Aspects of Microbiology 6

Bacterial Cell Structure

Rogers

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Foreword

The study of bacteria has been a major factor in our understanding of cell metabolism. They are conveniently grown in the laboratory and their rapid rate of growth and multiplication eases the work of the researcher. Many of the chemical structures and reactions that occur in bacterial biochemistry are common to other organisms, or at least have their close counterparts therein, and so it has been possible to use bacteria as the vehicle of a large part of biochemical research. Similarly bacterial genetics, although different from that of eukaryotes in important respects, has much in common with that of other forms of life and consequently bacteria have featured prominently in genetics research. Their rapid multiplication rate is of course a most important feature of such work.

For these reasons the biochemist has used mainly non-pathogenic bacteria for the development of his subject, and their importance in all of this should not be underestimated. In microbiology bacteria have been central to the subject since their first discovery. Moreover, their importance has grown considerably since the discovery of antibiotics. The effectiveness of antibiotics in clinical medicine has done nothing to diminish the need to study bacteria on the grounds that they no longer present a serious challenge to the clinician. On the contrary, the development of resistance towards antibiotics and other chemotherapeutic reagents and the need to understand the details of their mechanism of action have greatly stimulated work on bacterial genetics and biochemistry. More recently the interest in biotechnology and the enormous potential of genetic manipulation have stimulated further the study of bacteria.

Advances in all of these topics require a detailed knowledge of the molecular structure and often the biosynthesis of the components of the cell. Dr Rogers has described in this small book some of the main features of the chemistry and biochemistry of bacterial capsules, walls, membranes, genetic material, flagella and other appendages. Restrictions of space prevent a comprehensive treatment and the author has had to be selective. Further reference is given mainly to reviews so that the reader can discover more about the considerable knowledge that has been gained in this fascinating subject. It is obvious that the biochemistry of bacteria still offers much to the research worker. We know relatively little about the mechanisms of control of biosynthesis and degradation, the translocation of wall and capsular material, the multifunctional nature of such structures and their role in surface interaction and pathogenicity. Much more remains to be discovered about cell growth and division and the possibilities for the alteration of bacterial metabolism through recombinant DNA techniques are still in their early stages of development. It is to be hoped that this book will assist in the stimulation of further research as well as in informing readers of the considerable background that already exists.

James Baddiley

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1 Introduction

Ten thousand billion dried bacteria weigh about a gram and a million laid end to end measure only about two metres. Yet they grow, divide, respire, oxidize a truly vast variety of substrates, export toxins, antigens and polysaccharides, and many differentiate. This short book is about the ultrastructure and chemical designs of these small creatures. Considering their size the extent of our knowledge is no small tribute to the ingenuity of microbiologists and to the physicists and engineers who designed and constructed high resolution electron microscopes in the 1930s and 40s. Much of course had already been learnt during the 300 years of developing light microscopy about the shapes and arrangements of micro-organisms long before the advent of the electron microscope. Special staining methods such as that designed by Gram and the acid fast method designed by Ehrlich and Ziehl, both in the nineteenth century, allowed the differentiation of broad classes of micro-organisms. Some cell appendages such as flagella could also be stained but when attempts were made to examine internal structures, the resolution of the optical microscope was strained beyond its proper limits. This led to extensive controversy in many areas, for example, over the presence and behaviour of the nuclear material in bacteria, or over the nature of the walls and membranes.

Is it all worthwhile? The era in which workers tended to look at bacteria as very small bags of enzymes has long past. As soon as more certainty was required about arrangement of enzymes and other macromolecules in the cell morphological understanding became of vital importance. Knowledge of the structure and ultrastructure of larger creatures has made many critical contributions to our understanding of functions of whole organisms. One only has to think of the advances made possible by confirmation of the early 'Cell Theory' to be convinced of the truth of this. If this has been true for the tissues of larger forms of life and of their constituent cells, we would expect it to be equally if not more important for the minute bacteria. When cells from, say, the liver of a mammal are sectioned and examined with an electron microscope their complexity is immediately apparent. We can see mitochondria, themselves the size of bacteria with their own internal structure; systems of internal membranes, some of which bear protein-biosynthesizing ribosomes, and a nucleus wrapped in a complex separate membrane containing the genomic material which separates into chromosomes under the guidance of a spindle apparatus during cell division. Apart from these structures we have the outer bounding membrane, connected with the systems of internal membranes, as well as the membrane bound vesicles, the lysosomes. Bacteria like liver cells, generate energy by oxidizing molecules, pump metabolites from the exterior into the cytoplasm, and biosynthesize proteins as well as a range of other macromolecules. However, they have far fewer internally recognizable structures (Fig. 1.1). Moreover a bacterium, like all cells, must replicate its DNA and separate exactly equal genomes to the two new cells formed each time it divides. All this has to be achieved by cells of about the same size as only one mitochondrion in the liver cells. No well authenticated sub-cellular structures apart from ribosomes have been found in most bacterial species, although some such as the photosynthesizers are exceptions in having

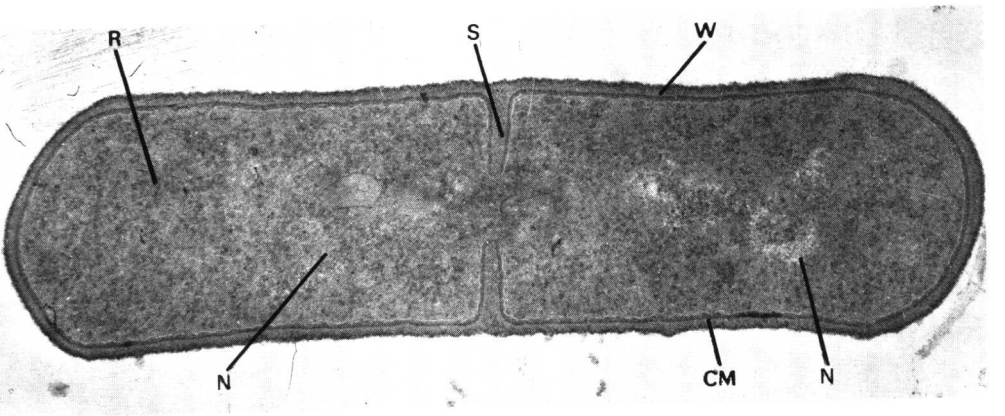


Fig. 1.1 A longitudinal section of *B. subtilis* showing the paucity of recognizable structures within the bacterial cell. W = wall, CM = cytoplasmic membrane, S = septum beginning to divide the cell, N = nuclear material, R = massed ribosomes in the cytoplasm. (Photograph kindly supplied by Dr Ian Burdett)

membranous residues of various shapes; the blue-green algae or cyanobacteria, although true prokaryotes, have an exceptional complexity that differs from both cells of higher organisms and from other bacteria. In some ways they form an intermediate group as far as ultrastructure is concerned. Some others, especially those with well developed specialized functions, such as nitrogen fixation or hydrocarbon utilization, also have relatively simple but plenteous internal membranes. In a number of examples these specialized membranes appear to have been derived by differentiation of the bounding cytoplasmic membrane. Many species also have very simple granules of various sorts packaged in highly specialized membrane-like material.

Unlike the liver cells no neatly packaged nucleus exists in bacteria. Instead of having a number of chromosomes wrapped in the specialized nuclear membrane, the DNA of bacteria exists as a much twisted single circle 'free' in the cytoplasm, often supplemented by smaller circles, the so-called plasmids. Although no specialized membrane surrounds bacterial DNA it is nevertheless probably attached to the cytoplasmic membrane which may form part of the mechanism allowing segregation of the two units of the replicated genome into daughter cells during division.

Space saving has to be effected and multipurpose structures must be used, which is perhaps best illustrated by the many functions undertaken by the simple-looking cytoplasmic membrane. This single structure in bacteria assumes the functions of the mitochondrion as energy generator, the boundary membrane in pumping metabolites in and catabolites out of the cells, the smooth endothelial reticulum and Golgi apparatus in synthesizing polysaccharides for export. It may also possibly serve as the nuclear membrane and spindle apparatus for genome separation. All in all the cytoplasmic membrane is a truly multipurpose organ.

Outside the cytoplasmic membrane is the wall. Terrestrial green plants have evolved thick, strong cell walls that enable them to cope with the force of gravity and to grow away from the earth, whereas bacteria have evolved strong walls presumably to protect the all important cytoplasmic membrane. They have to be able to

protect this membrane, not only against noxious physical or chemical agents in the environment but also against a strong internal cell pressure. One of the properties of the cytoplasmic membrane itself is, as has been mentioned, that of pumping a wide variety of low molecular weight metabolites from the external environment, where they may exist at very low concentrations, into the cytoplasm to give high internal concentrations. As a result, an osmotic pressure as large as 20 atmospheres presses on the membrane when some bacteria are surrounded by the usual growth media. This internal pressure must be resisted but at the same time the wall must be able to grow and allow the bacteria to divide. The necessary strength of the wall is nearly always provided by a special class of polymers, the peptidoglycans. Two general plans of wall architecture, both involving the peptidoglycans, have been evolved to provide different sorts of protection in Gram positive and Gram negative bacteria. We now know a good deal about the biosynthetic pathways that give rise to peptidoglycans but have little evidence as to how the walls expand and form septa to divide the bacteria.

Irrespective of whether the outermost layer of the cell is an extension of the bounding membrane as in mammalian cells, or the wall as in bacteria, or capsules which sometimes extend outwards from bacterial cell walls, it is this that makes first contact with the environment, and if very large molecules or even insoluble materials are involved they may be the only points at which interaction can occur. If, for example, bacteria are to adhere to solids, (which they often do in their natural habitats), react with immunogenic systems in host bodies or be able to interact with DNA from another bacterium, they must have surfaces prepared for the particular task in hand. The walls of bacteria are made not only of the necessary strengthening polymers but they also contain a variety of polysaccharides, lipopolysaccharides, proteins and polyol-phosphate compounds distributed to the outermost surfaces. A wide range of polysaccharides, proteins and polypeptides may be excreted to form the semi-organized capsules which extend well out into the environment. It is these components that may decide the nature of the interactions between organisms and their environment. Projecting from some species are a variety of filaments which in one way or another also appear to be involved in cell-cell or cell-environment interactions. Flagella are the propellers for movement and may be singularly attached at one end of the cells, or multiply attached all round them. They can spread bacteria through their environments as well as propel them towards desirable foodstuffs and away from noxious agents. Fimbrae of various sorts, on the other hand, are very thin projecting filaments, some of which are involved in sticking the bacteria to surfaces; somewhat larger filaments in some species form sex pilae that are important both in the transfer of chromosomal DNA from one to another as part of the sexual interactions between the bacteria and in the transfer of plasmids. Plasmids, among other activities, specify resistance to antibiotics. Fig. 1.2 shows a diagrammatic representation of a bacterial cell which summarizes some of the above information.

So far we have spoken of bacterial cells in a general sense. As far as possible this book will accordingly deal with bacterial structures in a general way. The pictures the book contains are of simple shaped rods and cocci. It would be misleading to leave the impression that all bacteria have such simple shapes. There are, for example, those with a spiral shape, the spirochaetes, those with quite complex life cycles each stage having a different shape like the rhodomicrobia or the caulobacters, and the cyanobacteria, which have complex internal structures more similar to eukaryote micro-organisms than to most other bacterial genera. It would require a separate

Bacterial Cell Structure

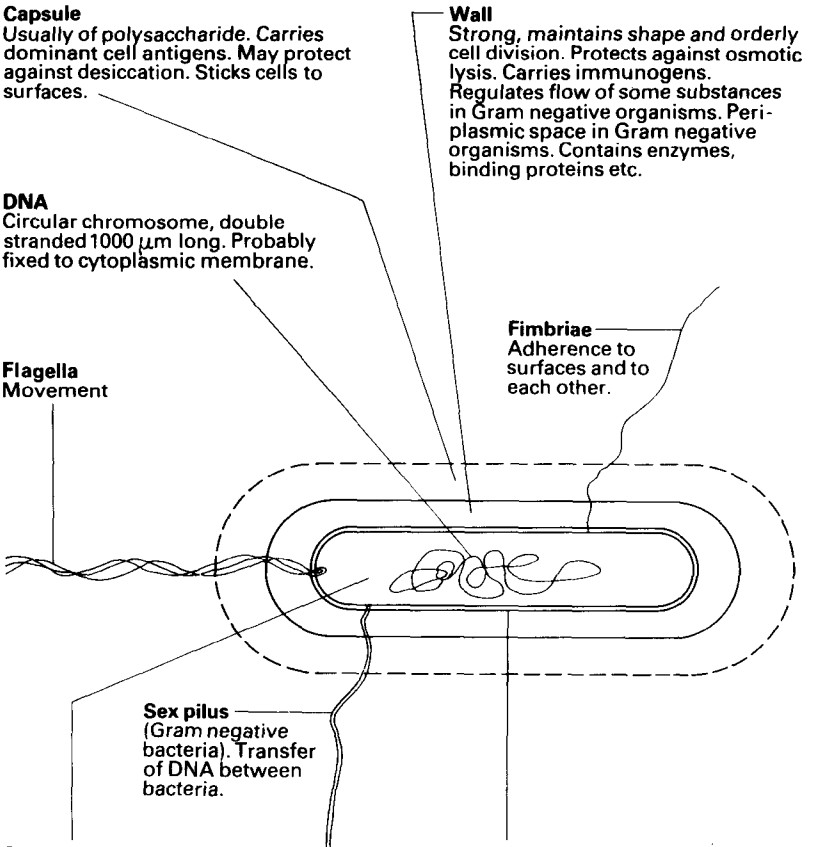


Fig. 1.2 General structure of the bacterial cell.

book however to deal with these more exotic organisms in detail. Nevertheless, as far as is known the basal components of all bacteria are built in the same general way irrespective of their shapes.

Scientific knowledge does not grow evenly within any subject, partly because of the different degrees of difficulty involved in understanding problems and partly because of the different degrees of effort that they have attracted. This frequently leads to paradoxes that may be difficult to understand for those not so familiar with the frequently hard and chancy business of extracting new information from nature. The studies described in this book are by no means free from such paradoxes. We have, for example, extensive and detailed knowledge about the structures of the polymers making up bacterial cell walls but no certain knowledge of how they are arranged. The circular nature of DNA is now well known, as is the arrangement of the genes on it. Yet we know little or nothing about how it is packaged in the cell or how the newly replicated genome is separated from the old. Our understanding of the arrangement of ribosomes within the bacterium is ill defined. When separated from the cell many ribosomes seem to be attached to messenger RNA, which in turn is attached to the DNA. Nothing of this can be seen in the ribosome-packed cytoplasm of the cell. Finally, although we have detailed knowledge about the chemistry of the large number of polysaccharides formed by bacteria as capsules and slimes we have virtually no understanding of the structure of capsules, or of the relationship between capsules and walls. We do not understand the mechanism of decision between the formation of capsules or the liberation of polysaccharide slimes.

Frustrating though these paradoxes may seem, they nevertheless are the very stuff of the excitement and fun of biological research. To watch and contribute to the piecemeal growth of answers to these problems is the joy of those involved in research.

2 The cell walls of bacteria

The appearance of bacterial walls

The specific shapes adopted by bacteria have been recognized from the earliest times of examination by the light microscope. Moreover since rod-shaped bacteria seemed inflexible, that is, when they bumped into objects they did not deform or bend, it was deduced that some sort of stiff layer surrounded them. When placed in strong solutions, the cytoplasm of some bacteria retracted giving further clear indications of a surrounding wall. Attempts, however, to design specific stains for such walls were not very successful, partly because the resolution of the light microscope was taxed too far. This situation was resolved by the advent of electron microscopes and of methods for fixing, staining and cutting sections of bacteria. It then soon became apparent that two basic types of organization of the outer bacterial layers existed and that these corresponded with the Gram staining reaction. This staining method, it will be remembered, consists of first treating a dried and fixed film of bacteria with gentian violet followed by a KI-I_2 solution to form a dark purple complex in the organisms. Subsequent treatment with polar solvents such as alcohol or acetone removes the complex from some species—the Gram negative ones—but not others—the Gram positive ones. Common Gram negative organisms are *Escherichia coli*, salmonellae and gonococci. Common Gram positive species are bacilli, streptococci and staphylococci. Certain exceptions to the general organization of walls exist, and will be dealt with later, but it widely applies and the two types will be described separately. It seems probable that it is indeed the properties of the walls in the two types of organism that lead to the different staining reactions. When walls are removed from bacteria the remaining protoplasts of all organisms stain Gram negative.

Walls of Gram positive bacteria Under the electron microscope, sections of organisms such as bacilli or streptococci from growing cultures, after fixing and staining with osmium tetroxide as described by Ryter and Kellenberg, show a wide, rather transparent layer about 30 nm thick surrounding them. The only infrastructures seen in this layer are two relatively dark bands, one on the innermost side and the other on the outer side (see Fig. 2.1). This difference can still be seen even when no heavy metal stain or fixative has been used to treat the bacteria. The walls dip in to form septa which divide new daughter cells, and outside they are roughened to different degrees. There has been much discussion about the significance of their banded appearance and it was at one time thought to correspond to a true chemical difference in the distribution of polymers. The walls usually consist of about 50 to 60% peptidoglycan, the remainder being made of teichoic and teichuronic acids and polysaccharides. The middle, more electron-transparent layer was thought to be enriched in peptidoglycan. The consensus of modern opinion, however, would be that the layered appearance corresponds to either or both a differing density in an otherwise homogeneous layer or a different penetrability of the stains into the porous electrically charged material.

Although a thickness of 30 nm has been quoted for walls of Gram positive organ-

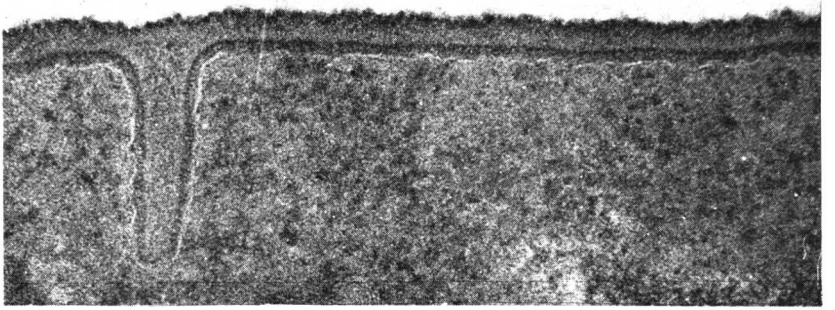
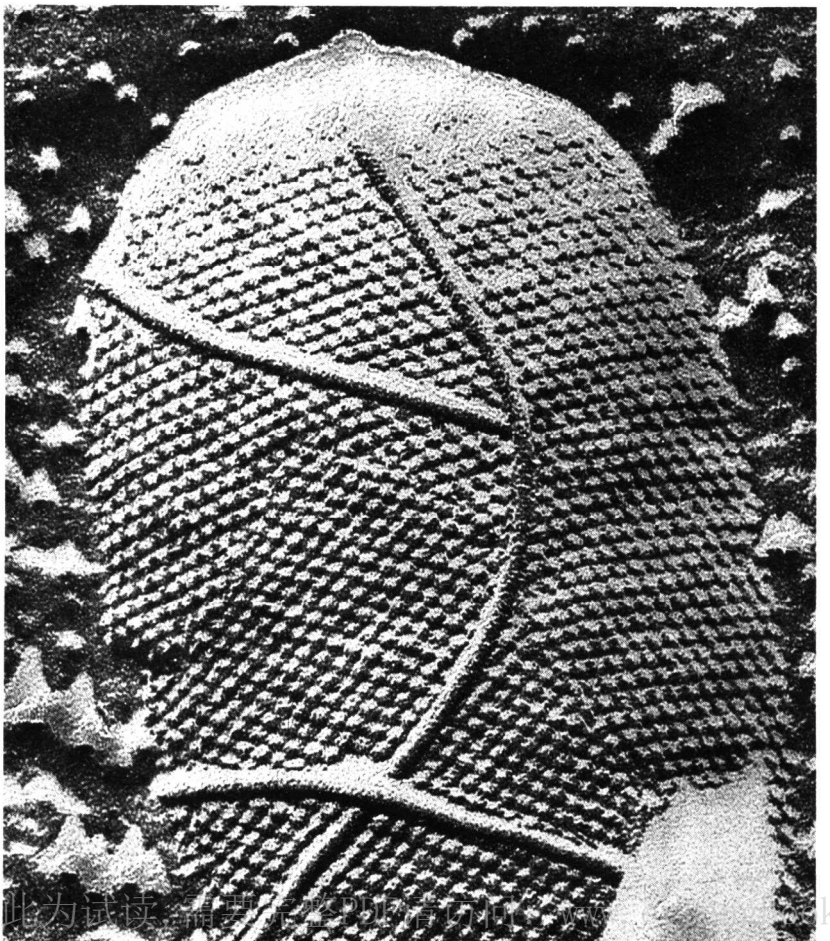


Fig. 2.1 A highly magnified part of a longitudinal section of *B. subtilis* showing the wall in detail with its more dense inner and outer layers. (Photo: Dr Ian Burdett)

Fig. 2.2 The patterned surface layer revealed by the freeze-etching technique on a strain of *Clostridium thermohydrosulfuricum*. (Photograph kindly provided by Dr Sleytr; from Sleytr, 1978)



isms, this can be greatly varied by altering growth conditions. If, for example bacteria are incubated under conditions that allow wall formation but not protein synthesis, uniform thickening over the whole surface occurs. The rate of formation of wall polymers is little affected either by antibiotics which inhibit protein synthesis, such as chloramphenicol or tetracycline, or by omission from media of a required amino acid present in proteins but not in walls. When staphylococci are incubated under these conditions, walls many times thicker than the diameter of the cytoplasm can be formed. The chemical composition of such walls appears to be identical to those around rapidly growing bacteria. Among other consequences of this relaxed control is that in non-steady state cultures, (e.g. older cultures where the bacteria are not growing exponentially), wall thickness may vary greatly.

The walls of bacteria are frequently regarded as the outermost layers of these organisms excluding capsules. The negative staining technique has shown however that this is not always true. In this procedure suspensions of organisms are dried on to electron microscopic grids along with reagents such as ammonium molybdate or sodium silico-tungstate. When specimens are thus treated highly organized patterned layers applied to outer surfaces of both Gram positive and Gram negative species can be seen. The freeze-fracture technique demonstrates these layers even more clearly (see Fig. 2.2). In the latter technique, introduced in the 1950s and perfected in 1961, the sample is frozen as rapidly as possible to a temperature of $-150^{\circ}C$; this produces only very small ice crystals. It is then transferred to a stage similarly cooled and contained in an apparatus designed so that the sample can be fractured with a sharp glass or diamond knife. More of the structure can be made accessible by subliming some of the ice from the specimen, the freeze-etching process. A thin layer of evaporated carbon or a heavy metal such as platinum is then deposited over the frozen specimen. The resulting replica can be floated off for examination under the electron microscope. The patterned layers seen by either of the above techniques are of protein and are not attached by covalent bonds to the rest of the wall since they can be removed by reagents such as solutions of urea or guanidine hydrobromide that do no more than weaken hydrogen bonding. Table 2.1 shows

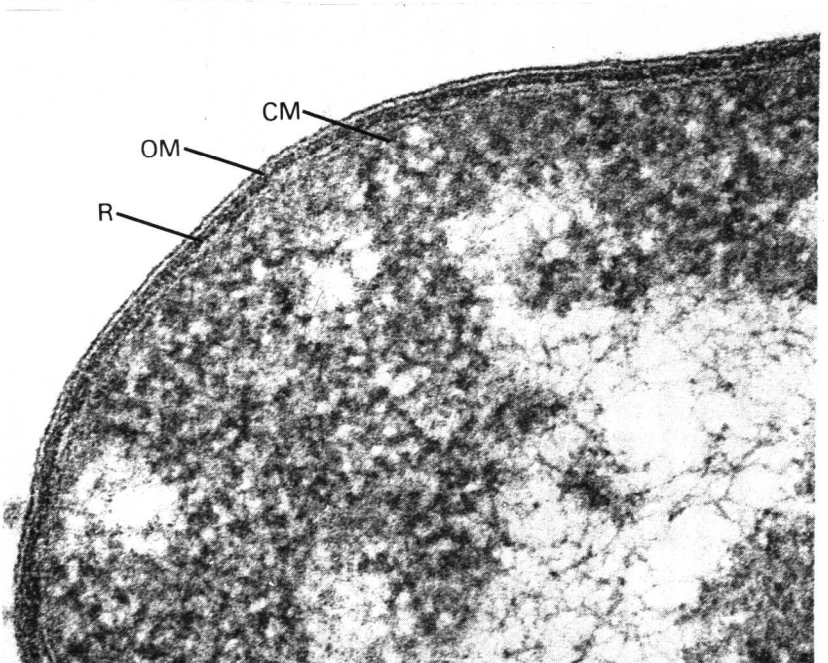
Table 2.1 Molecular weights of proteins constituting the patterned layers on the surfaces of bacteria

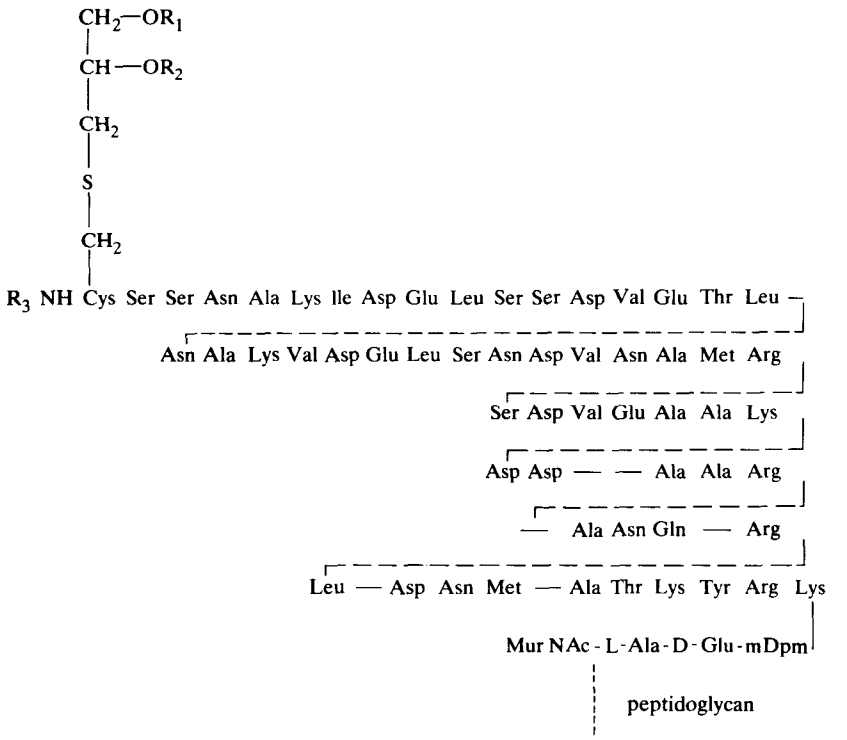
Micro-organism	Molecular weight (daltons $\times 10^{-3}$)
Acinetobacter strain MJT/F5/199A	67
Clostridium thermohydrosulfurium	140
Clostridium thermosaccharolyticum	140
Bacillus sphaericus (wild type) strain P1	140
Bacillus sphaericus (phage resistant)	
strain MBR 9	105
strains MBR 10–12	86–93
strains MBR, 3, 4, 14, 22, 38, 68	86

the molecular weights of some of the proteins that have been examined. Sheets of reaggregated material can be formed from such protein solutions, and if organisms that have already been stripped of their coats are treated with them together with an appropriate divalent cation such as Mg^{2+} or Ca^{2+}, the original patterned superficial layer can be reformed. The conditions for reattachment and the formation of sheets of the material are rather precise and frequently different. The function of these coats is not clear, although they have some relationship to bacteriophage attachment in *B. sphaericus*. Strains of the organism with different resistance to bacteriophages have coats made up of proteins with molecular weights that differ from those of the parent. However mutants without patterned coats were not found among the resistant strains.

Walls of Gram negative species Unlike the walls of Gram positive species, those of Gram negative organisms are clearly multilayered, with at least two layers that have been isolated and shown to have fundamentally different compositions and organizations. As shown in Fig. 2.3 a thin dense layer overlies the cytoplasmic membrane. This is the rigid or R-layer, and is strength-giving, consisting of peptidoglycan with lipoprotein attached to it. Peptidoglycan accounts for only 5 to 10% of the total dry weight of the wall, unlike Gram positive organisms where the figure is usually 50 to 60% and can be as high as 80 to 90%. The R-layer can be removed by treatment with lysozyme or with other enzymes specifically hydrolyzing peptidoglycan, in the presence of a chelating agent such as EDTA to increase the permeability of the outer membrane.

Fig. 2.3 A highly magnified part of a longitudinal section of *E. coli* showing the multilayered nature of the cell envelope. CM = cytoplasmic membrane, OM = outer membrane, R = rigid layer. (Photo: Dr Ian Burdett.)





R_1 and R_2 found to consist of 45% palmitic acid, 11% palmitoleic acid, 24% *cis*-vaccenic acid, 12% cyclopropylene hexadecanoic acid and 8% cyclopropylene octadecanoic acid

R_3 is 65% palmitic acid, 11% palmitoleic acid and 11% *cis*-vaccenic acid

Fig. 2.4 The sequence of lipoprotein attached to the peptidoglycan of *Escherichia coli*.

The lipoprotein fraction is covalently attached to the peptidoglycan by the $-NH_2$ of its terminal lysyl residue, whilst at its -COOH end a cystinyl residue is substituted by an esterified glycerol making it strongly hydrophobic (see Fig. 2.4). The lipophilic end of the protein is buried in the outer membrane (Fig. 2.3). It is almost certain that the lipoprotein thus functions as an anchor between the outer membrane and the peptidoglycan layer. Mutants unable to make it show ballooning of the outer membrane away from the rest of the wall.

Lipid A of lipopolysaccharide (see p. 19) is fixed in the outer membrane leaflet and hydrophilic polysaccharide chains extend well out into the surrounding membrane. These chains of the lipopolysaccharides define the immunological specificity of the

O antigens in such organisms as the salmonellae. The outer membrane itself, as we shall see, differs considerably in composition from more usual membranes, not only in its high content of lipid A, but also because of its protein composition which is dominated by the presence of four or five fractions in very much larger quantities than any others. The nature and function of some of these proteins will be discussed in Chapter 3.

If Gram negative bacteria such as *E. coli* are suspended in strong sucrose or salt solutions the protoplast contained by the cytoplasmic membrane shrinks away from the thin peptidoglycan layer. Such a phenomenon is called plasmolysis and can occur in most cells bounded by a wall. It is due to the removal of water from the cytoplasm by osmosis. The osmotic pressure within Gram negative species is, incidentally, much lower than that in Gram positive bacteria although plasmolysis is easier to see in the former. In a considerable number (about 200) of places the layers of the envelope in Gram negative species appear to be anchored together. If plasmolysis is extreme the cytoplasmic membrane is drawn out into long threads attached by one end to the undeformed peptidoglycan layer. These areas of adhesion or junctions may be very important since they correlate topographically with the points on the bacterial surface at which bacteriophages adsorb, lipopolysaccharide is excreted, and at which at least one of the major outer membrane proteins is inserted.

Polymers in bacterial walls

Peptidoglycans The only groups of bacteria that have so far been reported to be without peptidoglycans as supporting polymers in their walls are the archaebacteria, which are so called (see p. 25) to indicate their possible early and separate evolution; among them are the methanogenic bacteria and the halobacteria. The acholeplasma also have no recognizable walls but can perhaps only be regarded as honorary bacteria. Stable L-forms of bacteria are wall-less but are derived directly from normal bacteria, probably by genetic lesions in their wall biosynthetic pathways. Morphologically more complicated organisms such as the cyanobacteria and the actinomycetaceae do have a peptidoglycan layer in their walls.

The peptidoglycans are universally built of *N*-substituted glucosamine and muramic acid (3-0 lactylglucosamine) linked together by $1 \rightarrow 4\beta$ bonds. Very commonly the *N*-substituents are acetyl groups although in mycobacteria, *Nocardia* and *Micromonospora* species glycollyl groups are present and in strains of some species of bacilli high proportions of glucosamine with unsubstituted $-NH_2$ groups have been found. The polysaccharide chains are then linked together by short peptides constituted, in any one peptidoglycan, of a limited number of amino acids (Fig. 2.5). The peptides are linked to the carboxyl groups of the muramyl residues by amide or pseudo-peptide linkages. The amino acids in the peptides alternate between the L and D forms unless there is either a glycyl residue or an amino acid that has two active centres such as 2, 6-diaminopimelic acid. The latter is often found in the *meso* form. Variable proportions of these peptides are linked by their carboxyl termini, always represented by D-alanyl residues, often to amino groups of the L-centre of a diamino acid (e.g. lysine, diaminopimelic acid) in a neighbouring peptide chain which is usually supposed to be substituted on to a separate glycan strand. However, other types of cross-linking are known (see Fig. 2.6). Such peptide cross-linking of the glycan strands is illustrated in Fig. 2.5 and must build up a com-

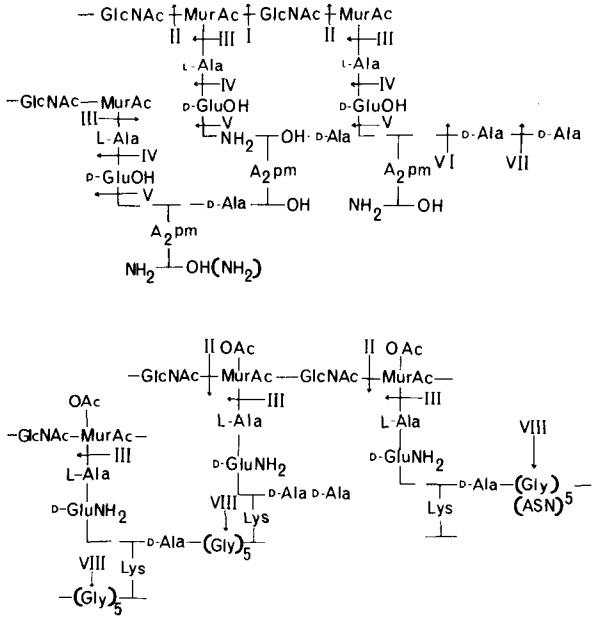


Fig. 2.5 Structure of the peptidoglycans from the walls of (**a**) many bacilli and Gram negative species, (**b**) *Staphylococcus aureus*, in which the crosslinking bridge between peptides consists of five glycyl residues (residues A_4, A_5, A_6 in Figure 2.6), and of *S. faecalis* in which it consists of one asparagine residue. The Roman numerals I, II, III and VIII represent bonds hydrolyzed by lysozyme (I), and naturally occurring autolysins (II, III and VIII) dissolving the walls. VI and VII are the points of attack of D-alanyl carboxypeptidases present in many organisms. IV and V are the points of attack of enzymes found in sporulating cultures.

plicated 'chain armour' around the organisms. Thus the peptidoglycans have a very special structure not easily attacked by enzymes which hydrolyse either common polysaccharides or proteins. Their strength and conformation are influenced by the nature of the cross-linked peptides as well by the $1 \rightarrow 4\beta$ bonds in the glycan. The latter are the same as those in other simple structural polysaccharides such as cellulose (poly $1 \rightarrow 4\beta$ glucose) or chitin (poly $1 \rightarrow 4\beta$-*N*-acetylglucosamine). It should be noted, however, that the substitution of the lactyl group in the three position of the muramyl residues in peptidoglycan prevents the formation of one of the intramolecular hydrogen bonds per disaccharide present in chitin.

Four basic types of peptidoglycan have so far been recognized (Ghuysen, 1968) that are differentiated by the way in which the peptides are linked together. These are illustrated in Fig. 2.6. The range of amino acids found in peptidoglycans from different species of bacteria is, however, large (Table 2.2). A more complicated classification of the different types of peptidoglycan has also been designed (Schleifer and Kandler, 1972). Apart from the variety of amino acids and types of cross-linkage,

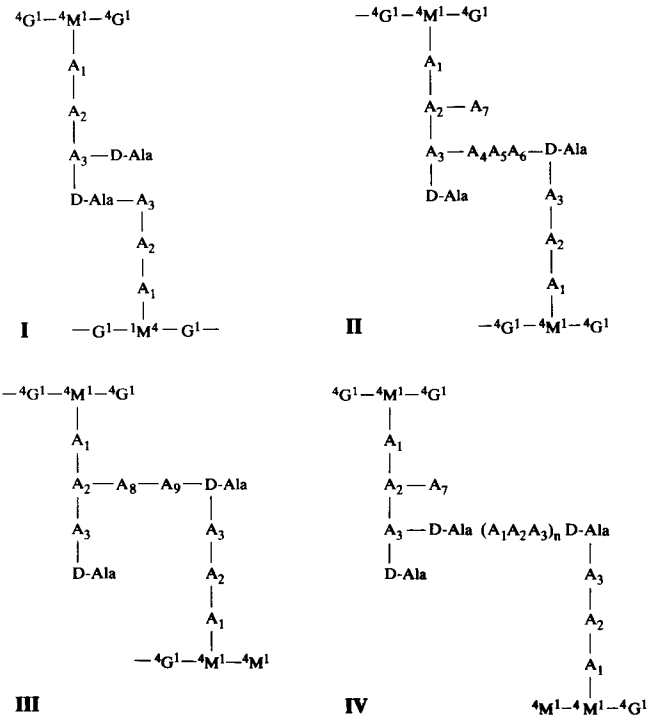


Fig. 2.6 Generalized structures of peptidoglycans G = *N* – acetylglucosamine, M = *N* – acetylmuramic acid, A_1 – A_9 are amino acids. A_1 is commonly L-alanine, A_2 is commonly D-iso-glutamate or D-iso-glutamine, A_3 may be a variety of diamino acids although L-lysine and meso-2, 6 diaminopimelic acid are common. A_4, A_5 and A_6 are commonly glycine, serine or threonine (see Table 2.2).

Table 2.2 The amino acids present in all different known peptidoglycans (from Rogers, 1974)

Position	Amino acids that have been identified
A_1	L-Ala(++), Gly, L-Ser
A_2	D-iso-Glu(++), 3-Hyg
A_3	L-Lys(++), meso-DAP(++), DD-DAP, L-DAB, L-Hse, LL-DAP L-Ala, L-Glu, L-Orn, meso-HyDAP, L-Hyl, Nγ-acetyl-L-DAB
A_4, A_5, A_6	Gly(++), L-Ala(++), L-Thr(++), L-Ser(++), D-Asp(++) D-Ser, D-Glu, D-Glu(NH_2), D-Asp(NH_2), L-Lys
A_7	Gly, Gly(NH_2), D-Ala(NH_2)
A_8, A_9	D-Lys, L-Lys, D-Orn, Gly, D-DAB

The positions of A_1 to A_9 are shown in Figure 2.6. The sign (++) after an amino acid indicates that it has been found in the peptidoglycan of many different micro-organisms. A_4 to A_6 and A_8 and A_9 are bridge amino acids. In any given organism, the bridge consists of one or two repeating residues, except for certain micrococci, in which three amino acids are present. When D-Asp, D-Asp(NH)$_2$ or D-Glu(NH_2) form the bridge they do so as single residues. Most of these data have been summarized from the review of Schleifer and Kandler. Key: 3-Hyg, threo-3-hydroxy-glutamic acid: DAP, 2,6-diaminopimelic acid; DAB, 2, 4-diaminobutyric acid; HyDAP, meso-2, 6-diamino-3-hydroxy-β-pimelic acid.