Whitby & Hynes

MEDICAL BACTERIOLOGY

Including Elementary Mycology and Parasitology

Eighth Edition

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Including Elementary Mycology and Parasitology

EIGHTH EDITION

By

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PREFACE TO THE EIGHTH EDITION

THE three years which have passed since publication of the Seventh Edition of *Medical Bacteriology* have predictably seen a continued advance in knowledge of viruses. Their nature and mode of action is more clearly understood, and enough has been learnt of their structure to permit a morphological classification. The text has accordingly been revised.

Less predictably, immunology has entered into a renaissance. On the one hand the demonstration of immunological tolerance, and on the other the development of surgical techniques making organ grafts possible, have combined to give a new importance to tissue immunology. Appropriate changes are recorded in Chapters 7 and 8.

Artificial modifications of the penicillin molecule have produced new antibiotics, but many other antibiotics which were thought to be new have recently been shown to be little different from older ones. Their position in the glossary makes this clear.

Although the scope and aims of this volume remain as hitherto, minor changes of emphasis and the advent of new knowledge have together entailed numerous small additions and alterations throughout this edition.

M. H.

London, 1964

The Classification of Bacteria of Importance in Medicine

SCHIZOMYCETES

Family	Genus	Example of Species	Synonyms
Order Eubacteriales			
Micrococcaceæ	Staphylococcus	*Staph. aureus*	*Staph. pyogenes, Micrococcus pyogenes*
	Micrococcus	*M. ureæ*	
	Sarcina	*Sarc. ventriculi*	
Lactobacillaceæ	Streptococcus	*Str. pyogenes*	
	Diplococcus	*Dip. pneumoniæ*	Pneumococcus, *Str. pneumoniæ*
	Lactobacillus	*L. acidophilus*	
Neisseraceæ	Neisseria	*N. gonorrhœæ*	Gonococcus
Enterobacteriaceæ	Proteus	*P. vulgaris*	
	Escherichia	*E. coli*	*Bact. coli*
	Klebsiella	*Kl. pneumoniæ*	*Bact. friedländeri*
	Salmonella	*Salm. typhi*	*Eberthella typhosa*
		Salm. paratyphi B	
	Shigella	*Sh. flexneri*	*B. dysenteriæ Flexner*
Pseudomonadaceæ	Pseudomonas	*Ps. pyocyanea*	*B. pyocyaneus, Ps. æruginosa*
Spirillaceæ	Vibrio	*V. choleræ*	
	Spirillum	*Sp. minus*	
Brucellaceæ	Hæmophilus	*H. influenzæ*	Pfeiffer's bacillus
	Bordetella	*Bord. pertussis*	*H. pertussis*
	Brucella	*Br. melitensis*	
	Pasteurella	*Past. pestis*	*B. pestis*
Bacillaceæ	Bacillus	*B. anthracis*	
	Clostridium	*Cl. welchii*	
Order Actinomycetales			
Actinomycetaceæ	Actinobacillus	*Actinobac. lignieresi*	
	Actinomyces	*Actinomyc. bovis*	
	Erysipelothrix	*Ery. rhusiopathiæ*	
Mycobacteriaceæ	Mycobacterium	*Myco. tuberculosis*	*B. tuberculosis*, Koch's bacillus
	Corynebacterium	*C. diphtheriæ*	*B. diphtheriæ*, Klebs-Loeffler bacillus
	Fusiformis	*F. necrophorus*	
	Pfeifferella	*Pf. mallei*	
Order Spirochætales			
Spirochætaceæ	Treponema	*Trep. pallidum*	*Spirochæta pallida*
		Trep. recurrentis	*Borrelia recurrentis*
	Leptospira	*Lepto. ictero-hæmorrhagiæ*	

CONTENTS

Chapter 1

THE GENERAL PROPERTIES OF BACTERIA

Classification

THE bacteria do not fit into the rigid classifications of either the botanist or the zoologist: indeed, it is not finally agreed to whose province they belong. Certain forms exhibit botanical characters and appear to be most nearly related to the fungi; others have features resembling the protozoa. The earliest classifications were purely morphological, and many of the names then introduced are still used for their descriptive value.

Cocci are spherical bodies with an average diameter of 1 μ (1/1,000 mm.), and are named according to their method of division. Cocci which divide in one axis only and adhere to one another to form a chain are known as *streptococci* (Fig. 19); cocci which divide irregularly and adhere to one another giving rise to a cluster comparable to a bunch of grapes are known as *staphylococci* (Fig. 18); cocci in which the cells tend to be arranged uniformly in pairs are known as *diplococci* (Fig. 20); cocci which divide regularly in two planes at right angles to one another result in collections of four organisms, and are known as *tetrads* or *tetracocci* (Fig. 21); others which divide in three planes at right angles forming cubes of eight or multiples of eight are known as *sarcinæ*: the latter are usually larger than 1 μ.

Bacilli are straight, or only slightly curved, rod-shaped organisms. They vary considerably in length, but are usually about 1 μ broad. Many bacilli are motile from the presence of one or more flagella (p. 2). In some species spore formation occurs. Occasionally true branching is found, and such organisms form a connecting link between the lower and higher bacteria.

Vibrios or **Spirilla** consist of definitely curved rods, often described as of " comma shape." They are usually actively motile (Fig. 28).

Spirochætes consist of thin, flexible filaments twisted into a spiral shape (Fig. 45). They are actively motile in either direction (having no " head " or " tail ") but have no flagella. They are in many respects nearer than other bacteria to the protozoa.

Viruses and **Rickettsiæ** are minute organisms which can multiply only within living cells. They are too small to be observed by the

microscope, ranging from about 10 mμ to 300 mμ in diameter (1 mμ = 1/1,000,000 mm.).

The modern classification of bacteria is more expedient than logical. It takes account of the biochemical properties, antigenic structure and ecological relationships of bacteria, as well as of their morphology. Each organism has a generic and specific name, the first distinguished by a capital letter and both written in italics, *e.g.*, *Pasteurella pestis*. More colloquial terms, such as the plague bacillus for this example, are quite legitimate.

The Structure of Bacteria

Some 70 or 80 per cent of the bacterial substance consists of water, and the remainder is composed of protein, carbohydrates, lipoids and waxes with a high proportion of ribonucleic acid. There is a great variation in the relative proportions of these constituents, not only between species but also between cultures of the same strain grown on different media. The protoplasm of bacteria is enclosed in a relatively rigid *cell wall* consisting of a muramic acid-peptide base overlaid by a complex of proteins, lipids and polysaccharides (the bacterial surface antigens). Like other cell boundaries the bacterial wall plays an active part in maintaining the organism's internal environment and in the absorption of metabolites. Bacteria can sometimes exist without their cell walls as so-called L-forms (p. 3). It is possible by special techniques to demonstrate in at least some bacteria a discrete *nuclear apparatus* which divides before the rest of the cell and transmits genetic characters to the daughter-cells. Granules of glycogen, starch, or lipoids occur in many species. *Volutin* granules are characteristic of certain genera such as *Corynebacterium*: their high nucleoprotein content causes them to stain a reddish purple with such dyes as polychrome methylene blue—the so-called *metachromatic staining* (p. 214). The cytoplasm of the *Mycobacteria* contains a large amount of a waxy material which imparts characteristic staining properties (p. 229).

Flagella. Motile bacteria possess delicate, hair-like elongations of their cytoplasm known as flagella which are demonstrable only by special techniques (p. 51). *Monotrichate* organisms have a single terminal flagellum, *amphitrichate* organisms a terminal flagellum at each pole, *peritrichate* organisms have many flagella disposed all round the bacterial body, and *lopotrichate* organisms have tufts of flagella at one or both ends. Bacterial flagella are usually regarded as true motile organs analogous to those of protozoa. Nevertheless, certain experimental observations suggest

that bacteria move by gyratory undulations, and that flagella are no more than remnants of the " slime layer " of the bacterial wall which has become tattered by the friction of movement. Spirochætes certainly move in this way, and not by flagella.

Capsules. Many species of bacteria have a definite outer envelope which appears in stained or unstained preparations as a clear zone around the bacterial cell (Fig. 20, p. 159). This capsule can be stained by appropriate methods (p. 50). The capsular material is probably a secretion of the organism rather than a thickening of the cell-membrane; it is usually composed of complex polysaccharides but sometimes of polypeptides.

Capsules are most marked when bacteria grow in animal tissues and they develop prominently in media with a high protein content. They exert a definite protective function both as a mechanical hindrance to the defence mechanisms of the animal body and as a covering to the more susceptible bacterial antigens (p. 94).

Spores. Bacteria of the genera *Bacillus* and *Clostridium* can form spores when conditions are unsuitable for multiplication. Bacterial spores are concerned with survival, not reproduction; they constitute a resting phase which is highly resistant to heat, drying and chemicals. They may even survive on stained slides. A spore is formed within the body of a bacterium by the collection of the protoplasm into a compact mass, usually round or oval in shape; around this mass is formed an extremely resistant capsule. When circumstances are again favourable for bacterial multiplication the spore capsule ruptures, and the organism resumes its bacillary form. Spores may be spherical or oval in shape and central, terminal, or subterminal in position. They may be of the same diameter as the bacterial cell or larger. They are difficult to stain (p. 50).

Involution Forms. Grotesque and distorted forms of bacteria are common in old cultures when many organisms are degenerate and dying. Involution forms are sometimes, as in the case of the plague bacillus (p. 278), characteristic of the species.

L-forms. Abnormal forms appear when bacteria are exposed to inhibitory agents, especially penicillin. The bacteria first swell into large bodies which may either revert to the original bacterial form, or may give rise to strands of small granules from 0·2 μ upwards in diameter. The small forms usually reproduce themselves as such on solid media (the colonies are likewise minute), but in broth they usually revert to the original morphology. Sometimes, as in certain *Salmonellæ*, the change to small forms is irreversible. The abnormal forms, both small and large, are

apparently bacteria which have lost their cell walls. Thus they are resistant to penicillin, which acts on the cell wall (p. 127), but they have the same sensitivity to other antibiotics and the same antigenic composition as the parent bacteria.

Variation in Micro-Organisms

Spontaneous mutations occur in bacteria and viruses with much the same frequency as in higher organisms. The new variants quickly outgrow the parent strain if they have any substantial advantage in their particular environment. Thus the tubercle bacillus is normally inhibited by streptomycin (p. 233), but readily gives rise to streptomycin-resistant mutants, which often replace the original strain in patients under treatment with this antibiotic. Among the more important mutations, which are described later, are loss of pathogenicity in artificial culture (p. 62), variations of virulence in epidemics (p. 63), and the development of resistance to chemotherapeutic agents and antibiotics (p. 128).

The cultural environment of an organism can be deliberately designed to favour the growth of a particular mutant. If, for example, penicillin be added to the medium, only resistant mutants will grow. One may speak colloquially of " training " a culture to grow under the stipulated new conditions, but it must be understood that the new characters arise by chance mutation and not by any influence of environment on heredity.

Genetic Recombination. The inheritable characters of bacteria are carried by discrete genes, each with a definite locus within the bacterium. Bacteria have no sexual mode of reproduction for the regular transfer and recombination of genes, but in exceptional instances there can be a partial transfer of genetic material from one organism to another.

Conjugation. When certain strains of *Escherichia* (or of a few other genera) are mixed together, a few members of one strain will pair with members of the other. During this period of contact genetic material passes from one organism to the other, the donor's genes displacing some of the acceptor's. A new genetic race of bacilli is thus produced, with characters derived from both parents.

Transformation. In some strains of pneumococci, *Neisseria*, *Hæmophilus* and few other genera, genetically active desoxyribonucleic acid (DNA) escapes into the culture medium. Strains lacking this genetic material may absorb the DNA from solution; thus avirulent pneumococci can be made virulent by the DNA secreted by virulent strains (p. 161).

Phage Transfers. There are a few instances in which a phage acts as a gene, conferring definite characteristics on bacteria infected by it. Thus an appropriate infection of non-pathogenic diphtheria bacilli may

turn them into toxin-producing strains (p. 408). If the phage infection dies out, toxigenicity is lost. In other instances, a phage may incorporate some of the host's genes in its substance and carry them to a new host (*transduction*, p. 408). In this case the genes enter permanently into the new host's genome, and remain even when the phage is lost.

The Multiplication of Bacteria

Bacteria divide by simple binary fission. Cocci may divide in any diameter (p. 1) whereas bacilli always divide transversely and never longitudinally. Daughter-cells may remain attached one to another by the incompletely divided cell-membrane after division, giving rise to such characteristic aggregates of bacteria as staphylococci and the " Chinese letters " of diphtheria bacilli.

Some authorities have described complex *life-cycles* for various bacteria, usually including filter-passing forms. Their views are not, however, generally accepted.

The Phases of Growth. When a culture medium is inoculated with bacteria their number remains constant for a few hours, then rapidly increases in geometric progression for a few more hours, and finally reaches a nearly constant level. These phases of growth are known respectively as the lag, logarithmic and stationary phases. The proportion of dead bacteria in the culture remains nearly constant during all these phases, but then increases steadily until, after a period ranging from a day or so to many weeks, no more viable organisms can be found.

The Lag Phase. The lag phase is a period of intense metabolic activity, during which the individual bacteria increase greatly in size even though they do not divide. They seem to undergo a process of rejuvenescence, losing some of their resistance to such inimical agents as heat and disinfectants and at the same time acquire the power of rapid multiplication. Old bacteria may require a period of adjustment in the new medium before they show the increased activity characteristic of the later lag phase.

The Logarithmic Phase. After the lag phase the bacteria as they grow begin to divide rapidly, usually some three times every hour. The number increases in geometric progression, one organism becoming successively 2, 4, 8, 16, 32. . . . When the logarithm of the number is plotted against time the result is a straight line, hence the name of the phase. This ability to divide rapidly is a property of the bacteria, not of the medium, for if organisms in the logarithmic phase are transferred to a new medium they continue to divide without any lag phase.

The Stationary Phase. After the logarithmic phase the number of living bacteria in the medium remains constant for hours or days, the rate of formation of new bacteria equalling the rate of dying. The medium can support no more than a given density of living organisms, the maximum at which the oxygen supply is adequate for growth. The nutritive properties of the medium are not exhausted nor, except in a few special cases, do the bacteria produce toxic metabolites, for if the medium be freed from bacteria it will again support growth to the same level.

Factors Influencing Bacterial Growth

The cultivation of bacteria on artificial culture media is fundamental to their study in the laboratory. A proper understanding of nutritive requirements and other factors influencing the growth of bacteria is therefore of the greatest importance.

Food Supply. Water forms the greater part of bacteria and is essential for their growth, as well as being the vehicle by which soluble foodstuffs are enabled to diffuse through the cell membrane. The simplest, *autotrophic*, organisms can synthesize their protoplasm from CO_2, ammonia, and the mineral salts of the soil. The pathogenic bacteria, however, are much poorer in enzyme systems, perhaps because they have come to depend on those of their hosts, and can multiply only when they are supplied with amino-acids. These latter often supply carbon and nitrogen as well as radicles which the organism needs but cannot synthesize. Much detailed work has been done on this subject, but it has served rather to emphasize the diversity of the essential nutrients of different species rather than to establish any general principles. The media commonly used in practice (Chapter 4) are a complex mixture of protein substances derived at one or two removes from animal sources.

Accessory Growth Factors. Minute traces of a variety of substances, including metallic ions, certain amino-acids, and the animal vitamins are necessary for the growth of bacteria. Most of these accessory growth factors seem to be necessary for the function or construction of enzyme-co-enzyme systems. The animal vitamins can be assayed by observing their effect on suitable bacteria in a defined medium, but the exclusion of other growth-promoting principles from the material under test is a matter of considerable technical difficulty.

Atmosphere. Some bacteria are able to flourish only in an atmosphere containing a free supply of oxygen, and are known as *obligatory aerobes*. Others are unable to grow in the presence of free oxygen, and are known as *obligatory anaerobes*. The majority of bacteria, though preferring an atmosphere containing oxygen, are yet able to grow, perhaps with difficulty, under anaerobic conditions (*facultative anaerobes*). Many aerobes and facultative anaerobes give a more luxuriant growth when the oxygen tension is somewhat lower than that of the atmospheric (*microaerophiles*). Certain bacteria such as the gonococcus and *Br. abortus* require 5 to 10 per cent of CO_2 in the atmosphere for growth when first isolated.

Reaction. The majority of bacteria prefer a slightly alkaline medium of pH 7·2–7·6, but most pathogenic species can grow between pH 5·0 and pH 8·0. An acid reaction is particularly liable to stop growth, and carbohydrate-fermenting bacteria may produce enough acid in culture to kill themselves. The *Lactobacilli* (p. 176) are very tolerant of acid and will even grow at pH 4·0, whereas, at the other extreme, the cholera vibrio (p. 207) will grow at pH 9·6.

Temperature. Each species of bacterium grows best at an optimal temperature which usually approximates to that of its natural habitat. Thus the human pathogens grow best at about 37° C. although growth is usually possible from 15° to 40° C. Low temperatures merely inhibit the growth of an organism, whereas temperatures above the optimum may first change its properties, particularly its pathogenicity, and then kill it. Certain bacteria, known as *thermophiles*, have optimal temperatures of 55° C. or higher, and may even grow at 75° C. Such organisms are a source of difficulty in pasteurization (p. 12) and similar processes. At the other extreme bacteria may be found which grow well at 0° C. Sterilization by heat is considered on pp. 10–18.

Biochemical Activities

Bacteria, like other living cells, obtain their energy by breaking down relatively complex compounds into simpler substances. The process depends on respiratory enzyme systems similar to those of the animal cell, with hydrogen transport as the fundamental mechanism. The side-results of these metabolic processes are of great practical importance both for the identification of bacteria and for the commercial production of such substances as alcohol and vinegar.

Fermentation of Carbohydrates. Most bacteria of medical interest are able to break down certain carbohydrates or related alcohols into simpler organic compounds, carbon dioxide and hydrogen. The ability to ferment a particular sugar is often a very constant property of a bacterial species so that related organisms can often be differentiated by observing their action on a judicious selection of carbohydrates and hexahydric alcohols. The fermentation produces *acid*, which is detected by incorporating an indicator in the medium, and sometimes *gas* (chiefly CO_2 and H_2) which is collected in an inverted Durham's tube (p. 34). The student of physiology will be familiar with the mechanism of the anaerobic dissimilation of glucose in muscle. Bacteria likewise split sugars by this means as well as by aerobic mechanisms.

Bacterial fermentation is used commercially for the manufacture of alcohol, lactic acid, vinegar, and many other organic chemicals, as well as for the production of foodstuffs such as cheese. (The holes in Gruyère cheese are gas bubbles produced by fermentation.)

Proteolysis. The familiar process of putrefaction is due to the degradation of protein by bacterial activity. Many bacteria have no proteolytic activity and even those, mostly anaerobes, which are proteolytic can attack the more complex proteins only if simpler compounds such as amino-acids are present to assist their metabolism. A complex system of proteolytic enzymes splits protein molecules into poly- and di-peptides and eventually into amino-acids, with H_2S, CO_2, H_2 and indole as by-products. The amino-acids may be split further, even down to NH_3, CO_2, H_2 and CH_4. The foulest odours of putrefaction are due to mercaptans produced by anaerobic proteolysis.

The demonstration of proteolytic activity may be an aid to the identification of bacteria. It is most clearly shown by the liquefaction of gelatin or coagulated serum, but the digestion of meat or milk, or the production of H_2S or indole may also be used to indicate proteolysis.

Lipases. Many bacteria display lipolytic activity. Some are used for making butter and cheese; others make foodstuffs rancid.

Pigment Production. A few species of bacteria may be readily identified by the brightly coloured pigment they produce. The pigment, as in *Staph. aureus* and the *Chromobacteria*, is usually intracellular, but it sometimes diffuses into the medium, as in the case of *Pseudomonas pyocyanea* (p. 178).

Other Activities of a Chemical Nature. The medical bacteriologist inevitably tends to regard bacteria as inimical to life, yet none of the higher plants or animals could survive without their assistance. The decay of plants and animals, which returns their elements to the soil, is effected by bacteria, and the autotrophic bacteria finally reduce the degradation products of proteins to forms assimilable by plants.

The Nitrogen Cycle. At least one genus of bacteria, the *Azotobacter*, can obtain nitrogen for synthesis of protoplasm directly from the air. Energy is obtained by the aerobic degradation of carbohydrates, derived in the soil from decayed plants. A species of *Clostridium* can similarly fix atmospheric nitrogen under the anaerobic conditions which obtain in swampy soils. Bacteria of the genus *Rhizobium*, which live in symbiotic association with the roots of leguminous plants and supply them with nitrogen from the air, are of great agricultural importance. The end-point of protein decay is ammonia, and certain soil bacteria use this substance as their source of energy, oxidizing it to nitrates (*nitrification*) or nitrites (*nitrosification*), which in their turn are utilized by plants. The reverse process of *denitrification* is effected by other bacteria which use nitrates or nitrites as hydrogen receptors, reducing

them to ammonia and thus perhaps impairing the fertility of the soil.

The Sulphur Cycle. The decay of the sulphur-containing moiety of protein terminates in reduced compounds such as H_2S and mercaptans, whereas plants can utilize sulphur only as the highly oxidized sulphates. The colourless sulphur bacteria of the genus *Thiobacteriales* use H_2S as a source of energy, oxidizing it to sulphur and the sulphur to sulphates. Other species of this genus contain a green pigment, *bacteriochlorophyll*, which effects the photosynthesis of CO_2 to carbohydrates. H_2S or organic compounds containing the $-SH$ radicle are used as hydrogen donors, just as H_2O is used in the photosynthesis of plants. The sulphur formed by the reaction is eventually oxidized to sulphates, but is often first stored in the bacteria as a food reserve. Members of the genus *Thiobacillus* obtain their energy by the oxidation of thiosulphates, H_2S, or sulphur, with sulphates as the terminal product. Free sulphur is often liberated in the surrounding medium, but is never stored within the bacilli. The formation of sulphuric acid by these bacteria in the soil may be of great importance in freeing phosphoric acid from insoluble calcium phosphates.

Other Soil Bacteria. Bacteria living in swampy soils, where organic decomposition is vigorous, often obtain their energy by strange means. Some oxidize hydrogen, some carbon monoxide, and some methane. Certain species can even oxidize petroleum products to obtain carbon and energy.

Iron Bacteria. Certain higher bacteria, the *Chlamydobacteriales*, enclose themselves in a mucilaginous sheath. Most are found in water rich in organic iron compounds (derived from decaying plants) which they break down, depositing the iron in the sheath. Here the iron is oxidized to ferric hydroxide and the organism is eventually so thickly enveloped in " rust " that it can no longer reproduce itself. The thick, ferruginous, slime formed by these bacteria may be a source of great embarrassment to water engineers.

Photogenesis. Several species of bacteria, mostly marine forms, emit light. They are responsible for the phosphorescence of certain fish.

Chapter 2

STERILIZATION

THE technique of sterilization is fundamental to the study and control of bacterial disease. In the laboratory all apparatus and media must be freed from contaminating organisms, which abound everywhere, before pathogenic bacteria can be isolated and identified. In the operating theatre and wards sterilization of instruments and dressings is the basis of the aseptic technique that has made modern surgery possible. The control of communicable disease depends in part upon killing the infecting organisms before they can spread outwards from the patient to infect others.

Sterilization by Heat

Effect of Heat on Bacteria. Bacteria demand an optimal temperature to grow at their best (p. 7) but a much higher temperature kills them by coagulating their protein. Different species vary greatly in their susceptibility—the gonococcus, for example, dies within a few minutes at 47° C., whereas fæcal streptococci resist 60° C. for 30 minutes and non-pathogenic thermophilic bacteria may actually grow at 75° C. Old bacteria are somewhat more resistant to heat than young, actively-dividing forms; and spores are vastly more resistant than either. Most spores can survive in boiling water for a few minutes and some will live for almost 24 hours.

The rate of sterilization may be greatly affected by relatively small changes in temperature. Thus a suspension containing 100,000 typhoid bacilli per ml. is sterilized in 2 hours at 47° C., in 18 minutes at 51° C., in 2½ minutes at 55° C. and in 21 seconds at 59° C., assuming these temperatures to be immediately attained. The effect of a given temperature is greatly affected by the immediate environment of the individual bacteria—hot water is more effective than hot air, death is quicker in slightly alkaline media than in neutral, and quicker still in acid media.

Dry Heat. Dry heat is a relatively inefficient method of sterilization, for air and many materials are bad conductors of heat, and the less its water content, the less readily is protein coagulated.

Flaming. Some incombustible laboratory apparatus, such as a wire loop, is sterilized immediately before use by being passed

through the Bunsen flame. Only surfaces actually touched by the flame are sterilized.

The Hot-Air Oven. Dry heat is now little used except for the sterilization of all-glass syringes and laboratory glassware. A temperature of 160° C. for 1 hour is necessary to kill spores (the accepted criterion of efficient sterilization). The oven consists fundamentally of a cabinet insulated against heat loss with

Table I. TIMES AND TEMPERATURES FOR STERILIZATION

	Temp.	Time
Hot-air oven	120° C.	4 hours
	160° C.	60 minutes
Radiant heat	180° C.	8 minutes
Boiling water	100° C.	20 minutes[1]
Steamer	100° C.	90 minutes
Steam pressure sterilizer: 15 lb. per sq. in.	121° C.	15 minutes
20 lb. per sq. in.	126° C.	10 minutes
30 lb. per sq. in.	134° C.	3 minutes

The times are those needed to kill spores after the indicated temperature is attained.

[1] Many non-pathogenic spores survives 20 minutes' boiling.

electricity or gas as a thermostatically controlled source of heat. It is important, but difficult, to ensure that the air temperature is uniform throughout the oven. The air itself is a bad conductor of heat, and a little cooling greatly increases its density. " Cold pockets " can thus readily persist at the bottom of the oven and in sheltered corners. Such effects are minimized by heating all walls to a uniform temperature, but a fan must often be installed to ensure thorough mixing of the air.

It should be observed that the time required to heat up the oven and its contents, often an hour or more, must be added to the period of sterilization (one hour at 160° C.). After this, the oven must be allowed to cool slowly with its door closed, otherwise glassware may crack.

Radiant Heat. Infra-red rays provide a highly penetrating and quickly acting source of heat. Glass syringes can be sterilized by passing through such a source on a conveyor belt; an exposure of 10 minutes to a temperature of 180° C. is generally used.

Moist Heat. Hot water kills spores and bacteria much more quickly than dry heat at a given temperature, largely because such chemical processes as active hydrolysis of protein are added