MACKIE & McCARTNEY'S

HANDBOOK OF BACTERIOLOGY

A Guide to The Laboratory Diagnosis and Control of Infection

Edited by

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PREFACE

It is 35 years since the first edition of this textbook was published. That "Mackie and McCartney" quickly became a most popular handbook of practical bacteriology was shown by the rapid succession of new editions and reprints, the latest of which appeared in 1956. This fruitful partnership was ended by the untimely death of Professor Mackie in 1955 and the recent retirement of Dr. McCartney from active laboratory work. As Professor Mackie's successor, I accepted, after careful consideration, an invitation to assume the editorship of new editions of this textbook, with the proviso that the task of revision would be shared with staff members of the Bacteriology Department of Edinburgh University. The names of the team of collaborators and the sections of this tenth edition for which they are mainly responsible are given in the front of the book.

Inevitably, with the many advances in microbiology, extensive revision and considerable expansions were necessary and this edition contains some 230 pages more than its immediate predecessor, in spite of the uniform use of smaller print for the technical methods in Part II. The number of chapters has been increased from 24 to 46, partly because of new material and partly by subdivision of composite chapters, particularly in Part III dealing with systematic microbiology. Thus, there are now eight chapters on viruses in place of one; the intestinal pathogens are considered in four separate chapters; and the space devoted to microbial biology, infection and immunity has been nearly doubled. Throughout Part III the practical aspects of the laboratory diagnosis of infection in the individual and of the epidemiology of specific infectious diseases in the community has been given fuller consideration in the hope that this textbook will become even more widely used by medical students and doctors in many lands as a laboratory guide to the rational treatment and prevention of infective disease. Fuller use has also been made of illustrative figures and diagrams than in earlier editions.

This textbook is used by students, both graduate and undergraduate, of veterinary medicine and we are indebted to Mr. G. Fraser, Ph.D., M.R.C.V.S., of the Bacteriology Department, The Royal (Dick) Veterinary College, for revision of the sections on animal infections.

The burden of preparing the index has fallen most heavily on Dr. R. R. Gillies, and we have had help in the proof reading from Drs. G. P. B. Boissard, J. G. Collee, and F. L. Constable of this Department. We have had much helpful advice from numerous other colleagues both in Edinburgh and further afield, and to all of them we would express our grateful thanks. We are also greatly indebted to Mr. C. Macmillan and Mr. J. Parker of E. & S. Livingstone, Ltd., for their patient helpfulness in the preparation of this edition.

1960 ROBERT CRUICKSHANK

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CHAPTER I

MORPHOLOGY OF BACTERIA

BACTERIOLOGY or Microbiology, as applied to medicine, embraces the study of the micro-organisms that are parasitic on man. Microbes are designated *parasites* when they obtain their nourishment from the body of a living host. Some parasites have the power to produce disease and these are described as *pathogenic*, while others are normally harmless and are described as *commensal*. The latter term (lit. " table companion ") properly applies to the numerous micro-organisms which live harmlessly in the mouth, throat and intestine, sharing the food eaten by their host. Its meaning, however, has been usefully widened to include harmless parasites living elsewhere in the body, *e.g.* in the nose and on the skin. The distinction between commensal and pathogenic varieties is not absolute; some normally commensal species are potential pathogens, able in certain circumstances to cause disease, while some pathogenic species occasionally assume a commensal role, *e.g.* in the so-called *healthy carriers* of infection.

In veterinary science, bacteriology is specially concerned with the micro-organisms responsible for disease in domesticated animals. As many infective diseases are common to man and animals, medical and veterinary bacteriology are closely related branches of the general subject. Pathogenic organisms show great diversity in their degree of adaptation to different host species. Some are infective for the human species only, others for certain animals but not man, and yet others for man as well as one or more animal species. The host range of the causative microbe is an important factor determining the epidemiology of a disease, since wild or domesticated animals of different species may act as the source of infection. (The term " source " of infection applies to the normal growth habitats of the microbe, *e.g.* the human or animal host; objects contaminated with live non-growing microbes are termed " vehicles " or " reservoirs " of infection.)

Many microbial species are *non-parasitic*, or *free-living*. These abound in soil, water, mud and other such natural habitats, and with rare exceptions are harmless to man and animals. They nourish themselves by a variety of mechanisms. The protozoa capture, ingest and digest internally solid particles of organic foodstuff such as bacteria and plant debris (holozoic nutrition).

The algae and photosynthetic bacteria absorb carbon dioxide and soluble inorganic substances, and gain their energy by photosynthesis from sunlight (holophytic nutrition). Certain bacteria, the *autotrophs*, also utilise carbon dioxide as the sole source of carbon, but gain their energy by oxidation of an inorganic substance. Finally, there are numerous species of bacteria and fungi, described as *saprophytic*, which utilise organic nutrient substances obtained from dead plant or animal matter; they absorb soluble nutrients through their cell surfaces, and when utilising solid material, must first decompose this externally into soluble products by the action of extracellular enzymes. Saprophytes are responsible for the decomposition and eventual mineralization of all organic waste, *e.g.* animal corpses and excreta, dead and fallen vegetation, and sewage. They are also responsible for the natural spoilage of food and fodder. A few species of saprophytic bacteria and fungi are able in special circumstances to infect the human or animal body, and cause disease (*e.g.* producing tetanus or gas gangrene when the tissue defence mechanisms are disordered by wounding). The term " saprophyte " is sometimes also applied to those commensal parasites which feed on dead organic matter within the body of a living host, for instance in the intestinal contents.

Micro-organisms may be defined as living creatures which are microscopical in size and relatively simple, often unicellular in structure. The diameter of the smallest body that can be resolved and seen clearly by the naked eye is about 100 μ (1 micron, or $\mu = 0{\cdot}001$ millimetres). All but a few of the micro-organisms are smaller than this and a microscope is therefore necessary for their observation. The light microscope under optimal conditions can resolve bodies down to 0·2 μ in diameter, and this includes all microbes except the viruses, most of which are still smaller. The electron microscope has a limit of resolution approaching 0·001 μ (*i.e.* 1 mμ or millimicron), and can resolve even the smallest viruses (0·01 μ diam.). It should be noted that when bacteria or fungi are allowed to grow undisturbed on a solid or semi-solid substrate, their numerous progeny accumulate to form " colonies " which are readily visible to the naked eye.

Living material, or protoplasm, is organised in units known as " cells ". Each cell consists of a body of protoplasm, the *protoplast*, enclosed by a thin semi-permeable *cell membrane* or *cytoplasmic membrane*, and also, in most cases, by an outer, relatively rigid *cell wall*. The protoplast is differentiated into a major part, the *cytoplasm*, and an inner body, the *nucleus*, which contains the hereditary determinants of character, the *genes*, borne on threadlike *chromosomes*. The bodies of higher plants and animals are multicellular, with interdependence and specialisation of function among the cells, the different kinds of cells being segregated in separate tissues. Many micro-organisms, on the other hand, are unicellular, existing as single self-sufficient cells, unattached to their fellows. Other micro-organisms grow as aggregates of cells joined together by their cell walls in clusters, chains, rods, filaments or mycelia (*i.e.* meshworks of branching filaments), and some grow as a plasmodium, or multinucleate mass of cytoplasm. Generally, these morphologically multicellular microbes are physiologically unicellular, each cell being self-sufficient and, if isolated artificially, able to nourish itself, grow and reproduce the species. Some specialisation of cell function, approaching that of true multicellular organisms, is encountered in colonies of moulds and higher bacteria; thus, certain cells comprising an aerial mycelium are specialised for the formation and dissemination of spores, and are dependent for their nutrition on the activities of other cells comprising a vegetative mycelium.

The majority of micro-organisms may be classified in the following large biological groups: (1) Algae, (2) Protozoa, (3) Slime moulds, (4) Fungi proper, or *Eumycetes*, including the moulds and the yeasts, (5) Bacteria or *Schizomycetes* ("fission fungi"), (6) *Rickettsiales* and (7) Viruses or *Virales*. Since the algae and slime moulds contain no species of medical or veterinary importance, they will not be dealt with in this book. The main differential characters of the other groups are as follows:

Protozoa.—Non-photosynthetic unicellular organisms (a few are colonial) with protoplasm clearly differentiated into nucleus and cytoplasm; holozoic, saprophytic or parasitic; regarded as the lowest forms of animal life, though certain flagellate protozoa are very closely related to photosynthetic flagellate algae in the plant kingdom; reproduce asexually by binary fission or multiple fission (schizogony), and in some cases also by a sexual mechanism. Some exhibit a definite life cycle with both sexual and asexual phases, and some form round, thick-walled resting cells, or "cysts".

Fungi.—Non-photosynthetic micro-organisms possessing relatively rigid cell walls; saprophytic or parasitic. *Mould forms.*—Grow as branching filaments (hyphae) which interlace to form a meshwork (mycelium); the hyphae are coenocytic (*i.e.* have a continuous multinucleate protoplasm), being non-septate or septate with a central pore in each cross-wall; reproduce by the formation of various kinds of sexual and asexual spores developed from the vegetative (feeding) mycelium or from an aerial mycelium which effects their air-borne dissemination. *Yeast* forms.—Ovoid or spherical cells which reproduce asexually by budding and also, in many cases, sexually with the formation of sexual spores; do not form a mycelium, although the intermediate *yeast-like fungi* may form a "pseudomycelium" of elongated cells.

Bacteria.—A varied group of small micro-organisms with primitive cellular organisation; generally unicellular, but the cells may grow attached to one another in clusters, chains, rods, filaments or, as in the "higher bacteria" (*Actinomycetales*), a mycelium. Their cells are smaller (mostly 0·4–1·5 μ in short diameter) than those of protozoa and fungi, in most cases have relatively rigid cell walls, are spherical (coccus), rod-shaped (bacillus), comma-shaped (vibrio), spiral (spirillum and spirochaete) or filamentous, and show little structural differentiation when examined by ordinary microscopical methods. Special staining methods show that they possess a central nuclear body which contains desoxyribonucleic acid and divides by simple fission without evidence of mitosis or chromosomes. They reproduce mainly by simple transverse fission. Certain species form endospores as a resting phase and some (actinomycetes) reproduce by formation of conidia (exogenously formed asexual spores). Many species are motile by means of flagella and some by active flexion of the cell body. Most are saprophytic or parasitic, and a few autotrophic or photosynthetic.

Rickettsiales.—Simple unicellular organisms which are rod-shaped, spherical or pleomorphic; smaller than most bacteria, though still resolvable by the light microscope (*i.e.* over 0·2 μ diam.) and mostly not filterable through bacteria-stopping filters; strict parasites which can grow only in the living tissues of a suitable animal host, usually intracellularly. A few exceptional species can grow in cell-free nutrient media containing body fluids.

Viruses.—The smallest and simplest of the micro-organisms; range between 0·3 μ and 0·01 μ in diameter, mostly being ultramicroscopic (*i.e.* smaller than resolvable by the light microscope) and filterable through bacteria-stopping filters; strict intracellular parasites capable of growth only within the living cells of an appropriate animal, plant or bacterial host, and never on inanimate nutrient medium. The viruses which infect and parasitise bacteria are named *bacteriophages* or *phages*.

The bacteria and viruses play the most important part in the causation of human infective disease. Protozoal infections are most prevalent in tropical and sub-tropical countries, while the common fungal infections are mainly superficial (*e.g.* skin infections) and of minor severity.

The remainder of this chapter will be devoted to the general biology of the bacteria. That of the other groups of micro-organisms will be dealt with in Chapters XXXV to XLV.

MORPHOLOGICAL STUDY OF THE BACTERIA

Microscopical examination is usually the first step taken for the identification of an unknown bacterium. The bacterium may be allocated to one or other of the major groups when its *morphology* and *staining reactions* have been observed. The morphological features of importance are the size, shape and grouping of the cells, and their possession of any distinctive structures such as endospores, flagella (or motility), capsules and intracellular granules. Staining reactions are observed after treatment by special procedures such as the Gram and Ziehl-Neelsen stains, the different kinds of bacteria being shown in separate colours due to their different permeability to certain decolorising agents. A preparation stained by one of these methods usually suffices for observation of the general morphology of the bacterium, but some morphological features can be demonstrated only by the application of further special stains.

Unstained Preparations of Living Organisms.—The morphology of bacteria can be studied in the first place by examining them microscopically in the unstained condition, suspended in a thin film of fluid between a glass slide and cover-slip (*i.e.* in an " unstained wet film "). In this way their general shape can be seen and their motility determined (pp. 18, 15). Certain very slender bacteria, however, such as the spirochaetes, are so feebly refractile that they can not be seen by the ordinary microscopic methods, and *dark-ground illumination* (p. 96) or *phase-contrast microscopy* (p. 103) is necessary for their demonstration.

Electron microscopy (p. 106) is now applied to the morphological study of bacteria. Although rarely available for routine diagnostic work, it is important in enabling demonstration of certain cell structures, *e.g.* fimbriae (p. 16), which cannot be observed by the light microscopes.

For the study of the development of individual organisms and the growth of bacteria in colonies (p. 30), the " agar-block " method Ørskov, and the microscope-incubator may be used (p. 265). These methods enable living bacteria to be observed at intervals during their actual growth on a suitable substrate, and present a more natural picture than other procedures involving manipulations which may sometimes create artificial appearances.

Stained Preparations.—The microscopical examination of fixed and stained preparations is usually an essential routine procedure. The bacteria are more readily discovered and studied when immobilised by fixation and darkly stained in contrast with the bright background. *Simple staining* is effected by the application of a watery solution of a single basic dye, *e.g.* methylene blue, methyl violet or basic fuchsin, or sometimes along with a mordant, *e.g.* dilute carbol fuchsin (p. 111). The coloured, positively charged cation of the basic dye combines firmly with negatively charged groups in the bacterial protoplasm, especially with the abundant nucleic acids. This staining is retained through subsequent washing with water to remove excess dye from the slide. Acidic dyes, having coloured anions, do not stain bacteria except at very acid pH values, and thus can be used for "negative staining" (see below) Cells or structures which stain with basic dyes at normal pH values are described as *basophilic* and those which stain with acidic dyes as *acidophilic*.

Prior to staining, the film or smear of bacteria must be fixed on the slide. *Fixation* is usually effected by heat; the slide is first thoroughly dried in air and then heated gently in a flame. Vegetative bacteria are thereby killed, rendered permeable to the stain, stuck to the surface of the slide and preserved from undergoing autolytic changes. Chemical fixatives are used for sections of infected tissue and films of infected blood, since they cause less damage to the tissue cells; they include formalin, mercuric chloride, methyl alcohol and osmic acid (p. 142 *et seq*)..

It should be noted that the bacterial cell wall (see below) is not stained by ordinary methods and the coloured body seen corresponds to the cell protoplasm only. This is usually much shrunken as a result of drying. Chains of stained bacteria thus show the coloured bodies separated by gaps which are the sites of unstained connecting cell walls.

Beaded and *Bipolar Staining.*—Certain bacteria do not colour evenly with simple stains. Thus, the diphtheria bacillus shows a "beaded" appearance, with alternate dark and light bars. The plague bacillus shows "bipolar staining", the ends being more deeply coloured than the centre. The uneven staining may be due to the manner in which the protoplasm shrinks when the cell is dried and fixed.

"*Negative*" or *Background Staining* is of value as a rapid method for the simple morphological study of bacteria. The bacteria are mixed with a substance such as India ink, or nigrosin (pp. 111, 122), which, after spreading as a film, yields a dark background in which the bacteria stand out as bright, unstained objects.

Silver Impregnation methods (p. 136) are utilised for the staining of spirochaetes, especially for demonstrating these organisms in tissues. The slender cells are thickened by a dark surface coating of silver deposit.

Impression Preparations (p. 127) are used for cytological studies when it is desired to avoid the distortion of cell structure and colonial arrangement inevitable in the normal procedure of preparing a smear, drying it and fixing it by heat. Bacteria newly spread on the surface of an agar medium, or grown on agar to form small colonies, are fixed *in situ*, without drying, to a slide or cover-slip. This is done by allowing a chemical fixative to diffuse through the agar from below. The fixed bacteria adhere to the glass when the agar is removed and can then be stained.

Staining Reactions.—The staining reactions of bacteria are of the greatest importance in their differentiation and identification. *Gram's staining reaction* (p. 112) has the widest application, dividing all bacteria into two categories named " Gram-positive " and " Gram-negative ", according to whether or not they resist decolorisation by acetone, alcohol or aniline oil after staining with a pararosaniline (triphenylmethane) dye, *e.g.* crystal or methyl violet, and subsequent treatment with iodine. The Gram-positive bacteria resist decolorisation and remain stained a dark purple colour. The Gram-negative bacteria are decolorised and then counter-stained light pink by the subsequent application of basic fuchsin, safranine, neutral red or dilute carbol fuchsin. In routine diagnostic work, a Gram-stained smear is often the only preparation examined microscopically, since it shows clearly the general morphology of the bacteria as well as revealing their Gram-reaction. It should be noted that species which are characteristically Gram-positive, may appear Gram-negative under certain conditions of growth; thus, some show an increasing proportion of partly or wholly Gram-negative cells in ageing cultures on nutrient agar. Gram-reactivity appears to reflect a fundamental aspect of cell structure and is correlated with many other biological properties. Thus, the different species of a single genus generally show the same reaction. Gram-positive bacteria are more susceptible than Gram-negative bacteria to the antibacterial action of penicillin, acids, iodine, basic dyes, detergents and lysozyme, and less susceptible to alkalies, azide, tellurite, proteolytic enzymes, lysis by antibody and complement, and plasmolysis in solutes of high osmotic pressure.

The mechanism of the Gram stain is not fully understood. Gram-positive organisms are able to retain basic dyes at a higher hydrogen-ion concentration than the Gram-negative species, showing an isoelectric point of pH 2–3 as compared with pH 4–5. The more acidic character of their protoplasm, which is enhanced by treatment with iodine, may partly explain their stronger retention of basic dye. It has also been suggested that the difference in Gram reaction depends upon a difference in the permeability of the cell wall or cytoplasmic membrane. After staining with methyl violet and treatment with iodine, a dye-iodine complex, or " lake ", is formed within the cell, which is insoluble in water but moderately soluble and dissociable in the acetone or alcohol used as the decoloriser. Under the action of the decoloriser, the dye and iodine diffuse freely out of the Gram-negative cell, but not from the Gram-positive cell, presumably because the latter's surface is less permeable to the decoloriser or its iodine solute. Whatever the complete mechanism of the reaction may be, Gram-positivity appears to depend upon the integrity of the cellular structure and the presence in the cell of a specific magnesium ribonucleate-protein complex (and maybe also of other specific compounds). Thus, Gram-positive bacteria become Gram-negative if they are ruptured mechanically, or if their magnesium ribonucleate is removed by autolysis or by treatment with bile salt or the enzyme ribonuclease. From cytological studies it seems that Gram-positive staining colours the whole cell, including the cell wall, but chemical analysis of isolated cell walls has not shown them to contain magnesium ribonucleate.

The *acid-fast staining reaction*, as revealed by the Ziehl-Neelsen method (p. 116), is of value in distinguishing a few bacterial species, *e.g.* the tubercle bacillus (p. 537), from all others. These " acid-fast " bacteria are relatively impermeable and resistant to simple stains, but when stained with a strong reagent (basic fuchsin in aqueous 5 per cent. phenol, applied with heat), subsequently resist decolorisation by strong acids, *e.g.* 20 per cent. sulphuric acid. Any decolorised non-acid-fast organisms are counter-stained in a contrasting colour with methylene blue or malachite green.

The acid-fast bacteria have an exceptionally rich and varied content of lipids, fatty acids and higher alcohols, and their acid-fastness has been attributed to this. When the lipids, including those firmly bound in the protoplasm, are removed by treatment with suitable solvents, the cells are no longer acid-fast. One of the lipids peculiar to acid-fast bacteria exhibits the property of acid-fastness in the free state; this is *mycolic acid*, a high molecular weight hydroxy acid containing carboxyl groups. The mere presence of such a substance in the cell is not by itself sufficient to explain acid-fastness, since the character is lost when the cell is ruptured by mechanical means or autolysis. Acid-fastness therefore depends on the structural integrity of the cell, its content of lipids and, possibly, a special anatomical disposition of the lipids.

Bacterial Protoplasm and Nuclear Bodies.—The protoplasm of the bacterial cell is a viscous watery solution, or soft gel,