Pharmaceutical Microbiology

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Preface to the Sixth Edition

We were delighted to be asked to produce a sixth edition of *Pharmaceutical Microbiology* and we thank the publishers for their considerable input. With the willing cooperation of our co-authors, we have been able to update and modify our text. Several chapters are under new authorship in an attempt to produce a fresh approach. Some chapters have been streamlined but others expanded to take into account the rapid changes and progress being made in certain areas. A new chapter on vaccination and immunization has been introduced to act as a link with the updated chapters on the principles of immunity and the production of immunological products. The chapter on antibiotic assays has been deleted from this edition because it was considered not only that few developments had taken place in this field during the past few years but also that the topic had been comprehensively dealt with in the previous edition.

We hope that this edition will satisfy the needs of pharmacy students, now that the pharmacy degree has been extended to 4 years, and that it will also be of value to pharmacy graduates in hospital, industry and general practice as well as to microbiologists working in the pharmaceutical industry.

W. B. Hugo
A. D. Russell

Preface to the First Edition

When we were first approached by the publishers to write a textbook on pharmaceutical microbiology to appear in the spring of 1977, it was felt that such a task could not be accomplished satisfactorily in the time available.

However, by a process of combined editorship and by invitation to experts to contribute to the various chapters this task has been accomplished thanks to the cooperation of our collaborators.

Pharmaceutical microbiology may be defined as that part of microbiology which has a special bearing on pharmacy in all its aspects. This will range from the manufacture and quality control of pharmaceutical products to an understanding of the mode of action of antibiotics. The full extent of microbiology on the pharmaceutical area may be judged from the chapter contents.

As this book is aimed at undergraduate pharmacy students (as well as microbiologists entering the pharmaceutical industry) we were under constraint to limit the length of the book to retain it in a defined price range. The result is to be found in the following pages. The editors must bear responsibility for any omissions, a point which has most concerned us. Length and depth of treatment were determined by the dictate of our publishers. It is hoped that the book will provide a concise reading for pharmacy students (who, at the moment, lack a textbook in this subject) and help to highlight those parts of a general microbiological training which impinge on the pharmaceutical industry.

In conclusion, the editors thank most sincerely the contributors to this book, both for complying with our strictures as to the length of their contribution and for providing their material on time, and our publishers for their friendly courtesy and efficiency during the production of this book. We also wish to thank Dr H. J. Smith for his advice on various chemical aspects, Dr M. I. Barnett for useful comments on reverse osmosis, and Mr A. Keall who helped with the table on sterilization methods.

W. B. Hugo
A. D. Russell

Contents

Part 1
Biology of Microorganisms

Pharmaceutical microbiology is one of the many facets of applied microbiology, but very little understanding of its posed and potential problems will be achieved unless the basic properties of microorganisms are understood.

This section considers, in three separate chapters, the anatomy and physiology of bacteria, fungi and yeasts, and viruses, together with a survey of the characters of individual members of these groups likely to be of importance to the applied field covered by this book. Additional information is provided about more rapid methods for detecting bacteria. The final chapter in this section (Chapter 4) considers the principles of microbial pathogenicity and epidemiology.

The treatment is perforce brief, but it is hoped that the material will give an understanding of the essentials of each group which may be amplified as required from the bibliographic material listed at the end of each chapter.

1 Bacteria

1 Introduction

Bacteria share with the blue–green algae a unique place in the world of living organisms. Formerly classified with the fungi, bacteria were considered as primitive members of

Table 1.1 The main features distinguishing prokaryotic and eukaryotic cells

Feature	Prokaryotes	Eukaryotes
Nucleus	No enclosing membrane	Enclosed by a membrane
Cell wall	Peptidoglycan	Cellulose
Mitochondria	Absent	Present
Mesosomes	Present	Absent
Chloroplasts	Absent	Present

the plant kingdom, but they are now called *prokaryotes*, a name which means primitive nucleus. All other living organisms are called *eukaryotes*, a name implying a true or proper nucleus. This important division does not invalidate classification schemes within the world of bacterial, animal and plant life.

This subdivision is not based on the more usual macroscopic criteria; it was made possible when techniques of subcellular biology became sufficiently refined for many more fundamental differences to become apparent. Some of the criteria differentiating eukaryotes and prokaryotes are given in Table 1.1.

Recently, a third class must be added to the bacteria and blue–green algae. Organisms in this class have been named the Archaebacteria; they differ from bacteria and blue–green algae in their wall and membrane structure and pattern of metabolism. They are thought by many to be the first living organisms to have appeared on earth.

2 Structure and form of the bacterial cell

2.1 Size and shape

The majority of bacteria fall within the general dimensions of 0.75–4 μm. They are unicellular structures which may occur as cylindrical (rod-shaped) or spherical (coccoid) forms. In one or two genera, the cylindrical form may be modified in that a single twist (vibrios) or many twists like a corkscrew (spirochaetes) may occur.

Another feature of bacterial form is the tendency of coccoid cells to grow in aggregates. Thus, there exist assemblies (i) of pairs (called diplococci); (ii) of groups of four arranged in a cube (sarcinae); (iii) in a generally unorganized array like a bunch of grapes (staphylococci); and (iv) in a chain like a string of beads (streptococci). The aggregates are often so characteristic as to give rise to the generic name of a group, e.g. *Diplococcus* (now called *Streptococcus*) *pneumoniae*, a cause of pneumonia; *Staphylococcus aureus*, a cause of boils and food poisoning; and *Streptococcus pyogenes*, a cause of sore throat.

Rod-shaped organisms occasionally occur in chains either joined end to end or branched.

2.2 Structure

Three fundamental divisions of the bacterial cell occur in all species: cell wall, cell or cytoplasmic membrane, and cytoplasm.

2.2.1 *Cell wall*

Extensive chemical studies have revealed a basic structure of alternating *N*-acetylglucosamine and *N*-acetyl-3-*O*-1-carboxyethyl-glucosamine molecules, giving a polysaccharide backbone. This is then cross-linked by peptide chains, the nature of which varies from species to species. This structure (Fig. 1.1) possesses great mechanical strength and is the target for a group of antibiotics which, in different ways, inhibit the biosynthesis occurring during the cell growth and division (Chapter 8).

This basic peptidoglycan (sometimes called murein or mucopeptide) also contains other chemical structures which differ in two types of bacteria, Gram-negative and Gram-positive. In 1884, Christian Gram discovered a staining method for bacteria which bears his name. It consists of treating a film of bacteria, dried on a microscope slide, with a solution of a basic dye, such as gentian violet, followed by application of a solution of iodine. The dye complex may be easily washed from some types of cells which, as a result, are called Gram-negative whereas others, termed Gram-positive, retain the dye despite alcohol washing. These marked differences in behaviour, discovered by chance, are now known to be a reflection of different wall structures in the two types of cell. These differences reside in the differing chemistry of material attached to the outside of the peptidoglycan (Fig. 1.2).

In the walls of Gram-positive bacteria, molecules of a polyribitol or polyglycerol-phosphate are attached by covalent links to the oligosaccharide backbone; these entities

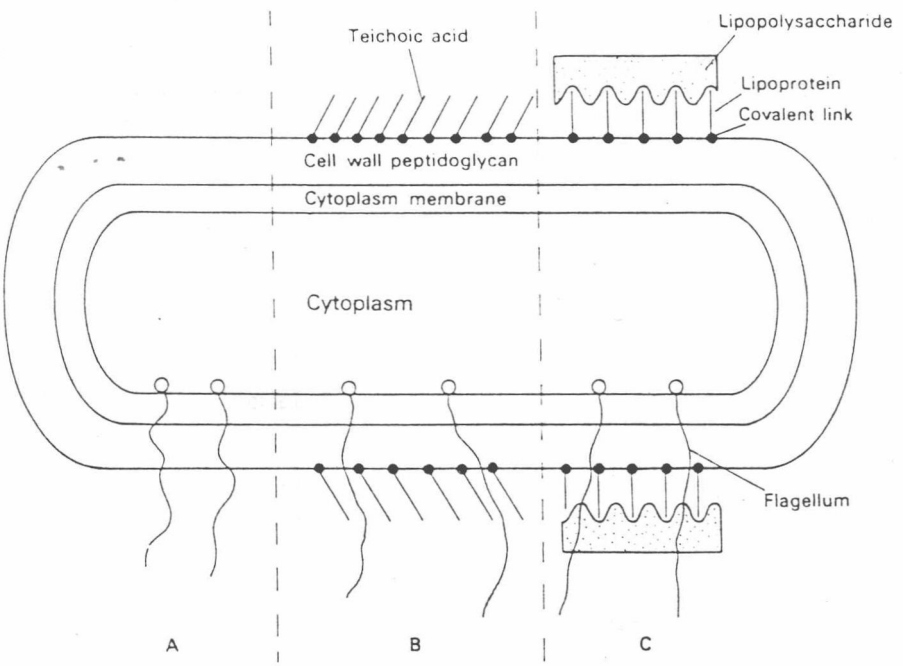

Fig. 1.1 Diagram of the bacterial cell. A, the generalized structure of the bacterial cell; B, Gram-positive structure; C, Gram-negative structure.

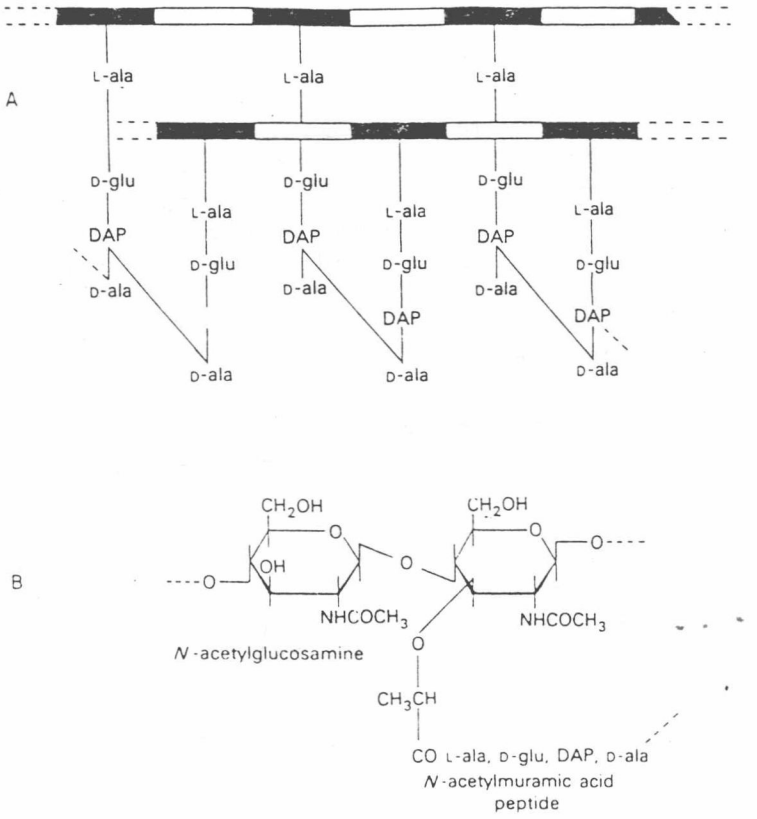

Fig. 1.2 A, peptidoglycan of *Escherichia coli*. ■, *N*-acetylmuramic acid; □, *N*-acetylglucosamine. B, repeating unit of peptidoglycan of *E. coli*. L-ala, L-alanine; D-glu, D-glutamine; DAP, diaminopimelic acid; D-ala, D-alanine.

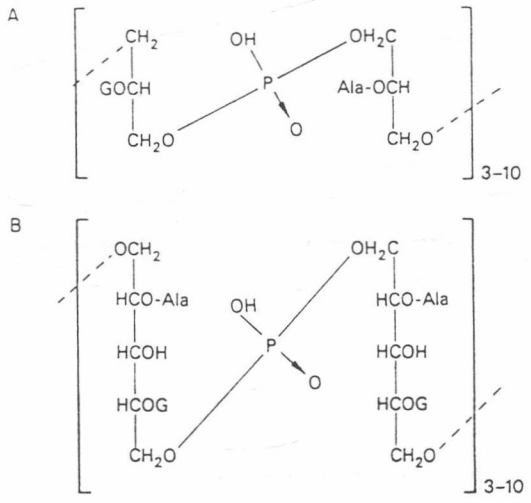

Fig. 1.3 A, glycerol teichoic acid; B, ribitol teichoic acid; G, glycosyl; Ala, D-alanyl.

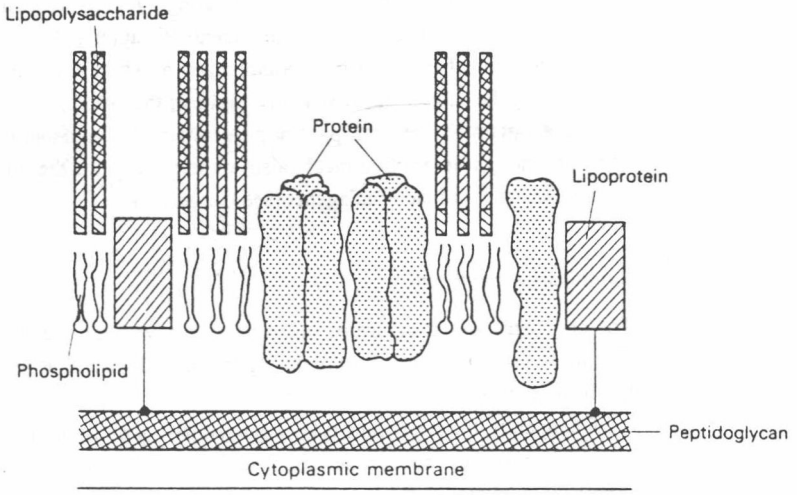

Fig. 1.4 Diagram showing detailed structure of the envelope of Gram-negative bacteria.

are teichoic acids (Fig. 1.3A, B). The glycerol teichoic acid may contain an alanine residue (Fig. 1.3A). Teichoic acids do not confer additional rigidity on the cell wall, but as they are acidic in nature they may function by sequestering essential metal cations from the media on which the cells are growing. This could be of value in situations where cation concentration in the environment is low.

The Gram-negative cell envelope (Fig. 1.4) is even more complicated; essentially, it contains lipoprotein molecules attached covalently to the oligosaccharide backbone and in addition, on its outer side, a layer of lipopolysaccharide (LPS) and protein attached by hydrophobic interactions and divalent metal cations, Ca^{2+} and Mg^{2+}. On the inner side is a layer of phospholipid (PL).

The LPS molecule consists of three regions, called lipid A, core polysaccharide and O-specific side chain (Fig. 1.5). The O-specific side chain comprises an array of sugars that are responsible for specific serological reactions of organisms, which are used in identification. The lipid A region is responsible for the toxic and pyrogenic (fever-producing) properties of this group (see Chapter 18).

The complex outer layers beyond the peptidoglycan in the Gram-negative species, the outer membrane, protect the organism to a certain extent from the action of toxic chemicals (see Chapter 13). Thus, disinfectants are often effective only at concentrations higher than those affecting Gram-positive cells and these layers provide unique protection to the cells from the action of benzylpenicillin and lysozyme.

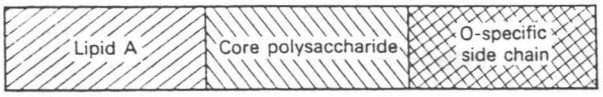

Fig. 1.5 Lipopolysaccharide structure in Gram-negative bacteria.

Part of the LPS may be removed by treating the cells with ethylenediamine tetra-acetic acid (EDTA) or related chelating agents (Chapter 12).

The proteins of the outer membrane, many of which traverse the whole structure, are currently the subject of active study. Some of the proteins consist of three subunits, and these units with a central space or pore running through them are known as porins. They are thought to act as a mechanism of selectivity for the ingress or exclusion of metabolites and antibacterial agents (see Chapter 8).

2.2.2 *Cytoplasmic membrane*

The chemistry and structure of this organelle have been the subject of more than a century of research, but it is only during the last 20 years that some degree of finality has been realized.

Chemically, the membrane is known to consist of phospholipids and proteins, many of which have enzymic properties. The phospholipid molecules are arranged in a bimolecular layer with the polar groups directed outwards on both sides. The structures of some phospholipids found in bacteria are shown in Fig. 1.6. Earlier views held that the protein part of the membrane was spread as a continuous sheet on either side of the

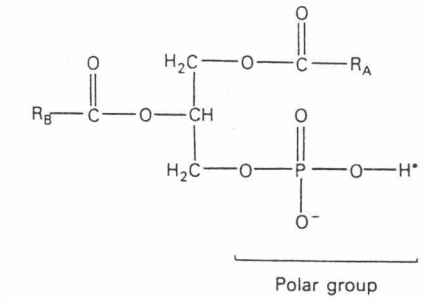

Fig. 1.6 The structure of some phospholipids found in *E. coli*. A, the structure of phosphatidic acid. H* of this structure is replaced by grouping B–D to give the following phospholipids: B, phosphatidylethanolamine; C, phosphatidylglycerol; D, diphosphatidylglycerol (cardiolipin). R_A.COO and R_B.COO are fatty acid residues.