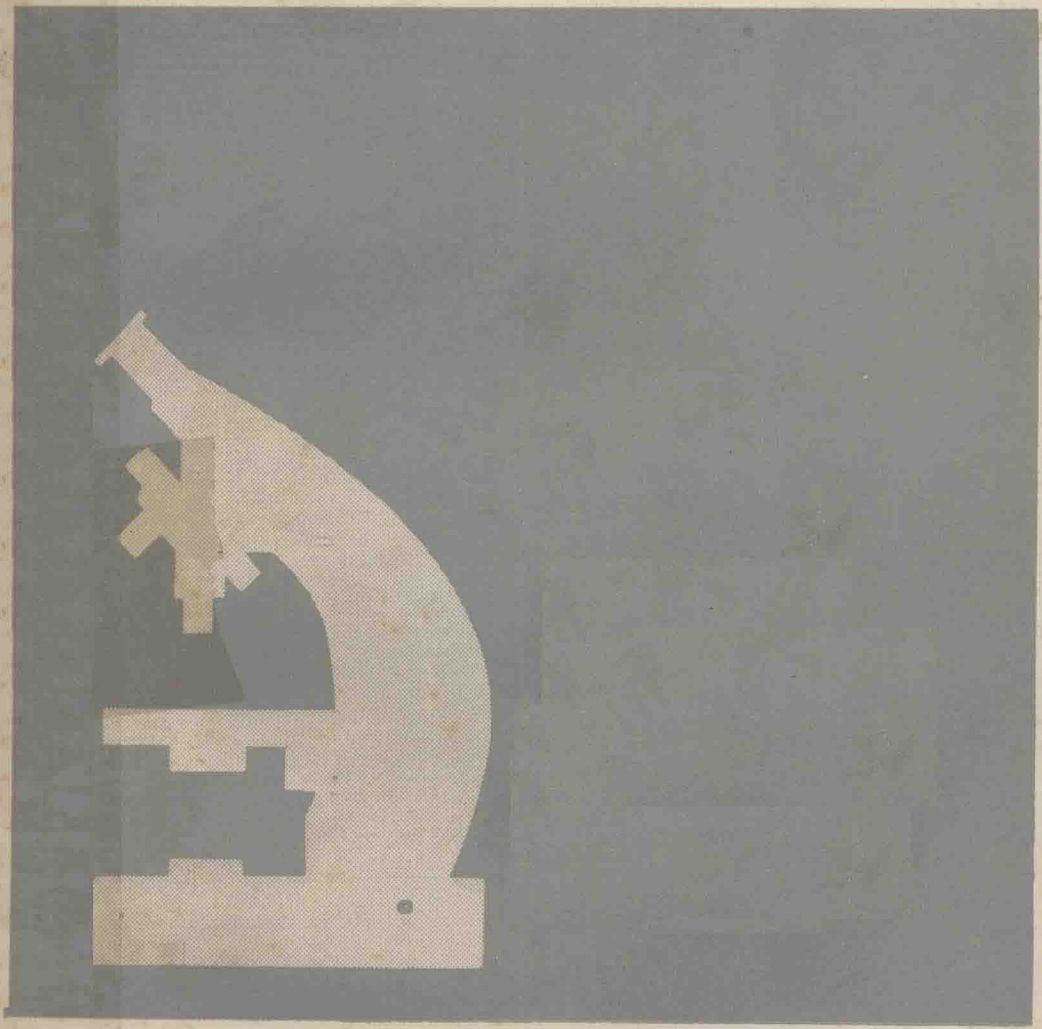
Ian W. Dawes &
Ian W. Sutherland

Microbial Physiology



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Microbial physiology

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Preface

Most prefaces begin with 'This book is . . .', followed by an apology for its existence, a statement of the aims of the authors, an acceptance of responsibility for all errors and omissions, and a grateful acknowledgement of the patience of the authors' spouses.

The title of this book is obvious, and we have no intention of putting off potential customers by apologising for any shortcomings. Errors are inevitably the result of the other author. The size was set by our genial publisher who must therefore accept all kudos for the delightful brevity of the book, its lack of boring detail, and of course any omissions. Our wives are no more, nor less, patient than they were before we began and we remain happily married to them.

A final word of warning to our readers. This book is intended (it had to come in somewhere) as an introduction to microbial physiology to cover a broad spectrum of course requirements. It provides a foundation upon which to build by judicious reading of other sources. We trust our readers find the book useful and we would be delighted to receive any comments in case we have the opportunity to prepare another edition.

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Edinburgh 1976

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Introduction

Physiology, according to the *Concise Oxford Dictionary*, is the science of normal functions and phenomena of living things. Even when restricted to microorganisms the present knowledge is very extensive, and like microorganisms in a nutrient culture, is increasing exponentially. There are many reasons for this interest. For some, trying to understand the basics of life processes, microorganisms have become experimental tools largely because they can be grown and manipulated with relative ease. For many, the choice of experimental organism has been just one bacterial species, *Escherichia coli*, since biochemical and genetic techniques have been developed to a level of sophistication unmatched in any other organism. There are however a number of major differences between prokaryotic and eukaryotic organisms, and a number of eukaryotic microorganisms (notably the slime mould *Dictyostelium*; the yeast *Saccharomyces*; fungi such as *Neurospora* and *Aspergillus*; algae *Chlamydomonas* and *Chlorella*, and the protozoan *Tetrahymena* are receiving increasing attention as representative eukaryotes amenable to detailed study.

E. coli, moreover, does not typify the prokaryotic cell. Different groups of bacteria have adapted to grow in different environments to the extent that they can be isolated from almost any ecological niche, including such unexpected places as the mouths of geysers and the fuel tanks of aeroplanes. This diversity is reflected in the range of activities to be found in different groups, and in their response to particular environmental conditions. Bacteriologists, ecologists, many industrial microbiologists, taxonomists and developmental biologists are therefore more likely to be interested in the differences between various microorganisms, as well as their similarity to *E. coli* or their conformity to the notion of a 'generalised microbial cell'

In this book there is an attempt to strike a balance between the two approaches of studying a few organisms in depth, and discussing the different activities to be found amongst different groups of microorganisms. Where possible eukaryotes are introduced into the discussion to emphasise the differences between them and prokaryotes. It is assumed that the reader has a basic knowledge of biochemistry so that less space has been given to common biological processes, and more to those aspects in which microorganisms differ from plant and animal cells.

The astute student will realise that any book of this size can only act as an introduction to the subject and provide a basis for further reading. For those wanting to obtain a broader coverage of the topics outlined here, or to delve into other developing areas of microbiology, a list of references to useful review articles

is provided at the end of the book. In addition to these, the following review journals are very important sources of information and act as indicators of current trends in the study of microbiology: *Annual Reviews of Microbiology, Symposia of the Society for General Microbiology, Bacteriological Reviews, Advances in Microbial Physiology,* and *Critical Reviews in Microbiology.*

1 Chemical cytology of the microbial cell

Microbial cells, even those of eukaryotes, are usually so small that important structures cannot be resolved by light microscopy. Consequently, much of our present knowledge of cell structure is the result of electron microscopy. Specimens for electron microscopy often require extensive pretreatment before they can be visualised satisfactorily, and care must be taken in interpreting electron micrographs. During the preparation of thin sections many compounds are inactivated and there are problems in trying to locate some labile chemical components, such as enzymes, within particular structures by cytochemical staining.

Techniques have been developed for disrupting cells so that some organelles or other particles can be isolated in a state which retains at least some of the *in vivo* structure and activity. Particular structures can usually be isolated from cell lysates by zonal centrifugation through sucrose density gradients. Here also care should be taken in interpreting the results, since disruption of cells often involves harsh physical treatments needed to overcome the strength of the cell wall surrounding most microorganisms. Methods used to break cells include grinding with glass beads or sand, exposure to ultrasonic vibration or sudden changes in pressure, or to more gentle osmotic lysis after removing the cell wall with lytic enzymes.

Before discussing general cytological features found in microorganisms it is well to recall that there are many fundamental differences between prokaryotes and eukaryotes. These are summarised in Table 1.1, and Fig. 1.1 illustrates structures which can be found in prokaryotic and eukaryotic microorganisms. There is a very wide range of cytological features found in microbes, and those outlined in Fig. 1.1 and in the subsequent discussion need not occur in all species. There may also be extensive differences between cells of the same species grown under different physiological conditions, and where quantitative results are important they must include a definition of the culture conditions used.

CELL SURFACE AND ITS APPENDAGES

Flagella and Cilia

Cell motility is found in both eukaryotic and prokaryotic microorganisms and is usually dependent on specialised organelles protruding from the cell surface. In bacteria these are flagella; in eukaryotes the terms flagella and cilia have both been used, although the structures are essentially similar.

Table 1.1 Brief comparison of prokaryotic and eukaryotic cells

Characteristic	*Prokaryotic cell*	*Eukaryotic cell*
Size	Usually around 1–5 μm	Usually greater than 5 μm
Movement	Flagellar or gliding motion, simple fibrillar arrangement of each flagellum	Flagellar or amoeboid motility, complex fibrillar arrangement of flagellum
Wall structure	Usually contains several polymers, almost always peptidoglycan	Contains a variety of organic or rarely inorganic polymers, never peptidoglycan
Vacuoles	Rarely present, if so gas vacuoles	Often present, range of different types and functions
Arrangement of nuclear DNA	No delineating nuclear membrane, single circular chromosome attached to cell membrane or mesosome	Within membrane-bound nucleus as several linear chromosomes. DNA complexed to basic proteins, histones
Replication of DNA and segregation	Bidirectional from single replication origin. Amitotic segregation	Bidirectional replication from multiple origins. Limited to part of cell cycle and segregation by mitosis or meiosis
Protein synthesis	Translation simultaneous with transcription. Only one RNA polymerase known, with modifying proteins. Ribosomes are 70 *S* and inhibited by a group of antibiotics specific for prokaryotes.	Translation of nuclear genes occurs in cytoplasm. Three RNA polymerases. Cytoplasmic ribosomes are 80 *S* and inhibited by cycloheximide. Mitochondrial and chloroplast ribosomes resemble those of prokaryotes
Energy production	Respiration, fermentation or photosynthesis. Wide range of substrates. Respiratory chain associated with plasma membrane, Photosynthesis on invaginations.	Some fermentative, but usually either respiratory or by photosynthesis. Respiration in mitochondria, photophosphorylation in chloroplasts
Reproduction	Asexual, by binary fission. Conjugation is rare, leads only to partial diploids and is not associated with reproduction	Sexual or asexual. Many ways, including budding, binary fission, hyphal extension and sporulation. Conjugation part of reproduction and leads to diploids

Bacterial Flagella

Flagella are helical structures several times the length of the bacterial cell (Fig. 1.2) and if sheared off by ultrasonic treatment motility is lost, but is regained as new flagella are resynthesised. The pitch and wavelength, and the arrangement of flagella on the cell surface are characteristic to each species. In *Pseudomonas* species there may be one or two polar flagella, while in other genera they may be found in large numbers over the whole surface of the cell. In *spirochaetes* a modified type of flagellum is present as an *axial filament*; two sets of fibrils are fixed at the poles of

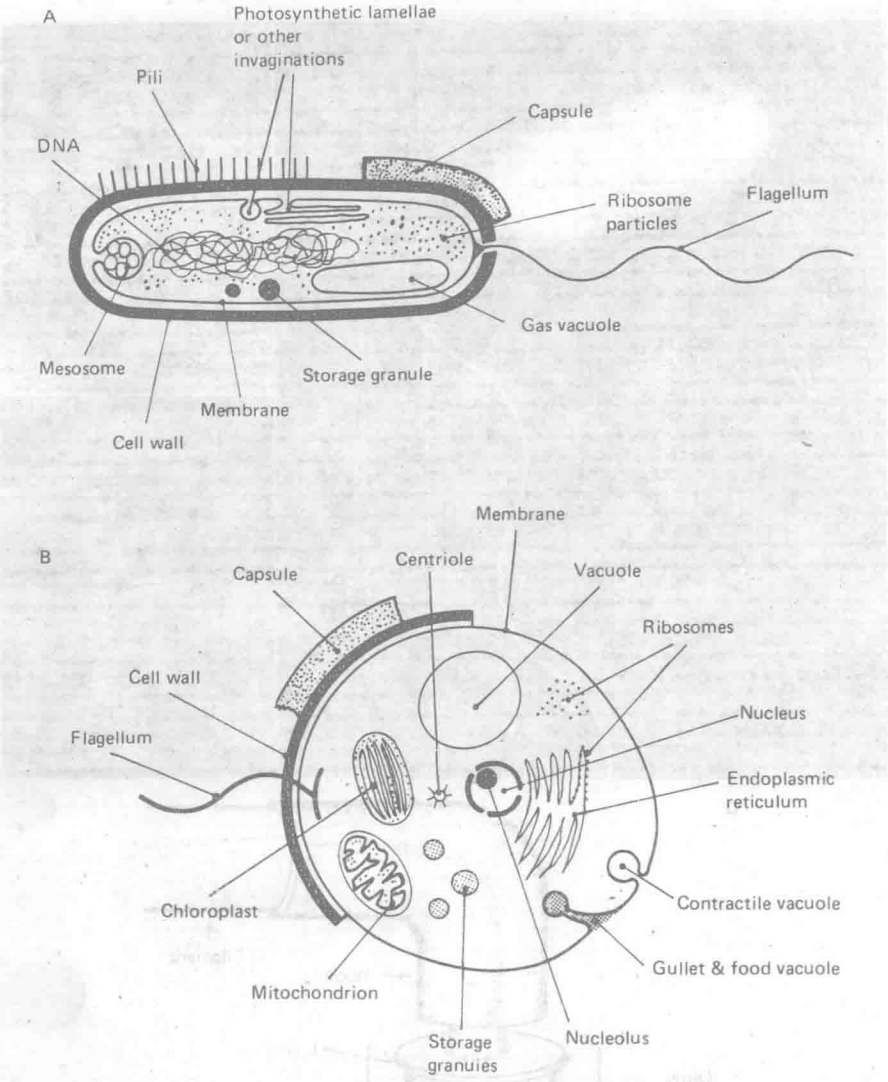
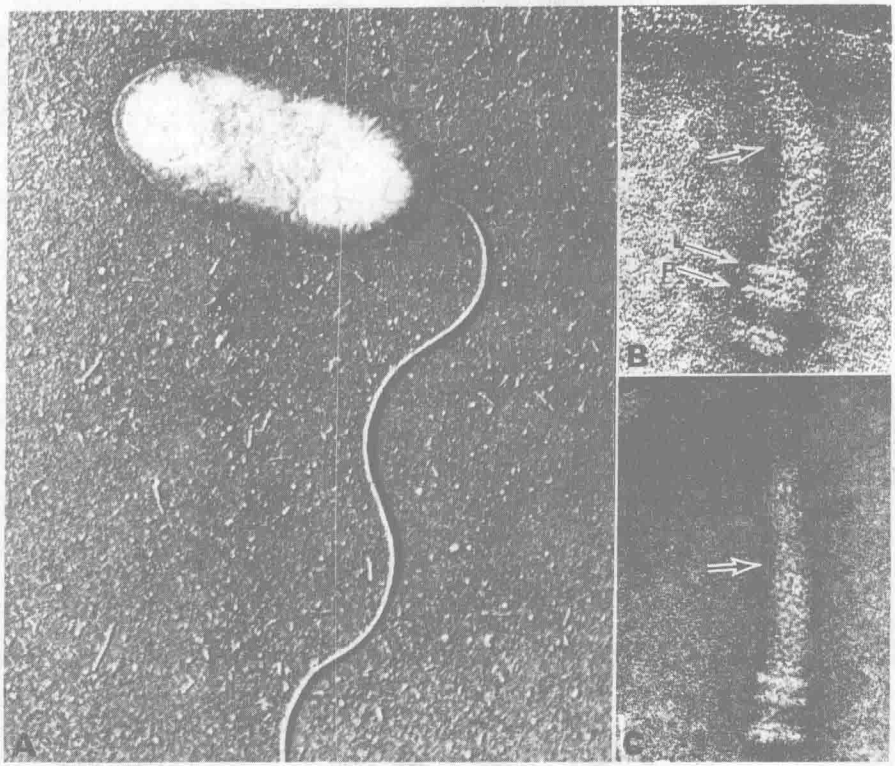


Figure 1.1 'Representative' A, prokaryotic; and B, eykaryotic cells. Some structures shown are dispensable, including capsules, flagella, membrane invaginations, pili, storage granules, vacuoles and chloroplasts. Obviously these do not represent form and shape of cells, merely the main structures.

the cell and run the length of the cell enclosed within the outermost layer of the cell surface. This arrangement enables these spirally shaped organisms to move by a flexing motion.

. Flagella are composed of subunits of a single protein, *flagellin*, which in the electron microscope are seen to be aggregated into chains of molecules arranged helically. Flagellins from different species differ in their amino acid composition



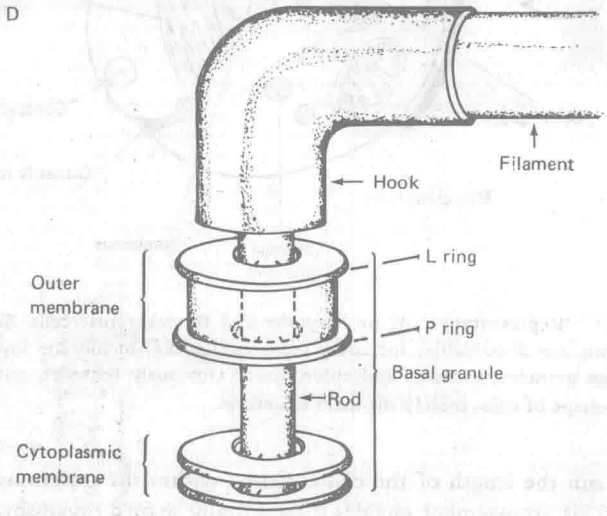


Figure 1.2 The bacterial flagellum. A, Cell with a single polar flagellum, electron micrograph of a shadowed cell. B, Basal granule, negatively stained. C, Some subunit structure of the flagellum revealed by negative staining. D, Interpretation of the basal body structure. (A, by courtesy of Professor J. P. Duguid; B, C and D, by courtesy of Dr. Julius Adler and the *Journal of Bacteriology*)

and serological properties and in some species (*Salmonella* species and *Spirillum serpens*) contain an unusual amino acid, ε-*N*-methyllysine.

Flagellar assembly provides a simple and interesting model of how biological structures are assembled from their chemical components. Under acid conditions *Bacillus pumilis* flagellar filaments can be dissociated into their subunit polypeptide of molecular weight 30 000 to 40 000. On slowly raising the pH these reaggregate to give straight filaments as well as the wavy flagellum-like structure. The straight form undergoes rearrangement to the more stable wavy form. Experiments using *p*-fluorophenylalanine, which leads to formation of abnormal flagella, have shown that they grow by condensation of subunits at the tip distal to the cell membrane, and raises the intriguing question of how the subunits are transported there – through the hollow central core of the flagellum?

The filament of each flagellum is attached to the cell membrane at a structure known as the *basal granule* or disc. The precise structure varies between species, but a typical example is shown in Fig. 1.2. A filament is joined by a hook to a rod and set of rings located in the cell wall and the cell membrane. This basal granule is probably involved in the transduction of energy from the cytoplasm or membrane to the flagellum. The complete assembly and functioning of the bacterial flagellum is quite complicated: in *E. coli* and *Salmonella typhimurium* some twenty genes are involved including that for flagellin, and those determining rotation of the 'motor' including whether or not it turns the flagellum, and if so, whether it can go both clockwise and anticlockwise.

Eukaryotic Flagella and Cilia

Eukaryotic flagella or cilia are much larger and more complex than the corresponding prokaryote organs and are always attached to cylindrical basal bodies within the cytoplasm. An extension of the plasma membrane surrounds the flagella and encloses a system of microtubules (each resembling a bacterial flagellum) arranged as two central ones surrounded by a further nine pairs (Fig. 1.3). The only

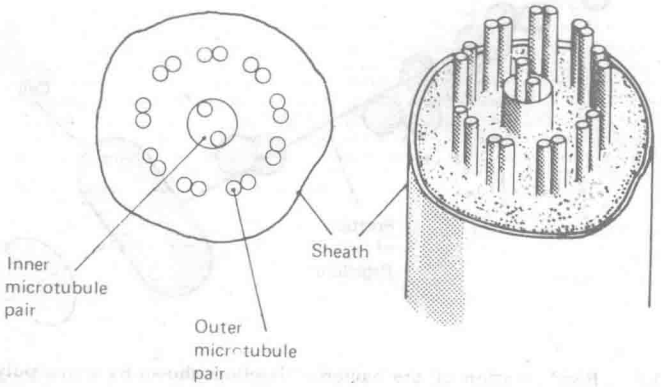


Figure 1.3 Crosssection and diagrammatic representations of a eukaryotic flagellum

difference between flagella and cilia lies in their length, up to 200 μm and up to 10 μm respectively, and in the much greater number of cilia found on the cell.

Mechanism of Motility

How do flagella move cells? In electron micrographs they appear as sinusoidal structures, but this is really a two-dimensional visualisation of a three-dimensional shape. There are several ways such helices can move cells, and it appears that eukaryotes and bacteria differ in this respect. Eukaryotic flagella contain complicated bending machinery, and an input of energy at one end, causing a slight perturbation of flagellin subunits at the base, leads to a helical wave travelling the length of the flagellum.

Bacterial flagella, on the other hand, appear to rotate rigidly. When the distal end of a straight filament is fixed to a glass slide with an anti-filament antibody, the cell rotates at several revolutions per second. Similarly, after fixing polystyrene beads to mutant cells with a straight filament the beads rotate about the axis of the filament in one direction while the cells rotate in the other (Fig. 1.4).

At the moment it is not clear how energy is supplied to either system, although in eukaryotes ATP may be involved since detached eukaryotic flagella beat if ATP is added. This ATP is hydrolysed by an ATPase thereby affording a source of energy. Bacterial flagella do not possess ATPase activity, and although ATP does cause an interconversion between two arrangements of flagellin subunits isolated from *S. typhimurium*, it is not hydrolysed. The bacterial 'motor' may well reside in the cell membrane with the basal body acting as an energy transducer. Located in the bacterial membrane is the respiratory chain generating energy for transport across membranes and oxidative phosphorylation of ADP to ATP (pp. 64, 83) and

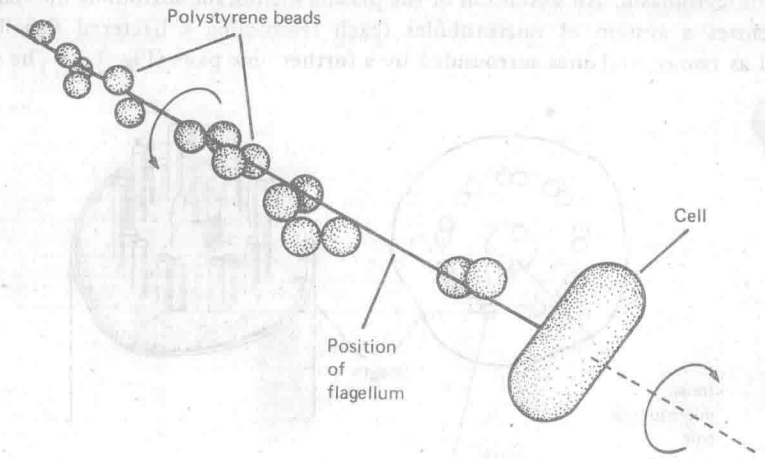


Figure 1.4 Rigid rotation of the bacterial flagellum shown by fixing polystyrene beads to the straight flagellum of an *Escherichia coli* mutant. (By courtesy of Dr. H. C. Berg and *Nature*)

is also generates the energy for flagellar rotation. This coupling of energy released in the respiratory chain does not require ATP as an intermediate; rather, as we will see later, solute and ion transport, ATP biosynthesis and flagellar rotation are alternatives for linking into the respiratory chain.

Most bacteria move steadily in almost a straight line, then alter course abruptly. In some organisms, including *E. coli* and photosynthetic bacteria, the choice of a new direction seems random, but can be influenced by chemicals (or for photosynthesisers, light) acting as attractants or repellants.

Another form of motility, gliding movement, is found in blue-green algae and related bacteria, and in myxobacteria. The cells move slowly over solid surfaces although no recognisable organs of locomotion can be seen. The only common property found in such cells which might be involved in this movement is the secretion of mucilage over the exterior of the cells and onto the surface of the medium. In eukaryotes *amoeboid* movement is found in slime moulds and some protozoans. This is the result of *cytoplasmic streaming* in organisms without a rigid cell wall, and occurs on solid surfaces.

Pili and Fimbriae

At the surface of many Gram-negative bacteria may be found numerous filamentous appendages called *pili* or *fimbriae* which are up to 3 μm long and have a diameter of 5 to 10 nm (Fig. 1.5). A second type of pilus is found in several bacteria which undergo conjugation; this 'sex pilus' is much larger than common 'type I' pili or fimbriae. It is 25 to 30 nm thick and intermediate in length between common fimbriae and flagella. Only one or two sex pili are present on each cell.

Pili or fimbriae resemble flagella in being composed of a protein which can be disaggregated and show spontaneous self-assembly, but they are readily distinguishable from flagella by their smaller diameter and absence of wave structure. All types of pili are hollow; during bacterial conjugation genetic material passes through a sex pilus from donor to recipient cell. The sex pili are also the site of adsorption of a group of highly specialised bacteriophages, some of which adsorb at the tip and others along the length of the pilus. Pili or fimbriae may play a part in adhesion of bacterial cells either to other cells or particulate material, which is obviously important for the utilisation of solid substrates and in the much more specialised process of bacterial conjugation and transfer of genetic material. They also provide a means of attachment in aqueous environments.

Capsules and Slime

Many microorganisms have a discrete external layer of mucilaginous material called a *capsule* which completely surrounds the cells and occludes the cell walls. The size of the capsules and the amount of capsular material produced is markedly dependent on the cultural conditions and is often favoured by a high degree of aeration and a high carbon to nitrogen ratio in the growth medium. In a few

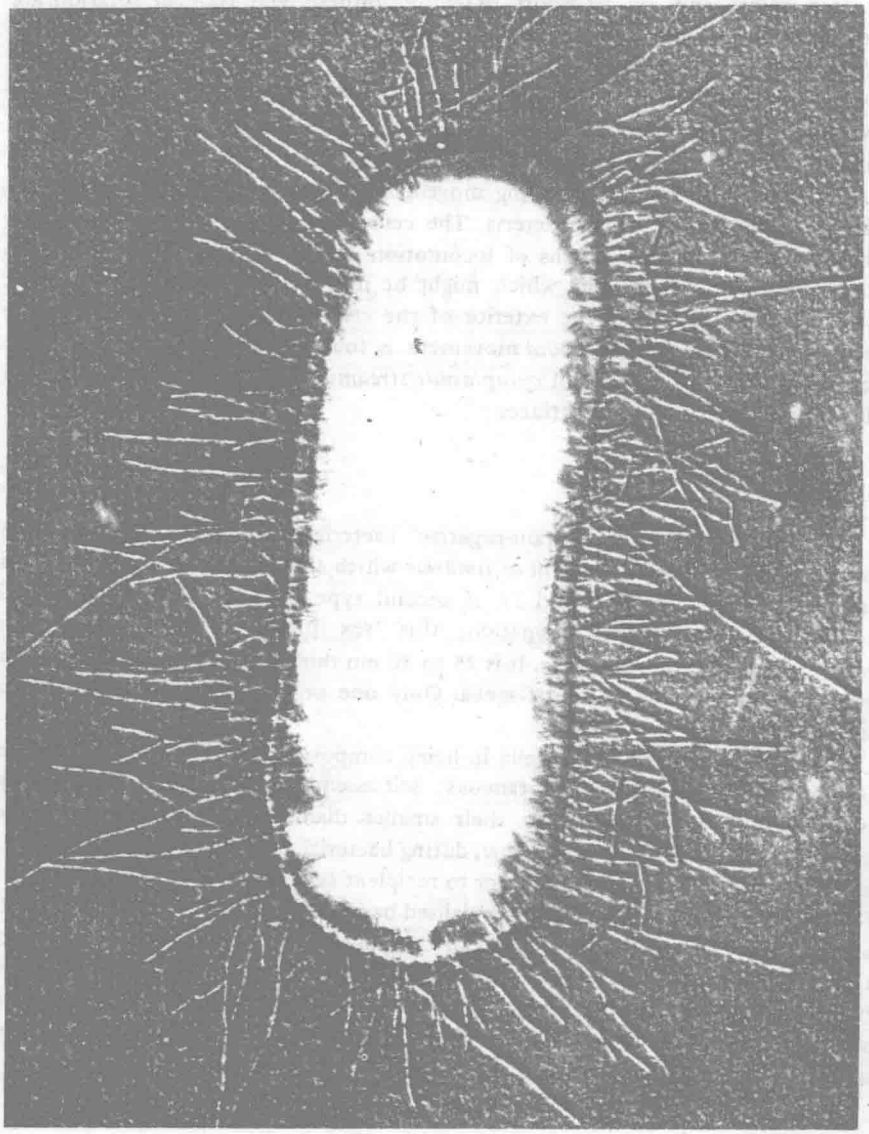


Figure 1.5 Pili at the surface of *Escherichia coli*. Shadowed electron micrograph. (By courtesy of Professor J. P. Duguid.)

Table 1.2 Chemical composition of microbial capsules and slime

Organism	*Nature of polymer*	*Capsule/slime*	*Monomers*
Bacillus anthracis	Polypeptide	Capsule	D-glutamic acid
Leuconostoc mesenterioides	Homopolysaccharide (dextran)	Slime	D-glucose
Streptococcus pneumoniae type 3	Heteropolysaccharide	Capsule or slime	D-glucose, D-glucuronic acid
Klebsiella aerogenes type 54	Heteropolysaccharide	Capsule or slime	D-glucose, L-fucose, D-glucuronic acid
Escherichia coli strain K12	Heteropolysaccharide	Slime	D-glucose, D-galactose, L-fucose, D-glucuronic acid, acetate, pyruvate
Cryptococcus neoformans	Heteropolysaccharide	Capsule	D-xylose, D-mannose, D-galactose, D-glucuronic acid

species, there is no capsule and only cell-free slime is formed; capsules and slime in most microbial species are polysaccharides, termed *extracellular* polysaccharides to distinguish them from others found within the cell walls.

Microbial extracellular polysaccharides may be homopolysaccharides formed from a single sugar, or heteropolysaccharides composed of two or more sugars. A large number of different monosaccharides have been identified in capsular and slime polysaccharides including: neutral sugars – D-glucose, D-galactose and D-mannose (all hexoses); L-fucose and L-rhamnose (methylpentoses); amino sugars – *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine; and uronic acids – D-glucuronic acid and D-galacturonic acid (Table 1.2). Pentoses such as D-ribose and D-xylose are seldom found in bacterial capsules and slime but are frequently components of the extracellular polysaccharides of yeasts and algae. In addition, numerous extracellular polysaccharides contain combined phosphate, acetate, formate or pyruvate.

There may be many possible functions of microbial capsules but non-capsulate variants grow as well if not better under laboratory conditions as capsulate wild-type strains. In its natural environment a capsule may, for instance, protect an organism against desiccation, phage infection or phagocytosis. For pathogenic bacteria, capsule formation is associated with virulence, affording protection against attack by both antibodies and by macrophages.

Cell Walls

The shape of most microbial cells is due to the presence of a rigid cell wall, which has the major function of protecting the fragile protoplast (cell membrane and its contents) from osmotic lysis. Microorganisms usually live in an environment which is hypotonic to the cell cytoplasm, and unless the protoplast is supported by the

cell wall it expands and lyses. The rigidity of the cell wall is mainly con ..ed by a single polysaccharide or related component; other polysaccharides are present and these are generally characteristic to particular groups of organisms or even strains of one species. The polymeric wall components are organised into complicated multi-layer structures; some bacterial examples are shown in Fig. 1.6.

The wall has other functions, the highly charged polymers may provide an ion-exchange mechanism assisting in the uptake of ions and nutrients. It is also effectively a molecular sieve providing a barrier to entry of some molecules, and retaining proteins found in the *periplasm*, the region between the wall and membrane in Gram-negative bacterial cells.

Prokaryotic Cell Wall

Apart from *Mycoplasma* species, which lack cell walls, and *Halobacteria*, which have an unusual wall structure, bacteria and blue-green algae can be grouped into one of two groups, Gram-positive or Gram-negative, according to their wall structure and composition. This difference was initially based on the ability of the organisms to retain crystal violet during Gram's staining procedure, but with electron microscopic and chemical analysis of wall structures it became clear that the staining reflected an intrinsic difference in the chemistry and arrangement of the wall layers between the two groups.

Cytological differences between Gram-positive bacteria and Gram-negative bacteria are indicated in Fig. 1.6. Gram-positive walls are thicker and appear relatively amorphous, whereas Gram-negative 'envelopes' are much more complicated and show a multi-layered structure. This is seen also in the chemical composition of the walls (Table 1.3) and in the localisation of polymers (Fig. 1.6). Several of the polymers found in bacterial cell walls are unique to prokaryotes. These are discussed below.

Peptidoglycan Peptidoglycan is found in almost all bacteria (except *Mycoplasma* and *Halobacteria*) and is not only unique in itself to the prokaryotic cell, but contains up to three components not found in eukaryotic polymers: D-amino acids, muramic acid and diaminopimelic acid. Peptidoglycan is the polymer conferring rigidity on the bacterial cell, determining shape and resistance to osmotic lysis. When peptidoglycan is removed by lysozyme in hypotonic medium the cells lyse. In hypertonic media, rounded spheroplasts or protoplasts are formed depending on whether part or all of the wall is removed. Peptidoglycan is essentially a linear polymer of alternating residues of *N*-acetyl-D-glucosamine and *N*-acetyl-D-muramic acid with separate chains crosslinked to varying degrees by short peptide bridges. The polysaccharide backbone of the molecule shown in Fig. 1.7 is ubiquitous to prokaryotes containing the polymer. Attached to the lactyl moiety of the muramic acid residues are peptide side chains containing four amino acids in a characteristic sequence, L-alanyl-D-glutamyl-X-D-alanine; X may be neutral (e.g. homoserine) but