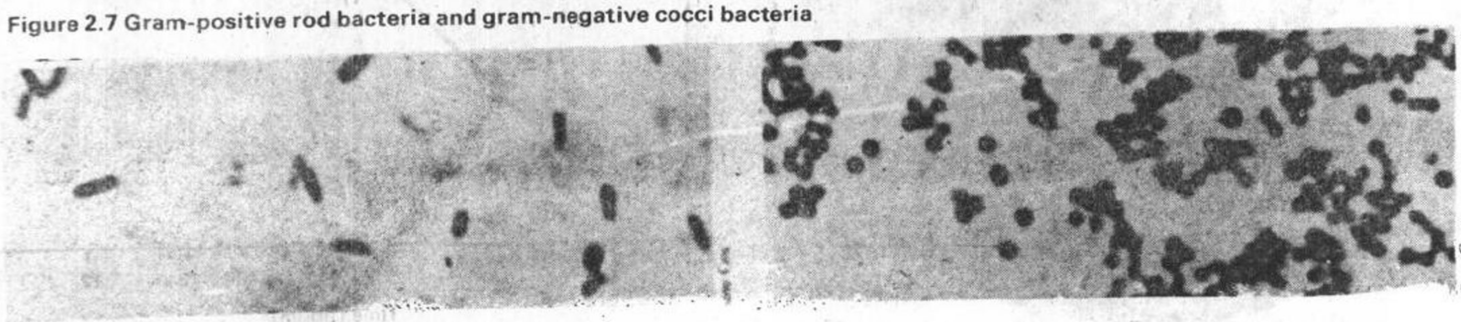
Usually bacteria have to be stained to be identified, using an air-dried smear on a slide. A staining technique, first used by Christian Gram in 1884, distinguishes two types of bacteria: **gram-positive** and **gram-negative**.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Bacteria in the air-dried smear appear colourless	The smear is treated with crystal violet (a basic stain). All cells appear violet when the stain is washed from the slide	The smear is flooded with Lugol's iodine (a mordant treatment to combine the dye to those bacteria with which it will react)	The smear is now treated with a decolourising solution of acetone and alcohol. This removes the violet dye from those cells with which it has not reacted. **Gram-positive bacteria remain purple**	Finally the red dye safranin is briefly added as a counter stain. It is taken up by the colourless bacteria of the treated smear. **Gram-negative bacteria now appear red**. Gram-positive bacteria remain purple

The staining properties of gram-positive and gram-negative bacteria are due to differences in the chemical composition of the walls. **Gram-positive bacteria** have hardly any lipid in the wall. They are bacteria that may produce endospores (see page 9). Examples are *Staphylococci*, *Streptococci* and many others.

Gram-negative bacteria have chemically complex walls. There is less mucopeptide, more lipid (up to 20%) and protein. They are not affected by the naturally occurring enzyme *lysozyme* (e.g. in human tears). Lysozyme causes lysis: dissolving of the walls of many gram-positive bacteria so that they swell by osmosis and burst. Gram-negative bacteria may have flagella inserted at the end(s) of the cell (called polar flagella). Examples include *Salmonella* spp and many others.

Figure 2.7 Gram-positive rod bacteria and gram-negative cocci bacteria



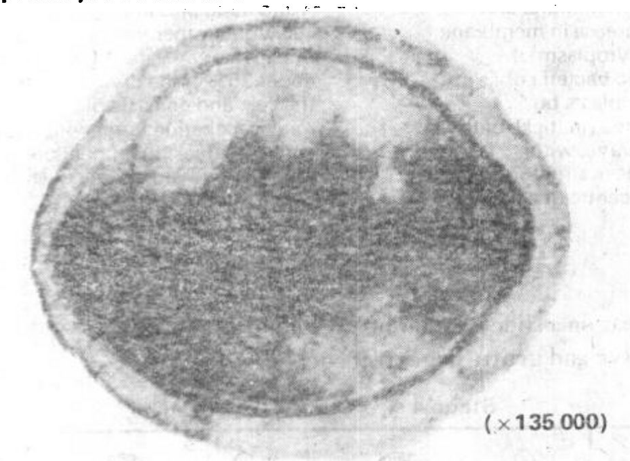
Growth of a bacterial colony

Bacteria grow and multiply extremely rapidly. In ideal conditions cell division may be as frequent as once every 20 minutes which, in theory, would enable a single cell to give rise to 100 000 000 cells in 15 hours. This volume of cells would occupy a large colony spot on the surface of a nutrient agar plate on which bacteria are often grown. This rate cannot be maintained indefinitely, but bacteria do occur in huge numbers. For example:

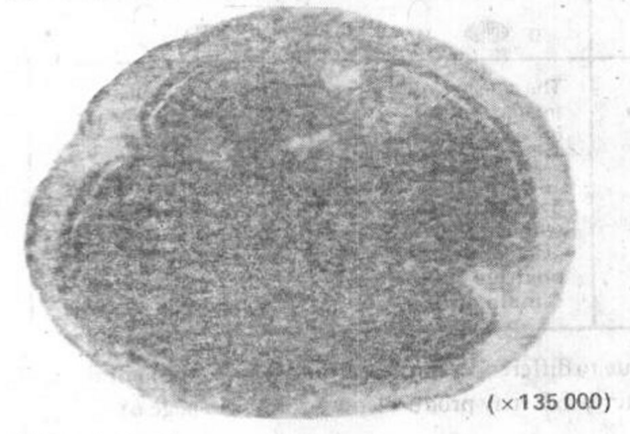
1 g of garden soil may contain	10 000,000 000 bacteria
1 cm^3 fresh milk may contain	10 000 bacteria
1 cm^3 sour milk may contain	33 000 000 000 bacteria

Because of the very large numbers of bacteria, populations have to be estimated from known dilutions from a measured sample in a method called the dilution plate technique (see facing page: 'Outline of Techniques').

Figure 2.9(i) Transmission electron micrograph of a section of a *Staphylococcus aureus* cell, showing its prokaryotic structure



(ii) Early stage in cell division



(iii) Cell division completed

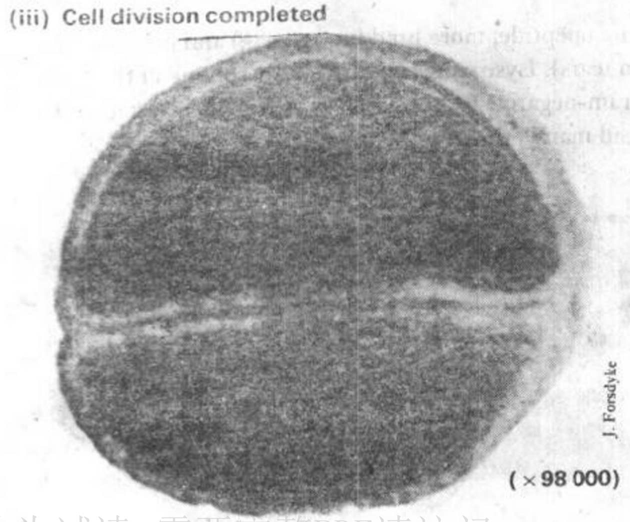
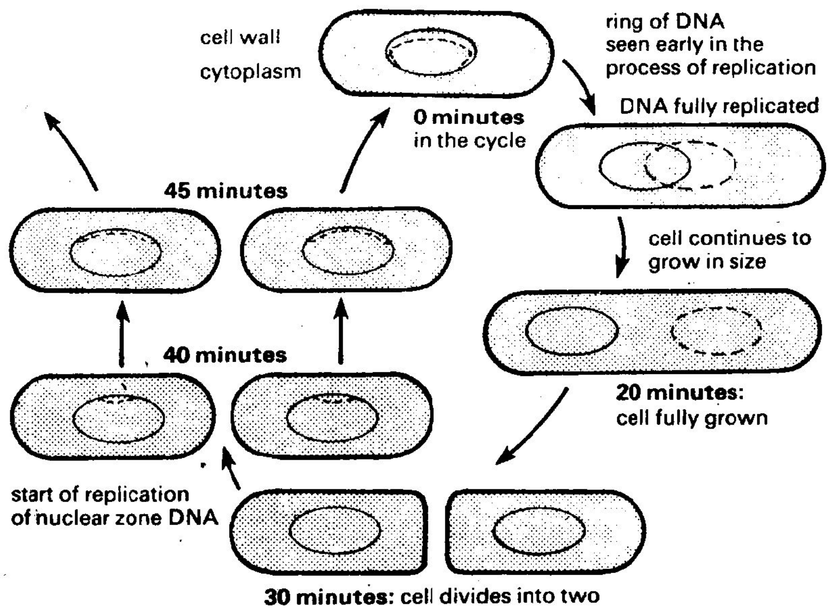


Figure 2.8 Reproduction in bacteria: their cell cycle. A diagrammatic representation of the process of asexual reproduction by cell division in the bacterium *Esherichia coli*, taking approximately 45 minutes

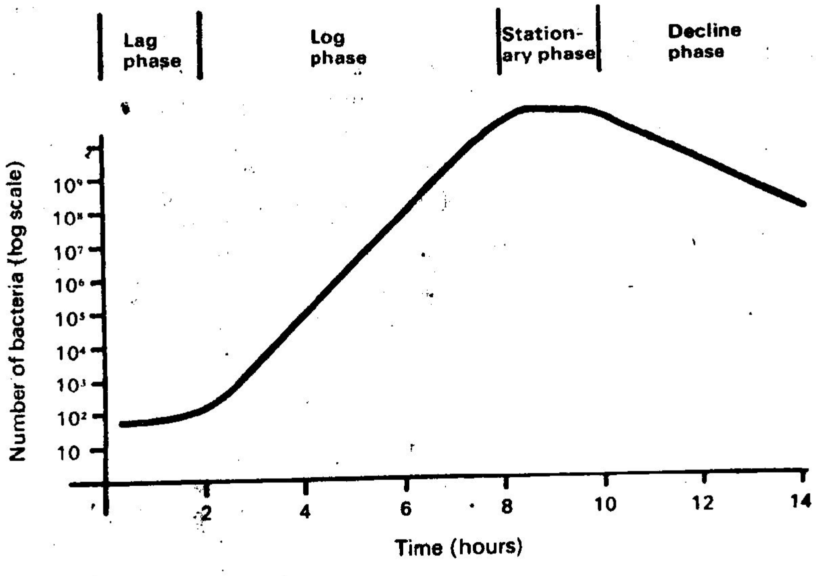


Bacterial growth is dependent upon adequate water and an appropriate food supply. In addition, the following external factors are important.

pH	Temperature	Air with oxygen
Most bacteria are favoured by slightly alkaline conditions (pH 7.4). A few tolerate extremes of acidity or alkalinity	The range 25 to 45°C is favourable for the majority of bacteria. The extremes of survival are −5 to 80°C or higher	Most bacteria flourish in air (aerobes), but many can survive in the absence of oxygen (facultative anaerobes). Some flourish in anaerobic conditions (obligate anaerobes)

When a new medium is colonised there may be a lag phase (i.e. little or no reproduction) whilst the bacteria adjust to the new medium. During this time new enzymes are synthesised; it is a period of intense metabolic activity. There follows an exponential or logarithmic (log phase) of growth when the most spectacular growth-rate occurs. With time, the food store may be used up, excretory products accumulate, and the growth rate declines. Eventually, autolysis occurs leading to death of the colony.

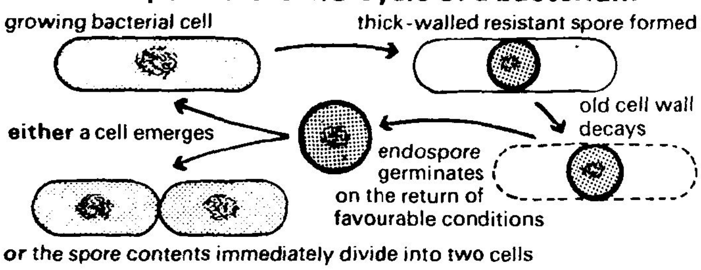
Figure 2.10 Growth of a bacterial colony with time, following inoculation of a fresh medium



Endospores

Some bacteria produce very resistant spores during unfavourable conditions. The spores have great resistance to cold, heat, pH change, desiccation and the effects of chemicals. Only certain gram-positive bacteria produce endospores (see page 7 and the photograph on page 14, figure 2.16).

The endospore in the life-cycle of a bacterium

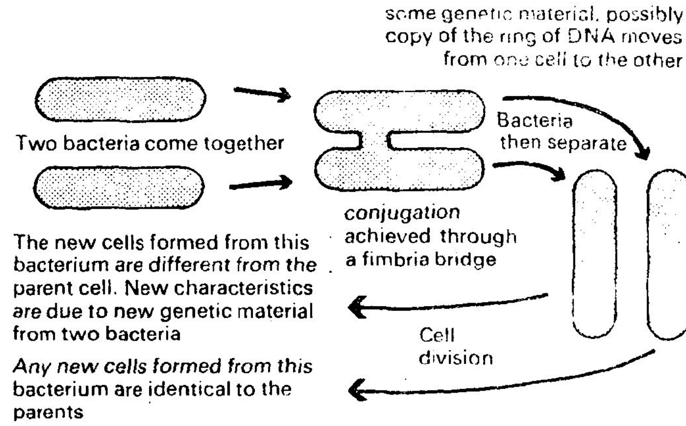


Feeding

Bacteria require an energy source and a source of raw materials (nutrients). **Energy** may come from the metabolism of sugars or fats, from light, or from chemical reactions. The **raw materials** needed for growth and reproduction include **carbon**, which may come from sources as diverse as jet fuel, engine oil, wood, sugar or gaseous CO_2 (substances which can be utilised by bacteria are termed biodegradable). Other requirements are **nitrogen**, possibly from N_2 gas, ammonium salts, amino acids or proteins; **inorganic salts**, usually PO_4^{3-}, SO_4^{2-}, Mg^{2+}, K^+, Ca^{2+}, and Fe^{3+} ions; **water**; and occasionally metabolities such as vitamins. These nutrients may be obtained in two different ways.

Sexual reproduction

Certain bacteria reproduce by conjugation.



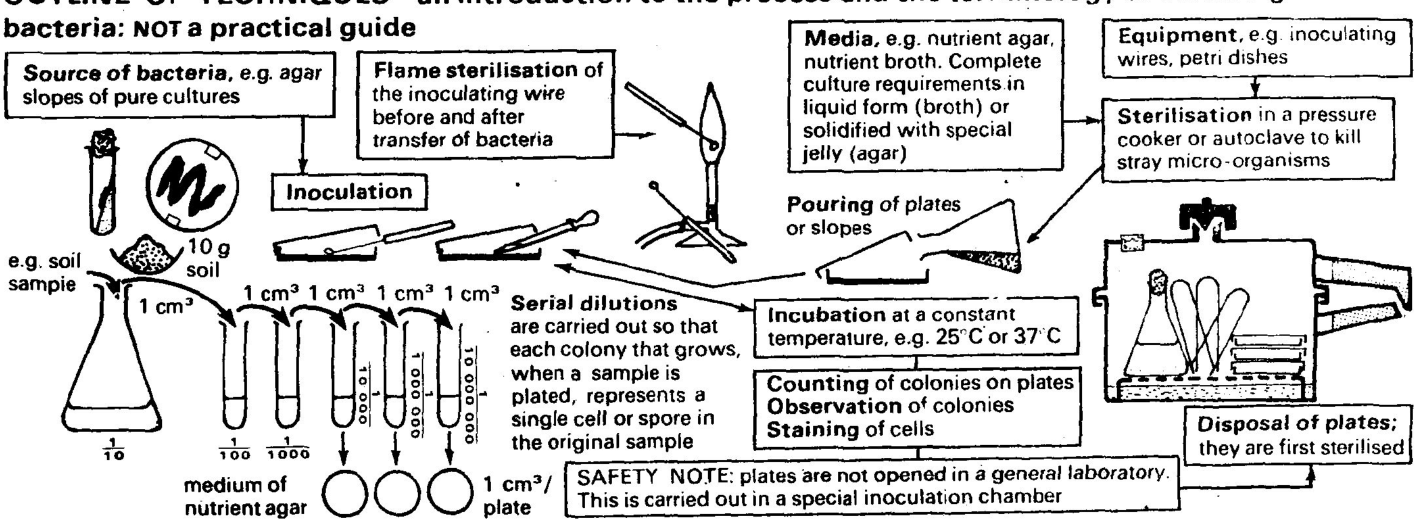
Autotrophic bacteria use light energy or the energy from chemical reactions to combine carbon (usually from CO_2) and hydrogen (usually from water) to produce sugar. With this sugar and certain ions they manufacture all other requirements. Photosynthetic autotrophs include the green sulphur bacteria and the purple sulphur bacteria. Chemosynthetic autotrophs include *Nitrosomonas* and *Nitrobacter* (see page 10) and *Thiobacillus ferro-oxydans* (see page 12).

Heterotrophic bacteria consume dead or living organisms or other organic matter such as rubbish or fuels etc. as a source of energy and nutrients. These bacteria secrete digestive enzymes into their food; digestion occurs outside the cell wall and the products are selectively absorbed into the cell.

Culturing bacteria

Bacteria are cultured (grown) on, or in, media that are designed to supply the cells with all their nutritional requirements (that is a complete culture medium). Aseptic techniques, in which apparatus and equipment are kept free of stray micro-organisms, are used to prevent contamination of bacterial cultures and of the surrounding environment. Although most bacteria are harmless, bacteriologists treat all bacterial cultures as potentially dangerous. A few are extremely dangerous.

OUTLINE OF TECHNIQUES – an introduction to the process and the terminology of culturing bacteria: NOT a practical guide



Why some bacteria are dangerous

The human body is continuously exposed to contamination by micro-organisms, but very few harmful organisms are able to establish themselves. This is due mainly to:

(a) the inhibitory effects of our natural secretions,

(b) the invading bacteria having to compete with harmless micro-organism populations already resident in the body.

Thus we are usually infected with micro-organisms, but only rarely are we diseased.

Some bacteria are pathogenic because

- **Capsule**, secreted around cell wall, prevents the bacteria being engulfed by, for example, polymorphs (leucocytes) of the blood
- **Pili or fimbriae** enable bacteria to adhere to host cells
- **Exotoxins** and enzymes that overcome host-cell defences. Secreted by the bacteria they kill or injure the cell. These secretions are all proteins
- **Endotoxins** are proteins, lipids or polysaccharides from the bacterial cell wall, liberated only on the decay of the bacterial cell. They cause fever and shock

Examples of exotoxins

Clostridium botulinum on decaying food in the absence of air produces one of the most potent poisons known to man. *Staphylococcus aureus* produces heat-stable toxins that attack the gut and cause 'food poisoning'.

Examples of endotoxins

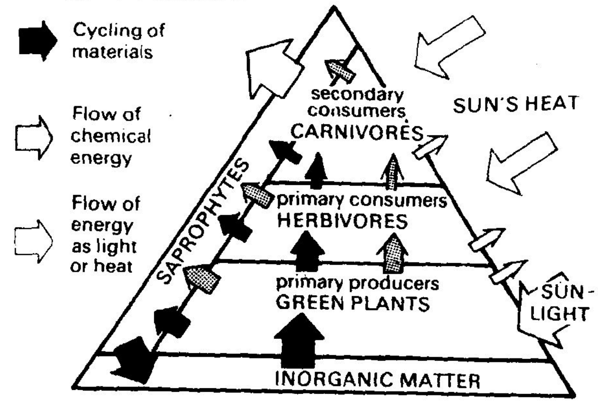
The large molecules of lipopolysaccharide (lipid and carbohydrate combined together) that are a part of the outer wall of gram-negative organisms such as *Salmonella typhi*, which causes the fever and shock of typhoid fever.

Examples of useful bacteria

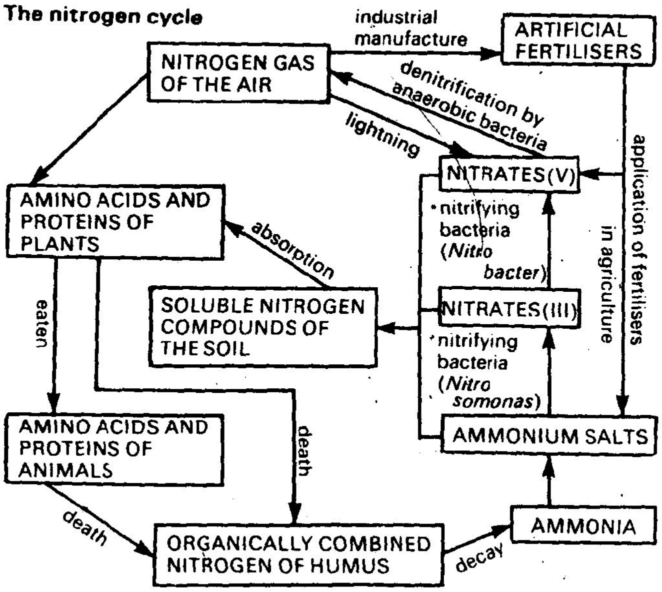
1. Bacteria in the cycling of nutrients

Plants and animals use elements and nutrients that occur at or near the earth's surface, and supplies of these nutrients are limited. For survival of life the materials in one organism must eventually be made available to other organisms. Elements such as carbon, nitrogen, sulphur, phosphorus and iron (along with many other substances found in minute amounts) are cycled, and these cycles are 'driven' directly or indirectly by energy from the sun. These processes are summarised in the Ecological Triangle, figure 2.11. The breakdown by saprophytes of green plants, their products and their predators (herbivores and carnivores) makes the nutrients and elements available for re-use. These saprophytes include many fungi (see page 45) and many bacteria, and the process is illustrated by the nitrogen cycle and carbon cycle.

Figure 2.11 Ecological triangle showing the pyramid of biomass and both the cycling of materials and the energy flow within it



The nitrogen cycle

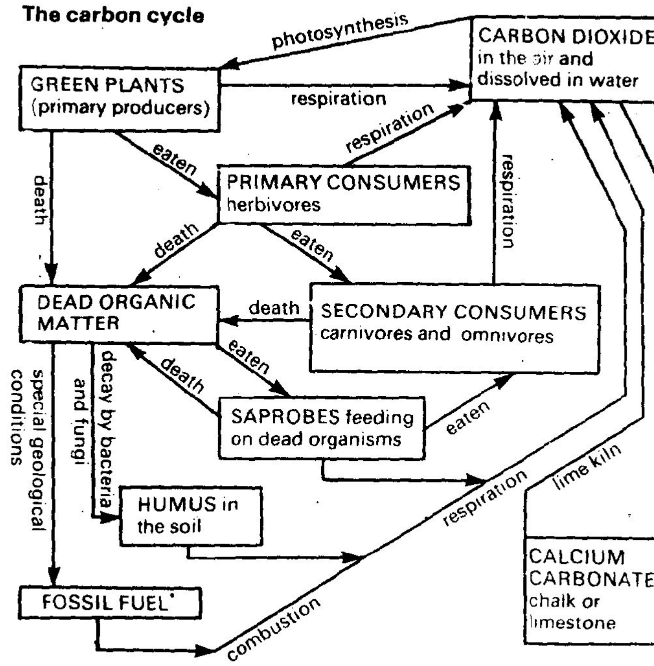


* Nitrifying bacteria are chemosynthetic autotrophs, using energy from exothermic chemical reactions,

e.g. $2NH_3 + 3O_2 \xrightarrow{\text{by } \textit{Nitrosomonas} \text{ spp}} 2HNO_2 + 2H_2O + \text{energy}$

e.g. $2HNO_3 + O_2 \xrightarrow{\text{by } \textit{Nitrobacter} \text{ spp}} 2HNO_3 + \text{energy}$

The carbon cycle

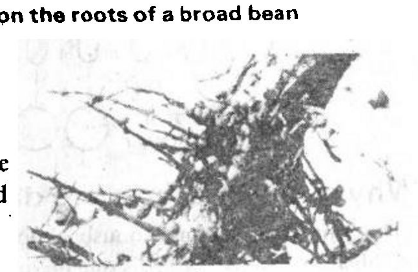


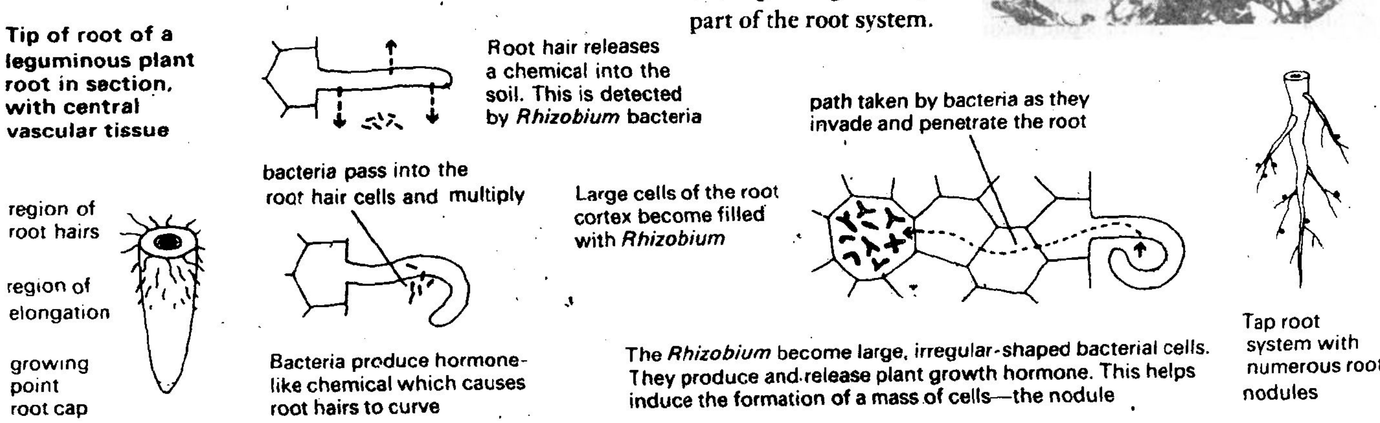
The formation and functioning of leguminous root nodules

Bacteria of the genus *Rhizobium* occur in nearly all types of soil. Many leguminous plants (family Leguminosae, e.g. clover, peas, beans) become hosts to species of *Rhizobium* during the growing season, forming root nodules which contain these bacteria within the host cells.

Figure 2.12 Root nodules on the roots of a broad bean

Root nodules form on the lateral roots after *Rhizobium* bacteria have entered from the soil. Here nodules are seen at the end of the growing season, on part of the root system.





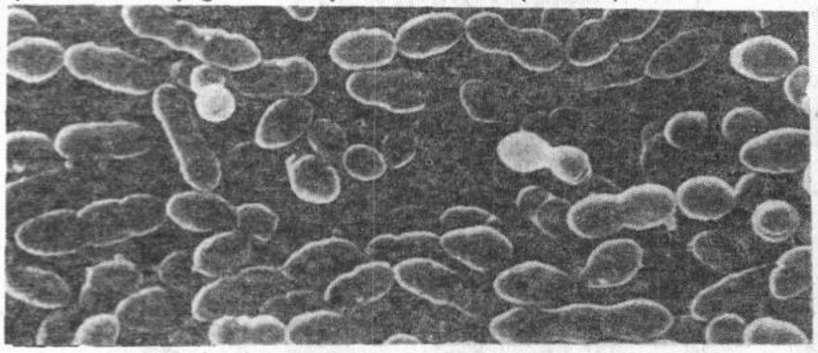
2. The bacteriology of milk and its products

Milk is liquid food produced by female mammals for their young. It provides a balanced diet, low only in vitamin C and iron.
As formed in the cow's udder, it is biologically sterile (in a healthy animal).
As it leaves the mammary gland via the teat, the milk quickly acquires a characteristic flora of non-pathogenic bacteria. If these bacteria are not checked by low temperatures and pasteurisation they quickly cause the milk to go sour. Milk passing into dairy equipment is raw and untreated. It may acquire further bacterial contamination if the equipment is unclean.
Untreated milk may contain harmful bacteria, e.g. the brucellosis causing bacteria *Brucella abortus*, causing contagious abortion in cattle and undulent fever in humans.

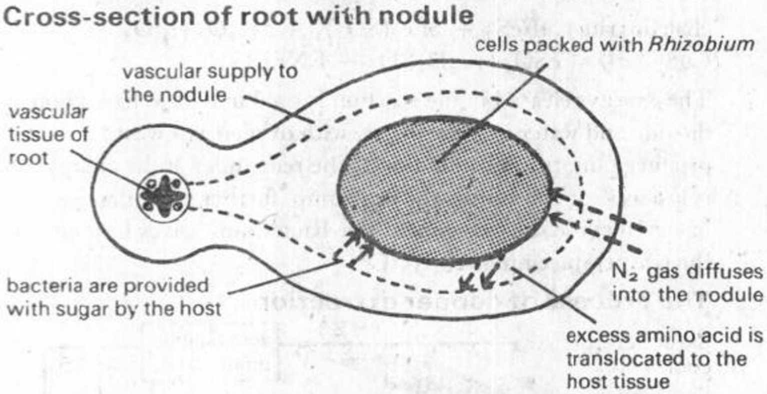
TREATED MILK

Pasteurisation	Sterilisation	Ultra high temperature (UHT)
Milk is heated to 72°C for 20 seconds and then quickly cooled to below 10°C. This destroys the majority of the bacteria without harming milk quality.	Milk is filtered, violently mixed to disperse the cream into tiny droplets (homogenised), bottled and then heated to over 100°C for about 25 minutes. There is a loss of flavour and some colour change.	Milk is heated to 132°C for one second and then placed in a sterile container under aseptic conditions. This kills all bacteria and spores. There is little loss of flavour.

Figure 2.13 *Lactobacillus bulgaricus*, a bacterium used as a 'starter' in the production of yoghurt from pasteurised milk (× 4 375)



Cross-section of root with nodule



Nitrogen from the air is reduced to ammonia by *Rhizobium*, using the enzyme nitrogenase (see page 18). Ammonia is combined with organic acid to make amino acids. The nodules are pink/red due to haemoglobin that is formed in the nodule by the host (leguminous) cells. Nitrogenase enzyme is inhibited (poisoned) by oxygen. The haemoglobin may regulate or control the oxygen concentration, and facilitate nitrogen fixation

At the end of the growing season the nodules break down, releasing bacteria and ammonium compounds into the soil. In this way **leguminous plants increase the fertility of the soil for all plants.**

Pasteurised milk is drunk as it is or used in the manufacture of yoghurt, cheese, butter, or milk powder.

(a) Yoghurt. This is a culture of two different bacteria which is added to milk at 40–45°C to ferment lactose to lactic acid and to curdle and sour the milk. One of the bacteria used is shown in figure 2.13.

(b) Cheese. To pasteurised milk are added:
(i) rennet, a preparation of calf stomach, containing renin to coagulate casein (milk protein),
(ii) 'starting culture' (inoculum) of *Streptococcus lactis*, to ferment lactose to lactic acid. The pH drops from 7 to 4.5.
This causes the milk to separate into CURD and WHEY.

CURD (solids) of protein and fat
↓
Salt added for flavour and as a preservative → 'Soft cheese', e.g. cottage cheese
↓
Pressed to dry it further → Turned into 'hard cheeses'

Further bacterial and fungal action is required to introduce the characteristic flavour and aroma of particular cheeses.

WHEY (93% water, 5% lactose) → Dehydrated for animal feed

(c) Butter. Cream is separated from the milk, churned to change the fat globules into granules, and the solid fraction is treated with starting cultures of *Streptococcus lactis* and *Leuconostoc lactis* to sour the lactose and produce flavour and aroma by bacterial enzyme action.

(d) Dried milk powder. This is used for cooking or to make feed for babies. It is a versatile product with keeping quality but little flavour.
Powdered milk for babies is useful where breast milk is not available. The problems with bottle feeding arise from:
(i) bacterial contamination; careful sterilisation of equipment is essential,
(ii) malnutrition; the powder must be made up into milk of the correct strength.
The misuse of powdered milk has reached tragic proportions in some Third World countries where people have been persuaded by advertisements to change to this method of baby feeding unnecessarily.

3. Bacteria and genetic engineering

The nuclear material in the bacterial cell is a single strand of DNA in the form of a ring or helix. Prior to cell division this strand of DNA (it is of the same chemical composition as in eukaryotes) replicates, and one copy passes into each daughter cell. (This is shown in figure 2.8, page 8.) The unusual feature of bacterial DNA is that it can be changed by addition of short lengths of DNA from the cell of a closely related bacterium. The process may be brought about by an invading bacterial virus (called a phage, see page 15).
DNA controls and directs the biochemical activity of the cells by directing protein and enzyme synthesis. In this way DNA controls growth, development and the functioning of the whole organism. Because bacterial DNA does not occur in pairs as it does in eukaryotic chromosomes (the eukaryotes have two alleles to every gene) it means that any changes that are brought about in the bacterial DNA may result in more or less immediate changes in the functioning of the bacterial cell. These naturally occurring changes or accidents of DNA addition have been much studied, and techniques for engineering such changes in bacteria, using unrelated DNA from viruses, other bacteria, and from eukaryotic cells have been developed (called genetic engineering or recombinant genetics). Bacterial strains have been made which permit relatively inexpensive synthesis of vitamins, hormones (e.g. human insulin for diabetics), antibiotics and drugs such as human interferon, an anti-viral agent.
The danger associated with genetic engineering, is that, inadvertently, some very harmful gene may be added to the DNA of a ubiquitous and normally harmless bacterium of the human micro-flora, which might then escape into the population and multiply. Stringent safeguards have been laid down to prevent such an event, e.g. genetic engineering being limited to species that only prosper under abnormal physiological conditions, not occurring in the human body.

4. Vaccines

Robert Koch (1843–1910) isolated *Bacillus anthracis* from anthrax sufferers and established the rules (now known as Koch's postulates) by which it is judged when an organism is a pathogen and not just a casual member of the normal body flora.

Koch's postulates

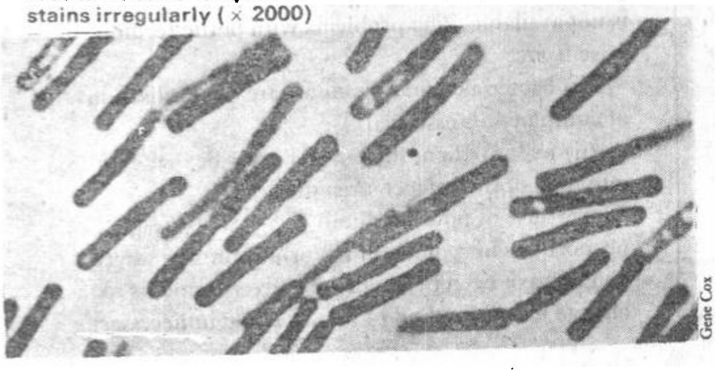
(a) The same organism must be found in all cases of the disease.

(b) The organism must be isolated and grown in pure culture from the infected host.

(c) The organism from the pure culture must reproduce the disease when inoculated into a susceptible host.

(d) The organism must be isolated in pure culture from the experimentally infected animal.

Figure 2.14 *Bacillus anthracis*: **a large, broad-celled gram-positive bacterium that requires aerobic conditions. The rods have almost square corners and cytoplasm that stains irregularly (× 2000)**



It was also with *B. anthracis* that the first systematic vaccinations were conducted.
A vaccine is a suspension of killed, or living but inactivated organisms which, when inoculated into the body, act as an antigen causing development of antibodies that render the body immune or, at least, resistant to infection by that organism. Humans can be infected with the anthrax bacillus by contact with hides of infected animals. Anthrax is primarily a disease of sheep, cattle and other herbivorous animals. An infection occurs in the skin, but the bacterium invades the blood and produces a toxin which acts on the central nervous system causing breathing to cease. Louis Pasteur (1822–95), working with farm animals, kept some anthrax bacilli at 42°C (i.e. at above body temperature) and produced a less virulent (attenuated) strain. This, when injected into the body, was no longer capable of causing anthrax, but it did induce immunity to the disease. When the virulent strain was injected subsequently, the animals did not contract anthrax.
From earlier times people were aware that some individuals have, or acquire, immunity to certain diseases. Edward Jenner (1749–1823) observed that many patients who were in contact with cattle had apparently acquired immunity to the usually fatal disease smallpox. He believed that this immunity was acquired following an attack of the similar but much milder disease cowpox; the cowpox being transmitted to people from the cows they worked with. It was Jenner who first used the technique of inoculation. He gave people cowpox and then showed that they subsequently acquired immunity to smallpox.

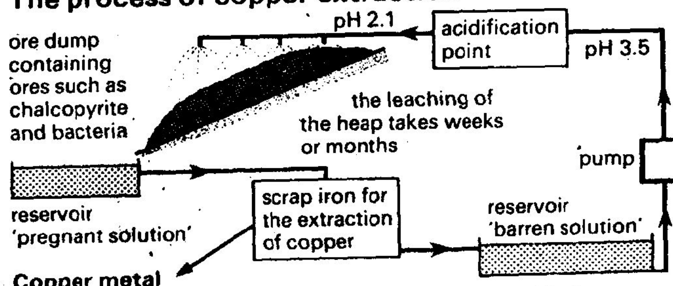
5. Microbial mining

It is now necessary to use lower and lower grade ore as the explosion in demand for metals for industrial and manufacturing uses has already outstripped the supply of accessible, high-grade deposits of appropriate ores. The deliberate use of bacteria to assist in recovery of metals from low-grade ores is a quite recent development. In the bacterial leaching of copper for example, the metal is being economically recovered from old mine waste containing only 0.25–0.50% copper.
The tiny, rod-shaped *Thiobacillus ferro-oxidans* occurs naturally in ore dumps in the absence of light and in an acid medium. The bacterium 'eats rock', i.e. it is chemolithotrophic. It oxidises sulphides to sulphates and iron(II) to iron(III) ions.

chalcopyrite $CuFeS_2 + 2Fe_2(SO_4)_3 + 2H_2O + 3O_2 \rightarrow CuSO_4 + 5FeSO_4 + 2H_2SO_4 +$ ENERGY

The energy released in the reaction is used in part to fix carbon dioxide and water to make sugar, with oxygen as a waste produce (autotrophic nutrition). The remainder of the energy is lost as heat and warms the ore dump, further speeding the bacterial extraction of copper. The bacterium thrives best in the temperature range 20–35°C.

The process of copper extraction



$Cu^{2+} + SO_4^{2-} + Fe\,(metal) \rightarrow Cu(metal) + Fe^{2+} + SO_4^{2-}$

The bacterium consumes the ore by transferring electrons from iron ions and sulphide ions making the ore more soluble. The electrons are transferred to oxygen to produce water and give up energy which is coupled to ATP synthesis.

Bacteria in water and sewage

Sewage is a mixture of organic matter in water, rich in bacteria which aerobically digest sewage. This process needs to be contained and completed at a sewage plant because if raw sewage is discharged into rivers the bacterial consumption of dissolved oxygen is so great that other organisms are deprived of oxygen and die of asphyxia. Oxygen gas is only slightly soluble in water and it is shortage of oxygen due to excessive bacterial activity rather than the presence of a poison in sewage that kills larger animals in sewage polluted rivers and streams. This feature of water quality is known as its Biological Oxygen Demand (BOD). It is defined as the amount of oxygen absorbed biologically by one litre of the water sample in five days at 20°C.

Treatment of sewage

Bacteria, with other micro-organisms, turn sewage into re-usable and inoffensive substances.

Primary treatment: mechanical

Sewage in-flow → **Screening off** of coarse materials; grit is removed by grids.

Secondary treatment: microbial

Sedimentation tank
Solids settle down

Solid matter to anaerobic digestion tank
Carbon and carbon dioxide from the solid organic matter is reduced to methane by bacteria
$C + 4(H) \rightarrow CH_4$
e.g. *Methanobacterium* spp
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$.

Methane gas is then burnt to power the sewage plant

Liquid has air pumped through.
Bacteria, e.g. *Bacillus* spp
Pseudomonas spp
Zooglea spp
and protozoa, e.g. the ciliates
Vorticella spp
under aerobic conditions, oxidise all organic matter to CO_2, H_2O and NH_3

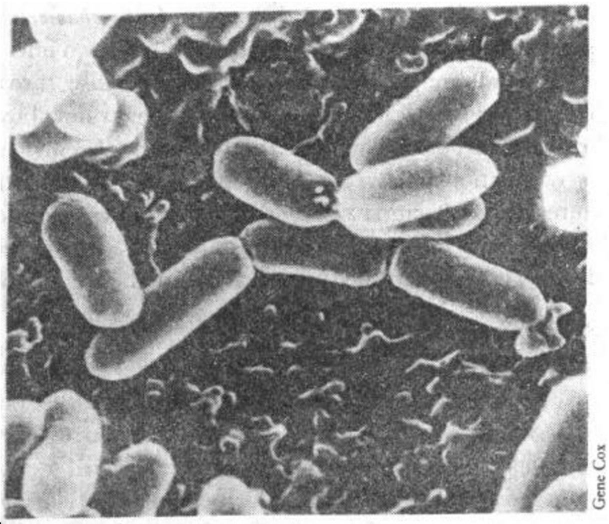
Clean water is discharged into rivers

Remaining solid matter is flash-dried over a gas flame to dry and sterilise; it is then sold as **fertiliser**

Contamination of drinking water by sewage

The human small and large intestines house a huge and characteristic microflora. They are mostly commensal bacteria, but a few bacteria live mutualistically, providing their host with vitamins, e.g. vitamin K and vitamin B complex. *Escherichia coli* is an extremely common component of the human intestines, and it is responsible for faecal odour. (Human faeces are 50% bacteria.) Water purity tests check for the presence of this bacterium as evidence of contamination of the drinking water supply by sewage.

Figure 2.15 ***Escherichia coli*, a bacillus that is part of the normal flora of the human intestinal tract (×16 600).** Rods show fimbriae (pili), filamentous appendages which are shorter and smaller than flagella. They cause bacteria to adhere, and they may also be involved in the sexual transfer of genetic material in conjugation (see page 9)

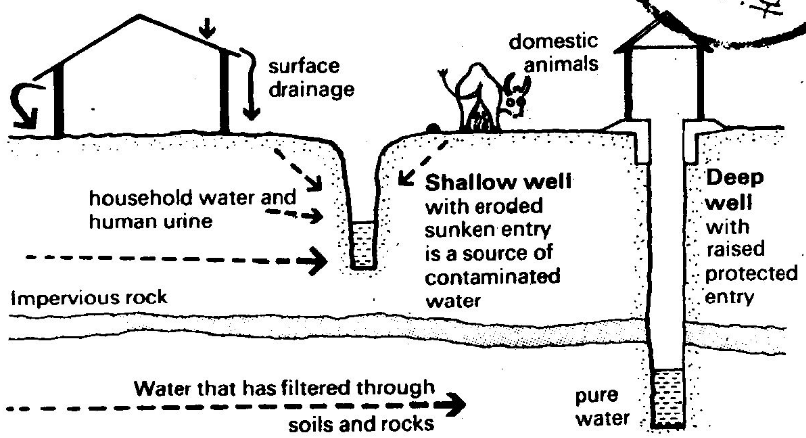


Gene Cox

Control of water-borne disease

1. In Third World countries

A very deep well surrounded by a concrete lip to exclude surface contamination is the safest source of drinking water.



2. In Developed countries

In situations where urban conurbations are crowded together, the water supply to one city (e.g. London) is the river that drains sewage works of towns up-stream (e.g. Reading). The steps in purification are:

Untreated water

AERATION	oxidation of organic matter by micro-organisms
COAGULATION	aluminium sulphate is added, causing particles to clump together
SEDIMENTATION	coagulated material settles out
FILTRATION	using sand
CHLORINATION	at quite low concentration destroys all living things

Tap water

Water-borne diseases of consequence include:

Cholera

Cholera is caused by *Vibrio cholerae*. This is very common amongst the people in Bangladesh and the Far East; it is said to be endemic there. It is spread by people where the sewage is not correctly disposed of and contaminates the water supply. The patient vomits and has severe diarrhoea. Death can be quick due to dehydration and shock. The bacterium produces a powerful toxin that inflames the lining of the intestines.

Typhoid

Typhoid is caused by *Salmonella typhi*. This is typically a disease of overcrowded communities, where the people have become weakened by the effects of poverty and starvation. It causes high fever, slow pulse-rate, irritation of the intestine and diarrhoea. Healthy carriers occur and can pass the infection by handling food for other people.

Some bacterial diseases of Man

1. Botulism

Botulism is a poisoning due to a nerve toxin called botulin, produced by the rod-shaped bacterium *Clostridium botulinum* under anaerobic conditions. The bacterium occurs in soil, but the danger to man arises if, in the canning of food, the temperature is not raised high enough to destroy any endospores which may have got in. Today cases of the disease are very rare. Botulin is one of the most poisonous toxins known, but it is not produced in food if the food is:

(a) in the presence of air,
(b) vero acid, that is below pH 5,
(c) preserved in 8% or greater sodium chloride solution,
(d) preserved in 50% or greater sugar solution.

Furthermore, proper cooking inactivates any of the botulin that is present, although botulin can occur in incorrectly canned foods, such as asparagus, corn, peas, pepper, spinach, ham or sausage.

2. Diphtheria

This disease is caused by the bacterium *Corynebacterium diphtheriae*. It is spread by droplet infection (e.g. sneezing) from infected to healthy people, and produces a local infection of the nose, throat and larynx. The bacterium releases a powerful toxin that can kill by damage to the heart. In developed countries this disease has been virtually eradicated by vaccination. The vaccine is prepared by extracting the toxin from a culture of the bacteria and rendering it harmless with formalin. When the vaccine is injected into a human (diphtheria is purely a human disease) it causes antibody production and hence immunity.

3. Tetanus

The tetanus-causing bacterium *Clostridium tetani* is an obligate anaerobic bacillus that exists in soil and in the gut of man. The bacterium is sensitive to penicillin, but it produces extremely resistant endospores. Deep body wounds may be deprived of oxygen owing to damaged blood supply and to the metabolism of the contaminating bacteria. Such wounds may be contaminated with *C. tetani*. Under anaerobic conditions the bacterium grows and releases a powerful toxin into the bloodstream. This is transported around the body and interferes with impulse conduction at the synapses of nerves causing convulsive contraction of voluntary muscles. Tetanus is most prevalent among those wounded in war, and in children, particularly infants in rural Asia, where the baby's umbilical cord stump may be contaminated with soil or faeces. Tetanus prevention is easier than treatment. In developed countries children are treated with tetanus toxin which has been modified in a laboratory so that its poisonous properties are lost but its antigenic properties remain (called tetanus toxoid). Immunity acquired in this way is not permanently retained by the body, and booster doses to re-acquire immunity become necessary from time to time.

Figure 2.16 *Clostridium tetani*, **the agent of tetanus (lockjaw) (×4 200).** These bacteria are obligate anaerobes; they cannot grow in the presence of oxygen. They are large, motile gram-positive rods that produce extremely resistant endospores. These spores occur as terminal swellings that make the whole structure resemble a miniature drumstick


Gene Cox

4. Tuberculosis (TB)

Tuberculosis is caused by *Mycobacterium tuberculosis* which may be inhaled or swallowed. It attacks the lungs, and in elderly, ill or malnourished people, it leads to serious illness and death due to progressive destruction of the lung tissue.

It causes a major health problem world-wide since there are so many people who are malnourished and who live in cramped unsanitary homes on the edge of cities. In developed countries there is good control of TB. This may be attributed to:

(a) improvements in the general standard of living and nutrition,
(b) eradication of TB in cattle and the pasteurisation of milk,
(c) development of drugs for the control of the disease in those affected,
(d) early detection of infection by mass radiographic screening,
(e) the introduction of effective BCG (Bacillus-Calmette-Guérin) vaccination which involves inoculating 12 to 13-year-olds with an injection of living but inactivated (attenuated) cells. Before treatment of an individual with BCG, it is necessary to test whether they are already suffering from TB or have natural immunity. The reaction of the skin to the products of dead bacilli is tested; those whose skin reacts strongly (their skin swells and reddens at the test site) either have immunity or the disease.

5. Whooping cough (Pertussis)

Whooping cough is an acute infection of the respiratory tract. It leads to violent coughing and fever. It is caused by a small, gram-negative coccobacillus (broad oval bacteria – see pages 6 and 7) called *Bordetella pertussis*. It does not invade the bloodstream but toxins from the bacteria cause the fever, and as the organism grows, a thick ropey mucus is produced from the lungs.

Whooping cough was once a common infectious disease of children causing very high mortality in infants under one year of age. It is hard to treat, but vaccination with an injection of killed bacteria, administered in the second or third month of life is totally effective in prevention. The vaccine, of dead bacilli, is administered together with diphtheria and tetanus toxoid. A total of three, monthly injections is given. Occasionally whooping cough vaccine causes allergic convulsions and very rarely may produce permanent brain damage. The number of cases of brain damage after vaccination is 1 in 50 000 vaccinations. The risks of contracting whooping cough are far greater than that of immunisation. However, children with another illness must not be vaccinated until they are well again. Children who suffer from convulsions should not be treated at all, and those who show symptoms of nervous system disorder during the vaccination sequence should have no further injections of the vaccine.

6. Venereal disease

Venereal diseases are infections which are spread from person to person during sexual intercourse or other sexual contact. Some babies may be infected in the womb or at birth by an infected mother. The most common venereal diseases are syphilis and gonorrhoea. These diseases can be cured with antibiotics, provided thorough treatment is given at an early stage.