AIDS TO BACTERIOLOGY

H. W. SCOTT-WILSON

NINTH EDITION

STUDENTS' AIDS SERIES

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PREFACE TO NINTH EDITION

THE ninth edition of "Aids to Bacteriology" is designed primarily to provide the medical student with a survey of the bacteria, viruses, fungi and protozoa of medical importance. It is hoped that the non-specialist practitioner also will find it a convenient guide to present-day bacteriology.

The general arrangement and scope of this edition are similar to those of the eighth, but the text has again been revised and brought up to date. Although it is becoming increasingly difficult to do so, it has been possible to find room for the necessary amount of new matter, without any substantial increase in size, by compression or omission of less important, older material.

Nomenclature is still a difficulty. In general, that given in Bergey's Manual is employed, but in some cases, where that terminology is not in common use in this country, the more generally recognized names are adopted.

An Appendix has been added in which the more commonly encountered bacteria have been grouped according to their outstanding characteristics. The tables thus formed are designed for the easier identification of such organisms, and it is hoped that they may prove to be useful to the student.

My grateful thanks are again due to Dr. Frank Kellett and Dr. J. H. Scott-Wilson for their helpful advice and criticism.

H. W. SCOTT-WILSON.

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CHAPTER I

THE GENERAL BIOLOGY OF BACTERIA

BACTERIA are minute vegetable organisms, each individual consisting of one cell only. All species perpetuate themselves by a process of fission; this property, and the fact that they contain no chlorophyll, are the features by which botanists assign bacteria their position in the scale of plant life (Schizomycetes).

Partly by force of circumstances, partly for convenience, the science of bacteriology is not restricted to the study of bacteria. So in Chapter XXII the moulds and yeasts, and in Chapter XXIII some lowly forms of animal life, are dealt with.

Anatomy and Composition.—Three fundamental forms are recognised—cocci (spheroidal forms), bacilli (straight rods) and spirilla (curved rods).

The bacterial cell contains four different structures —cytoplasm, cytoplasmic membrane, cell wall and capsule. The cytoplasmic body is composed of colloidal protoplasmic material surrounded by a cytoplasmic membrane consisting partly of lipoproteins (Knaysi, 1944). This cytoplasmic membrane can be photographed with the electron microscope. The cell wall, which can also be seen with the electron microscope, surrounds the cytoplasmic membrane and gives rigidity and elasticity to the cell. Its chemical composition is not fully known, but may vary with the species, being carbohydrate in some and nitrogenous in others (Knaysi, 1944).

Capsules.—The cell membrane in many species (pneumococcus, Friedländer's pneumobacillus, etc.) secretes a *capsule*. The capsules of the pneumococci and Friedländer's bacilli are composed of complex polysaccharides, while the capsules formed by the anthrax bacillus consist of a polypeptide (Bovarnick, 1942). Capsule formation is best seen in preparations derived from animal bodies; in cultures on artificial media they tend to disappear.

Bacteria may cluster together in a jelly-like mass known as a *zooglœa.* But capsulated bacteria are not all "slime-formers" and, conversely, a zooglœa may be composed of bacteria which, so far as is known, do not form capsules.

Chemical Composition.—The constituents found by chemical analysis are: (1) protein (globulins and albumens), (2) nucleins and nucleoproteins, (3) carbohydrates, (4) fats and waxes, (5) lipoids and (6) inorganic salts, in very variable proportions.

Scattered through the cell or massed at the poles of certain bacteria are granules that stain more deeply than the remainder of the cell (metachromatic granules). The metachromatic granules of bacteria of the diphtheria group consist of volutin (Fleuche and Henneberg, 1934), while granules of fat, starch and glycogen are known also to occur. They are probably reserve food substances.

Nuclei.—Special staining methods and electron microscope photographs show that nuclear material is present, either scattered through the cytoplasm or in the form of a definite nucleus dividing with the cell (Knaysi and Baker, 1947).

Movement.—Many bacteria, particularly bacilli and spirilla, are capable of motion, produced by little protoplasmic threads (flagella). The exact relation of these flagella to the structure of the bacterial cell is not yet known.

Some organisms, such as the cholera vibrio, have a single flagellum at one end (*monotricha*), in others there is one at each pole (*amphitricha*); the flagella may assume the form of a tuft at one pole (*lophotricha*), or they may be scattered round the cell (*peritricha*). The position of the flagella is always the same in each species but, in those species having more than one flagellum, the number is not always constant and depends on the health of the culture. Flagellated organisms are not always motile, but go through a resting stage. The largest number of flagella is found in the cells of young (twenty-four hours) cultures in suitable culture media. Flagella are not visible when the organism is examined under the microscope in the usual way, but they can be observed in specimens specially stained, also by dark-ground microscopy and the electron microscope. Motility must not be confused with "Brownian" movement which is an oscillatory movement shown by small, solid particles (including bacteria) suspended in a fluid medium. It is due to molecular motion in the fluid. True motility causes bacteria to alter their relative positions in the surrounding medium.

Size.—The unit of measurement adopted is the "micron," which is equal to 0·001 millimetre, and is represented by the letter μ. The majority of bacterial species do not show great differences in size, most of the bacilli, for instance, measuring about 2 μ to 4 μ in length and the cocci about 0·7 μ to 1 μ. Larger forms, however, do occur; the spirochaete of relapsing fever may attain a length of 40 μ.

Smaller forms occur in gradation through the *Rickettsia* and larger viruses, which are just visible through the optical microscope (limit 0·2 μ), to the minute virus of foot and mouth disease (0·01 μ).

Reproduction.—Bacteria are asexual and propagate by transverse fission. There is no conclusive evidence of the occurrence of other modes of reproduction. When a cell has attained the maximum size for its species, it elongates, with constriction round its middle, followed by a simple partition. Hence the family name of

Schizomycetes. Two young cells are thus formed from the mother cell. This process may be repeated as often as once in twenty minutes if conditions are favourable. An increase in geometric progression is not consistently maintained, however, for when the bacterial concentration reaches the maximum that the medium can support, the number of living organisms tends to become stationary. This is probably due to exhaustion of oxygen supply rather than of the nutritive properties of the medium or the production of toxic metabolites.

Bacteria do not always separate after fission. Division may occur in one plane with the formation of a chain (streptococci, bacterial chains); in two planes, producing tetrads (*Gaffkya tetragena*); in three planes, producing cubical packets (sarcina); or irregularly, producing a cluster (staphylococci).

When growing on a solid culture medium, the individual organisms are unable to separate and consequently become aggregated in the same place, forming a visible colony.

When bacteria have occupied the same site in the body, or lived in the same culture for a long period, or if conditions for growth are otherwise unfavourable, abnormal shapes and sizes are produced (involution forms). These generally take stains less readily, or else do not stain at all. Other characters, such as pathogenicity or fermentative power, are likely to diminish, and there is general evidence of degeneracy.

Spore formation is not common. It occurs only in bacilli, not in cocci or spirochætes. When sporulation is about to take place a granule appears in the protoplasm, slowly enlarging to form a highly refractile, round or ovoid body enclosed in a tough membrane. Spores exhibit marked resistance to heat, desiccation and chemical agents. They are thus able to preserve their species through most disadvantageous circumstances. When favourable conditions recur, the spore loses its refractile appearance, elongates and bursts its membrane to extrude an organism which divides in the usual manner. Only one spore is formed in a cell, so that this cannot be regarded as a reproductive process.

Spores may be oval or round. In the aerobic sporing bacilli they are of smaller diameter than the body of the organism, but in the anaerobic *Clostridium* they are larger, causing a swelling of the cell. When terminal and round, such large spores will give a "drumstick" appearance to the organism. Spores are formed only when suitable conditions are present. The temperature must be favourable for growth; spores are not formed if it is too low. An increased oxygen supply favours sporulation and it tends to occur as the available food supply becomes exhausted, and in old cultures.

Nutrition.—In order to grow and multiply, all bacteria must be provided with the following requirements: (1) water, (2) energy supply, (3) nitrogen, (4) carbon, (5) inorganic salts, (6) in some cases growth factors (vitamins, etc.).

(2) *Energy Supply.*—In bacteria of the order Rhodobacteriinæ a chlorophyll-like substance is present, and energy obtained by photosynthesis. In all other aerobic bacteria energy is obtained by the oxidation of a variety of substances, varying with the species. Among inorganic substances, iron, sulphur, hydrogen, ammonia, nitrites or carbon monoxide may be oxidized, while organic substances ranging from methane, alcohol, carbohydrates to complex organic compounds may be utilized. The necessary oxygen is obtained from the atmosphere.

In anaerobic organisms (which cannot grow in the presence of oxygen) energy is derived from the simultaneous reduction and oxidation of organic substances by enzyme action, with release of energy.

(3) *Nitrogen.*—Organisms of the species *Azotobacter*

can obtain nitrogen from the atmosphere. Other organisms utilize nitrogenous compounds such as ammonia and amino-acids.

(4) *Carbon.*—Apart from the photosynthetic species which can reduce carbon dioxide, bacteria obtain their carbon from carbohydrates, alcohols or fatty acids.

(5) *Inorganic Salts.*—The exact requirements of bacteria in this respect are not known, but phosphorus, sulphur and iron appear to be the most important, although many other elements are frequently present. They are derived from salts in the media in which the organisms grow.

(6) *Growth Factors.*—These substances are necessary for the growth of some bacteria, but not of others. They may be vitamins (co-enzymes) of various types or amino-acids essential for the structure of the organism.

Growth on Artificial Culture Media.—In preparing culture media care must be taken to supply all the substances (including growth factors) required by the organism it is desired to grow. In some cases, however, the requirements of the organism are so complex that they can be supplied only by a living body, or "host." Such organisms are known as parasites.

Parasites may grow exclusively in animal bodies (obligate parasites) or may be capable of growing also on non-living matter (facultative parasites). In true parasitism the parasite benefits at the expense of the host. If bacteria grow on dead organic matter, they are called "saprophytes."

When the association of two different organisms is favourable to both, the condition is known as "symbiosis" ("mutualism" in America) and the organisms as "symbionts."

The reaction of the medium has an important effect on the growth of bacteria in it. Each species has an optimum *p*H at which growth is most prolific, and upper and lower limits beyond which growth will not take place.

Metabolism is the general term for all chemical processes associated with life. It is divided into:

(1) *Anabolism.*—The building up of the body of the organism from simpler food substances.

(2) *Catabolism.*—The breaking down of food substances to provide energy to carry out the anabolic processes. This is done by a variety of oxidation processes, activated by enzymes, and bacteria are divided into groups according to their oxygen requirements.

Aerobic bacteria require molecular oxygen and cannot grow in its absence.

Facultative anaerobes usually grow best in the presence of oxygen, but can grow without it.

Micro-aerophilic organisms grow best at a low oxygen tension.

Anaerobic bacteria cannot grow in the presence of free oxygen. They, in effect, obtain oxygen by the reduction of certain organic substances, the oxygen thus rendered available being utilized to oxidize other substances, the net result being that a balance of energy is rendered available to the organism.

Products of Metabolism.—These may be divided into: (1) the cell substance; (2) substances serving some useful purpose in the cell economy (enzymes, etc.); (3) waste and disintegration products formed by the breaking down of food substances.

Production of Heat.—All micro-organisms during growth produce heat. Thermophilic bacteria, some of which have an optimum temperature as high as 60° to 70° C., are not uncommon in manure, hay, cotton, etc., and may cause the temperature to reach 70° C. or higher. Under suitable conditions oxidation may then continue to raise the temperature further until the haystack or cotton bale is set on fire.

Photogenesis.—Phosphorescence is often due to bacteria. Numerous varieties of photogenic bacteria are known, many but not all of which are of marine origin. It is generally accepted that bioluminescence is caused by the action of an enzyme "luciferase" on a substance "luciferin" in the presence of oxygen and water, the luciferin being oxidized to "activated oxyluciferin." Energy is transferred from the activated oxyluciferin to the enzyme itself to form "activated luciferase" which emits the energy in the form of light and reverts to luciferase. The chemical nature of these substances is not known.

The phosphorescence seen on dead fish, meat, etc., is due to the growth of phosphorescent bacteria. One species (*Bacterium hæmophosphoreum*) is known to be pathogenic to insect larvæ, causing the infected larvæ to glow in the dark.

Pigment Formation.—A number of bacteria produce pigments. The *raison d'être* of these pigments is uncertain.

The pigment in some species remains in the bacterial cell, in others it is diffused into the surrounding medium. The colours vary, red (*Serratia marcescens*), brownish yellow (*Staphylococcus aureus*), violet (*Chromobacterium violaceum*) and blue (*Pseudomonas æruginosa*) being common types.

Fluorescence.—Some bacteria produce a fluorescent pigment soluble in the culture medium.

Production of Phenol.—Organisms capable of producing phenol have been isolated from the fæces. *Bacillus phenologenes* (Berthelot) with *l*-tyrosine as the only organic nutrient can produce 800 milligrammes of phenol per litre in fifteen days at 37° C.

Oxidation products are very numerous and formed from a large variety of organic and inorganic substances. The formation of alcohol, lactic and acetic acids and carbon dioxide from carbohydrates by species of *Lactobacillus* and the oxidation of ammonia to nitrites and nitrates by *Nitrobacterieæ* are examples.

Reduction.—An instance of this is the reduction of nitrates to nitrites by species of *Pseudomonas.*

Fermentation by bacteria.—Most bacilli and many cocci are capable of fermenting carbohydrates or related alcohols, the fermentation reactions of the bacilli of the coli-typhoid group being of special importance.

Specific enzyme production is an important aid to the identification of many organisms. A *coagulase* causing the coagulation of blood serum is produced by pathogenic staphylococci; the presence of *oxidase*, giving a purple colouration with tetramethyl-*p*-phenylenediamine, helps to indicate the presence of colonies of gonococcus in a mixed culture; while production of *urease* (converting urea to ammonia) and *catalase* (decomposing hydrogen peroxide) are other aids to identification.

Putrefaction.—Nitrogenous substances, such as the proteins, are decomposed by bacteria, particularly by those of the *Proteus* and *Clostridium* groups. The insoluble albumens, etc., are first converted into albumoses and peptones, then amino-acids are produced, with a variety of other substances, such as fatty acids, basic bodies and gases.

Indole.—Indole is one of the final products of the decomposition of proteins and is of importance in bacterial identification because organisms, otherwise very similar, may differ in regard to the production of this substance. To ascertain if an organism produces indole, it is inoculated into peptone water (2 per cent.) or into a glucose-free broth. The culture is incubated for twenty-four hours or longer and tested for the presence of indole. Ehrlich's reaction is reliable, the reagent being as follows: 4 grammes of paradimethylamidobenzaldehyde are dissolved in 380 millilitres of absolute alcohol and 80 millilitres of concentrated hydrochloric acid.

To 5 millilitres of the culture 1 millilitre of the reagent and of a saturated aqueous solution of potassium persulphate are added, when a rose-pink colour is produced if indole is present.

Salkowski's reaction (the formation of a red colour with sodium nitrite and sulphuric or hydrochloric acid) is not specific, being given also by indole-acetic acid.

The cholera vibrio is able both to produce indole from peptone and to reduce nitrates to nitrites. If grown in a nitrate-peptone medium Salkowski's reaction is given on the addition of acid alone (the cholera red reaction).

The production of indole depends on the presence of the tryptophane group in the culture medium.

Acetyl-methyl-carbinol.—Organisms of the genus *Aerobacter* produce acetyl-methyl-carbinol from glucose. On the addition of alkali, in the presence of air, acetyl-methyl-carbinol is oxidized to diacetyl, which reacts with peptone in the culture medium to give a red colour (Voges-Proskauer reaction).

Production of Acid and Alkali.—Many bacteria produce ammonia by the decomposition of nitrogenous matter, while the power of some to ferment sugars, with the production of acid, and sometimes of gas as well, is used for diagnostic purposes.

Antibiotic Substances.—A large number of bacteria and moulds have been found to produce substances which, in low concentrations, kill or injure other organisms, but do not injure the parent organism. Most of these are too toxic or unstable for use in medical treatment, but penicillin (from *Penicillium notatum*) and a number of other antibiotics are now widely used. (See Chapter xxiv.)

Ptomaines.—Among the substances formed during the bacterial decomposition of proteins are various amines which, under the name of ptomaines, were formerly considered to be causes of food poisoning. It is now known that these amines are relatively non-toxic and are present only in conditions of advanced decomposition when it would be unlikely that the food would be eaten.

Most instances of *food poisoning* are due to infection with some specific organism, such as *Salmonella enteritidis*, present in the foodstuff. Toxic substances having an irritant effect on the gastro-intestinal mucous membrane may, however, be produced by the growth of staphylococci in foodstuffs and also possibly by the growth of some organisms that may be otherwise non-pathogenic. The nature of these toxic substances is not known.

Toxins.—Bacteria capable of producing disease in most cases effect this by the production of "toxins." Toxins are supposed to be protein in nature, are soluble and non-crystallizable. They are probably not products of protein disintegration, like the ptomaines, but are specific metabolic products of the bacterial cell.

Toxins that diffuse readily from the bacterial cell into the surrounding medium are termed exotoxins. The diphtheria and tetanus bacilli produce such exotoxins, and, if a fluid culture be passed through a Pasteur filter, the toxins pass through, giving a toxic filtrate. Toxins retained in the bacterial cell as an integral part thereof are called endotoxins or intracellular toxins. The bacilli of typhoid, anthrax and cholera produce endotoxins, which on filtration are retained in the organisms on the filter, and the filtrates have but slight toxic properties.

Temperature Requirements.—As a rule, bacterial growth ceases at temperatures below 12° C. and above 42° C. For each species there is an "optimum" temperature at which it thrives best, and a range of temperatures at which growth will occur less profusely. The optimum temperature differs more or less according to the species. Normal inhabitants of the human body and