Stewart
& Beswick

# Bacteriology Virology and Immunity

*for students of medicine*

10th edition

Baillière Tindall

# BACTERIOLOGY VIROLOGY AND IMMUNITY

## For Students of Medicine

TENTH EDITION

F.S. STEWART MD, FRCPI, FCPath, MRIA

*Member of the Royal Irish Academy*
*Fellow Emeritus of Trinity College, Dublin*
*Formerly Professor of Bacteriology and Preventive Medicine, University of Dublin*

T.S.L. BESWICK MA, MSc, MD, FRCP, FRCPath

*Professor of Virology, University of Manchester*
*Honorary Consultant Virologist, Manchester North and Central Districts, Salford Area Health Authority and North Manchester Regional Virus Laboratory*

Baillière Tindall · London

A BAILLIÈRE TINDALL book published by
Cassell & Collier Macmillan Publishers Ltd
35 Red Lion Square, London WC1R 4SG
and at Sydney, Auckland, Toronto, Johannesburg
an affiliate of
Macmillan Publishing Co. Inc.
New York



First published 1925
Ninth edition 1968
Tenth edition 1977
ISBN 0 7020 0637 8

English Language Book Society edition (of Tenth edition) 1977

Printed in Great Britain by
The Whitefriars Press Ltd, London and Tonbridge

# *Bacteriology, Virology and Immunity for Students of Medicine*

*First published in 1925 as* A Handbook of Bacteriology for Students and Practitioners of Medicine, *this well known textbook was written by Professor Joseph W. Bigger, of the University of Dublin. It quickly achieved wide acceptance and was revised five times by the original author, the sixth edition appearing in 1949.*

*Professor F. S. Stewart, who had succeeded Professor Bigger as Professor of Bacteriology and Preventive Medicine in Dublin, then took over the book and was solely responsible for the seventh, eighth and ninth editions, this last being published in 1968 under the title* Bacteriology and Immunology for Students of Medicine.

*For the tenth edition Professor Stewart has been joined by Professor T. S. L. Beswick, Professor of Virology in the University of Manchester, and to indicate the scope of the book which has expanded with the subject the title has again been changed to include virology.*

*The immense advances that have taken place in recent years in microbiology and immunology have necessitated an unusually full revision for this edition and the sections covering immunity and virology have been almost entirely rewritten. Nevertheless, the book remains primarily a textbook for students of medicine; it should also be of value to undergraduates taking Honours Science courses, and as a work of basic reference for medical graduates.*

# *Preface*

In the nine years that have elapsed since the publication of the ninth edition of this book microbiology and immunology have continued to grow at an explosive rate and this has necessitated a very full revision of the text. As in previous editions, however, the emphasis is on the general principles of microbiology and immunity and on the aspects of these subjects that are of importance in relation to infectious disease. As a result there has been a further reduction in material of a purely technical nature, for which several excellent texts are currently available. Thus the systematic bacteriology has been reduced by about one-quarter and the chapters on diagnosis and on the microscopic examination and cultivation of bacteria have been combined in one. Nevertheless there has inevitably been some overall increase in the text owing to the incorporation of additional material, particularly in immunity, which has been virtually completely rewritten (by F.S.S.) and in virology (by T.S.L.B.), the areas in which growth has been most rapid. No part of the book, however, has escaped major revision. An important change has been its division into four sections, general microbiology, immunity, systematic bacteriology and virology. Although the book has been prepared with the requirements of the medical student particularly in view, it should also be of value to undergraduates taking honours science courses, to postgraduates working for higher degrees and as a work of reference for medical graduates.

The authors are indebted to many people for assistance in respect of certain sections of the text, particularly their colleagues and former colleagues in Dublin and Manchester. Professor McEntegart of the Department of Medical Microbiology in the University of Sheffield kindly offered the hospitality of his department to one of them (T.S.L.B.) during a two months leave of absence granted to him by the University of Manchester and devoted to a final revision of the text. The authors also wish to thank Dr W. Hodgkiss of the Torrey Research Station, Aberdeen, for a number of the electron micrographs and Dr R. Ollerenshaw and his colleagues, especially the late Mr J. Kilshaw, of the Department of Medical Illustration in Manchester, and Mrs F. S. Stewart for new line-drawings. Finally we would like to thank the editorial staff of Baillière Tindall for their patience and understanding during the long period in which the work was in preparation.

*February 1977*

F. S. STEWART
T. S. L. BESWICK

# *Contents*

# *Colour Plates*

*between pages 24 and 25*

# Part I

# GENERAL MICROBIOLOGY

# I.1

# *The General Properties of Bacteria*

The term bacteria is applied in a general sense to a somewhat heterogeneous collection of minute unicellular organisms of relatively simple structure. As a group bacteria are closely related both to the simpler plants (the microscopic algae and fungi) and to the simpler animals (the protozoa) but may be regarded as even simpler and more primitive. On the whole their resemblance to the algae and fungi is greater than to the protozoa and they are therefore usually classified in the plant kingdom in which they are assigned to the class Schizomycetes or fission fungi.

In certain respects, however, bacteria obviously possess properties normally associated with the animal kingdom, many being actively motile and owing their motility to the possession of organelles (flagella) similar to those found in motile protozoa. In fact van Leeuwenhoek, the discoverer of bacteria, referred to them because of their frequent motility as little animals or 'animalcules'. In order to deal with this dilemma some microbiologists, following the proposal of Haeckel, a nineteenth-century biologist, assigned bacteria and unicellular micro-organisms in general to a kingdom distinct from both the animal and plant kingdoms—the protista. The protista have in turn been divided into higher and lower protists, differentiated by the presence or absence of a nuclear membrane. Protozoa and fungi which possess this membrane are classified as higher protists, while others, such as bacteria and blue–green algae, are classed as lower protists.

The number of types of bacteria is enormous. The majority are free-living organisms which play a vital role in the economy of nature—in the circulation of carbon, nitrogen and other elements. In fact it can be said that without the activities of the free-living bacteria, other forms of life would be impossible. They are also the agents in a diversity of natural processes of great social and economic importance—the souring of milk, the ripening of cheese, the curing of tobacco. Many of the free-living bacteria have been exploited by man because of the diversity of metabolic end-products they produce—acetic acid, citric acid, butanol, acetone and antibiotics, to name but a few. Here, however, we are concerned with the parasitic bacteria whose life is associated with the animal body and, more particularly, with the pathogenic bacteria which produce disease; these constitute only a small minority of the total bacterial population.

The classification of the various types of bacteria presents considerable difficulties and will be considered more fully in a later chapter (Chapter II.1). For the moment, however, we may note that on purely morphological grounds they may be subdivided into six main groups.

1. *Bacteria proper or true bacteria.* These are rigid unbranched free-living organisms. To the medical bacteriologist they are the most important of the Schizomycetes. They may be further divided according to their shape into cocci, bacilli, vibrios and spirilla.

Cocci are more or less spherical organisms. They are given descriptive names depending on the ways in which the individual organisms are arranged. Diplococci are arranged in pairs; streptococci in chains and staphylococci in clusters like bunches of grapes. Tetrads are cocci arranged in groups of four cells and sarcinae in cubical packets of eight cells.

Bacilli are rod-shaped bacteria whose length is at least twice their width. Some of the smaller bacilli may, however, assume an almost coccal

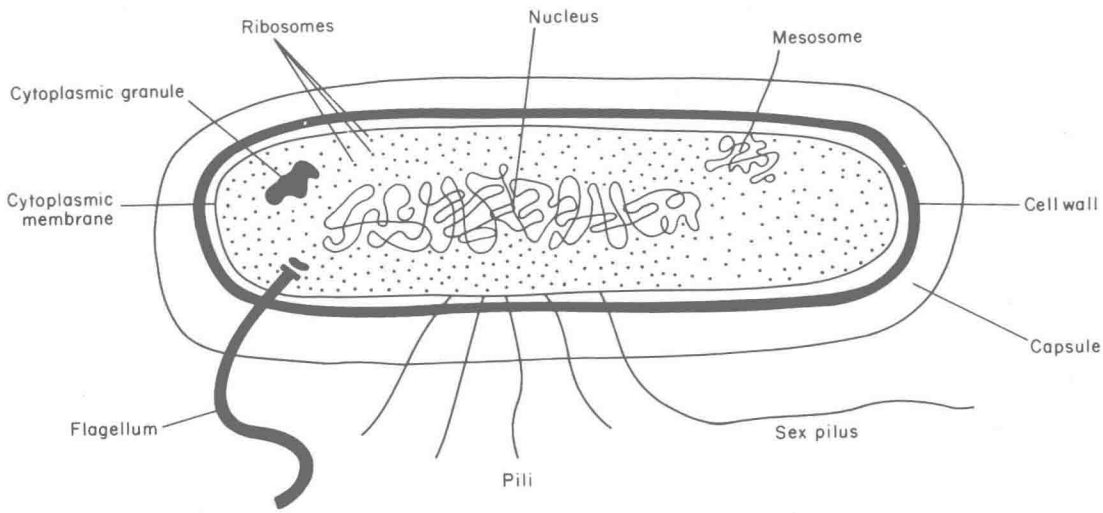


Fig. 1.1. The structure of a bacterial cell.

appearance. Vibrios are curved rods the curve of which forms less than one complete spiral. Longer rigid curved organisms usually with several spirals are known as spirilla.

2. *Actinomycetes*. These, like the bacteria proper, are rigid organisms but resemble the fungi in that they may show true branching and in tending to form filaments.

3. *Spirochaetes*. These are flexuous non-branched organisms of spiral shape.

4. *Mycoplasmas*. These are highly pleomorphic organisms of indefinite shape. In general they resemble bacteria which have been deprived of their rigid cell walls (p. 5).

5. *Rickettsiae and chlamydiae*. These are very small organisms which were at one time regarded as being intermediate between viruses and bacteria. The chlamydiae have frequently been described as large viruses, but are now recognized as bacteria, though differing from the true bacteria in being so highly parasitic that they can multiply only within other living cells. This obligatory parasitism is due to a deficiency in the full complement of metabolic enzymes necessary for the maintenance of a free-living existence. Nevertheless, though highly parasitic, these organisms have some independent metabolic capability, and can be regarded as living organisms, in a sense in which viruses (p. 337) manifestly cannot. It seems likely that both rickettsiae and chlamydiae have developed from true bacteria by a process of retrograde evolution.

6. *Miscellaneous higher bacteria*. These have been very inadequately studied and are of doubtful status. They are free-living organisms of unusual morphology, comprising stalked, sheathed, budding and slime forms. Though of great intrinsic interest, they are of no importance in medical microbiology.

## STRUCTURE OF BACTERIA

Bacteria are the smallest free-living organisms; their size is measured in micrometres (μm) 1 μm being $10^{-6}$ m (1/25 000 in). Because of this a microscope affording a considerable degree of magnification is necessary for their demonstration. Most of the cocci have a diameter of about 1 μm in length and from 0·5 to 1 μm in width. Different species of bacteria tend to have characteristic average dimensions but individual strains may show appreciable deviation from these.

When examined in the living condition bacteria are seen to be transparent, colourless and homogeneous or finely granular. In unstained wet preparations the contrast between bacteria and background is poor. They are therefore usually examined in stained preparations. Staining not only increases contrast and therefore facilitates the visualization of bacteria but also, when stains specific for these are employed, permits the

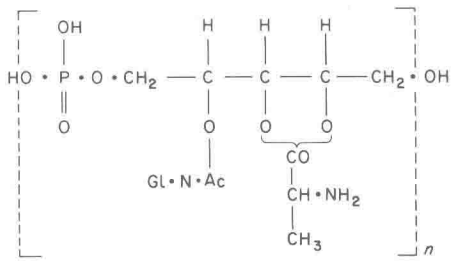


Fig. 1.2. Ribitol teichoic acid from a strain of *Staphylococcus aureus*. The molecule contains one alanine residue for every two ribitol residues. Gl.n.Ac=N-acetyl glycosamine. $n=12–16$.

recognition of certain structural features. For general purposes the stain most frequently employed is Gram's stain (p. 26). On the basis of their reactions in this stain bacteria may be classified into two large groups—Gram-positive and Gram-negative. This classification is of great importance in relation to bacterial identification.

In spite of their small size bacteria have a well organized cellular structure. Like the cells of higher organisms the bacterial cell possesses a *nucleus*, a *cytoplasm* and a *cytoplasmic membrane*. The true bacteria and the actinomycetes resemble plant cells in possessing, in addition, a *cell wall* which is distinct from the cytoplasm and cytoplasmic membrane.

The cell wall is a complex rigid structure which gives bacteria their definite shape. It must possess considerable strength so as to enable it to withstand the osmotic pressure—in some cases this is as much as 20 atmospheres—of the intracellular constituents. Were it not in fact for the cell wall, bacteria would burst, since the delicate cytoplasmic membrane would by itself be unable to withstand this high internal pressure. The relationship of the cell wall to the underlying cytoplasmic membrane may be thought of as being like that of a bicycle tyre to its inner tube. The thickness of most bacterial cell walls under normal conditions of growth is in the range of from 10 to 20 nm, the cell walls of Gram-positive bacteria being in general somewhat thicker than those of Gram-negative bacteria.

The rigidity of the bacterial cell wall is due to the presence of a basal three-dimensional enveloping structure known as *peptidoglycan* or *murein*. The cell wall peptidoglycans so far examined have been found to be composed chemically of a backbone consisting of the amino sugars N-acetyl glucosamine and N-acetyl muramic acid, to the muramic acid residues of which are attached peptide side-chains comprising a limited number of amino acids, D- and L-alanine, D-glutamic acid and either L-lysine, diaminopimelic acid, diaminobutyric acid or ornithine. The latter two dibasic amino acids have only recently been recognized in cell walls. In some species either glycine or aspartic acid is also present. In the intact cell the three-dimensional structure of the peptidoglycan is completed by extensive cross-linking between neighbouring peptide chains. In *Staphylococcus aureus* this appears to occur through a glycine pentapeptide, linking lysine and terminal D-alanine residues.

In the case of certain bacteria, notably *Bacillus* species, *Micrococcus lysodeikticus* and *Str. faecalis*, the wall mucopeptide of the intact cell is susceptible to the action of lysozyme. As a result of this action the wall is broken down leaving a structure known as a *protoplast*, which is bounded only by the cytoplasmic membrane. Protoplasts are osmotically sensitive and undergo rapid lysis in isotonic or hypotonic solutions. Because of this they can only be maintained in intact form in hypertonic solutions. Protoplasts may also be obtained from certain bacteria by growing them in the presence of penicillin or other antibiotics which are capable of inhibiting cell wall synthesis. Protoplasts are much more readily prepared from Gram-positive than from Gram-negative bacteria. This is not surprising, as the peptidoglycan is a relatively minor component of the Gram-negative cell wall. In fact, protoplasts obtained from Gram-negative organisms appear to retain a good deal of the cell wall lipopolysaccharide and lipoprotein.

Peptidoglycan is the major constituent of the cell walls of Gram-positive bacteria constituting from 50% to as much as 90% of the wall. In Gram-negative species, however, mucopeptide constitutes only from 5 to 10% of the wall, but even under these circumstances it is responsible for the osmotic stability of the cell. This is because the osmotic pressure of the Gram-negative cell appears to be considerably less than that of the Gram-positive cell. In addition to mucopeptide, Gram-positive cell walls contain polysaccharides and teichoic acids. The latter are polymeric complexes of ribitol phosphate or glycerol phosphate with simple sugar or amino sugar

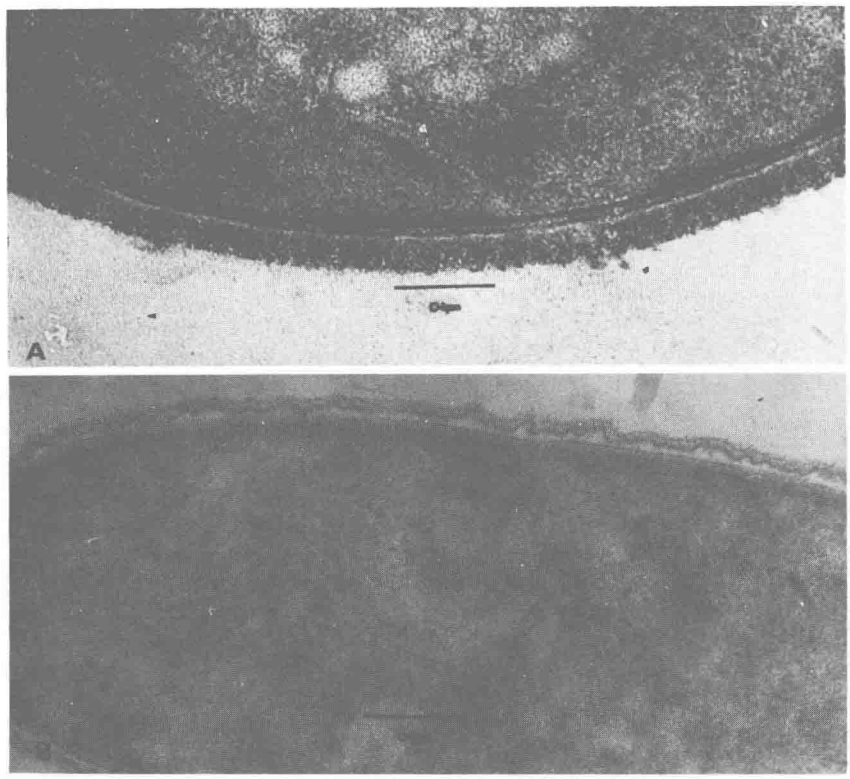


Fig. 1.3. Electron micrographs of the cell wall in Gram-positive (A) and Gram-negative (B) bacteria. *(W. Hodgkiss.)*

residues, together with alanine in the unnatural D-configuration. It now seems possible that some at least of these additional components are bound to the mucopeptide by covalent bonds. Their precise significance in cell wall structure is at present unknown. Gram-positive cell walls do not appear to contain protein as an internal component of the wall but in some cases protein may be present in a microcapsular or capsular layer, e.g. the M proteins of the streptococci (p. 213), and the glutamyl polypeptides of *Bacillus* species (p. 226).

Gram-negative cell walls are considerably more complex biochemically than Gram-positive cell walls, and unlike the latter contain a high concentration of lipid and protein. The precise anatomical arrangement of the various components in the Gram-negative cell wall is not fully understood. It would appear, however, that the cell wall mucopeptide occurs as an inner layer, outside which is the lipoprotein and lipopolysaccharide, each possibly occurring as further distinct layers.

From the marked difference in cell wall composition shown between the Gram-positive and Gram-negative bacteria it might be expected that the chemical composition of the cell wall could be useful as a taxonomic criterion. That this is the case was first shown by Cummins and Harris who found that the sugar composition of the cell walls of various Gram-positive bacteria was of particular value for differentiation at both generic and species level. Thus the cell walls of *Staph. aureus* contain hexosamine but no simple sugars. Rhamnose is found in the cell walls of many streptococci but is absent from the pneumococcus cell wall, and arabinose is apparently a universal component of the cell walls of the corynebacteria. Recent evidence suggests that the presence of diaminobutyric acid may be associated significantly with plant pathogenicity in the corynebacteria.

Beneath the cell wall, as an anatomically distinct

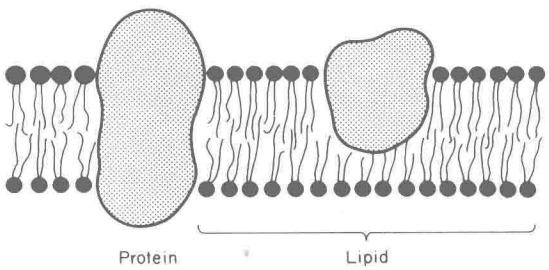


Fig. I.4. A cross-section of a cytoplasmic membrane.

structure, is the cytoplasmic membrane. The membrane is semi-permeable, allowing the passage of water but impermeable to large molecular weight compounds and also to many small molecular weight compounds, e.g. simple sugars and amino acids. Because of this it constitutes the osmotic barrier of the cell, controlling the passage of nutrients into the cytoplasm and of end products of metabolism out of it.

Compared with the cell wall the cytoplasmic membrane is very thin, from 6 to 10 nm in cross-section. The structure of the cytoplasmic membrane as proposed by Singer and Nicolson is now generally accepted. This envisages the membrane as having a bimolecular leaflet structure consisting of a sandwich of two layers of lipid, the hydrophobic side-chains of which are on the inside and the hydrophilic residues on the outside, in contact on the one hand with the external environment and on the other with the cytoplasm. Interspersed in the lipid bilayers are molecules of protein with their hydrophilic residues projecting on the outside and inside of the membrane. The molecules of which the membrane is composed have considerable translational mobility through the membrane and also rotational mobility in the transverse axis of the membrane. The entire structure is consequently described as being a fluid mosaic.

There is considerable evidence that the cytoplasmic membrane, together with its cytoplasmic extensions (see below), may be the functional equivalent of the mitochondria of higher cells. Thus in *Staph. aureus* and *Micrococcus lysodeikticus* it has been found to be the main location of Krebs cycle enzymes (p. 18), of the electron transport chain and of oxidative phosphorylation. It also contains *permeases* which are necessary for the active transport of many small molecular weight organic compounds across the membrane. In addition, recent evidence indicates that, at least in Gram-positive cells, the membrane participates in the synthesis of the cell wall.

Morphologically, the bacterial cytoplasm is much simpler than the cytoplasm of higher organisms. Electron micrographs of bacteria have failed to reveal structures analogous to mitochondria and the complex membranous system of higher organisms known as endoplasmic reticulum and the Golgi apparatus are notably absent. However, certain bacteria, particularly Gram-positive bacteria, contain invaginations of the cytoplasmic membrane which have sometimes a lamellar and sometimes a vesicular form. These structures, known as *mesosomes*, can sometimes be seen attached to the bacterial chromosome; they are often closely related to the septum of dividing cells. It is thought that they may have a regulatory role in cell division, ensuring that each daughter cell receives a nucleus. Some may be involved in the secretion of extracellular products.

The main structural components of the bacterial cytoplasm would appear to be the ribonucleoprotein granules, known as *ribosomes*. These measure from 10 to 20 nm in diameter, and are the sites of protein synthesis. It has been estimated that a single bacterium may contain upwards of 10 000 ribosomes. The ribosomes have been shown to occur in groups, known as *polysomes*, linked together like beads on a chain by messenger RNA (p. 19). The individual ribosomes have a sedimentation coefficient of 70 Svedberg units. Each 70S ribosome is a complex of two smaller units with sedimentation coefficient of 50S and 30S. In the presence of magnesium these form stable complexes to yield a 70S ribosome. In low magnesium concentrations, however, they dissociate and in the absence of magnesium further degradation occurs. Ribosomes appear to be the points of attack of certain antibiotics which act on bacteria by interfering with protein synthesis (Chapter I.5).

Although it was for a long time disputed, bacteria are now accepted to possess a structure which is the morphological and functional equivalent of the nucleus of higher organisms. Like the latter it carries the genetic blueprint of the cell coded in the nucleotide sequence of its desoxyribonucleic acid (DNA).

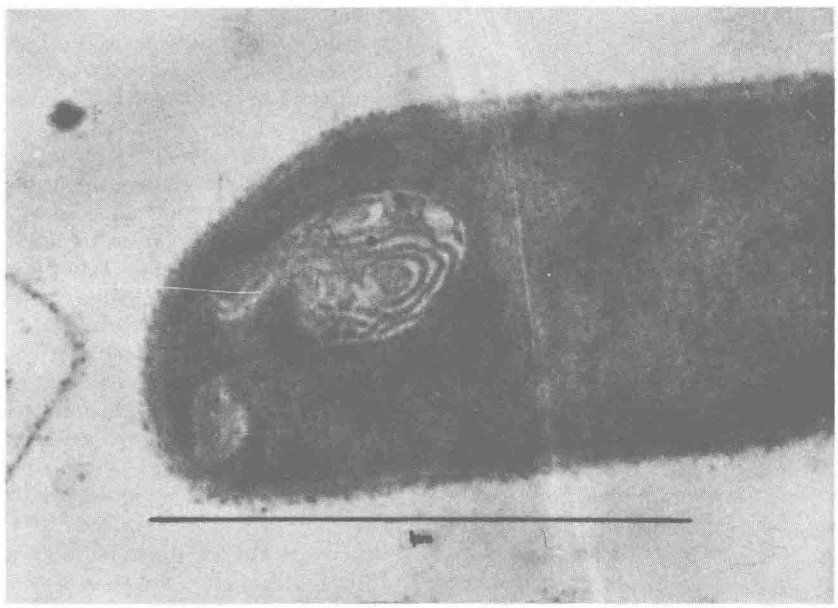

Fig. I.5. The mesosome of *Clostridium bifermentans*. *(W. Hodgkiss.)*

As seen in electron micrographs of very thin sections of bacteria, nuclei appear as central areas of lower electron density than the rest of the cell. These areas consist of interlacing bundles of very thin fibrils, about 2 to 6 nm in diameter, in a clear structureless matrix. There is convincing autoradiographic evidence that, in the case of *E. coli* and probably other organisms as well, the nuclear DNA occurs in the form of a single chromosomal thread of about 2000 μm in length. Except when mobilized for transfer, it exists in the cell as a circular closed loop. One can imagine the coiling necessary to fit this long thread into the nuclear area of a cell which is in itself only 2 to 3 μm in length. It is this folding which gives the appearance of bundles of fibrils seen in electron micrographs. This concept of a single chromosomal thread is in agreement with the genetic evidence indicating the determinants of transmissible characters on a single linkage group. The bacterial cell thus differs genetically from the cells of other organisms in being genetically haploid and in possessing only a single chromosome. There is, therefore, no necessity for the occurrence in bacteria of the complicated series of changes associated with mitosis and meiosis in higher organisms.

**Table I.1. DNA and protein composition of an average bacterial cell**

| *DNA* | *Protein* |
|---|---|
| Single molecule 2000 μm long | $2 \times 10^6$ molecules |
| Weight $5 \times 10^6$ daltons | Weight $5 \times 10^{10}$ daltons |
| $1{\cdot}6 \times 10^7$ nucleotides | $8 \times 10^8$ amino acids |
| 2500 nucleotides per gene | 400 amino acids per molecule |
| 6400 genes | Possibly 1000 molecular species |

Volume $10^{-12}$ ml; dry weight $1{\cdot}5 \times 10^{-11}$ daltons.

The bacterial nucleus differs from the nucleus of higher cells in two further respects:

1. It does not possess a nuclear membrane, and is therefore in direct contact with the cytoplasm of the cell. This may well facilitate rapid transfer of messenger RNA from the nucleus to the ribosomes. Cells lacking a nuclear membrane are sometimes referred to as *procaryotic* in contrast to *eucaryotic* cells which possess a nuclear membrane.
2. The bacterial DNA does not appear to be

associated with a basic protein, though the presence of some protein in the nucleus has not been excluded.

Many bacteria possess in addition to the above structure *flagella, fimbriae* or *pili, capsules, spores* and various types of *granules*. Since these structures are found only in certain species their demonstration is of importance in bacterial identification.

*Flagella* are the locomotor organs of bacteria. They are long, contractile hair-like filaments measuring from 12 to 19 nm in diameter, and usually several micrometres in length. In some bacterial species, e.g. the enterobacteriaceae, they are arranged along the sides of the organism. This is known as *peritrichous* flagellation. In others, such as *Pseudomonas,* flagella occur singly or in tufts at one or both ends—*polar* flagellation. Individually, flagella are too thin to be seen by ordinary light microscopy but they can be stained by silver impregnation methods. They are best demonstrated by electron microscopy when they appear, particularly those from peritrichously flagellated bacteria, as helical or wavelike structures. In electron micrographs the flagella can be seen to pass through the cell wall and to originate either from the cytoplasm or from the cytoplasmic membrane. Though they are not attached to the cell wall, flagella can apparently initiate movement only when the cell wall is present. Thus protoplasts prepared from *Bacillus* species by lysozyme treatment and possessing apparently normal flagella, are non-motile.

From studies with a variety of peritrichously flagellated species it would appear that the standard method of attachment is through a hook at the proximal end of the flagellum. The hook appears to be attached to a disc-like or spherical basal structure, the whole complex being closely associated with, and probably directly attached to, the cytoplasmic membrane. The basal structure is responsible for the motility of flagella in a manner which has not been fully explained. This appears, however, to be due to the first instance to the passage of helical waves of contraction down the flagellum, the effect of which is to propel the bacteria forward. Relative to their size, the speed with which the bacteria can be propelled is considerable; actively motile species are capable of travelling at up to 50 μm/sec. For a typical Gram-negative bacillus of approximately 5 μm in length, this would be equivalent to a man performing a 100 m dash in 5 sec.

Chemically, flagella are composed mainly, if not entirely, of protein. They consist of strands wound round each other in helical forms which in certain species, e.g. *Vibrio*, are surrounded by sheaths. The strands are made up of a globular protein called *flagellin*, disposed helically around a hollow core. Under acid conditions the subunits dissociate, but on reversal of these conditions they reaggregate to form intact flagella. This process is analogous to the self-assembly of viral capsids and suggests that under *in vivo* conditions flagella may spontaneously assemble from preformed units.

*Fimbriae* or *pili* are hair-like processes arising like flagella from granules in the cytoplasmic membrane and are only found in certain bacteria, particularly in Gram-negative bacilli. They differ from flagella in being shorter and thinner, straight, and play no part in the motility of the organism. Fimbriae cannot be demonstrated with the light microscope but can be seen in suitably prepared electron micrographs. They are best developed when the bacteria are grown in fluid media. They are probably structures by which bacteria can attach themselves to cells and to other particulate material. Certain bacteria possess specialized fimbriae or pili which are longer and thinner than the common type. These appear to be hollow and to constitute conjugation tubes through which DNA is transferred from one organism to another during conjugation; they are consequently referred to as sex pili.

Bacteria of the genera *Bacillus* and *Clostridium* produce highly resistant dormant forms known as *spores*. These appear first as round or oval bodies which form within the cytoplasm of the organism. They are highly refractile but stain with great difficulty. In simple Gram-stained preparations they appear as clear unstained areas. When stained, however, e.g. by Möller's method, they hold the stain firmly and are not readily decolorized. The spore may be situated in the centre of the bacillus equatorial, near one end-subterminal, or protruding from one end-terminal, the precise location being constant within a single species. In the *Bacillus* genus the diameter of the spore is normally less than that of the bacterium but in the genus *Clostridium* the spore is usually wider than the bacterium.

The first stage in the formation of the spore is a condensation of chromatin, apparently

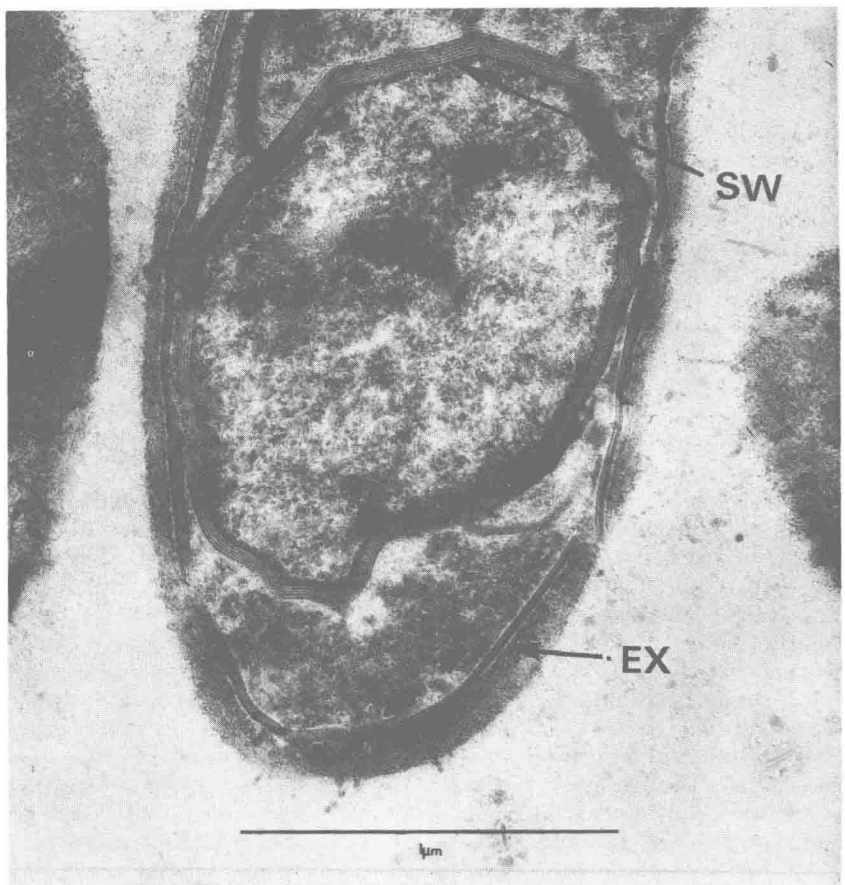


Fig. 1.6. The developing spore and exosporium of *Clostridium pasteurianum*. EX, exosporium; SW, spore wall. *(W. Hodgkiss.)*

representing half the cellular DNA, in an area at one end of the bacterium known as the *spore field* which is destined to be the site of formation of the new spore. At the same time a transverse septum derived from the cytoplasmic membrane forms by a process of invagination, and divides the developing spore, now known as the *forespore*, from the rest of the bacillus, known as the *sporangium*. From this stage on, the two areas of the cell are functionally distinct, the sporangium continuing to produce components characteristic of the vegetative form, and the forespore producing components characteristic of the spore. The dividing septum eventually completely encircles the forespore as a double-layered membrane, the inner layer of which becomes the spore wall. The spore *cortex* is then laid down between the inner and outer layers and the process completed by transformation of the outer layer into the spore coats and *exosporium*.

Finally, when the spore is fully formed, the body of the organism degenerates and the spore is set free, the entire process culminating in the release of the mature spore taking from four to eight hours to complete. During sporulation major changes are observed in energy metabolism. Thus in *Bacillus* species there is an activation of the enzymes of the tricarboxylic and glyoxylic acid cycles which are present, though dormant, in the vegetative cells. The primary function of this activation appears to be the provision of Krebs cycle intermediates for the synthesis of the characteristic spore component, *dipicolinic acid*. In this process poly-$\beta$-hydroxy