Topley and Wilson's

Principles of bacteriology, virology and immunity

Seventh edition in four volumes

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Volume 2

Systematic bacteriology

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General Editors' Preface to 7th edition

After the publication of the 6th edition in 1975 we had to decide whether it would be desirable to embark on a further edition and, if so, what form it should take. Except for the single-volume edition of 1936, the book had always appeared in two volumes. We hesitated to alter this arrangement but reflection made us realize that a change would be necessary.

If due attention was to be paid to the increase in knowledge that had occurred during the previous ten years two volumes would no longer be sufficient. Not only had the whole subject of microbiology expanded greatly, but some portions of it had assumed a disciplinary status of their own. Remembering always that our primary concern was with the causation and prevention of microbial disease, we had to select that part of the newer knowledge that was of sufficient relevance to be incorporated in the next edition without substantial enlargement of the book as a whole.

One of the subjects that demanded consideration was virology, which would have to be dealt with more fully than in the 6th edition. Another was immunology. Important as this subject is, much of it is not directly concerned with immunity to infectious disease. Moreover, numerous books, reviews and reports were readily available for the student to consult. What was required by the microbiologist and allied workers was a knowledge of serology, and by the medical and veterinary student a knowledge of the mechanisms by which the body defends itself against attack by bacteria and viruses. We resolved, therefore to provide a plain straightforward account of these two aspects of immunity similar to but less detailed than that in the 6th edition.

The book we now present consists of four volumes. The first serves as a general introduction to bacteriology including an account of the morphology, physiology, and variability of bacteria, disinfection, antibiotic agents, bacterial genetics and bacteriophages, together with immunity to infections, ecology, the bacteriology of air, water, and milk, and the normal flora of the body. Volume 2 deals entirely with systematic bacteriology, volume 3 with bacterial disease, and volume 4 with virology.

To the last volume we would draw special attention. It contains 27 chapters describing the viruses in detail and the diseases in man and animals to which they give rise, and is a compendium of information suitable alike for the general reader and the specialist virologist.

The first two editions of this book were written by Topley and Wilson, and the third and fourth by Wilson and Miles. For the next two editions a few outside contributors were brought in to bridge the gap that neither of us could fill. For the present edition we enlisted a total of over fifty contributors. With their help every chapter in the book has been either rewritten or extensively revised. This has led to certain innovations. The author's name is given at the head of each chapter, and each chapter is prefaced by a detailed contents list so as to afford the reader a conspectus of the subject matter. This, in turn, has led to a shortening of the index, which is now used principally to show where subjects not obviously related to any particular chapter may be found. A separate but consequently shorter index is provided for volumes 1, 2 and 3, and a cumulative index for all four volumes at the end of volume 4. Each volume will be on sale separately. As a result of these changes we shall no longer be able to ensure the uniformity of style and presentation for which we have always striven, or to take responsibility for the truth of every factual statement.

We are fortunate in having Dr Parker, who has been associated with the 5th and 6th editions of the book, as the third general editor of all four parts of this edition and as editor of volume 2. Dr Geoffrey Smith with his extensive knowledge of animal disease has greatly assisted us both as a contributor and as editor of volume 3. Dr Fred Brown, of the Animal Virus Research Institute, has organized the production of volume 4, and Professor Heather Dick the immunity section of volume 1.

Two small technical matters may be mentioned. Firstly, in volume 2 we have retained many of the original photomicrographs and added others at similar magnifications because they portray what the student sees when he looks down an ordinary light microscope in the course of identifying bacteria. Elec-

tronmicrographs have been used mainly to illustrate general statements about the structure of the organisms under consideration. Secondly, all temperatures are given in degrees Celsius unless otherwise stated.

Apart from those to whom we have just expressed our thanks, and the authors and revisers of individual chapters, we are grateful to the numerous workers who have generously supplied us with illustrations; to Dr N. S. Galbraith and Mrs Hepner at Colindale for furnishing us with recent epidemiological information; to Dr Dorothy Jones at Leicester for advice on the *Corynebacterium* chapter and Dr Elizabeth Sharpe at Reading for information about *Lactobacilius*, to Dr R. Redfern at Tolworth for his opinion on the value of different rodent baits; to Mr C. J. Webb of the Visual Aids Department of the London School of Hygiene and Tropical Medicine for the reproduction of various photographs and diagrams, and finally to the Library staff at the London School and Miss Betty Whyte, until recently chief librarian of the Central Public Health Laboratory at Colindale, for the continuous and unstinted help they have given us in putting their bibliographical experience at our disposal.

GSW
AAM

Volume Editor's Preface

In this Volume we present descriptions of bacteria, and of other micro-organisms bearing a close resemblance to them, such as the spirochaetes, chlamydiae, rickettsiae and Mycoplasmatales, that may be encountered by medical and veterinary microbiologists. Our treatment of systematic bacteriology resembles fairly closely that of earlier editions of this book. The authors of individual chapters have endeavoured to strike a reasonable balance between giving suitable recognition to important earlier work and summarizing adequately the very large amount of information that has accumulated in recent years; with each succeeding edition this becomes a more difficult task. In their descriptions of individual bacteria, authors have given prominence to characters useful in classification and identification that can be detected by workers in moderately well equipped non-specialized laboratories, and to those that may be of interest to microbiologists concerned with disease in man or animals.

When presenting facts that may throw light on the ability of organisms to cause disease it has not always been easy to make consistent decisions about what should appear in this volume and what should be included in the accounts of the individual diseases in Volume 3. The general principle we have followed on the subject of pathogenicity is to describe the effect on small animals in this volume, and in Volume 3 the diseases to which the organisms give rise in man and the larger animals. Exceptions, of course, have had to be made for such organisms as the diphtheria bacillus, the tetanus bacillus, and the cholera vibrio, where toxin production and pathogenicity are so closely related as to render some repetition inevitable. In such cases cross-references are given so as to ensure that the reader is made fully aware of all the information we provide on the subject.

In order for the reader to trace any particular organism we have included in the index a list of bacterial genera and the chapter number in which each will be found, together with a list of bacterial species with a note of the genus to which each belongs.

In separating our description of the organisms themselves from the diseases to which they give rise we have adhered to the practice decided upon in the first edition of the book in 1929. At that time medical and veterinary workers were interested in bacteria almost entirely on account of their ability to cause disease. Little attention was paid to the nature and properties of the bacteria themselves. Topley, however, influenced to some extent by the botanists, concluded that bacteriology should be regarded as a branch of biological science and that bacteria should be studied in their own right. This decision, novel as it then appeared to the writers of medical textbooks, attracted some criticism, but it was soon justified by the rapid growth of bacterial chemistry and the many contributions this made to our knowledge of the growth, survival and death of bacteria, of their pathogenic activities, and of ways of identifying them. Opinion has now moved so much further in the same direction that it is important to remember that bacteria are not simply collections of enzymes and other macromolecules, or carriers of interesting pieces of nucleic acid, but are fellow members of the living world. In this volume we have endeavoured to keep this delicate balance.

1983 MTP

Contents of volume 2

Systematic bacteriology

Contents of volumes 1, 3 and 4

Contents of volume 1
General microbiology and immunity

Contents of volume 3
Bacterial diseases

Contents of volume 4
Virology

20

Isolation, description, and identification of bacteria

M. T. Parker

Obtaining pure cultures of bacteria

The first essential in the study of bacteria is to obtain them in pure culture. During the sixties and seventies of the last century, micro-organisms were perforce cultivated in liquid media, and the preparation of pure cultures under these conditions was difficult. The only method available was to dilute the cultures with a sterile fluid until there was only about one organism in each two drops and to seed a number of tubes of liquid medium each with one drop (Lister 1878); but this afforded no certainty that the cultures so obtained were pure. The introduction of solid media by Robert Koch in 1881 rendered possible the easy separation of different organisms, because most of them formed discrete colonies, and serial subculture could be made from single colonies. Originally the medium was spread in the melted state on glass slides and allowed to set. Later, the petri dish was introduced and agar replaced gelatin as the solidifying agent in the medium; the resultant technique has since remained virtually unchanged.

Collecting suitable samples

To isolate the desired organisms, we must ensure that a suitable sample of the material to be examined is provided. This will often not be collected by a

laboratory worker; we should therefore be prepared to spend time educating clinical and environmentalist colleagues in the correct methods of obtaining samples and transmitting them to the laboratory. These methods depend to some extent on the material sampled, the bacteria being sought and the practicability of rapid transport to the laboratory.

In many instances we are seeking to isolate pathogenic bacteria, which may not be present in large numbers. The site sampled must be one at which the pathogen is known to be harboured, and the sample should be of adequate size; these are matters upon which the laboratory worker should advise his clinical colleagues. In general, a quantity of pus or a piece of tissue, when obtainable, is preferable to a swab. When swabbing a dry surface, for example, the anterior nares or the skin, it is advisable first to moisten the swab (with peptone water, not water or saline). The swab should be rubbed vigorously on the site to be sampled, but care should be taken to avoid contact with adjacent areas likely to yield large numbers of irrelevant organisms; for example, when swabbing the throat, contact with the mouth and tongue should be avoided.

Blood for culture should be collected from a vein by means of a dry-sterilized needle and syringe or similar closed device immediately after disinfection of the skin (Chapter 4), and never through an existing intravenous catheter. It may be transferred to a specimen tube containing an anticoagulant, preferably liquid (sodium poylanethol sulphonate), because this neutralizes the bactericidal power of fresh blood (von Haebler and Miles 1938). Alternatively, bottles of medium may be taken to the bedside; several bottles should be inoculated with a total of 10 ml or more of blood, and the volume of medium should always be at least 10 times the volume of blood added. In most instances, one of these bottles should contain a medium suitable for the growth of fastidious anaerobes. (For blood-culture media, see Chapter 57). Specimen tubes and bottles of medium should be provided with a diaphragm covered by a removable plastic cap, so that the blood can be added without opening them.

At necropsy, solid organs should be sampled *in situ* after searing the surface with a hot iron; portions of tissue should be removed with sterile instruments. When this is impracticable, reasonably good results can be obtained by removing a large portion of tissue (at least a 30 mm cube), taking this immediately to the laboratory, plunging it into boiling water for 30 sec and sampling it from the centre. A method for sampling vegetations on heart valves removed at necropsy is described by Tyrrell and his colleagues (1979).

The difficulty of obtaining samples uncontaminated by normal flora has led to the introduction of various 'invasive' techniques of specimen collection. For example, lung puncture and transtracheal aspiration are used to sample the lower respiratory tract, and suprapubic puncture of the bladder is used to obtain uncontaminated samples of urine. These are valuable methods, but are not entirely without risk, so they should be used only when the information to be obtained is likely to benefit the patient, and by skilled operators.

For the collection of urine samples, see Chapter 57, and for further information about the collection of samples from patients, see Tyrrell *et al.* (1979) and Stokes and Ridgway (1980).

Samples for quantitative culture

For solid or liquid materials, the absolute and relative numbers of bacteria may be measured either by plating measured dilutions on to solid media and counting the colonies or by diluting to extinction by various modifications of Lister's (1878) method. Quantitative sampling of surfaces by means of swabs does not give results in absolute numbers, because only a proportion of the organisms is picked up and many of these remain attached to the fibres of the swab on plating or elution; nevertheless, valuable comparative results can be obtained if the method is carefully standardized. Alternatively, surfaces can be sampled by applying a measured volume of fluid, detaching the organisms by a standard procedure, and performing colony counts. A quite different method of sampling surfaces, by making impression-preparations, is widely used; agar medium may be applied directly to the surface (ten Cate 1959), or a moistened carrier, such as velvet, may be used to transfer the inoculum to an agar surface (Holt 1966). The resultant colony counts bear no relation to those obtained by swabbing or elution; they give some indication of the distribution of organisms on the surface, but none about the numbers present at the point at which a colony appears.

None of these cultural methods measures the number of viable bacterial cells, because bacteria tend to form clumps or chains. What we are measuring is the significant viable unit, which for colony-counting methods is the *colony-forming unit*. Attempts should therefore be made to standardize the degree of bacterial aggregation by using a constant method of shaking and a dispersing agent such as Triton X100.

(For a critical assessment of quantitative methods for the sampling of surfaces, see Favero *et al.* 1968. Methods for sampling air, water, milk, foodstuffs and environmental surfaces were discussed in Chapter 9).

Transport to the laboratory

Every effort should be made to ensure that the desired organism is viable when it reaches the laboratory. Organisms may die out in specimens for a variety of reasons: bacterial multiplication may cause an adverse change in pH; a number of organisms are very sensitive to oxygen and some to drying. Thus, no one set of conditions is likely to be optimal for all pathogens. Rapid transport of samples to the laboratory is always desirable, and in a few instances there is no alternative

to it except the inoculation of media directly from the sampling site. Swabs, whether made of cotton or synthetic fibres, tend to contain inhibitory material (see Chapter 4 and Dadd *et al.* 1970); these may be removed or neutralized by boiling in phosphate buffer, by impregnating with horse serum (Rubbo and Benjamin 1951) or bovine albumen when viruses are also being sought (Bartlett and Hughes 1969), or by coating the swab with powdered charcoal.

Several general-purpose transport media have been designed in which most pathogens will survive without being overgrown by more robust organisms. The most successful are semisolid media containing thioglycollate but little nutrient (Stuart 1959, Amies 1967, Gästrin *et al.* 1968). For the survival of the more fastidious anaerobes, swabs should be submerged deeply in a long column of semisolid thioglycollate medium (see also Hill 1978). Material from transport media is seldom suitable for direct microscopic examination. Some pathogens, such as streptococci, survive better when completely dry than at intermediate humidities. Strips of filter paper may be seeded from a swab and air-dried before despatch (Hollinger *et al.* 1960) or the swab may be placed in contact with a dehydrating agent (Redys *et al.* 1968). Virus-transport media often contain antibiotics and are thus unsuitable for the isolation of bacteria, rickettsiae and chlamydiae. There is little advantage in refrigerating specimens during transit to the laboratory, except when quantitative culture is to be performed after significant delay. Specimens for the culture of *Chlamydia* should be transported in liquid nitrogen if it is impracticable to inoculate media directly from the patient (Tyrrell *et al.* 1979).

Plating on solid medium

This is usually the first step in obtaining a pure culture. The media chosen will depend upon what bacteria are sought; guidance on this is given in the appropriate chapters of this book. After the inoculum has been seeded on to a plate, careful streaking with a loop or bent glass rod to spread it in an even gradient of decreasing concentration is essential if well spaced colonies are to be obtained.

Attention must be given to providing the optimal temperature and gaseous atmosphere for growth. Most pathogens will grow at 37°, but some do rather better at a slightly lower temperature; 36° is therefore preferable for routine diagnostic bacteriology. Some organisms that grow in the presence of oxygen do so only when CO_2 is added; now that CO_2 incubators are generally available, an increasing proportion of primary cultures are made in air + 5–10 per cent of CO_2. Growing interest in the non-sporing anaerobes, and the recognition that some of them die rapidly when exposed to oxygen, have led to the design of very elaborate systems for anaerobic culture, in which all operations are performed in a special cabinet that has been repeatedly flushed with oxygen-free gases and in which media are pre-reduced and sterilized anaerobically (Moore 1966). Some workers (e.g., Drasar 1967, Moore *et al.* 1969, Peach and Hayek 1974) claim to have obtained much better results in this way than by conventional anaerobic-jar techniques. Others report equally good results with anaerobic jars when sufficient attention is given to the maintenance and regular testing of jars (Watt *et al.* 1976), when freshly poured medium is used and CO_2 is added to the oxygen-free atmosphere (Watt *et al.* 1974), and when the jar is not opened until it has been incubated for 48 hr (Wren 1977).

After the primary plate has been incubated, it is inspected under magnification and a representative of each colonial type is subcultured with a needle. Careful picking usually results in pure cultures, but this cannot be guaranteed. It is therefore advisable to replate supposedly pure cultures at least once and to inspect the resultant colonies for homogeneity. The plates should be fairly dry; if there is a layer of moisture on the medium, organisms are apt to form a film of confluent growth over the whole surface. This often leads to difficulties in obtaining pure cultures of organisms, such as many of the clostridia, that tend to spread unless the surface of the medium is very dry. The pour-plate method is, in general, of less value. Deep colonies are not usually as characteristic as surface colonies, and subculture from them carries greater risks of contamination.

Single-cell methods Growth of a culture from a single cell provides certainty that it is pure. Briefly, single cells are identified microscopically in tiny liquid droplets or on an agar surface, and are transferred to a liquid medium or removed to a sterile area of the agar remote from all other bacteria by means of a micro-manipulator (see Johnstone 1973).

Isolating organisms from mixtures

Numerous methods have been devised to isolate organisms that form a minority of a bacterial population. These methods seldom yield pure cultures in the first instance; their use must be followed by subculture from a single well spaced colony.

Firstly, the material itself may be treated in such a way that the unwanted organisms are destroyed or are separated physically from the organisms to be cultured. *Heating* a mixture to 80° for 10 min will destroy all the vegetative bacteria but not the spores. This method is used in the purification of clostridia. *Chemicals* with a germicidal action are useful in destroying susceptible organisms while leaving the more resistant unaffected. For example, tubercle bacilli are more resistant than are most other vegetative organisms to chemical disinfectants. A common method of isolating them is to treat sputum with acid or alkali strong enough to kill the accompanying bacteria. *Filtration* may be used to separate viruses from bacteria. L-forms and small bacteria such a *Campylobacter* can grow slowly through a membrane filter and so be separated from larger organisms. The membrane is placed on the surface of a suitable medium and a drop of fluid containing the organisms to be isolated is placed on it; after incubation, the membrane is removed and the plate re-incubated.

Various methods have been devised for separating motile from non-motile or less motile organisms. The device most often used is that described by Craigie (1931). It consists of a test-tube of semisolid agar medium containing a piece of glass tubing which projects above the surface of the agar. The medium in the inner tube is seeded with the culture under test. Motile organisms pass down the inner tube and up the outer tube, from the top of which they may be subcultured after incubation (see also Tulloch 1939). Flagellar agglutination may be used to inhibit the motility of one organism in the presence of another, as in separating the phases of diphasic salmonellae (see Chapter 37). Although strict aerobes will not migrate through semisolid agar, these methods can be applied to pseudomonads if nitrate is added to the medium (see Chapter 31).

Secondly, the conditions of growth may be so arranged that the desired organism is favoured. It is sometimes possible to make use of the *optimum temperature for growth* of an organism. Thermophilic bacteria may be separated from others by growth at 60°. Again, certain bacteria will not grow at 22°, whereas others will. In this way *Branhamella catarrhalis* may be separated from the meningococcus. *Aerobic and anaerobic* culture may be used to separate some groups of bacteria. Incubated aerobically the strict anaerobes will not grow, but many of them, e.g. most clostridia, will survive; incubated anaerobically most of the strict aerobes will not grow, but some of them (see Chapter 31) can obtain oxygen by denitrification and will therefore grow unless the nitrate content of the medium is very low. Many facultative anaerobes do not grow as well under anaerobic as under aerobic conditions.

The addition of *chemical substances* to a medium may facilitate the isolation of an organism by stimulating its growth or by inhibiting the growth of other organisms that may be present in larger numbers. If either type of substance is added to a liquid medium, the desired organism increases in numbers both absolutely and relatively to other organisms. Such a liquid is known as an *enrichment medium*. If, on the other hand, a substance that inhibits the growth of some organisms is added to a solid medium, the colonies that form will be mainly of other organisms that are resistant to the substance. Such a solid medium is known as a *selective medium*. It is common to use the two types of medium in conjunction, inoculating the material first into enrichment medium and later subculturing from this on to a selective medium. The colonies on a selective medium are, however, not necessarily pure. At the base of the colony other organisms may be present which, though unable to develop on the selective medium, will grow when transferred to a non-selective medium. It is therefore wise to replate organisms from a selective medium on to a plain medium before beginning to study them.

Enrichment media containing substances that are a source of energy only for some organisms have been widely used by soil microbiologists. Another example of the same principle is the use of tetrathionate broth for the enrichment of salmonellae, which obtain energy by means of a tetrathionate reductase (Pollock *et al.* 1942); but some other enterobacteria, including *Proteus* and *Citrobacter*, also form this enzyme (L. Minor and Pichinoty 1963), so it is advisable also to include selective chemicals in tetrathionate broth.

A novel selective procedure is to add to the medium a substance that is not harmful to bacteria but may be metabolized by some strains with the production intracellularly of a toxic substance. Thus *Esch. coli* and other organisms that form β-galactosidase will hydrolyse phenylethyl β-D-galactopyranoside with the release of phenylethyl alcohol; organisms, such as salmonellae of sub-genus I, which lack this enzyme, are unaffected (Johnston and Thatcher 1967, Johnston and Pivnick 1970).

Selective substances used either in enrichment broths or in solid selective media include aniline dyes, metallic salts and bile salts. In recent years, the use of antibiotics in selective media has greatly extended their range and effectiveness. Many examples will be found in the chapters devoted to the description of individual organisms.

Thirdly, an *indicator* may be added to the medium, which changes colour when a certain organism or group of organisms develops. Thus, the diphtheria bacillus reduces sodium tellurite, whereas many of the organisms likely to be associated with it in a throat swab do not. When this substance is added to the medium, the colonies of the diphtheria and of the diphtheroid bacilli are coloured black; those of the streptococci and numerous other organisms are colourless. Indicators are frequently used to detect the production of acid from a carbohydrate incorporated in the medium. Blood is a very useful indicator. Some organisms produce no alteration in it, others form from it a green pigment, and others lyse it completely.

Selective agents and indicators are frequently included in the same medium. Thus in MacConkey's medium, bile salts are added to inhibit the growth of organisms other than those capable of multiplying freely in the intestine, and lactose and neutral red are added to distinguish the lactose-fermenting coliform organisms from the non-lactose-fermenting group.

Finally, use may be made of the fact that certain *pathogenic organisms* are invasive and when injected into a susceptible laboratory animal can be isolated from an organ distant from the site of injection. The introduction of this method of separating organisms from one another we owe to Koch (1880). As examples, we may quote the tubercle bacillus in pus, the pneumococcus in sputum, and Whitmore's bacillus in surface waters. The contaminating organisms are rapidly killed in the animal body, whereas the pathogenic organism multiplies and can be recovered in pure culture from the tissues. This method is not without danger. If the animal happens to be suffering from a latent infection with some organism, this organism may be isolated in culture and thought mis-

takenly to have been derived from the material injected into the animal (see Wilson 1959).

Maintenance of pure cultures

Having obtained an organism in pure culture, the next task is to keep it in that state while it is being studied. If a reasonably good technical standard is maintained this should seldom prove difficult. But the possibility of contamination, or the inadvertent substitution of one culture by another, should always be kept in mind. When an unexpected result is obtained, this should be an indication for replating the culture and comparing the appearances of the colonies with that recorded earlier.

Protracted biochemical and genetic studies are particularly liable to be marred by contamination. Before they are begun, therefore, freeze-dried stocks of the cultures to be used should be prepared, samples of them should be checked for purity, and a full cultural and biochemical examination of the organisms should be made. When possible, they should be typed serologically or by means of phage. These tests should be repeated at intervals during the experiments. Particular care should be taken to avoid errors due to contamination or substitution when organisms are re-isolated after injection into animals and when they are being repeatedly selected on plates containing inhibitory agents. Suspicion should be aroused when 'variants' are isolated that appear to differ from the parent organism in several phenotypic characters.

Identification of bacteria

This is performed within the framework of *bacterial classification*, in which species and genera have been carefully defined and comprehensively described, and are each represented by a type culture; order is preserved by the use of an agreed system of *bacterial nomenclature* (Chapter 21). Much help can be obtained by carrying out a direct comparison of the unknown organism with type cultures of the organisms it appears to resemble, but we must always remember that no two bacterial strains are identical in all respects.

Ideally, the characters of the unknown organism should be compared with those of all recognized groups of bacteria, but limitations of personal experience and time make this impracticable. Most of us work with material from a limited environment; the medical bacteriologist is familiar with organisms isolated from man and mammals, and from their immediate surroundings, but the plant pathologist and the marine bacteriologist, for example, work with quite a different range of organisms. Therefore, a number of almost independent systems of bacterial identification are used in practice. This is satisfactory most of the time, but occasionally an 'intrusion' from an unfamiliar environment may not be recognized, such as the soil and water organisms that are now an important cause of infection in hospital patients.

In the classification of organisms (Chapter 21) we take into consideration all their ascertainable characters, but in bacterial identification we are more selective. We learn from experience, or we are taught, which tests are most useful in distinguishing one organism from another, and we deploy these tests in such a way as to arrive at an identification in the most economical manner. We are usually identifying an organism for a purpose, and the information is often needed quickly. Sometimes we need to know only whether the organism in question does or does not belong to a particular group or species. In these cases our system of 'identification' may be very simple indeed. If we are dealing with a culture from a familiar source we may pick out a characteristic colony from a primary plate and apply to it a small number of tests—or even a single test.

When confronted with a quite unfamiliar organism we have to engage in a more comprehensive process. We begin by examining a few of the basic characters of the organism. In medical bacteriology these would probably include: a Gram and an acid-fast stain; a motility test; a spore stain and a test for resistance to 80° for 30 min if the organism was a gram-positive rod; a comparison of growth in the presence and absence of air; an oxidase and a catalase test; and a test for the ability to oxidize or ferment glucose. With this information we should be able to allot the organism tentatively to one of the main groups of organisms of medical importance and then proceed to a series of secondary tests appropriate for the group in question (see Cowan 1974).

We shall now discuss in turn the various sorts of tests that may be used for the identification of bacteria. It is no longer practicable to include technical details of the tests; reference should be made to one of the books devoted to this subject, for example, those of Skerman (1969) and Cowan (1974). Only widely applied tests are mentioned; others used for the identification of particular organisms are described in the appropriate chapters in this volume.

Morphological appearances

Under this heading we include the shape and size of the organism, its arrangement and its motility, the number, distribution and shape of flagella, the presence of fimbriae, the shape and situation of spores,