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The Bacterial Cell

Bacterial Structure

Bacteria are prokaryotic cells. The term 'prokaryote' includes the bacteria, the Archaea and blue-green algae. The distinguishing feature of a prokaryote is that its nucleus is not surrounded by a nuclear membrane, but the nuclear material (DNA) is free in the cytoplasm of the cell. In addition, there is no nucleolus, mitotic spindle or (usually) any separate chromosomes. Bacterial cells are distinctively smaller in size than eukaryotic cells of plants, animals and fungi (Figures 1.1 and 1.2).

Shape

Individual bacteria have characteristic shapes. The cells may be spherical (coccus), rod shaped (bacillus), comma shaped (curved rod), spiral (spirochaete) or filamentous. Bacterial shape differs to some degree with the growth conditions (e.g. whether in the body or artificial medium of one kind or another). In some species, therefore, a bacterium may appear as long rods in lab culture, but as short rods or coccobacilli in the body when causing disease. Nevertheless, the shape of most bacteria can be seen in the light microscope and is an important clue to their identity (Figure 1.3).

Anatomy of the Bacterial Cell

The bacterial cell consists of the protoplast containing numerous organelles, which is bounded by a thin, elastic, semi-permeable cytoplasmic membrane supported by the porous, relatively permeable rigid cell wall which bears a number of other structures (Figure 1.4).

Cytoplasmic Structures: Ribosomes, Nuclear Body

The cytoplasm is a gel containing organic and inorganic solutes, enzymes, ribosomes and the nucleic acids DNA and RNA. The ribosomes of prokaryotic cells are smaller than those of eukaryotic cells (plants, animals, fungi). They are known as 70S rather than the 80S ribosomes found in eukaryotic cells. This reflects a size difference because the Svedberg unit (S) is a unit of sedimentation and 80S ribosomes have a greater sedimentation rate than 70S. Both prokaryotic and eukaryotic ribosomes function to synthesise peptides (proteins), but they are sufficiently different organelles in the two groups for them to respond differently to inhibitors of protein synthesis such as some antibiotics which selectively disrupt the function of the ribosome.

The nuclear material (the DNA) is not a true nucleus. It is sometimes referred to as the nuclear body in bacteria because it is effectively free-floating in the cytoplasm. Bacterial cells are haploid (one copy of each gene) and the DNA is arranged in a single closed circular molecule of about 1000 μm in length. The bacterial chromosome is not bound to protein histones

Fundamentals of Veterinary Microbiology, First Edition. Andrew N. Rycroft.

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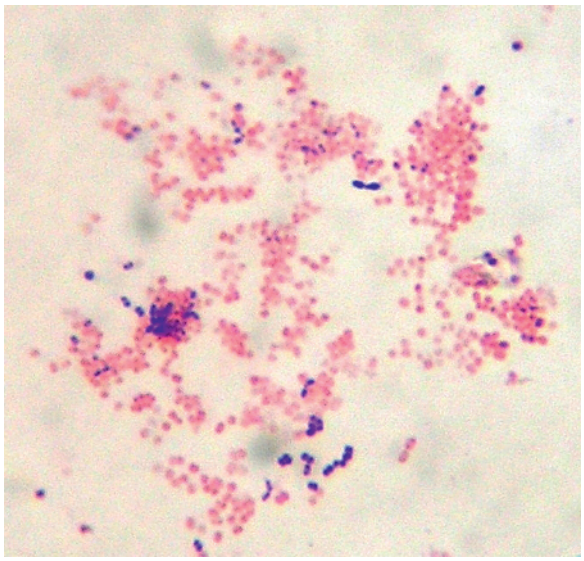

Figure 1.1 Bacterial cells stained by Gram stain and seen by light microscopy. The shape and arrangement are clear, but the resolution of the cells is limited by the light microscope.

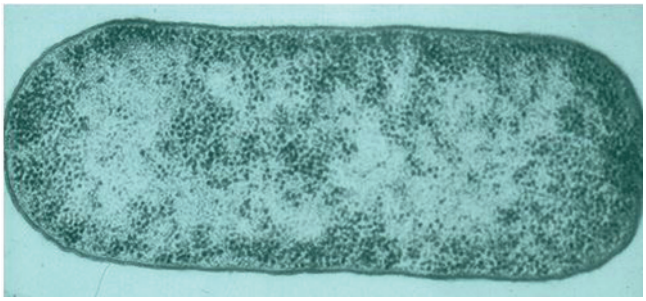

Figure 1.2 Transmission electron microscope picture of a bacterial cell. The small granular organelles scattered throughout the cell are ribosomes; lighter regions are due to nuclear material.

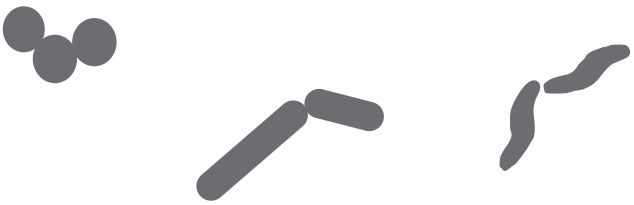

Figure 1.3 Bacteria show different shapes: cocci, rods and curved rods.

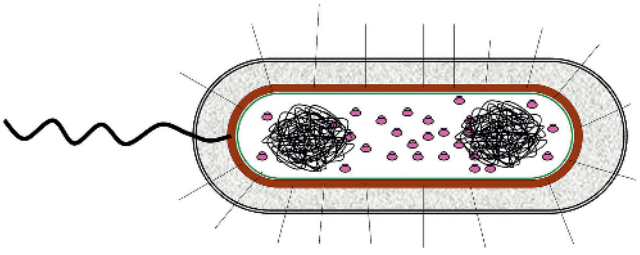

Figure 1.4 Prototypic bacterial cell to show the common subcellular features.

as it is in eukaryotic cells, and it does not stain like a mammalian chromosome. In a section through a bacterial cell in the electron microscope, it appears as complex folds. Two or even four nuclear bodies may be seen in a bacterial cell as DNA replication and segregation occurs before cell division.

Multiplication of bacteria is by simple growth and fission, not by mitosis. It is now recognised that bacteria have a cytoskeleton, which is needed for successful cell division. When a bacterial cell grows to sufficient size, the FtsZ protein forms a ring structure in the middle of the cell, known as the Z-ring. This apparently constricts or contracts to make a pinch point or septum for cell division. FtsZ also acts to organise other cell division proteins at the site of septum formation, so it is likely to have a complex role. Other cytoskeletal proteins are necessary for positioning of the septum, involved in the shape of the bacterial cell and in the successful partitioning of the daughter chromosomes into separate ends of the cell following DNA replication (Egan et al. 2020).

The cytoplasmic membrane (or plasma membrane) limits the cytoplasm. It is a typical fluid-mosaic model lipid bilayer about 9 nm across. It is composed of phospholipid and protein. The phospholipids are primarily phosphatidyl ethanolamine, with smaller proportions of phosphatidyl glycerol and cardiolipin (Figure 1.5).

Sterols are absent in almost all bacteria, but some *Mycoplasma* species, which have no cell wall, require sterols for growth and incorporate these into their membrane where they are essential for membrane stability. The membrane is flexible and is usually supported by a cell wall to maintain its integrity. The cytoplasmic membrane is the site of active transport via specific permease proteins. Its integrity is also essential for the maintenance of the proton gradient which is the driving force of electron transport and hence oxidative phosphorylation. Electron carriers of the respiratory chain, and ATPase, are located on the cytoplasmic membrane.

The Bacterial Cell Wall

The structures external to the cytoplasmic membrane constitute the bacterial envelope. One of these, the bacterial cell wall, provides the characteristic shape of the organism and prevents osmotic lysis of the cytoplasmic membrane. If the wall is ruptured, the cytoplasm expands through the gap and the cytoplasmic membrane bursts, killing the organism. This breakdown process is called lysis and can be caused by a number of agents: the enzyme lysozyme, some antibiotics, enzymes produced by bacteriophages (bacterial viruses) or enzymes produced by bacteria themselves (Figure 1.6).

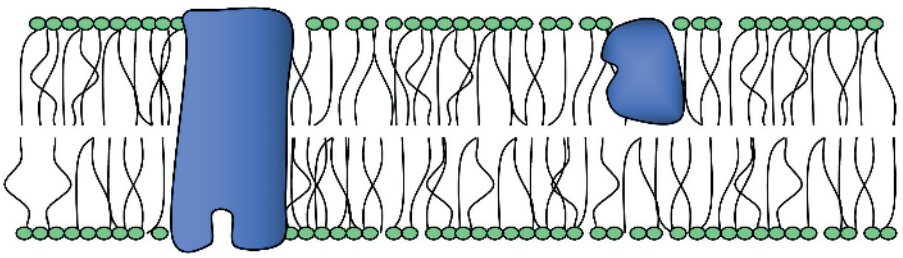

Figure 1.5 The cytoplasmic membrane: phospholipid bilayer embedded with protein molecules.

When the cell wall is weakened or lost due to one of these agents in a situation where osmotic lysis does not occur (hypertonic solution), the shape of the organism may change. Spheroplasts (from Gram-negatives) and protoplasts (from Gram-positives) are formed. If bacteria lose their cell wall *in vivo* (in the body of an animal), they are known as L-forms which may be a means by which some bacteria persist in the body during infection.

The cell wall of bacteria is a crucial structure as it is the site of action of some important groups of antimicrobials and the location of certain important antigens utilised both in identification of bacteria and in the immune response of the body to bacterial infection.

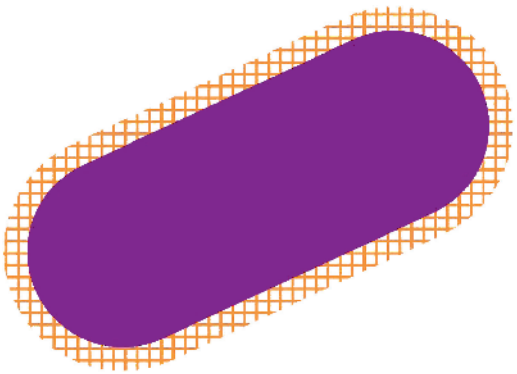

Figure 1.6 The cell wall peptidoglycan surrounds the cytoplasmic membrane as a tough sack-like structure.

Peptidoglycan

Bacterial cell walls are quite different from those of eukaryotic cells, and they contain substances unique to bacteria. Peptidoglycan, formerly known as mucopeptide or murein, is the most important component of the cell wall. It is common to both Gram-positive and Gram-negative cells. It surrounds the cell, external to the cytoplasmic membrane, as a single bag-like molecule. It is composed of linear glycan chains of alternating residues of *N*-acetylglucosamine and *N*-acetyl muramic acid. These are linked together by short peptide bridges to form a cross-linked insoluble polymer (Rohs and Bernhardt 2021).

The peptide bridges vary between different organisms, but they are known to contain biologically exotic substances including meso-diaminopimelic acid, D-alanine and D-glutamic acid. The peptidoglycan is a rigid structure which gives shape and strength to the bacterial cell wall.

Peptidoglycan forms the basic structure of the bacterial cell wall and similar peptidoglycan is found in the unconventional, obligate intracellular bacteria: *Chlamydia* and *Rickettsia* (Figure 1.7).

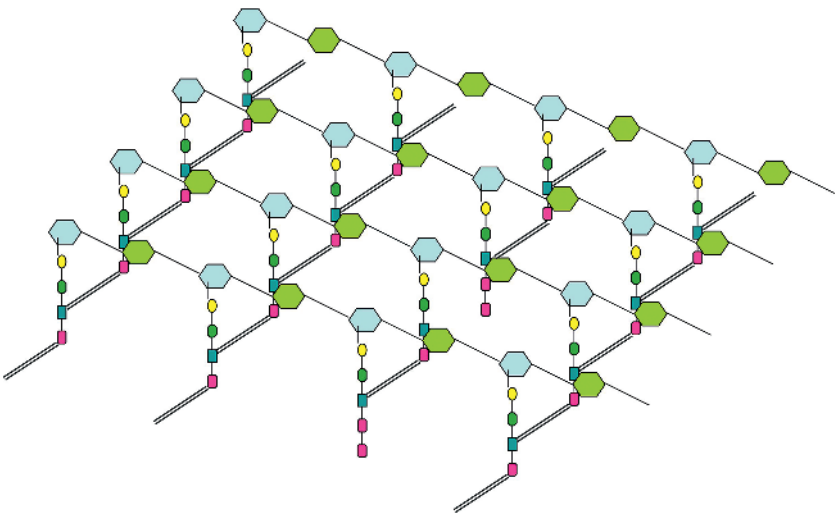

Figure 1.7 Peptidoglycan: long, linear glycan chains cross-linked by short peptide bridges to form the tough cell wall polymer.

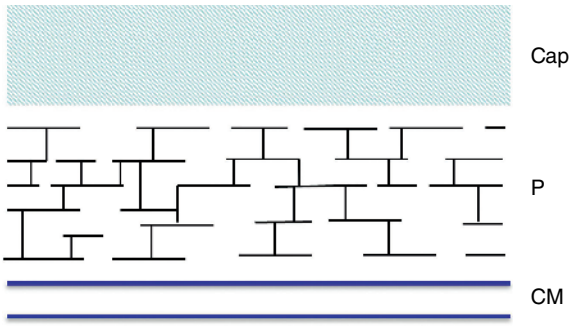

Figure 1.8 Simplified Gram-positive cell envelope structure: thick peptidoglycan layer.

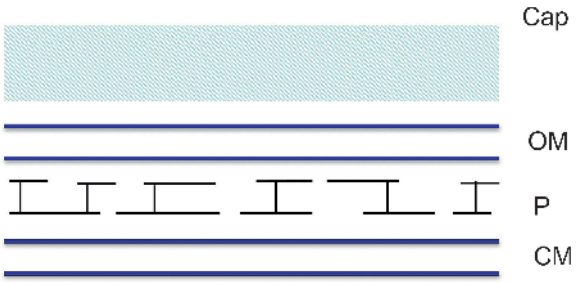

Figure 1.9 Simplified Gram-negative cell envelope structure: thin peptidoglycan and a second membrane – the outer membrane.

In addition, other accessory polymers are also found in most bacteria. Gram-positive organisms, such as staphylococci, contain teichoic acids composed of either poly-glycerol phosphate or poly-ribitol phosphate. These occur both within and on the surface of the cell wall and may account for 20–50% of the dry mass of the cell wall. On the streptococci, teichoic acids are sometimes the Lancefield group 'carbohydrate' antigens used in their classification and identification (Figure 1.8).

In Gram-negative cells, the peptidoglycan is much thinner than in Gram-positive organisms. Outside the peptidoglycan lies a second lipid bilayer membrane, the outer membrane (OM). This is a similar membrane to the inner, cytoplasmic membrane but it contains different proteins and, in addition to the phospholipids of the cytoplasmic membrane, a component unique to Gram-negative bacteria: lipopolysaccharide (LPS). This is located in the outer leaflet of the outer membrane. The outer membrane functions to protect Gram-negative bacteria against a harsh environment. It acts as a barrier and yet it allows molecules through via general porins (outer membrane proteins which act as a diffusion pore for small molecules) and substrate-specific porins (Figure 1.9).

Lipopolysaccharide

Lipopolysaccharide has three regions to the molecule. The lipid region (lipid A) is relatively invariable and contains 3-hydroxy fatty acids linked to a diglucosamine backbone. These fatty acids are hydrophobic and intercalate into the phospholipid bilayer of the OM (Putker et al. 2015) (Figure 1.10).

Linked to the lipid A is a short oligosaccharide which is variable between bacterial types, and which contains very unusual sugar residues. This is known as the LPS core region that protrudes into the environment. In some bacteria, the LPS stops at this point, and they are known as R-form or 'rough' bacteria. Such bacteria will auto-agglutinate in saline (unless other polysaccharides are external to the LPS) and these bacteria are often of low virulence. However, in many bacteria there is a third region, the O-side chain. This is a repeating oligosaccharide (perhaps five or six sugars linked together in the same pattern) with as many as 50 or 80 repeat units of this extending into the external environment of the bacterium. This makes the surface of the bacterium hydrophilic, and the O-side chain is highly antigenic, being the O or somatic antigen of Gram-negative bacteria. With a full O-side chain, bacteria are termed S-form or "smooth", and virulent bacteria are often of this type.

The LPS also has biological properties. The lipid A part of the molecule was thought to disrupt mammalian cell membranes. It is now known to act in a more subtle way to cause a host of biological effects upon the body, including pyrogenicity (raised body temperature) and the manifestations of Gram-negative septicaemia and circulatory collapse. The LPS is therefore also termed 'endotoxin' because it is toxic and yet a part of the bacterial cell and not secreted. In fact, we can think of the LPS as a signal, alerting the body to the presence a foreign invader. LPS becomes associated with the LPS binding protein. It then interacts with tissue macrophages through the CD14 protein, Toll-like receptor 4 (TLR4) and an associated protein called MD-2. Through cell signalling pathways, this leads to the release of potent cytokines such as IL-1

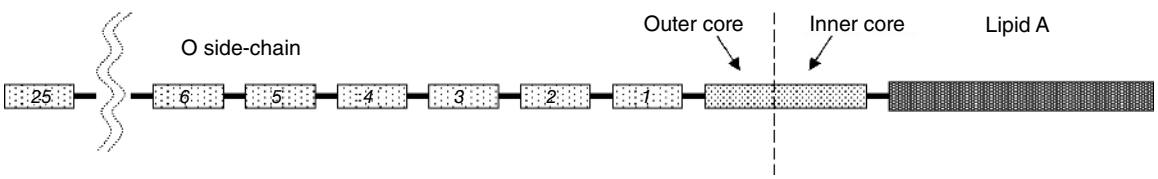

Figure 1.10 Lipopolysaccharide structure.

and TNF-α. In turn, these act on the hypothalamus to cause increased body temperature (fever) and other pro-inflammatory biological effects including vasodilation and hypotension.

In mycobacteria, which are Gram-positive organisms, wax-like mycolic acids are covalently linked to the peptidoglycan. These make the bacteria 'acid fast', resistant to desiccation, some disinfectants and most of the body's immune defence mechanisms.

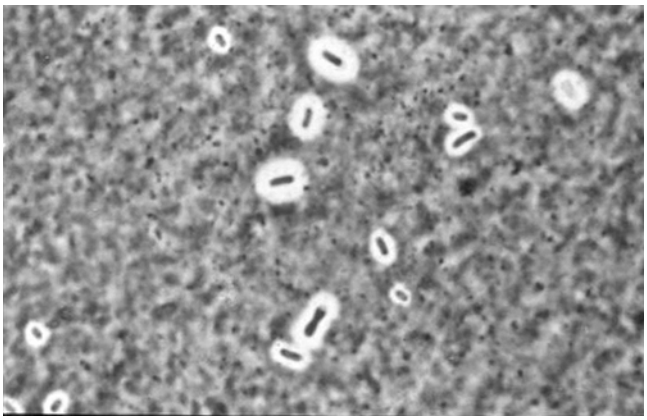

Figure 1.11 Negatively stained image of capsules of *E. coli*. Particles of India ink are excluded from the clear polysaccharide capsule which shows up as a bright area surrounding the dark bacteria.

Capsule

Many bacteria are surrounded with a layer of polysaccharide material known as the capsule (Figure 1.11).

Capsules may be either homopolysaccharide, being composed of a polymeric form of a single sugar type, or heteropolysaccharide, having two or sometimes more sugar residues. Very unusual sugar residues may be found quite often. In some cases, the capsule may be thick and visible in the light microscope (using special stains) and makes the bacterial colony viscous and slimy. In others, only a very thin layer, known as a microcapsule, is present. This means that the polysaccharide can be detected only by chemical or serological means or by electron microscopy (Orskov et al. 1977).

Most capsules are antigenic but some are relatively non-antigenic, presenting a surface which the body and the biochemical components of the immune system fail to recognise as foreign. Furthermore, the capsular antigen may mask other, deeper antigens in the cell envelope such as the teichoic acids or LPS which are easily 'seen' as foreign.

The function of capsules, at least in the body, is to evade phagocytosis and some of the most important pathogens have capsules which are essential for their ability to cause disease. One such organism is *Bacillus anthracis*. This has a most unusual capsule in that it is not polysaccharide but poly-amino acid: poly D-glutamic acid. This capsule is essential for *B. anthracis* to survive in the body and cause the disease anthrax. It is also important in the identification of *B. anthracis* in the blood of a fallen animal. The capsule shows a characteristic pink or mauve colour surrounding the bacterial cell (M'Fadyean reaction) when stained with polychrome methylene blue. This is diagnostic. It is also an important statutory examination before the removal and disposal of the carcass of a sudden death case in a large animal can be carried out.

Flagellae

Bacteria also carry surface appendages. Flagellae are protein structures which function in the movement of bacteria – motility (Figure 1.12).

They are composed of protein subunits (flagellin), and these are important antigens in the identification of some bacteria (e.g. the H antigens of *Salmonella*). Bacterial flagellae are carried as a polar flagellum at the end of the cell (such as on *Pseudomonas aeruginosa*) or they may be present over the surface of the bacterium which are referred to as peritrichous flagellae (such as on *Proteus mirabilis*). The flagellae of spirochaetes are specialised in being located not externally, but within the periplasmic space (between the inner and outer membranes). Here, they are termed 'axial filaments' but serve the same function.

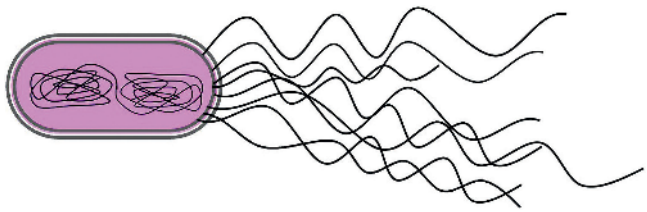

Figure 1.12 Bacterial flagellae are spiral structures attached at the cell envelope.

Flagellae cause bacteria to be motile by rotating (Bardy et al. 2003). Because it is curved, the filament of a flagellum acts as a propeller driving the organism through the

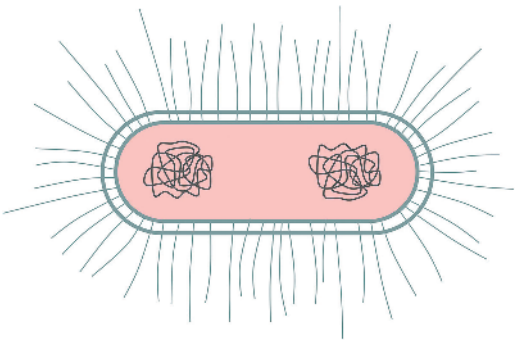

Figure 1.13 Bacterial fimbriae. Very fine surface appendages.

medium. The energy for this rotation is derived from the proton motive force (PMF) or proton gradient at the cytoplasmic membrane by a molecular electric motor. In this, protons pass through the mechanism across the membrane and bring about a part turn of the flagellum.

Many bacteria are chemotactic, reacting positively to some chemical stimuli by moving towards them and/or negatively to others.

Fimbriae

Fimbriae are also protein surface appendages. They are sometimes referred to as pili and are composed of subunits of pilin. They are thinner and shorter than flagellae and can only be seen by electron microscopy (Figure 1.13).

With a few exceptions, fimbriae are only present on Gram-negative bacteria. Their function in nature is to adhere to surfaces but different bacteria carry different fimbriae that adhere to different surfaces – some through extremely specific interactions. In some cases, this is a mucosal surface such as the small intestine or the urinary tract of an animal. Fimbriae in pathogenic bacteria may function as colonisation factors without which they would not cause disease. Antibody to fimbrial antigens can be protective in preventing attachment of the pathogen. Thus, fimbrial protein is the basis for a number of new or experimental vaccines against a surprising variety of diseases.

Sex pili are specialised fimbriae involved in the process of conjugation or transfer of plasmid genes between bacterial cells.

Spores

Bacterial spores are produced by only two genera: *Bacillus* and *Clostridium*. They are correctly known as endospores and are formed inside the mother cell in response to adverse conditions. They are not reproductive, only one spore being produced per bacterium and only one bacterium being produced by the germination of a spore. Spores are able to tolerate heat, desiccation, cold, radiation and chemical treatments that vegetative bacteria cannot survive (Nicholson et al. 2000).

Bacterial spores comprise the genomic DNA of the cell, surrounded by the cytoplasmic membrane and a layer of 'normal' peptidoglycan. External to this is the cortex, a specialised thick layer of peptidoglycan that has a much looser, less cross-linked structure. It is known to be responsible for the dehydration of the spore's core and is probably the structure which confers resistance to heat, desiccation and radiation. The coat protein is a keratin-like, very thick, highly resistant protein stabilised by disulfide (—S—S—) bonds. It probably confers chemical resistance on the spore (Figure 1.14).

Bacterial spores may remain viable for many years. They are reawakened by favourable environmental conditions. However, some spores require an activation step such as heat-shock or boiling to trigger germination. Germination is the

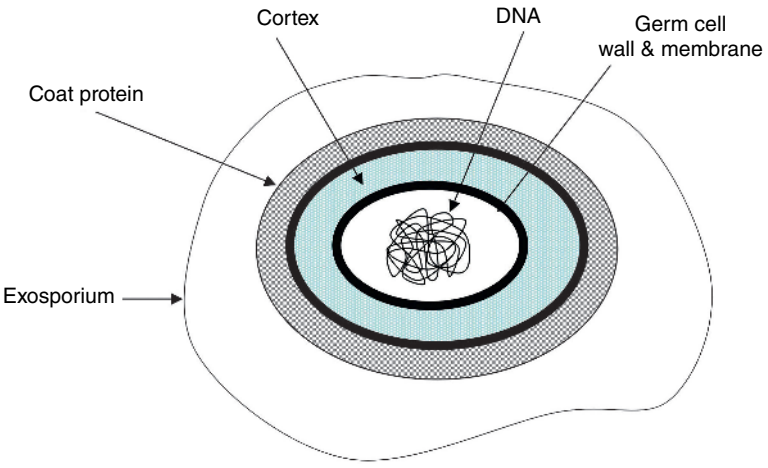

Figure 1.14 Bacterial endospore structure.

resynthesis of metabolic enzymes, and the degradation and removal of spore-specific components and outgrowth is the return to life as a vegetative bacterial cell.

Spores are important because they are the microorganisms that are most difficult to destroy and because they are formed by a number of very important veterinary bacterial pathogens. These include the agents of clostridial diseases of sheep, tetanus, botulism, blackleg of cattle and also anthrax.

The position of a spore within the mother cell tends to be characteristic of a particular species. Spores are produced at the end of a bacterium (terminal spore), as by *Clostridium tetani*, or within the centre of the cell (central spore). Similarly, a clostridial spore will bulge the mother cell and distend it considerably outside the normal bounds of the bacterium; the spores of *Bacillus* are more confined to the bounds of the bacterial wall (Figures 1.15 and 1.16).

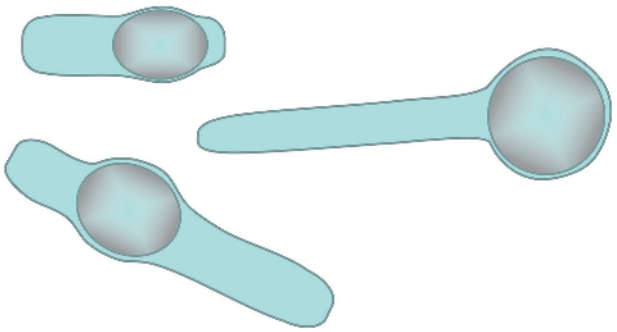

Figure 1.15 Morphology of bacterial endospores formed within a 'mother' cell. These vary according to the species of bacteria producing them.

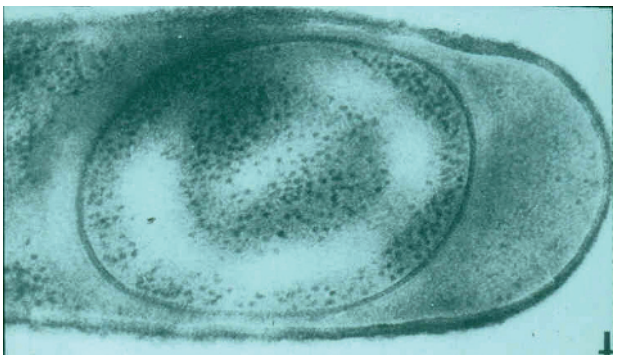

Figure 1.16 Transmission electron microscopic image of an endospore formed within a vegetative 'mother' cell.

Other Forms of Bacteria

L-forms of bacteria are sometimes generated during infection by adaptation of pathogens such as streptococci. These are produced during treatment with antibiotics which damage cell walls. They lose their cell wall peptidoglycan and remain viable in protected areas of the body. This may allow infection to persist in spite of antibiotic treatment, and by reverting to the normal (cell-walled) form of the pathogen they can produce relapses of infection. While L-forms may be responsible for some cases of chronic or unexplained recurrent infection, in practice they are rarely detected in veterinary infections.

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